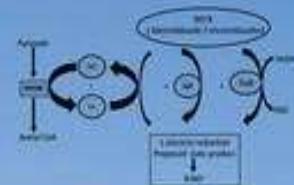


# A CENTURY OF PARASITOLOGY



Systematics and Diversity



Host Parasite Interactions



Ecology and Life History

Discoveries, ideas and lessons learned by scientists who published in *The Journal of Parasitology*, 1914-2014

EDITORS

**JOHN JANOVY, JR. AND GERALD W. ESCH**

**WILEY**



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Figure 2 Robert Hegner. In early mid-career (ca. 1920) at the Marine Biological Laboratory, Woods Hole, Mass. (left, from the Copeland/Bloom photograph album, History and Philosophy of Science Repository, URI: <http://hdl.handle.net/10776/3270>, Licensed as Creative Commons) and 16 years later as President of the American Society of Parasitologists (right, from Hegner, R. W. 1937. Parasite reactions to host modifications. *Journal of Parasitology* **23**: 1–12). Reproduced with permission of Allen Press Publishing Services.

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A century (1914–2014) of studies on marine fish parasites published in The Journal of Parasitology

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An overview of the history and advances in the population ecology of parasites

Figure 1 Schematic representation of the gains and losses in numbers of individuals for a direct life-cycle macroparasite (*P*) in a hypothetical host (*H*). Gains and losses in a hypothetical parasite population are defined by parasite birth rate ( $\lambda$ ), parasite death rate ( $\mu$ ), transmission rate ( $\beta$ ) and by the death of hosts due to infection ( $\alpha$ ) and other causes ( $b$ ). (Figure courtesy of Lori Goater, from Goater, Goater & Esch 2014. Reproduced with permission of Cambridge University Press.)

Figure 2 Relationship between parasite intensity and total body lipid (a), and parasite intensity and survival following onset of dropping water temperature (b) for bluegill (*Lepomis macrochirus*) from Reed's Pond, November 1981 through October 1982. Data are for fish held in outdoor aquaria at ambient temperature or in live boxes in the littoral zone. All fish were naturally infected with *U. ambloplitis*. The dotted line indicates the maximum intensity observed for bluegill that overwintered successfully in Reed's Pond. (Reprinted from Lemly and Esch (1984); Reproduced with permission of Allen Press Publishing Services.)

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History of microevolutionary thought in parasitology: The integration of molecular population genetics

Figure 1 I conducted a Web of Science search on the terms “microsatellite” and either “fish” or “parasite” on March 6, 2014. The search was for each year separately. Extreme caution is advised in strictly interpreting results. All papers may not actually be population genetic studies or may not be using microsatellites in the respective organisms. Indeed, I know the three papers in 1995 under “parasite” are not studies on the population genetics of parasites two are not even on parasites. The Figure is mainly of exploratory value and, while the exact numbers are incorrect, I suspect an approximate 10-year lag would be present even if proper scientific scrutiny were used. Keep in mind the search was only for one group of vertebrates as no one uses “free-living” as a keyword. Inclusion of other vertebrates would only increase the discrepancy. Thus, if anything, I suspect the Figure gives a gross underestimate of parasites relative to free-living animals. I

was not able to do a similar analysis for allozymes as Web of Science does not have abstracts or author provided keywords for most papers prior to 1995; thus, word searches will be less inclusive based on titles alone.

The worm's eye view of community ecology

Figure 1 Degree of infection of an individual fish. Redrawn from Figure 1 of Cross (1934) Journal of Parasitology 20: 244–245. Reproduced with permission of Allen Press Publishing Services.

Figure 2 Effects of concurrent infection on the intraintestinal distribution of *Hymenolepis diminuta* and *Moniliformis dubius*. Light bars are single infections; dark bars are concurrent infections. Redrawn from Figure 1 of Holmes (1961) Journal of Parasitology 47: 209–216. Reproduced with permission of Allen Press Publishing Services.

Figure 3 Parasite community assembly is influenced by processes operating at a range of spatial and temporal scales. Parasite species are found within a regional species pool that is constrained by evolutionary processes. A subset of the species from the regional pool will colonize a particular site depending on dispersal and exposure probability. This, in essence, suggests that the observed parasite community within a host is the result of infective stages passing through abiotic and biotic filters. Modified from Hille Ris Lambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. Ann. Rev. Ecol. Evol. Syst., 43: 227–248.

The iron wheel of parasite life cycles: Then and now!

Figure 1 Life cycle of *Haematoloechus parvplexus* (=*Pneumonoecus parvplexus*). (A) 32-day-old adult *H. parvplexus* from the lungs of the bullfrog, *Rana catesbeiana*. (B) Bullfrog, showing the escape of the metacercariae from the dragonfly in the stomach and their migration to the lungs; adult worms depositing eggs and the route of the eggs to the external environment. (C) Egg. (D) *Gyraulus parvus* eating eggs; releasing cercariae. (E) Sporocyst from the digestive gland of *Gyraulus parvus* with cercariae in various stages of development. (F) Cercaria showing body, tail, stylet, oral sucker, pharynx and ceca, ventral sucker, and excretory bladder. (G) Larva of the eastern pondhawk dragonfly, *Erythemis simplicicollis*, showing the swimming cercariae being taken into the branchial basket respiratory organ of the larva. (H) Lamella from the branchial basket of eastern pondhawk dragonfly larva containing two encysted metacercariae. (I) Teneral eastern pondhawk dragonfly with encysted metacercariae within the vestige of the branchial basket of the larva. (J) Encysted metacercaria. Drawings not to scale. All drawings are original but modified after Krull (1930).

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host life cycle using a tadpole as a transport host. This transmission strategy is used to infect adult bullfrogs. Adapted from Bolek, Snyder & Janovy, Jr (2009a). Reproduced with permission of Journal of Parasitology, Allen Press Publishing Services.

Transmission of *Toxoplasma gondii*—From land to sea, a personal perspective

Figure 1 Sugar fecal float of cat feces showing unsporulated oocysts of *T. gondii* (arrows), *Cystoisospora (Isospora) felis* (f), and *C. rivolta* (r).  
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Giardia intestinalis biochemistry and regulation: An evolutionary tale

Figure 1 Giardia glucose metabolism in trophozoites. 1. hexokinase; 2. glucose phosphate isomerase; 3. pyrophosphate-dependent phosphofructokinase; 4. fructose biphosphate aldolase; 5. glycolytic enzymes- (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase); 6. pyruvate kinase; 7. Pyruvate: orthophosphate dikinase; 8. Phosphoenolpyruvate carboxyphosphotransferase (GTP dependent); 9. aspartate aminotransferase; 10. malate dehydrogenase; 11. malate dehydrogenase (decarboxylating); 12. Alanine aminotransferase; 13. pyruvate: ferredoxin oxidoreductase; 14. acetyl-CoA synthetase; 15. primary alcohol dehydrogenase (NAD); 16. hydrogenase. Abbreviations used: oxidized ferredoxin (Fdo), reduced ferredoxin (Fdr)

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Figure 4 Giardan synthesis pathway. 1. glucosamine 6-P deaminase; 2. glucosamine 6-P N-acetylase; 3. phosphoacetylglucosamine mutase; 4. and UDP-N-acetylglucosamine pyrophosphorylase; 5. UDP-N-

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Figure 6 Graphic representation of alanine, ornithine and acetate concentrations from *Giardia* trophozoites metabolism as determined by GC-MS/MS analysis of growth medium after 8hr incubation. Each data point was compiled from a minimum of five independent replicates. Each spot represents one isolate and the two spots circled are results obtained from two metronidazole resistant isolates (determined by MIC). The data from these two are significantly different ( $P = 0.01$ ). Alanine and acetate are products of glucose fermentation in *Giardia* and ornithine is produced via the arginine dihydrolase pathway. Values presented are in nmol h<sup>-1</sup> 10<sup>-6</sup> cells.

The early history of immunoparasitology in the United States

Figure 1 (A) Emil von Behring, (B) Paul Ehrlich, (C) Ilya Mechnikov, (D) George Nuttall, and (E) Almroth Wright.

Figure 2 Robert Hegner (*Journal of Parasitology* 23:1 (1937). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Figure 3 William Walter Cort (*Journal of Parasitology* 39:4 (1953). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Figure 4 Norman R. Stoll (*Journal of Parasitology* 33:1 (1947). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Figure 5 William H. Taliaferro (*Journal of Parasitology* 20.3 (1934). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Figure 6 Asa C. Chandler. (Courtesy of Woodson Research Center Fondren Library, Rice University. Reproduced with permission.)

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Tick paralysis: Some host and tick perspectives

Table 1 Tick species reported to cause tick paralysis.\*



# **A Century of Parasitology**

**Discoveries, Ideas and Lessons Learned by Scientists  
Who Published in *The Journal of Parasitology*, 1914–  
2014**

**EDITED BY**

**John Janovy, Jr and Gerald W. Esch**

**WILEY**

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As co-Editors, we are honored to dedicate this book to the memory of our mentor, Professor J. Teague Self, a long-time member of the Department of Zoology, University of Oklahoma. We were privileged to secure our M.S. and Ph.D. degrees with the guidance of this man. Yes, he taught us a lot about parasitism and parasitology. But, one of our favorite memories was the real excitement of attending our annual ASP meetings because we knew Dr. Self would offer to introduce us to our heroes, e.g., people like Ray Cable, Norman Stoll, William Trager, Clark Read, Theodore von Brand, and so many others of their generation. It was always such a huge treat!

We also agree that his wisdom in dealing with people and his consistency as a professional were equally important to whatever success we have had over the past 50+ years. He was a true "giant" in our careers, and we thank him for helping us to focus in the right direction.

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## Preface

The idea for this book, as well as for the reviews published in Volume 100 of *The Journal of Parasitology* (2014), originated with Gerald Esch, editor of the Journal for 20 years. Dr. Esch picked a group of potential authors and subjects, a list that expanded and contracted over a period of several months as we contacted individuals, answered their questions, and at times twisted their arms (gently). He envisioned a celebration of the long shelf life and intellectual breadth of this journal, along with the rich history of parasitology, especially as manifest by American parasitologists and their colleagues from around the world. An initial gathering of potential authors was held in 2013 in Quebec City, during the annual American Society of Parasitologists meeting. There was considerable discussion about the scope of such an edited volume, and during the next few months there were extensive communications between authors and editors on a variety of topics. Dr. Esch initiated discussions with Ward Cooper, Commissioning Editor at Wiley, and eventually those talks led to a draft contract for the project. That's when the real work started.

Both of us express our deepest appreciation to the parasitologists who contributed to this book and who put up with the requests involved. Everyone associated with this project has learned quite a bit about the history of parasitology, as well as the history of ideas, the manner in which technology has shaped research in our discipline, and how research experiences lead to life lessons. Thus after reading the initial contributions, we decided to ask authors for comments on lessons learned, not only from their own work, but from an examination of history. The 2014 ASP President's Symposium in New Orleans consisted of papers delivered by the authors, and their chapters are elaborations of those talks.

All of the contributed chapters except the last one by Timothy Geary, Judy Sakanari, and Conor Caffrey are preceded by what the authors believed to be the first paper published in *The Journal of Parasitology* (*JP*) in their particular subject areas. We were all impressed with the insight, and sometimes foresight, of these parasitologists, some whose work appeared in Volume 1. Because *JP* is such a visually rich publication, we also looked for some representative first figures, for example, the first drawing of a new species, or the first transmission electron micrograph, and have included a number of those figures along with their original legends and some commentary.

We would also like to thank Mike Sukhdeo and Vickie Hennings for help with material derived from *The Journal of Parasitology*, and in particular the chapter entitled "Antihelmintic drug discovery: Into the future," by Timothy Geary, Judy Sakanari, and Conor Caffrey. After reading the initial draft of their manuscript, we both felt it would be an excellent review article in *JP*, so recommended that it be expedited through the publication process. We also felt that this final chapter was such a logical extension of the one by Bill Campbell that it didn't need a "first *JP* paper" beyond the one by Maurice Hall.

While reading through this volume, including the Table of Contents, you may find scientific names that are not italicized in some of the publication titles. In reprinting those original papers from *JP*, we kept the fonts, spelling, and nomenclature exactly as they were published in the journal when we listed those items in the Table of Contents.

The Wiley editor who took over this project from Ward Cooper is Kelvin Matthews. He has been a patient, communicative, and helpful editor and we greatly appreciate his work on this book.

Finally, we would like to thank Talia Everding, an undergraduate at the University of Nebraska-Lincoln, who served as an editorial assistant, reading all the chapters, often more than once, doing research in the back issues, and suggesting numerous ways to clarify the wording. We may have turned her into a parasitologist, in spirit if not in kind, with this assignment!

John Janovy, Jr and Gerald W. Esch



# **Chapter 1**

## **A century of parasitology: 1914–2014**

John Janovy, Jr

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The hundred years between 28 June 1914, when the assassination of Austrian Archduke Franz Ferdinand precipitated World War I, and 27 July 2014, closing day of the American Society of Parasitologists' 89th annual meeting, represent one of the most stressful, complex, yet in some ways wondrous, periods of human history. Samantha Power (2002) called this time the “Age of Genocide;” Albert Einstein's equations completely re-structured our image of the universe; nuclear weapons entered our political negotiations; and, molecular biologists obliterated some of our most cherished views of nature. A decade after the Wright brothers' first sustained flight in a heavier-than-air craft, in December 1903, military airplanes took to the skies over Europe; today, some authors claim that airports are our current versions of the thirteenth-century cathedrals (Binney, 1999). Robert Goddard began his experiments with solid-fueled rockets in 1915 (Lehman, 1963); by 2014, intercontinental ballistic missiles were hiding in silos scattered across the Great Plains of North America, an International Space Station circled Earth, and the first *Apollo* moon landing was a mostly forgotten historical event. Even as we spent the past century obliterating much of Earth's terrestrial biological diversity by clearing tropical forests, satellite telescopes were discovering exo-planets at an increasing rate. In 1914, a successful scientist like H. B. Ward, founder of the American Society of Parasitologists as well as driving force behind a new scientific journal, *The Journal of Parasitology*, could buy a new Royal Model 10 typewriter; a century later we stop teaching cursive to elementary school students largely because kids are communicating via QWERTY keyboards (designed in 1873) on hand-helds with temperature-sensitive screens. Senior citizens today can recite, largely from personal experience, the origin and impact of what we now call the “Information Age.”

The new century in which we live should be an interesting one too, with projected climate change potentially wreaking havoc on coastal ecosystems and human populations expected to (phrased euphemistically) “level off.” Some things have not changed very much, however; parasitic organisms still infect not only humans and our domestic animals, but also, to our knowledge, virtually every eukaryotic species on planet Earth. Despite Ronald Ross' (1902) claim, in his Nobel Prize acceptance speech—“It is my privilege in this lecture to describe particularly the steps by which this great problem has at length received its full solution”—malaria remains one of humanity's most persistent scourges. Schistosomiasis, filariasis, and geohelminth infections still cause untold misery, along with their protistan counterparts such as leishmaniasis and amebiasis, especially in the tropics. But these infectious diseases also have inspired generations of parasitologists to apply their time, talents, and intellectual resources to find cures, or develop control

methods, and thus provide relief from the economic and social burdens caused by parasitic organisms (Kuris, 2012; Loker, 2013).

In their quests to develop treatment and control technologies, parasitologists have indeed produced some major successes over the past century, but in the process they also have made conceptual contributions that might arguably be described as “metaparasitology”—an intellectual realm that includes the “rules” for pursuing the discipline. Although it may not always have been their intent, parasitologists have done research that in turn shapes our *ideas* about interactions between hosts and parasites. Excellent examples, among many, of concepts published very early if not originally in *The Journal of Parasitology*, include molecular mimicry in schistosomes (Damian, 1962, 1964, 1987), the relative immortality of cestodes (Read, 1967), and amphiparatenesis in *Alaria marcianae* as demonstrated by Shoop and Corkum (1987), who then extended the concept to those nematode species known to exhibit developmental arrest and transplacental transmission. With time, sometimes a surprisingly short time in historical terms, these kinds of contributions become principles of parasitism—the most common way of life among animals and animal-like eukaryotes.

Our goal in assembling this volume of contributed chapters is to bring the phenomenon of concept-driven research to the forefront, especially in the minds of younger readers. We also hope to provide historical perspective in the form of lessons learned from both successful and unsuccessful research endeavors. Thus, our contributing authors have been asked to step outside their immediate comfort zones, those places so often constructed and constrained by legitimate demands of proper methodology, statistical analysis, correct identification, and anonymous reviewers, and instead reflect on the historical development of their subjects. To quote from an early memo to our authors:

In most of the correspondence and discussion so far, we've mentioned the hope that these chapters would be heavy on ideas, and that authors would show us how research has inspired further work, how concepts demonstrated by particular papers have served a heuristic role in parasitology, and how historical precedents have been established. Our hope is that this volume will be unique in its role as a demonstration of how parasitologists think about their discipline and sub-disciplines, and how our material provides so many research opportunities yet can be quite uncooperative in sometimes unexpected ways. In the best of all worlds, students read this book, and come away with new ideas about their current research, an expanded view of how parasitologists pursue their careers, and a feeling that their own work, sometimes with obscure organisms that have little economic importance, has the potential to open up new areas of investigation. In other words, we understand that the subject is science, but we encourage all of you to think in terms of the history of science and what we have learned about how to do our science from having done it for years, if not decades.

Nobody needs reminded that mid-career scientists are fully occupied, and that statement certainly applies to the authors who have contributed to this volume. It is true, as Asa Chandler noted in his Presidential address during the 1945 American Society of Parasitologists meeting (Chandler, 1946), that parasitologists are “slow in going to seed,” so even our retired colleagues are busy with projects that consume their time and energies. Therefore, as must be the case for all such ambitious endeavors, this particular volume is not as inclusive as it might have been. But in our defense, after reading the initial chapter drafts, we editors came to the conclusion that it would require a whole shelf of such books to truly do the subject justice. We expect that some of you will take on this future task!

We also hope that this book sells enough copies to generate some net income. By written agreement between the editors and publisher, such “profits” have been assigned to the American Society of Parasitologists for support of *The Journal of Parasitology*, and especially to defray page charges for authors who have accepted papers, but are not in a position to pay costs for longer articles or essential color figures. *The Journal of Parasitology* is indeed an amazing publication with very long shelf life, rather like some of the authors who have published in it. If a student is able to read through a single issue, for example, and both understand and appreciate most of the work reported, that student will be broadly educated in a decidedly empowering manner. So our real dream, beyond some welcome support for authors who publish in the *Journal*, is that these chapters, which are mostly senior scientists' reflections on how our research has been shaped by ideas, will serve as conversation starters for younger scientists.

## Literature cited

- Binney, M. 1999. *Airport builders*. Academy Editions, Chichester, U.K., 223 p.
- Chandler, A. C. 1946. The making of a parasitologist. *Journal of Parasitology* **32**: 213–221.
- Damian, R. T. 1962. A theory of immunoselection for eclipsed antigens of parasites and its implications for the problem of antigenic polymorphism in man. *Journal of Parasitology* **48**: 16.
- \_\_\_\_\_. 1964. Mimicry: Antigen sharing by parasite and host and its consequences. *American Naturalist* **98**: 129–149.
- \_\_\_\_\_. 1987. The exploitation of host immune response by parasites. *Journal of Parasitology* **73**: 1–13.
- Kuris, A. M. 2012. The global burden of human parasites: Who and where are they? How are they transmitted? *Journal of Parasitology* **98**: 1056–1064.
- Lehman, M. 1963. *This high man; The life of Robert H. Goddard*. Farrar, Straus, New York, NY, 430 p.
- Loker, E. S. 2013. This de-wormed world? *Journal of Parasitology* **99**: 933–942.
- Power, S. 2002. “A problem from hell”: *America and the age of genocide*. Basic Books, New York, NY, 610 p.
- Read, C. P. 1967. Longevity of the tapeworm, *Hymenolepis diminuta*. *Journal of Parasitology* **53**: 1055–1056.
- Ross, R. 1902. Researches on malaria. In *Nobel lectures, physiology or medicine, Nobel Foundation*. Elsevier, Amsterdam, The Netherlands, p. 26–116.
- Shoop, W. L., and K. C. Corkum. 1987. Maternal transmission by *Alaria marcianae* and the concept of amphiparatenesis. *Journal of Parasitology* **73**: 110–115.



# **Part I**

## **Systematics and Diversity**



## Chapter 2

# Some New Gregarine Parasites from Arthropoda\*

Minnie Elizabeth Watson

For several years I have been studying a number of gregarines parasitic in various arthropods. The literature apparently contains no record of most of them and consequently they are here described as new species. A few of the species studied were already known, but I am able to give new records on distribution and additional data on biology and life-history. Careful attention was devoted to the biology of the forms studied and extended experiments were conducted on life-history problems. In this paper is presented a brief account of these studies, which will be published in full at a later date. Especial attention is called here to the observations on movement in gregarines and on cyst formation. The descriptions of new species though concise have been worked over so carefully that it is thought they will be ample for accurate determinations.

### BIOLOGICAL OBSERVATIONS

The polycystid gregarines have a septum which divides the cell into two or more distinct compartments, and the species described in this paper are all of this type. Polycystid gregarines inhabit chiefly the mid-intestines and intestinal diverticula of arthropods and are often found in large masses comprising many hundred parasites. In some genera the adult animals are solitary; in others they are attached one behind the other. Most of the latter are biassociative, but a few genera occur in chains of from three to ten or twelve individuals.

The sporonts, or adult animals, move about freely in the lumen of the intestines or lie inert between the lobes. The trophozoites, or young individuals, live either entirely within the epithelial cells, as in the family Stenophoridae, or attached to the free ends of the cells by means of variously shaped epimerites, globular in the genus *Gregarina*. When a trophozoite has absorbed sufficient nourishment from the host cell, either directly through its walls or by means of the epimerite, it breaks forth from the shriveled cell and becomes a sporont, living free in the intestine, and the useless epimerite, if present, is gradually lost. The animal now receives its food entirely by absorption of the digestive juices direct from the host intestine.

Movement of the free individuals takes place by a gradual progression and by bending of any part of the deutomerite. There are two structures necessary for motion. Running crosswise in the outer part of the endocyte is a delicate network of fibrillae, the myonemes, sometimes seen with a rather low power in individuals nearly devoid of protoplasm. In the outer portion of the epicyte, the outside layer of the body, there are found very fine longitudinal striations visible only with an oil-immersion lens. In the furrows between these striations there are minute pores (Schewiakoff, 1894) through which a gelatinous material is exuded. The animal progresses by contracting a few myonemes on that side of the body which happens to be ventral and this causes a minute undulatory motion against the slide. At the same time the animal secretes mucus, which enables it to move forward against friction, much as a slug moves forward by a wave-like motion on its ventral side. The trail of mucus left on the slide is the now useless material which has gradually been pushed backward through the longitudinal furrows by the progressing animal. Bending movement is effected by a contraction and expansion of the myonemes in any part of the deutomerite.

As the beginning of cyst-formation, two individuals, either associative or solitary, commence to revolve in a large circle. If the animals are solitary, an individual is drawn into the vortex of one which has started to revolve alone. If of a biassociative type, the two commence to revolve together. The spiral gradually becomes smaller as they continue in motion and the animals come to lie in contact laterally. Motion still continues and becomes rotary. As the two sporonts are forming a compact sphere, a thick, transparent covering is being laid down on the outside of the cyst which consists of as many thin layers of gelatinous material exuded from the posterior end of the moving animals as there are rotations before the animals come to rest. The fully formed cyst, sometimes still rotating, now passes from the mid-intestine of the host to the rectum and is given out with the feces.

Finding suitable moisture, the cyst develops within from 24 to about 48 hours with the formation and growth in many genera of as many as fourteen enormously long spore ducts. Each sporont breaks up into gametes, the gametes from one sporont uniting when fully formed with those from the other to form zygotes; and when the resultant spores have become mature they are forced out violently through the spore ducts into the surrounding medium. They become scattered and if accidentally eaten by an insect of the same species as the host, the outer spore wall is dissolved in the intestine, releasing eight active sporozoites. The latter pass to the epithelial cells

and either become attached or completely embedded, when the life-cycle begins anew.

There is evidence to indicate that auto-infection may occur by the cysts ripening in the intestine, and this would account for the enormous number of parasites often found within a single host.

The arthropods from which the parasites were taken include the Diplopoda, Orthoptera and Coleoptera, and the species are grouped in this order in the text. The gregarines described are members of the following genera: *Amphoroides*, *Steinina*, *Stenophora*, *Gregarina*, and a new genus which is here designated *Leidyana*.

#### GREGARINES IN THE DIPLOPODA

Infection in the diplopods is fairly heavy and about three fourths of the individuals examined at Urbana were parasitized. Parasites were abundant in the early spring as well as in the fall. Some species were nearly always found to be infected, others never.

*Stenophora diplocorpa* n. sp. (Fig. 1): Sporonts solitary, elongate. Maximum length  $360\mu$ , width  $15\mu$ . Ratio, length protomerite : total length :: 1:16 to 1:25. Ratio, width protomerite : width deutomerite :: 1:2 to 1:3. Protomerite dome shaped, widest at posterior margin and as wide as long. Slight constriction at septum. Deutomerite slender, elongate, incompletely divided into two nearly equal parts by a crosswise constriction, widest just anterior to this constriction. Cylindrical behind the constriction and broadly rounded at posterior end. Protomerite nearly transparent, deutomerite pale tan, not opaque. Nucleus visible in vivo, situated just behind constriction in the deutomerite, spherical, and containing one karyosome. Cyst and spores unknown.

Taken at Urbana, Illinois. Host: *Euryurus erythropygus* (Brandt). Habitat: intestine.

*Stenophora impressa* n. sp. (Fig. 2): Sporonts solitary, ellipsoidal. Maximum length  $375\mu$ , width  $48\mu$ . Ratio, length protomerite : total length :: 1:12. Ratio, width protomerite : width deuteromite :: 1:2.3. Protomerite conical, dilated in posterior half, as wide as high. An apparent pore at anterior end. Constriction at septum not deep. Deutomerite ellipsoidal, widest through central part, posterior extremity blunt or rounded. Endocyte of protomerite nearly transparent, of deutomerite opaque. Nucleus spherical with one large karyosome. Cysts spherical,  $160\mu$  in average diameter. Spores not known.

Taken at Urbana, Illinois. Host *Parajulus impressus* (Say). Habitat: intestine.

*Stenophora lactaria* n. sp. (Fig. 3): Sporonts solitary, elongate, ellipsoidal. Maximum length  $480\mu$ , maximum width  $39\mu$ . Ratio,

length protomerite : total length : : 1 : 10 to 1 : 16. Ratio, width protomerite : width deutomerite : : 1 : 1.2. Protomerite conical, dilated above base and tapering to a point. An apparent pore at apex. As broad as high. Constriction at septum. Deutomerite ellipsoidal, widest in anterior third, tapering to an acute, rounded extremity. Endocyte of protomerite nearly transparent, of deutomerite opaque. Nucleus ellipsoidal, twice as long as wide. Cysts spherical, 150 to 170 $\mu$  in diameter. Spores not known.

Taken at Urbana, Illinois. Host: *Callipus lactarius* (Say). Habitat: intestine.

*Amphoroides calverti* (Crawley) (Fig. 4): Sporonts solitary, elongate. Maximum length 1670 $\mu$ , average length 1400 $\mu$ , average width 120 $\mu$ . Ratio, length protomerite : total length : : 1 : 47. Ratio, width protomerite : width deutomerite : : 1 : 2.5 to 1 : 3. Protomerite greatly compressed in sporonts, shallow, five times as wide as high. Deep crater within top. Constriction at septum sharp and deep. Deutomerite elongate, widest in anterior third, tapering to a sharp point. Endocyte of protomerite tan in color, not dense; of deutomerite opaque, white. Nucleus small, spherical, not visible in vivo. Myocyte well developed. Cysts spherical, averaging 380 $\mu$  in diameter. Dehiscence by simple rupture. Spores not known.

Taken at Urbana, Illinois. Host: *Callipus lactarius* (Say). Habitat: intestine.

This species was described by Crawley (1903a) as *Gregarina calverti*, but the elongate shape of the sporonts, great size, dehiscence of the cysts by simple rupture, and the fact that all the animals are solitary prove that the species is not a member of the genus *Gregarina*. I place it in the genus *Amphoroides* because of the crateriform protomerite.

#### GREGARINES IN THE COLEOPTERA

The following nine species have been found in beetles and beetle larvae in the two general localities mentioned. In no instance has a complete life-history been established, but the generic position is determined beyond doubt by known characters. The members of the genus *Gregarina* are superficially very similar, but a close inspection yields points of difference sufficient to indicate the individuality of each species. While the primitives of several species are similar, the satellites are dissimilar and afford one means of differentiation. The relative sizes of the species and the color and density of the protoplasm afford other means of identification. The visibility of the nucleus is important in identification. The literature has been carefully investigated in the anticipation that some of the species, especially those in

the Elateridae and the Tenebrionidae had been previously described. All are, however, new.

*Gregarina katherina* n. sp. (Fig. 5): Sporonts biassociative, ellipsoidal. Length of associations 96 to 150 $\mu$ . Sporonts 45 to 70 $\mu$  long, 20 to 34 $\mu$  wide. Ratio, length protomerite: total length primite :: 1:6. Ratio, width protomerite: width deutomerite :: 1:7. Protomerite of primite dome shaped, of satellite flattened. Deutomerite ellipsoidal. Nucleus spherical, one large karyosome. Epimerite large, sessile, a hyaline knob. Cyst and spores not known.

Taken at Oyster Bay, Long Island, N. Y. Host: *Coccinella novum-notata* Herbst. Habitat: intestine.

*Gregarina barbarara* n. sp. (Fig. 6): Sporonts biassociative, ovoidal to subspherical. Length of association (average) 250 $\mu$ . Sporonts (primites) average 145 $\mu$  long, 90 $\mu$  wide. Ratio, length protomerite: total length primite :: 1:6. Ratio, width protomerite: width deutomerite :: 1:2.2. Protomerite hemispherical in primite, flattened in satellite, six times as wide as high, deutomerite ovoidal in primite, widest part in central region. Deutomerite of satellite widest in anterior third, no constriction at septum, contour here perfectly smooth. Nucleus small, spherical, with one karyosome. Body practically transparent. Cyst and spores not known.

Taken at Oyster Bay, Long Island, N. Y. Host: *Coccinella* sp. Habitat: intestine.

*Gregarina globosa* n. sp. (Fig. 7): Sporonts biassociative, sub-globose. Length of associations 435 $\mu$ . Length of sporonts 260 $\mu$ , width 180 $\mu$ . Ratio, length protomerite: total length :: 1:8.6. Ratio, width protomerite: width deutomerite :: 1:2.4. Protomerite hemispherical, broadest at base, no constriction at septum. Deutomerite nearly spherical. Protoplasm dense, dark gray to black in primite, lighter in satellite. Nucleus spherical. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Coptotomus interrogatus* Fab. Habitat: intestine.

*Gregarina monarchia* n. sp. (Fig. 8): Sporonts biassociative, elongate cylindrical. Length of associations 570 $\mu$ , width 130 $\mu$ . Ratio, length protomerite: total length :: 1:7. Ratio, width protomerite: width deutomerite :: 1:1.2. Protomerite subspherical, widest through middle portion, constriction at septum. Deutomerite elongate cylindrical, equal in width throughout, broadly rounded posteriorly. Deutomerite dense, black in transmitted light. Protomerite nearly transparent. Nucleus not visible in vivo. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Pterostichus stygicus* Say. Habitat: intestine.

*Gregarina intestinalis* n. sp. (Fig. 9): Sporonts biassociative, broadly ellipsoidal. Length of associations  $320\mu$ . Maximum length of sporonts  $160\mu$ , maximum width  $80\mu$ . Ratio, length protomerite : total length :: 1:5. Ratio, width protomerite : width deutomerite :: 1:2. Protomerite subspherical, widest through middle portion, deep constriction at septum. Deutomerite broadly ellipsoidal, protoplasm dense, dark gray. Nucleus not visible in vivo. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Pterostichus stygicus* Say. Habitat: intestine.

*Gregarina gracilis* n. sp. (Fig. 10): Sporonts biassociative, elongate ellipsoidal. Maximum length of associations  $370\mu$ ; maximum length of sporonts  $190\mu$ , maximum width  $75\mu$ . Ratio, length protomerite : total length :: 1:8. Ratio, width protomerite : width deutomerite :: 1:2. Protomerite hemispherical. Deutomerite elongate ellipsoidal. Color gray. Nucleus not visible in vivo, spherical, small, with one karyosome. Cysts average  $90\mu$  in diameter. Spores not known.

Taken at Urbana, Illinois. Host: larvae of Elateridae. Habitat: intestine.

*Gregarina tenebrionella* n. sp. (Fig. 11): Sporonts biassociative, subglobose, very small. Maximum length of association,  $140\mu$ , average length  $125\mu$ . Ratio, length protomerite : total length :: 1:4. Ratio, width protomerite : width deutomerite :: 1:1.7. Protomerite dome shaped, deutomerite nearly spherical in primitive, ellipsoidal in satellite. Nucleus small, spherical. Protoplasm gray. Cyst and spores not known.

Taken at Urbana, Illinois. Host: larvae of Tenebrionidae. Habitat: intestine.

*Gregarina fragilis* n. sp. (Fig. 12): Sporonts biassociative, ellipsoidal. Length of associations  $200\mu$ . Maximum length of sporonts  $110\mu$ , maximum width  $60\mu$ . Ratio, length protomerite : total length primitive :: 1:5. Ratio, width protomerite : width deutomerite :: 1:2. Protomerite dome shaped, cylindrical in posterior third. Protomerite of satellite same shape but slightly flattened anteriorly. Deutomerite ellipsoidal. Nucleus small, spherical, with one karyosome. Body practically transparent. Cyst and spores not known.

Taken at Urbana, Illinois. Host: *Coccinella* sp. Habitat: intestine.

*Steinina rotunda* n. sp. (Fig. 13): Sporonts solitary, globose. Maximum length  $250\mu$ , maximum width  $130\mu$ . Ratio, length protomerite without epimerite : total length :: 1:2.3. Ratio, width protomerite : width deutomerite :: 1:1.1. Protomerite conoidal, dilated

at beginning of posterior two thirds, constricted at septum. Protomerite densest in posterior half. Deutomerite spherical to obovate, posterior end either rounded or slightly pointed. Nucleus large with one large karyosome in young, with many chromatic bodies in adult. Endocyte light brown. Epimerite spherical, hyaline, persistent on large animals free in lumen of intestine. Cyst and spores not known.

Taken at St. Joseph, Illinois. Host: *Amara angustata* Say. Habitat: intestine.

#### GREGARINES IN THE ORTHOPTERA

Five of the following species are new, one representing a newly created genus. New distribution records and new measurements are given for three species which are already known in the literature.

*Gregarina nigra* n. sp. (Fig. 14).—Sporonts biassociative, cylindrical. Maximum length of associations,  $1000\mu$ . Maximum length of sporonts  $530\mu$ , maximum width  $180\mu$ . Ratio, length protomerite : total length primite :: 1:4. Ratio, width protomerite : width deutomerite :: 1:1.4. Protomerite a truncate cone angular at the free corners. Width equal to height. Widest at base, no constriction or a very slight constriction at septum. Protomerite of satellite scarcely flattened. Deutomerite cylindrical, broadly rounded posteriorly. Endocyte black. Nucleus not visible in vivo, spherical, containing many small karyosomes. Cysts and spores not known.

Taken at Urbana, Illinois. Hosts: *Melanoplus femur-rubrum* (deGeer); *M. differentialis* (Uhler); *Encoptolophus sordidus* (Burmeister). Habitat: intestine.

*Gregarina stygia* n. sp. (Fig. 15): Sporonts biassociative, obese. Maximum length of associations  $360\mu$ , length sporonts  $180\mu$ . Primite and satellite of approximately the same length. Maximum width of primite  $100\mu$ . Ratio, length protomerite : total length primite :: 1:6. Ratio, width protomerite : width deutomerite :: 1:1.6 to 1:2. Protomerite hemispherical in primite, flattened in satellite. Deutomerite of primite broadly ellipsoidal, nearly as wide as long; of satellite widest in anterior half, tapering slightly. Nucleus small, spherical. Endocyte dark tan but not dense, nucleus visible in vivo in both primite and satellite. Sarcocyste thicker in both protomerites than in the deutomerites. Trophozoite with a simple, small, knobbed epimerite. Cysts  $150\mu$  in diameter. Spores not seen.

Taken at Cold Spring Harbor, Long Island, N. Y. Host: *Ceuthophilus stygicus* (Scudder). Habitat: intestine.

*Gregarina galliveri* n. sp. (Fig. 16): Sporonts biassociative, maximum length of associations  $590\mu$ ; maximum length of sporonts  $300\mu$ , width  $130\mu$ . Ratio, length protomerite : total length primite :: 1:5.

Ratio, width protomerite: width deutomerite :: 1.1:1. Protomerite flattened, broad and low. Three times as wide as high. Deutomerite of primitive vase shaped, constricted at top, widening in posterior half. Deutomerite of satellite subspherical to ovoidal. Endocyte very dense in both protomerite and deutomerite, dark brown in color. Nucleus small, spherical, not visible in vivo. Cysts spherical, 350 $\mu$  in average diameter. Spore ducts numerous. Spores not seen.

Taken at Oyster Bay, Long Island, N. Y. Host: *Gryllus abbreviatus* Serv. Habitat: intestine.

*Gregarina illinensis* n. sp. (Fig. 17): Sporonts biassociative, elongate cylindrical. Maximum length of associations 1100 $\mu$ ; length sporonts 550 $\mu$ , width 180 $\mu$ . Ratio, length protomerite: total length primitive :: 1:5. Ratio, width protomerite: width deutomerite :: 1:1.1 to 1.5. Protomerite dome shaped, slightly constricted at septum. Deutomerite elongate cylindrical, broadly rounded behind. Protomerite of satellite cupped at top for insertion of posterior end of primitive. Nucleus large, spherical, with many small chromidial bodies. Endocyte dense, black in both protomerite and deutomerite. Cysts and spores not recovered from the host.

Taken at Urbana, Illinois. Host: *Ischnoptera pennsylvanica* (deGeer). Habitat: intestine.

*Gregarina achetae-abbreviatae* Leidy (Fig. 18): Sporonts biassociative, obese. Maximum observed length 500 $\mu$ ; average sporonts 450 $\mu$  long, 225 $\mu$  wide. Ratio, length protomerite : total length primitive :: 1:3. Ratio, width protomerite : width deutomerite :: 1:1.1. Protomerite hemispherical to subglobose, width twice the height. Slight constriction at septum. Deutomerite stout bodied, nearly as wide as long. Widest at shoulder where it is very little wider than protomerite. Posterior end truncate. Epimerite undescribed. Endocyte dense in deutomerite, less so in protomerite. Nucleus not visible in vivo and not seen. Cysts spherical, 250 $\mu$  in average diameter. Spore ducts two to five in number, of maximum length 1000 $\mu$ . Spores barrel shaped, 4.5×2.25 $\mu$ .

Taken at Haverford, Pa., and Urbana, Illinois. Host: *Gryllus abbreviatus* Serv. Habitat: intestine.

*Gregarina rigida* (Hall) Ellis (Fig. 19): Sporonts biassociative, stout bodied. Maximum length of associations 1425 $\mu$ , average length 550 $\mu$ . Sporonts 250 to 750 $\mu$  long, 130 to 210 $\mu$  wide. Ratio, length protomerite : total length of primitive :: 1:3 to 1:6. Ratio, length protomerite : total length satellite :: 1:5 to 1:16. Ratio, width protomerite : width deutomerite :: 1:1.4. Protomerite somewhat flattened, width sometimes three times the height, generally less. Constriction at septum more or less indistinct. Deutomerite cylindrical or barrel

shaped, little wider than protomerite, ending in a broadly rounded or flattened square-cornered extremity. Endocyte very dense and brownish yellow in deutomerite, tan in protomerite. Epimerite a small, spherical, hyaline knob. Cysts yellow-orange,  $300\mu$  in average diameter, spore ducts short, ten or more in number. Spores extruded in chains, barrel shaped,  $5 \times 8\mu$ .

Taken at Lincoln, Neb., Colorado Springs, Colo., and Urbana, Ill. Hosts: *Melanoplus femur-rubrum* (deGeer); *M. differentialis* (Uhler); *M. coloradensis* (?); *Encoptolophus sordidus* (Burm.); *Schistocerca americana* Burm.; *Melanoplus bivittatus* (Say); and *Hesperotettix pratensis* Scudder. Habitat: intestine and pyloric caeca.

This species was first described by Hall (1907) as *Hirmocystis rigida*. Crawley (1907) found it shortly after and named the species *Gregarina melanopli*. Ellis (1913) changed the name to *Gregarina rigida*.

*Leidyana solitaria* n. gen., n. sp. (Fig. 20): Sporonts solitary, cylindrical. Maximum length  $500\mu$ , maximum width  $160\mu$ . Ratio, length protomerite : total length :: 1:5 to 1:7. Ratio, width protomerite : width deutomerite :: 1:1.3 to 1:1.7. Protomerite conical, dilated in middle portion, constricted deeply at septum. Protomerite slightly wider than high in adults. Deutomerite cylindrical to elongate ellipsoidal, sometimes tapering, rounded posteriorly. Endocyte of protomerite pale tan, translucent, of deutomerite very dense, black in transmitted light, the two parts very plainly demarcated, nucleus not visible in vivo, spherical, with one or two small karyosomes. Epimerite a large, globular, hyaline knob on a short, slender stalk. Cysts spherical,  $350\mu$  in diameter (including the transparent covering). Dehiscence by spore ducts one to twelve in number. Spores given out in chains, barrel shaped, 3 by  $6\mu$ .

Taken at Cold Spring Harbor and Oyster Bay, L. I., N. Y., Haverford, Pa., and Urbana, Ill. Host: *Gryllus pennsylvanicus* Burm. Habitat: intestine.

This species was described by Crawley (1907) under the name *Stenophora erratica*. The mode of cyst dehiscence, however, precludes the possibility of its belonging to the family Stenophoridae. I have placed it in a new genus under the family Gregarinidae, characterized as follows: *Leidyana* n. gen. Sporonts solitary, epimerite a simple globular knob, dehiscence by spore ducts, spores doliform.

I should restrict the genus *Gregarina* to biassociative sporonts only, the other characters being identical with those of the new genus.

#### LITERATURE CITED

- Crawley, Howard. 1903. List of Polycystid Gregarines of the United States. Proc. Acad. Nat. Sc., Philadelphia, 55: 41-58; 3 pl.

1907. The Polycystid Gregarines of the United States (Third Contribution). Proc. Acad. Nat. Sc., Philadelphia, 59: 220-8; 1 pl.

Ellis, M. M. 1913. A Descriptive List of the Cephaline Gregarines of the New World. Tr. Am. Micr. Soc., 32: 259-96; 4 pl.

Hall, M. C. 1907. A Study of Some Gregarines with Especial Reference to *Hirmocystis rigida*, n. sp. Univ. Studies (Lincoln, Neb.), 7: 149-74; 1 pl.

Schewiakoff, B. 1894. Ueber die Ursache der fortschreitenden Bewegung der Gregarinien. Zeit. wiss. Zool., 58: 340-54; 2 pl.

#### EXPLANATION OF PLATES 1 AND 2

Fig. 1.—Sporont of *Stenophora diplocorpa* n. sp. from camera lucida drawing of the original.

Fig. 2.—Sporont of *Stenophora impressa* n. sp.

Fig. 3.—*Stenophora lactaria* n. sp.

Fig. 4.—*Amphoroides calverti* (Crawley).

Fig. 5.—*Gregarina katherina* n. sp.

Fig. 6.—*Gregarina barbarara* n. sp.

Fig. 7.—*Gregarina globosa* n. sp.

Fig. 8.—*Gregarina monarchia* n. sp.

Fig. 9.—*Gregarina intestinalis* n. sp.

Fig. 10.—*Gregarina gracilis* n. sp.

Fig. 11.—*Gregarina tenebrionella* n. sp.

Fig. 12.—*Gregarina fragilis* n. sp.

Fig. 13.—*Steinina rotunda* n. sp., a trophozoite with epimerite

Fig. 14.—*Gregarina nigra* n. sp.

Fig. 15.—*Gregarina stygia* n. sp.

Fig. 16.—*Gregarina galliveri* n. sp.

Fig. 17.—*Gregarina illinensis* n. sp.

Fig. 18.—*Gregarina achetae-abbreviatae* Leidy.

Fig. 19.—*Gregarina rigida* (Hall) Ellis.

Fig. 20.—*Leidyana solitaria* n. gen., n. sp.

PLATE 1

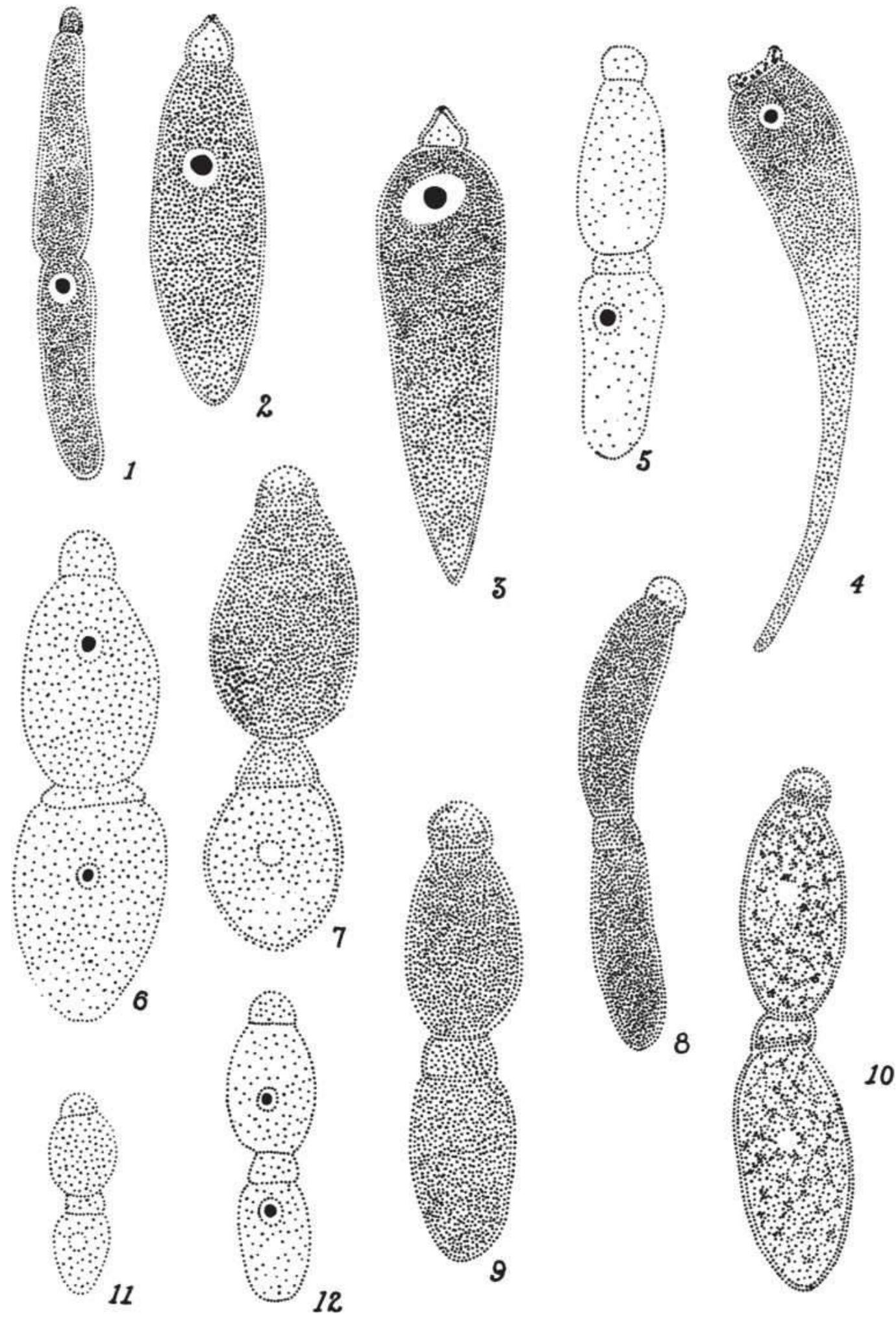
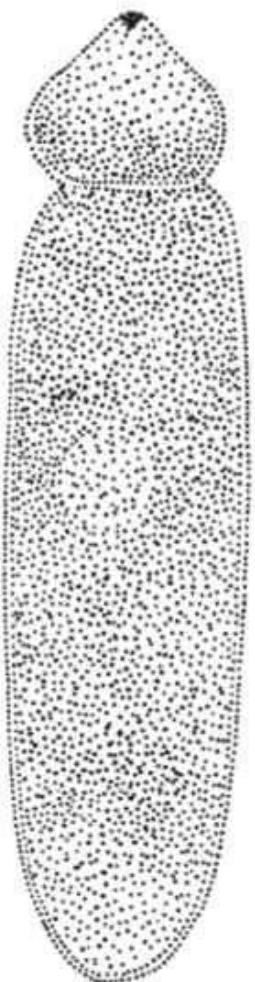
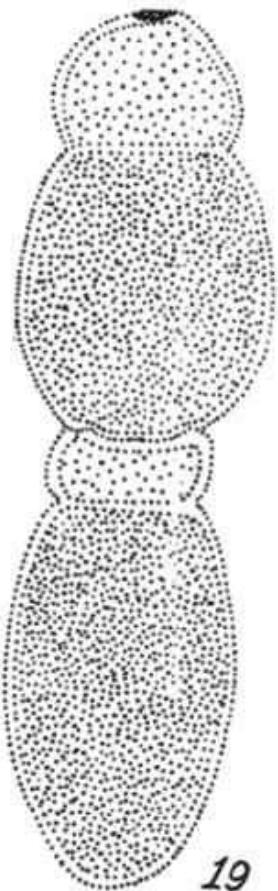
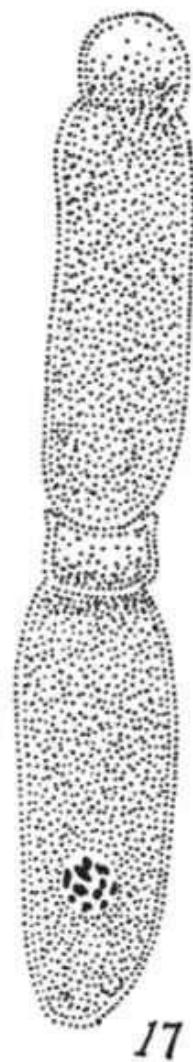
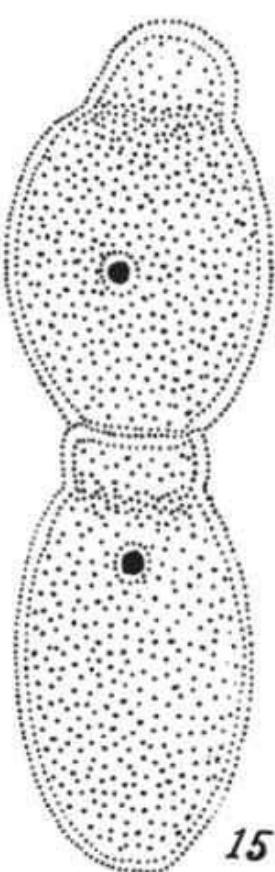
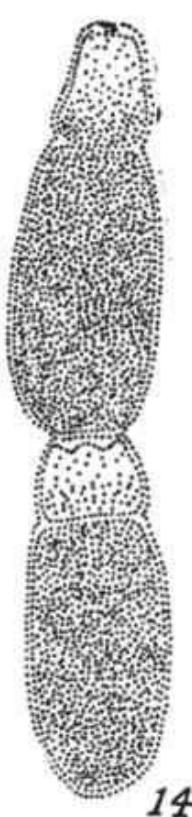
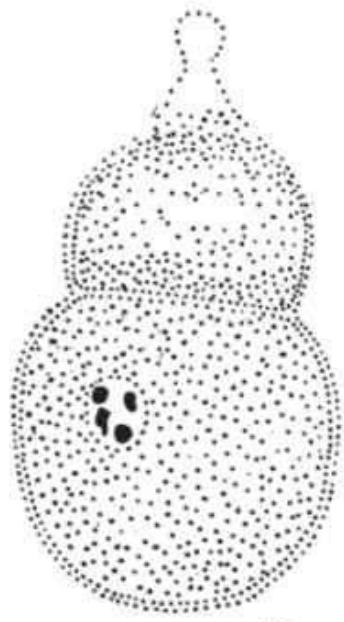




PLATE 2



under the direction of Henry B. Ward, No. 48.



# Parasitic protozoology and the scientific lessons of intellectual elegance

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After 30 years working on the septate gregarines, a ubiquitous, fascinating, and evolutionarily successful group with admittedly little monetary, medical, agricultural, social, or political importance or interest, I find myself reminding my students that helminthologists do not come to listen to protozoologists because they are interested in protozoans. They come because they are interested in protozoologists. They might find the protists under discussion bizarre, beautiful, or frightening, but what really draws them in is a curiosity to see what the protozoologists will do next. Given an impossible problem with an intractable system, any viable solution will always require intellectual elegance. And what scientist can resist a glimpse into such a system? The intellectual elegance of science is what draws most of us into empirical biology in the first place.

The premise of this centennial overview of research in parasitic protozoology for *The Journal of Parasitology* is that a strategic analysis of the intellectual design of research is more informative than a simple historical review of advances in the discipline. The critical insight for parasitologists in the last 100 years lies in an appreciation of how successful protozoologists designed their work.

First, some thoughts on protists, protozoans, and protozoologists. Genetic analysis has convincingly demonstrated that Protista is more a “lumber room of forms” than a monophyletic group. The question really seems to be: How many kingdom-level monophyletic groups are currently jumbled into the lumber room of Protista? I do not think we have enough homologous data across a large enough taxon sample to answer even this simple question with any confidence or predictability, although estimates range from a dozen to more than 30. I can usually default to Apicomplexa or even Alveolata for my own work, but, for our purposes, working definitions are in order. “Protista” encompasses all eukaryotes that are neither animals, nor plants or fungi. “Protoctista” is an admission that this group is a lumber room, so why not throw the algae in as well? “Protozoans” are heterotrophic protista that do not form filaments. Pragmatically for parasitologists, protozoans are organisms studied by parasitologists who refer to themselves as protozoologists. The organisms themselves don't know or care who studies them.

# A lesson learned from the history of parasitology

If nothing else, a century of parasitology teaches us that how we do our science is as important as the science itself. It follows that choosing a research system is the most critical decision of a research career. The choice dictates what sorts of intellectual questions we ask, what kind of tools we employ, what manner of methods we develop, and what kinds of intellectual discoveries and syntheses are even possible for us to conceive. Like so many choices in life, this one is made early and with little personal experience to guide the decision, but there is a century of experience for a young scientist to draw upon. From the first 100 years of *The Journal of Parasitology*, I present six vignettes of parasitologists that demonstrate six critical aspects of a productive research model, i.e., diversity, suitability, malleability, feasibility, comparability, and scalability.

## Diversity

The first quality of a research model is diversity, in which the great strength is opportunity. For a taxonomist, systems with high diversity are replete with what we often call “low-hanging fruit,” i.e., research problems that are readily accessible and conceptually interesting, and for the scientist willing to labor, there is a lot of work to be done. These systems tend to produce new taxonomic discoveries and publications at a fairly high rate. The weaknesses of highly diverse systems are two-fold. First, others generally have labored in the metaphorical orchard before, often with marginal skill and incomplete knowledge of the group. As a result, new work often requires recollection, redescription, and stabilization of existing taxa before new taxa can be recognized. Second, there is rarely a living expert conducting active work in the group. Accordingly, a new worker must resurrect a taxonomic group from a long hibernation in the literature, often designing new techniques and establishing a new systematic along the way. Despite popular notions, such low-hanging intellectual fruit requires significant pruning and cultivation before it can be harvested.

The first protozoologist to publish in *The Journal of Parasitology* provides an extraordinary illustration of the intellectual elegance of diversity. Minnie Elizabeth Watson published, “Some new gregarine parasites from Arthropoda” in Volume 2, of *The Journal of Parasitology*, in which she described 17 new species of North American gregarine parasites (Apicomplexa; Conoidasida; Eugregarinorida), roughly 25% of all known New World gregarine species at the time (Watson, 1915).

Minnie was born in 1886, in Fostoria, Michigan. She earned her B.A. degree at Olivet College in Michigan in 1909, and taught high school in Oyster Bay, Long Island, New York from 1909–1913. Several of the gregarines she described in the 1915 paper were collected during her years on Oyster Bay. She became a fellow of the Zoological Laboratory at the University of Illinois in 1913, where she worked with Henry Baldwin Ward, earning a M.Sc. in 1913, and a Ph.D. in 1915, for her work on gregarines (University of Illinois, 1918). Her graduate work culminated in two large monographic treatments of the septate gregarines. Her doctoral thesis, “Studies on gregarines: Including descriptions of 21 new species and a synopsis of the eugregarine records from the Myriapoda, Coleoptera and Orthoptera of the world,” was published in 1916.

In her dissertation, she integrated all of the known gregarine literature, beginning with Dufour in 1828, and ending with her own new species descriptions published in *The Journal of Parasitology* in 1915. This work was a monumental undertaking that included all known morphological, distributional, and host data for 54 genera and over 250 species of gregarines drawn from her own collections in the United States and an international literature base that included works in English, German, and French (Watson, 1916a). Published in 1922, her companion monograph, “Studies on gregarines II: Synopsis of the polycystid gregarines of the

world, excluding those from the Myriapoda, Orthoptera, and Coleoptera,” covered 64 genera and over 200 species, again drawing from her own collections and observations as well as an international literature base that incorporated works in English, German, French, Italian, and Portuguese (Kamm, 1922a). The second monograph concluded Minnie Watson’s review of all known gregarine species, but, more importantly, it proposed a complete, hierarchically structured, character-based revision of the systematic for the order Gregarinida, including superfamilies, families, subfamilies, and genera.

Minnie Watson married another University of Illinois research fellow, Oliver Kamm, in 1916. Kamm was among the first of America’s organic chemists and, with his colleagues at the University of Illinois, would later publish the seminal laboratory protocols for industrial organic synthesis, most of which are still in use today. As the lead researcher for Parke-Davis & Company in Detroit, Oliver Kamm became one of the founding pharmaceutical biochemists of the twentieth century, isolating both vasopressin and oxytocin from the human pituitary. After they were married, Minnie published her work under the name Minnie Watson Kamm. She published seven papers and two monographs on gregarines over a short 7-year period, essentially integrating and revising data for all known gregarine species and placing them in a single cohesive systematic, while conducting detailed studies of host-gregarine tissue association and gregarine life cycles (Watson, 1915, 1916a, 1916b, 1916c; Kamm, 1917a, 1917b, 1918a, 1918b, 1922a, 1922b).

After the 1922 monograph, “Studies on Gregarines II,” Minnie and Oliver moved to Detroit where he became the head of research for Parke Davis & Company. Minnie did not continue her gregarine research in Michigan, or, if she did, she never published on the group again. However, she demonstrated the fundamental organizing nature of the systematist’s mind, through her publication of nine illustrated volumes outlining a taxonomy of several hundred American pressed glass patterns, a body of work that remains in use by collectors today. She died in 1954.

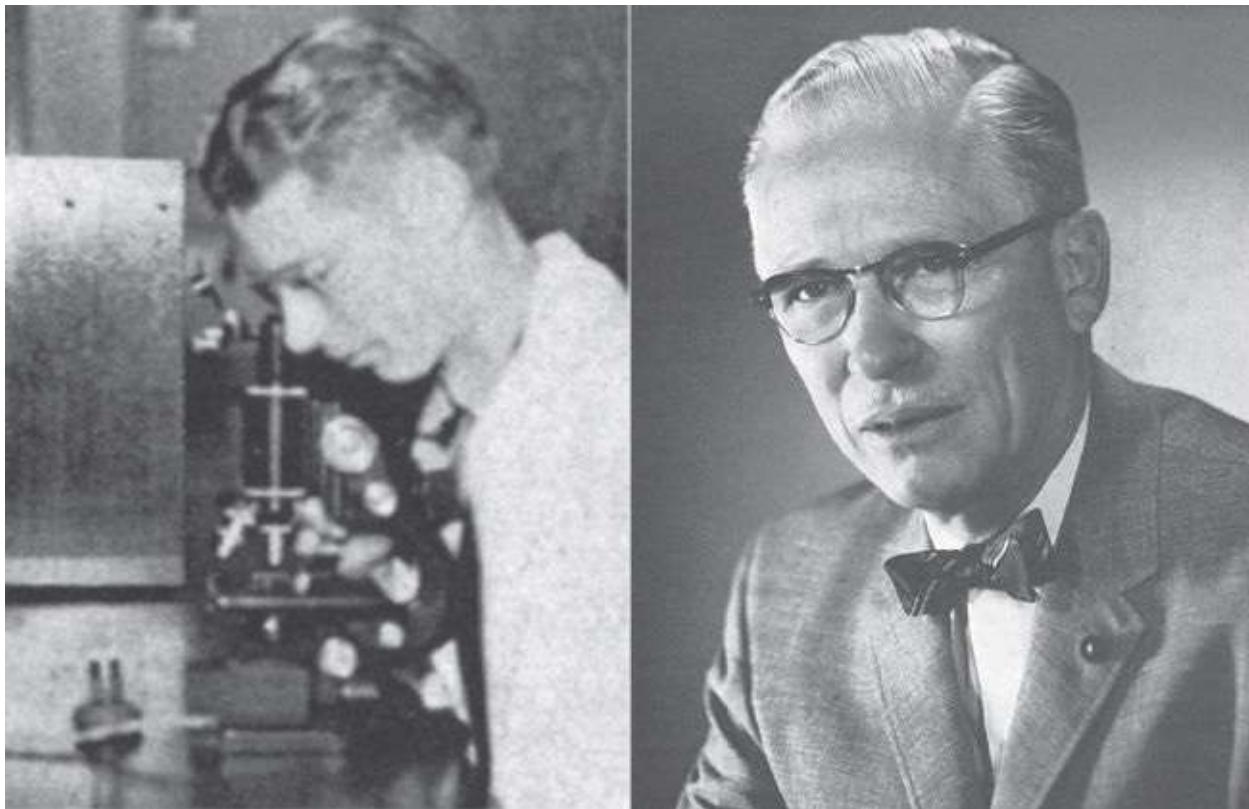
Minnie Watson Kamm had a meteoric parasitological career, exploiting the strengths of gregarine diversity by focusing her own work on the organizational weaknesses of the system. She is recognized by protozoologists as one of the most important apicomplexan systematists of the twentieth century. Despite her own scientific importance and her marriage to one of the seminal American biochemists of the twentieth century, curiously, no photograph of Minnie Watson Kamm is known to exist.

## Suitability

Suitability is a second important quality of a research system. Models usually provide an approximation of the problem under study. If they are appropriate, significant insight into the behavior of the system can be extrapolated from the behavior of the model. Thus, suitability is really a measure of how closely a system models or approximates the problem of interest. The most suitable systems aren't models at all; they are the system of interest. The advantage of a suitable model system is that it allows empirical investigation of a problem at low cost, as well as high replicability and throughput. The disadvantage to suitability is the difficulty of finding a suitable model system at all. This approach assumes that from amongst all known and unknown diversity in a group, at least one taxon is suitable as a subject for years, if not decades, of research. More importantly, it assumes that we can find that one taxon. For parasitologists, it is a “needle in a stack of needles” problem.

The work of G. Robert Coatney ([Fig. 1](#)) exemplifies the strategy of developing and using the suitable model. Coatney was born in Falls City, Nebraska in 1902. He earned his B.A. degree at Grand Island Baptist College in Nebraska under the parasitologist Frank Meserve in 1925 and his M.A. at the University of Nebraska in 1926, where he worked with another parasitologist, Franklin Barker. He earned his Ph.D. with Elery Becker at Iowa State University, where he settled on malaria as a topic of study (Pritchard, 1985). In 1932, Coatney acquired a faculty position at Peru State College in Peru, Nebraska. He described his impetus to search for a suitable system to study avian malaria:

At the time there was a broad interest in avian malarias but in-depth studies were limited to infections in the canary, which carried only a small amount of blood. This could be overcome with malaria in a larger bird. Peru was located on a major flyway and non-migratory birds were all about. One of them might be carrying a true malaria that would grow well in a larger bird attuned to laboratory conditions—a chicken or the common pigeon. I decided to study the bird parasites of that bird population with one object only: to find that parasite! (Coatney, 1985).



**Figure 1** G. Robert Coatney. Early in his career searching for avian malaria at Peru State College (left, from the 1936 *Peruvian*) and 40 years later as President of the American Society of Parasitologists (right, from Coatney, G. R. 1976. Relapse in malaria: An enigma. *Journal of Parasitology* **62**: 2–9). Reproduced with permission from: left—1936 *Peruvian*, the Yearbook of Peru State College; and right—with permission of Allen Press Publishing Services.

Coatney and his students at Peru State College studied bird blood parasites for the next 5 years, observing the course of infection of *Haemoproteus* in pigeons (Coatney, 1933), compiling taxonomic reviews of important avian blood parasites (Coatney, 1936, 1937), reporting parasites from 79 species of birds in Nebraska, plus describing 15 new species of avian blood parasites (Coatney and Roundabush, 1937; Coatney and West, 1937). He also conducted some preliminary chemotherapeutic tests using his avian blood parasite models that would provide the empirical groundwork for later drug testing and development against human malarias (Coatney, 1935; Coatney and West, 1937).

In the summer of 1937, Coatney found his malarial parasites in mourning doves and pigeons nesting in the wheel-house undercarriage of the college observatory on the roof of Hoyt Science Hall at Peru State (Coatney, 1938, 1985). Mary Hanson Pritchard (pers. comm.) recalls Coatney's own retelling of the event:

One night he and two student assistants climbed to the cupola of the observatory where a flock of pigeons was nesting. They collected half a gunny sack full of birds and took them to his laboratory. It was about 11 o'clock at night, and they were making blood smears at a great rate when the telephone rang. "The President says you must turn out the lights up there. You are wasting too much electricity." Remember that this was in the depths of the depression and that research was not the objective of a teacher's college. The next morning they finished the pigeons in the sack and began to stain the smears. It was in that lot of smears that the true avian malaria was found. (Pritchard, 1985).

When the United States Public Health Service sought Coatney's model for a federal research program on malaria, he replied that, "... if the Public Health Service wanted it, they could have it but they would have to take me too" (Coatney, 1985). By 1938, he had been assigned to the National Institutes of Health to test potential antimalarial compounds for the coming war. His pigeon model and drug-testing experiments at Peru State College made him not just the best, but the only qualified, person in the Public Health Service to head an antimalarial testing program.

With the onset of war in the Pacific, World War II became first a battle with malaria and secondarily a conflict among nations. For U.S. Forces, malarial attacks outnumbered combat casualties 5:1 (Heaton, 1963). By the fall of Bataan, 60–85% of American and Filipino troops were suffering acute malarial attacks. During the Guadalcanal Campaign where there were 1,800 cases of malaria per every 1,000 soldiers, malarial attacks outnumbered combat injury by a 6:1 margin (Heaton, 1963).

As part of the National Malaria Program, Coatney and his team developed and tested chloroquine, again using the most suitable system possible, i.e., *Plasmodium vivax*, *Plasmodium falciparum*, and *Plasmodium malariae* in human volunteers, first at the syphilis ward of St. Elizabeth's Hospital (a mental ward near Washington, D.C.) and later in large scale tests using prison volunteers at the Federal Correctional Institutions in Atlanta, Georgia and Seagoville, Texas (Coatney, 1985). Chloroquine effectively mitigated malaria in American troops in the Pacific theatre. The National Malaria Program was reinstated for the Korean War, producing primaquine, which also effectively ended the relapse problem. Chloroquine and primaquine were the drugs of choice for malarial control for nearly 50 years and remain the drugs of choice today in areas where *Plasmodium* species are not resistant.

No ethical complaints were lodged against the National Malaria Program, despite its size, scope, and subject. It was the only large-scale use of prison volunteers in the United States to solve an important medical problem. Axis war crimes against prisoners in Europe led to declaration of the Nuremberg Code in 1947, and subsequent passage of United States Code of Federal Regulations Title 45 Part 46

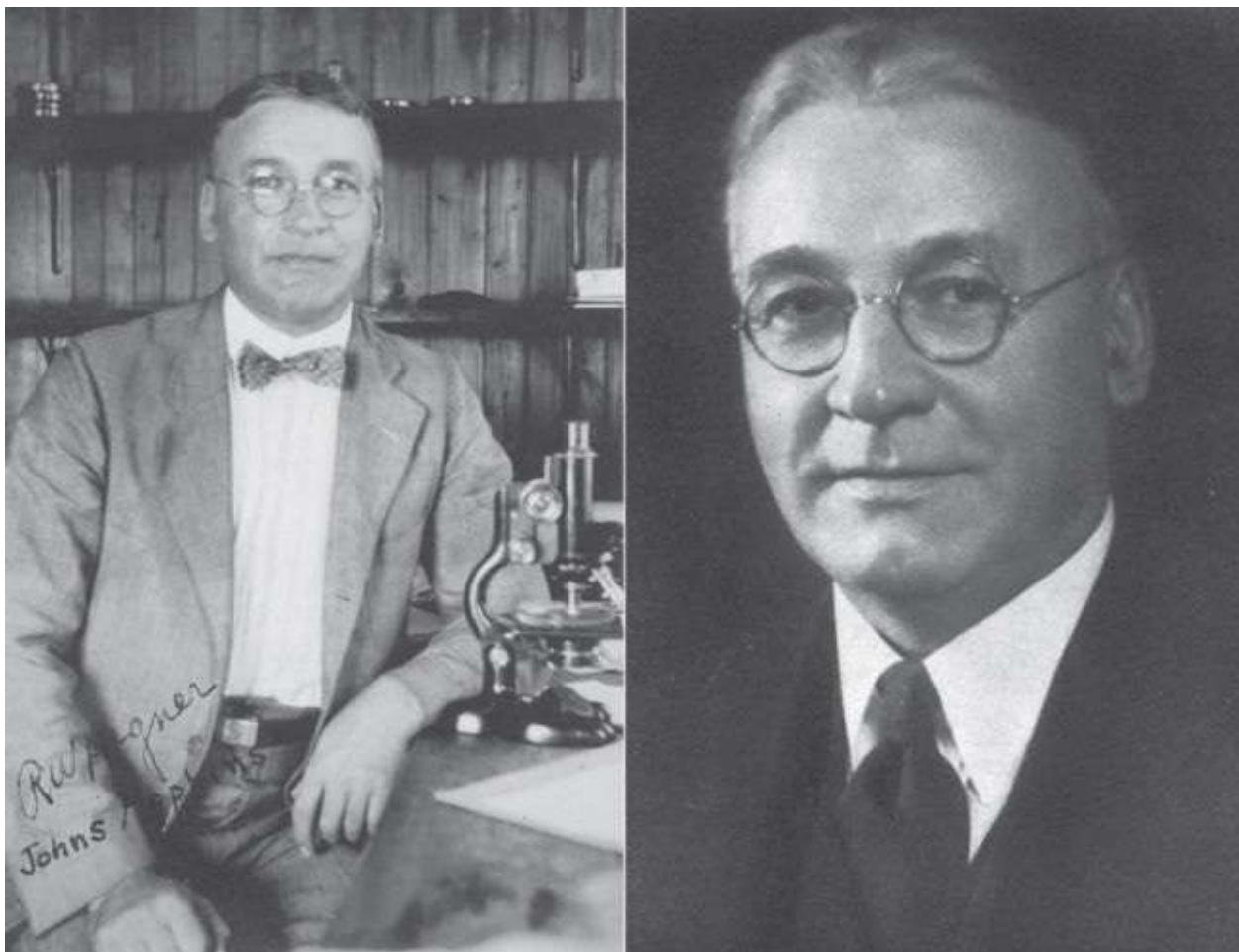
“Protection of Human Subjects,” making that particular model system difficult, if not impossible, to employ again.

Coatney's career is an iconic example of success driven by the suitability of a research system (Coatney, 1976). Using avian malaria in pigeons, he demonstrated the power of a program that is an almost perfect model for the actual dilemma of primate malaria in humans. His anti-malarial testing agenda depended on the low cost, high replicability, and high throughput of an even rarer, but more suitable, model, namely, actual primate malarias in human volunteers. In retrospect, he never solved the “needle in a stack of needles problem,” but what he did was almost more instructive. He chose to tilt the odds in his favor by looking for the right model in the right place at the right time. Then, he did what any good scientist would do; he took a chance and rolled the dice.

## Malleability

The cognitive scientist Douglas Hofstadter observed that, “variation on a theme is the crux of creativity” (Hofstadter, 1985). Malleability, or pliability, is the potential for variation and manipulation in a research system. This property represents the ultimate creative potential of a research system. Because the isolation and manipulation of variation lies at the heart of the scientific method, malleable systems are not only informative, they are usually also very productive.

Among parasitic protozoologists in the last century, Robert Hegner ([Fig. 2](#)) stands out as an example of choosing and exploiting systems for their malleability. He was born in Decorah, Iowa, in 1880. He earned the B.A. degree in 1903 and the M.Sc. degree in 1904, both at the University of Chicago, and then his Ph.D. at the University of Wisconsin in 1908. He spent nearly 10 years on faculty at the University of Michigan studying germ-cell development in insects, before taking a position in the newly formed School of Hygiene and Public Health of the Johns Hopkins University and turning his attention to parasitic protozoology (Cort, 1942).



**Figure 2** Robert Hegner. In early mid-career (ca. 1920) at the Marine Biological Laboratory, Woods Hole, Mass. (left, from the Copeland/Bloom photograph album, History and Philosophy of Science Repository, URI: <http://hdl.handle.net/10776/3270>, Licensed as Creative Commons) and 16 years later as President of the American Society of Parasitologists (right, from Hegner, R. W. 1937. Parasite reactions to host modifications. *Journal of Parasitology* **23**: 1–12). Reproduced with permission of Allen Press Publishing Services.

Hegner was interested in questions of host specificity, which had typically been viewed as a function of the parasite. He was interested in the degree to which specificity could be altered by changing the host. In other words, he asked: To what extent is specificity limited by a malleable variable in the host? Given a well-chosen system that allows direct transmission, the research becomes largely a matter of creativity and educated manipulation of system variables. Hegner and his students examined changes in parasite specificity with host manipulation in several systems, including intestinal opalinids in frogs and tadpoles (Hegner, 1922, 1932), trypanosomes in newts (Hegner, 1921), and flagellates, ciliates, and amoebae in chicks, among others (Hegner, 1933a, 1935). Hegner and Andrews' (1931) book, *Problems and methods of research in protozoology*, remains essential reading for any young parasitologists interested in the intelligent and malleable design of empirical research.

Two examples serve to illustrate the elegance and malleability of Hegner's research, i.e., trichomonads in rats (Hegner, 1933b; Hegner and Eskridge, 1935,

1937a, 1937b, 1937c) and *Plasmodium cathemerium* in canaries (Hegner and MacDougall, 1926; Hegner, 1929). Both of these research programs are distilled to their simplest form in Hegner's presidential address to the American Society of Parasitologists (Hegner, 1937).

Inheriting a group of experimental rats from a colleague at Johns Hopkins, Hegner noted that their caeca were free of the trichomonad infections typically found in the rat caecum. Upon inquiry, he discovered that these rats had been raised on a high animal protein diet rather than the high carbohydrate diet typical of laboratory rats. Hegner described his insight as follows:

It occurred to us that the intestinal protozoa we were supplying our students for laboratory study came principally from herbivorous animals, and a review of the literature soon revealed the fact that very few intestinal protozoa have been recorded from carnivorous animals. The evidence thus seemed quite convincing that for some reason a diet high in animal protein brings about a condition in the intestine that is unfavorable for the growth and reproduction of intestinal protozoa (Hegner, 1937).

Subsequent tests demonstrated that the suitability for trichomonad infections depended not only on the presence of protein, but also the protein type. When compared to control rats fed a normal high carbohydrate diet, cecal trichomonad intensity was reduced by 90% in rats fed a diet of beefsteak protein, and 97.4% in rats fed a diet of casein protein. In contrast, cecal trichomonad intensity nearly tripled in rats fed a diet of beef liver protein. In subsequent experiments, Hegner and Eskridge linked changes in diet to changes in the intensity of proteolytic anaerobic bacteria in the cecum and suggested that changes in diet led to changes in bacterial flora and thus changes in the trichomonad environment.

Hegner's work with *Plasmodium cathemerium* in canaries illustrates how both physiological and environmental manipulation could alter host suitability in very different ways. Once again, the work was inspired by a series of observations that led to the search for malleability.

The clues that led to our experiments came from several sources. Bass, in 1912, found that the addition of sugar to the culture medium was necessary to cultivate malaria parasites outside of the body. In 1913, Bass and Johns reported the cultivation of the organism of human malaria in the blood of a diabetic without the addition of sugar. Certain agents that have been found to be satisfactory as provocatives in bringing on a relapse in malaria, such as epinephrin, are known to increase the sugar content of the blood. These facts suggested that the blood stream can be improved as a culture medium for the growth and multiplication of malarial parasites by the addition of sugar (Hegner, 1937).

Hegner and MacDougall (1926) infected groups of canaries with *P. cathemerium*, manipulated their blood sugar with daily doses of sugar or insulin to increase or decrease blood sugar levels, respectively, and tracked parasitemia, relapse, and

host mortality. Manipulation of blood sugar altered host suitability across all measures. Birds receiving daily sugar doses had a higher parasitemia than those not receiving the sugar, and this parasitemia lasted longer than it did in the untreated group. Relapses occurred earlier in the course of infection and more frequently, and host mortality was higher than in the non-treated birds. Insulin-treated canaries displayed lower parasitemia and relapse rates than control birds, and suffered no mortality. In a similar experiment manipulating environmental conditions, experimentally infected birds held at increased temperatures and humidities also displayed higher parasitemia, extended courses of infection, and higher mortality.

Hegner's research career was built on collecting and synthesizing observations regarding systems and parasites, and then in choosing malleable systems that provided the opportunity for productive empirical testing of ideas and variables. His work clearly demonstrated that host susceptibility to parasitic infection, and perhaps host-parasite specificity, are stochastic, rather than fixed, properties. Although these observations seem obvious, even today most parasitological research assumes that susceptibility and specificity are phylogenetic rather than ecological properties. Without a doubt, understanding how host-parasite associations form and change over time requires careful empirical use of an appropriately malleable model system.

## Feasibility

Feasibility is the central requirement for a research system. This quality implies that any system that lacks feasibility cannot be studied. The truth is that it can be studied, but usually only indirectly and at great effort and expense. William Trager ([Fig. 3](#)) used to say, “You can't study something you can't grow” (Oransky, 2005). More than any other parasite protozoologist in the last century, Trager could induce parasitic protists to grow in culture, making suitable systems feasible for parasitologists worldwide.



[Figure 3](#) William Trager in his Rockefeller University Laboratory while President of the American Society of Parasitologists (right, from Trager, W. 1975. On the cultivation of *Trypanosoma vivax*: A tale of two visits in Nigeria. *Journal of Parasitology* **61**: 2–11). Reproduced with permission of Allen Press Publishing Services.

Trager was born in 1910, in Newark, New Jersey. He earned his B.Sc. in 1930, at Rutgers University and his M.Sc. and Ph.D. degrees at Harvard in 1931 and 1933, respectively, working on termite flagellates under L. R. Cleveland (Jensen, 2005). He spent a career spanning 60 years on the Princeton and New York campuses of Rockefeller University. Although he made many important contributions to parasitology, we are concerned with his two major ones, both resulting from his

efforts to make a malaria model feasible.

Trager's first major contribution was an *in vitro* system to culture *Plasmodium lophurae* from ducks (Trager, 1941, 1943, 1947; McGhee and Trager, 1950). Most malaria research in the United States at the time utilized avian models. Primate models were simply too expensive for routine research. However, if a *Plasmodium* species could be cultured *in vitro* then subsequent studies could manipulate media components in the search for chemotherapeutic targets. Trager succeeded in developing an *in vitro* culture method for *P. lophurae*, a parasite originally isolated from pheasants and subsequently maintained in ducklings, but the method was imperfect. Although the techniques allowed for prolonged cultivation of *P. lophurae* in an erythrocyte suspension, the method did not permit continuous culture. Nonetheless, the *P. lophurae* system allowed significant biochemical and nutritional studies to be conducted (e.g., Siddiqui and Trager, 1966; Trager, 1970) and led to a fundamental understanding of the nature of parasitism, namely that parasites lack specific biosynthetic pathways and depend upon their hosts for required nutrients. Thus, Trager and his colleagues established that parasitism reflects a nutritional dependency and not simply a resource preference.

Despite the development of an *in vitro* avian malaria model, the most desirable system for studying human malaria requires *Plasmodium* species specific for humans. Given the difficulty in procuring actual humans for the study of malaria, the best way to create a human malaria model is to develop a system that employs a continuous *in vitro* culture of a parasite species that naturally infects humans. Although many attempts had been made, Trager had the intellectual insight to simply culture the human red blood cells and allow them to sustain a *Plasmodium* species rather than trying to culture the parasite directly. Accordingly, Trager and a student, James Jensen, created a feasible model with their development of a "slow medium flow" culture system using *Plasmodium falciparum* and then perfected what they called the "candle jar method" for continuous culture (Trager, 1976; Trager and Jensen, 1976; Jensen and Trager, 1977).

The candle jar method itself is so uncomplicated that it is easily described here (based on Jensen and Trager, 1977). The technique requires RPMI-1640 (Roswell Park Memorial Institute medium), a readily available, sodium carbonate buffered defined medium supplemented with human serum obtained from outdated AB+ human blood purchased from a blood bank. Uninfected AB+ erythrocytes are obtained from the same lots of outdated blood. Infected erythrocytes are obtained from existing cultures or may be taken from new samples of infected human blood. Washed infected erythrocytes are added to suspended uninfected erythrocytes to produce an initial parasitemia of about 0.1%; then, 1.5-ml lots are dispensed into 35-mm plastic petri dishes. Culture dishes are placed in a glass desiccator with a stopcock. A candle is placed in the desiccator jar, lighted, and the candle jar is covered. When the flame extinguishes itself, the stopcock is closed. The method produces an inexpensive, predictable, oxygen poor, carbon dioxide

enriched atmosphere for erythrocyte culture. Each day the medium is aspirated off, replaced, and the culture is placed in a new candle jar. Thus, the serum supplemented RPMI medium and the culture atmosphere are replaced each day. Parasitemia in cultures increases about 60-fold over 96 hours (2 reproductive cycles). Malarial parasites produced in culture can be used to establish new cultures by the candle jar method; thus, the method is continuous and eliminates the need to passage the culture through a mosquito.

The simplicity and accessibility of the candle jar method enabled its immediate widespread adoption by researchers. James Jensen summed up the importance of the method almost 30 years after it was first published.

It has been estimated that malaria research between 1976 and 1986 was up more than 800% compared with 1966 to 1976. Undoubtedly, most of this increase in research was due to the fact that the parasites could be grown readily in nearly any moderately equipped laboratory (Oransky, 2005).

# Comparability

Comparability is the proclivity for predictive hypotheses to be formed and tested among taxa in a research system. This is a fundamental requisite for most parasite research systems because parasitology is a highly relative discipline. Comparison in parasite systems becomes increasingly powerful when host and parasite datasets are overlapped. This property is an essential component of host–parasite co-evolutionary studies. We usually credit von Inhering with the idea of host-parasite co-evolution, but the idea was independently developed, employed, and published by several other researchers in a short span of a few years (Metcalf, 1929). Clearly, Metcalf ([Fig. 4](#)) was among the earliest of these investigators and his work reflects the strength of comparability in a protozoan research model.



[\*\*Figure 4\*\*](#) Maynard Mayo Metcalf in a photograph distributed in the press by Science Service during the 1925 “Scopes Monkey Trial”. (Acc. 90-105—Science Service, Records, 1920s–1970s, Smithsonian Institution Archives.)

Metcalf may be more generally familiar as an early American proponent of evolution than as a parasitologist. He published the early and influential, “An Outline of the Theory of Organic Evolution,” in 1904 and was the only scientist to testify at the anti-evolution “Scopes Monkey Trial” (State of Tennessee vs. John Thomas Scopes, 1925).

Generally recognized as a zoologist, Metcalf spent most of his career studying the parasitic opalinid ciliates and their anuran hosts. Although he was especially interested in evolutionary and biogeographical questions, Metcalf was an early American proponent of co-evolutionary research and made a strong case for the power of what he called the, “host-parasite method.”

Metcalf earned his B.Sc. in 1889, at Oberlin College in Ohio and his Ph.D. at the Johns Hopkins University in 1893. He established the Department of Biology at Goucher College, Maryland in 1893, where he remained until his return to Oberlin College in 1906, to reorganize and head the Department of Zoology. Renovations in the college library to serve as a zoological laboratory were not completed until 1908, which meant that his research could not be accomplished locally.

Accordingly, Metcalf spent several years conducting research on opalinid ciliates parasitizing fish and frogs in Europe (Budington, 1941). While working at the Zoological Station in Naples, Italy, he discovered that alcohol preserved intestines of the marine sea bream, *Boops boops*, provided excellent specimens of opalinid ciliates (Metcalf, 1922). Realizing that already existing preserved materials could provide a geographic and taxonomic plethora of opalinid and host samples, far exceeding what could be collected by any one researcher, he undertook a global study of opalinid ciliates using both living and preserved host material (Metcalf, 1922).

As an aside, the taxonomic position of the opalinid protozoans remains controversial. The group has been placed in the Ciliophora, the Sarcomastigophora, and in their own phylum, Opalinata. Their placement remains controversial above the family level, Opalinidae. Rather than enter the systematic fray, I refer to the opalinids herein as “ciliates” because that is the way in which Metcalf referred them.

Metcalf quickly recognized that his opalinid parasite-host model could produce relatively independent data sets to test hypotheses of coevolution and biogeography. He outlined his basic concepts for coevolutionary studies in an early paper testing alternative hypotheses of convergence and common ancestry in New World leptodactylid frogs using the distribution of their opalinid parasites, and exhorted somewhat optimistically:

Every group of animals and of plants should similarly be studied with their parasites. In this way many questions of genetic relationship can be settled beyond doubt and many problems of geographical distribution and of migration routes can be solved. (Metcalf, 1920).

Given access and intestinal salvage privileges in the anuran collections of the United States National Museum, he would eventually dissect and collect opalinids from 1,079 preserved host specimens producing a comprehensive monograph that included extensive discussion and analysis of anuran-opalinid distributions and relationships (Metcalf, 1923a, 1923b; Hegner, 1940). His method depended primarily upon an appeal to parsimony in weighing hypotheses of convergence

and vicariance:

It is clear that the two lines of evidence together have many times the value possessed by either set of data alone ... it might perhaps be possible, though difficult, to regard the leptodactylids of Australia and South America as of distinct origin, their resemblance being due to convergence or parallel evolution ... such a belief becomes impossible in any instance in which we find that the apparently related Anura have closely similar or almost identical parasites. Parallel development, or convergent evolution of both the hosts and their parasites is too large a dose for even the most credulous to accept.  
(Metcalf, 1923a).

Metcalf's intellectual time was one in which the notion of ocean-spanning land bridges connecting static continents was favored over a dynamic notion of continental drift. (The theory of Continental Drift was proposed in 1912 [Wegener, 1912], but dismissed by the geological community for lack of a plausible mechanism until Tectonic Plate Theory was accepted in the early 1960s.) Metcalf was clearly bothered by the proposition of ocean-spanning land bridges, but found biogeographic merit in the theory of continental drift (Metcalf, 1923a, 1923b, 1928a, 1928b–1929). He was also interested in phylogeny and diversification. His studies of morphological variation across populations of species provided pioneering insights into the nature of shared characters and the logical problems of phylogenetic reconstruction. Nonetheless, his ideas were sometimes constrained by the orthogenic thinking of his time:

Is it the youth of the younger genera [of anuran opalinids] that accounts for their less diversified speciation? It may be. But a broad review of the whole animal kingdom from this point of view would, I think, show that, in many cases, groups destined to become highly diversified may acquire a high degree of diversification soon after their first appearance. One therefore suspects that *Zelleriella* is today a very compact genus, not merely because it is comparatively young, but because stability is in “the nature of the beast”  
(Metcalf, 1923a).

He was instrumental in the early development of host–parasite co-evolutionary and biogeographical studies, but was in many ways a man ahead of his time. In his day, no broadly accepted notion of Continental Drift or the Modern Synthesis was yet available to provide a context for using host-parasite associations to examine larger co-evolutionary questions. Metcalf's choice of a protozoan model is his most important lesson for a young parasitologist a century later. His inspiration was in choosing a model protozoan system that was amenable to a rapid, global study of host-parasite relationships. The choice of the protozoan model with high comparability allowed him to collect host-parasite data sets quickly and efficiently, resulting in significant systematic contributions while developing larger emergent innovations in co-evolutionary, biogeographical, and ecological thought based on the host-parasite method.

## Scalability

Scalability is the final representation of a research system; it reflects the ability of a research model to answer not just proximate questions, but to generate ultimate questions as well. More importantly, I suppose, scalability may be thought of as the proclivity of a research model to require us ask larger, more generalized, and ultimate questions. It was John Janovy, Jr ([Fig. 5](#)) who introduced me to the notion of proximate and ultimate questions as a doctoral student by asking, “So what do gregarines tell us about stuff?”



[Figure 5](#) John Janovy, Jr in the Swallow Barn Laboratory, Cedar Point Biological Station, Keith Co., Nebraska (Courtesy of John Janovy, Jr)

Proximate questions are the simple “what” and “how” questions of science. As a general rule, they are the fodder of simple empiricism, where proximate answers simply accumulate to eventually form the vast bedrock of the scientific record. Ultimate questions are the scientific “whys?” and the “so whats?” of science, which provide contextual meaning and broader scope of thought and applicability.

John Janovy, Jr was born in Houma, Louisiana, in 1937. He earned his B.Sc. in Math at the University of Oklahoma in 1959. He earned the M.Sc. and Ph.D. Zoology at the University of Oklahoma in 1962, and 1965, respectively. A protozoologist from the beginning, John Janovy's M.Sc. work on monstrosity in the ciliate, *Dileptus anser*, was directed by Harley Brown (Janovy, 1962, 1963).

His doctoral work on avian malaria in the Cheyenne Bottoms of Kansas was guided by J. Teague Self (Janovy, 1964, 1966a, 1966b) and his post-doctoral work at Rutgers on *Leishmania donovani* physiology was directed by Leslie Stauber. Janovy joined the faculty of the University of Nebraska-Lincoln in 1966, and, as of this writing, he continues daily research, mentoring, and writing as Professor Emeritus.

The progression of research models in Janovy's career bears consideration and should serve as a pragmatic lesson for young parasitologists. He began with ciliates in culture, moved to avian malaria in the field, returned to the laboratory to work on *Leishmania* species and other trypanosomatids in culture, and then spent nearly 30 years working on a variety of field systems with students, but always with a core interest in gregarines. His passion for field biology is well-known. Asked why he left field malaria studies for laboratory work on *Leishmania*, he replied that he, "didn't think he would be able to stay married if he spent that much time in the field." Asked why he abandoned the trypanosomatids over a decade later to spend 30 years as a field biologist, John replied that with the opening of Cedar Point Biological Station, he thought he could see a way to be a field biologist and keep his family. This exchange is a strong reminder that science is first and foremost a human endeavor, and the choice of a model system must always account for the human elements of a life.

The gregarines have been Janovy's "long-term" thread for over 30 years, largely because of their scalability. Given the relative specificity of gregarines and the diversity of their primarily insect hosts, it seems likely that there are well over a million species of gregarines in the world. Considering their host ties, gregarine systems offer an almost endless variety of specific host-parasite relationships for comparison. Given their ubiquity and prevalence of infection, gregarines are readily suited to study a wide variety of biological phenomena. There is a gregarine model for almost any question, place, habitat, relationship, or aesthetic sense, and over the years, John has best demonstrated the breadth of their utility as a model. He and his students have used gregarine systems to conduct studies ranging from morphology and anatomy (Cook et al., 2001; Hoshide and Janovy, 2002; Janovy et al., 2007), taxonomy and systematics (Richardson and Janovy, 1990; Clopton et al., 1991, 1992, 1993; Percival et al., 1995; Watwood et al., 1997; Wise et al., 1999; Janovy et al., 2007), ecology (Ruhnke and Janovy, 1989, 1990; Clopton et al., 1992; Clopton and Janovy, 1993; Logan et al., 2012; Bunker et al., 2013), host specificity (Clopton et al., 1992; Wise et al., 1999; Detwiler and Janovy, 2008), life cycle variation (Wise et al., 1999), and physiology (Schawang and Janovy, 2001; Schreurs and Janovy, 2008).

At the beginning of a century of *The Journal of Parasitology*, Minnie Watson Kamm documented the overwhelming diversity of gregarines. A century later, John Janovy Jr showed us what kind of questions you could ask with almost limitless diversity by reminding us that answering the question, "What is it?" is only the beginning.

## Conclusions and lessons

Any scientist worth their salt will readily admit that their organisms or system of choice is simultaneously both the best and the worst possible model for parasitological research. It's a two-fold recognition that no research model is perfect and the productive scientist learns to exploit strengths and minimize weaknesses inherent in their chosen system. Over a career, we readily see how much our work changes the way we view, understand, and manipulate our models, but too often we fail to acknowledge just how much our models change and manipulate our view of the world.

So what are the lessons of 100 years of parasitic protozoology for the next generation of parasitologists? First, choose your research system with great care—the system you choose will both enable and limit your research and your view of the world. The best systems instill a sense of wonder that keeps us engaged, a sense of opportunity that allows us to ask ultimate questions, and a sense of the intellectual elegance that draws us to and sustains us in a life of empirical science. Second, read *The Journal of Parasitology* with great care. As a young doctoral student, I was lucky to acquire Bronislaw Honigberg's almost complete run of *The Journal of Parasitology* on his death in 1992. After putting it on the shelf in my lab, I stared at the thing, all 297 linear centimeters of it, and thought, "Now what do I do?" I pulled down Volume 1, Number 1 (September 1914) and turned to page 1. Nearly 6 months later, I caught up in time to read Volume 79 issue 6 (December 1993) over the winter term break. It may have been the single most important education self-trajectory I ever undertook, if for no other reason, than for the exposure to the empirical design and intellectual elegance of my discipline. And, although *The Journal of Parasitology* now takes up 432 linear centimeters on my shelves, I still recommend a good thorough browsing for any young parasitologist with the pluck to take it on.

Oh, and gregarines are awesome.

## **Acknowledgments**

The author M. W. Kamm is the same individual as M. E. Watson; Minnie Elizabeth Watson married Oliver Kamm and continued to publish taxonomic work, although under her married name. We believe it is important that readers know this information because of the example set by this one person (see the section on Diversity, previously).

## Literature cited

- Budington, R. A. 1941. Maynard Mayo Metcalf. *Bios* **12**: 75–78.
- Bunker, B., J. Janovy, Jr, E. Tracey, A. Barnes, A. Duba, M. Shuman, and J. D. Logan. 2013. Macroparasite population dynamics among geographical locations and host life cycle stages: Eugregarines in *Ischnura verticalis*. *Journal of Parasitology* **99**: 403–409.
- Clopton, R. E., T. J. Percival, and J. Janovy, Jr 1991. *Gregarina niphandrodes* n. sp. (Apicomplexa: Eugregarinorida) from adult *Tenebrio molitor* (L.) with oocyst descriptions of other gregarine parasites of the yellow mealworm. *Journal of Protozoology* **38**: 72–479.
- \_\_\_\_\_, and J. Janovy, Jr 1993. Developmental niche structure in the gregarine assemblage parasitizing *Tenebrio molitor*. *Journal of Parasitology* **79**: 701–709.
- \_\_\_\_\_, \_\_\_\_\_, and T. J. Percival. 1992. Host stadium specificity in the gregarine assemblage parasitizing *Tenebrio molitor*. *Journal of Parasitology* **78**: 334–337.
- Clopton, R. E., T. J. Percival, and J. Janovy, Jr 1992. *Gregarina coronata* n. sp. (Apicomplexa: Eugregarinorida) described from adults of the southern corn rootworm, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae). *Journal of Protozoology* **39**: 417–420.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1993. *Nubenocephalus nebraskensis* n. gen., n. sp. (Apicomplexa: Actinocephalidae) from adults of *Argia bipunctulata* (Odonata: Zygoptera). *Journal of Parasitology* **79**: 533–537.
- Coatney, G. R. 1933. Relapse and associated phenomena in the *Haemoproteus* infection of the pigeon. *American Journal of Hygiene* **18**: 133–160.
- \_\_\_\_\_. 1935. The effects of atabrin and plasmochin on the *Haemoproteus* infection of the pigeon. *American Journal of Hygiene* **21**: 249–259.
- \_\_\_\_\_. 1936. A check-list and host-index of the genus *Haemoproteus*. *Journal of Parasitology* **22**: 88–105.
- \_\_\_\_\_. 1937. A catalog and host-index of the genus *Leucocytozoon*. *Journal of Parasitology* **23**: 202–212.
- \_\_\_\_\_. 1938. A strain of *Plasmodium relictum* from doves and pigeons infective to canaries and the common fowl. *American Journal of Hygiene* **21**: 380–389.
- \_\_\_\_\_. 1976. Relapse in malaria: An enigma. *Journal of Parasitology* **62**: 2–9.

- \_\_\_\_\_. 1985. Reminiscences: My forty-year romance with malaria. *Transactions of the Nebraska Academy of Sciences* **13**: 5–11.
- \_\_\_\_\_, and E. West. 1937. Some notes on the effect of atebrine on the gametocytes of the genus *Leucocytozoon*. *Journal of Parasitology* **23**: 227–228.
- \_\_\_\_\_, and \_\_\_\_\_. 1938. Some blood parasites from Nebraska birds II. *American Midland Naturalist* **19**: 601–612.
- \_\_\_\_\_, and R. L. Roundabush. 1937. Some blood parasites from Nebraska birds. *American Midland Naturalist* **18**: 1005–1030.
- Cook, T. J. P., J. Janovy, Jr, and R. E. Clopton. 2001. Epimerite-host epithelium relationships among eugregarines parasitizing the damselflies *Enallagma civile* and *Ischnura verticalis*. *Journal of Parasitology* **87**: 988–996.
- Cort, W. W. 1942. Robert William Hegner: 1880–1942. *Journal of Parasitology* **28**: 175–177.
- Detwiler, J., and J. Janovy, Jr 2008. The role of phylogeny and ecology in experimental host specificity: Insights from a eugregarine-host system. *Journal of Parasitology* **94**: 7–12.
- Heaton, L. D. 1963. Medical Department, United States Army, Preventive Medicine in World War II, Volume VI. Communicable Diseases: Malaria. [Online] <http://history.amedd.army.mil/booksdocs/wwii/Malaria/default.htm>. Accessed July 21, 2015.
- Hegner, R. W. 1921. Measurements of *Trypanosoma diemyctyli* from different hosts and their relation to specific identification, heredity and environment. *Journal of Parasitology* **7**: 105–113.
- \_\_\_\_\_. 1922. The effects of changes in diet on the incidence, distribution and numbers of certain intestinal protozoa of frog and toad. *Journal of Parasitology* **9**: 51–67.
- \_\_\_\_\_. 1929. Experimental studies of bird malaria. *The Quarterly Review of Biology* **4**: 59–82.
- \_\_\_\_\_. 1932. Observations and experiments on the opalinid ciliates of the green frog. *Journal of Parasitology* **18**: 274–277.
- \_\_\_\_\_. 1933a. Starch diets and infections with *Endamoeba histolytica* in chicks. *Journal of Parasitology* **20**: 57–58.
- \_\_\_\_\_. 1933b. The effects of a high vegetable protein diet on the trichomonad flagellates of rats. *American Journal of Tropical Medicine and Hygiene* **13**: 535–538.

- \_\_\_\_\_. 1935. Protozoa from other animals in the ceca of parasite-free chicks. *Journal of Parasitology* **21**: 59–60.
- \_\_\_\_\_. 1937. Parasite reactions to host modifications. *Journal of Parasitology* **23**: 1–12.
- Hegner, R. 1940. The contributions of Maynard M. Metcalf to protozoology. *Journal of Parasitology* **26**: 522–524.
- Hegner, R. W., and J. Andrews. 1931. *Problems and methods of research in protozoology*. The McMillan Company, New York, NY, 532 p.
- \_\_\_\_\_, and L. Eskridge. 1935. Effects of a high protein diet on *Trichomonas hominis* in rats. *Journal of Parasitology* **21**: 313.
- \_\_\_\_\_, and \_\_\_\_\_. 1937a. Effect of starvation on trichomonads in rats. *Journal of Parasitology* **23**: 225–226.
- \_\_\_\_\_, and \_\_\_\_\_. 1937b. Influence of liver diets on trichomonad infections in rats. *American Journal of Epidemiology* **26**: 127–134.
- \_\_\_\_\_, and \_\_\_\_\_. 1937c. Persistence in rats of human intestinal trichomonad flagellates. *American Journal of Epidemiology* **26**: 124–126.
- \_\_\_\_\_, and M. S. MacDougall. 1926. Modifying the course of infections with bird malaria by changing the sugar content of the blood. *American Journal of Hygiene* **6**: 600–609.
- Hofstadter, D. 1985. *Metamagical themes: Questing for the essence of mind and pattern*. Basic Books, New York, New York, 852 p.
- Hoshide, K., and J. Janovy, Jr 2002. The structure of the nucleus of *Odonaticola polyhamatus* (Gregarinea: Actinocephalidae), a parasite of *Mnais pruinosa* Selys (Odonata: Calopterygidae). *Acta Protozoologica* **41**: 17–22.
- Janovy, Jr, J. 1962. Observations on the size of the ciliate *Dileptus anser*. *Proceedings of the Oklahoma Academy of Science* **42**: 290–291.
- \_\_\_\_\_. 1963. Monsterism in *Dileptus* (Ciliata) fed on planarians (*Dugesia tigrina*). *Journal of Protozoology* **10**: 428–430.
- \_\_\_\_\_. 1964. A preliminary survey of blood parasites of Oklahoma birds. *Proceedings of the Oklahoma Academy of Science* **44**: 58–61.
- \_\_\_\_\_. 1966. Epidemiology of *Plasmodium hexamerium* Huff, 1935, in meadowlarks and starlings of the Cheyenne Bottoms, Barton County, Kansas. *Journal of Parasitology* **52**: 573–578.
- \_\_\_\_\_. 1966. Mosquitoes of the Cheyenne Bottoms Waterfowl management Area, Barton County, Kansas. *Journal of the Kansas Entomological Society* **39**:

- \_\_\_\_\_, M. G. Bolek, J. Detwiler, S. Schwank, A. Knipes, and G. Langford. 2007. *Gregarina niphandrodes* (Eugregarinorida: Septatorina): Oocyst surface architecture. *Journal of Parasitology* **93**: 714–716.
- \_\_\_\_\_, J. Detwiler, S. Schwank, M. G. Bolek, A. K. Knipes, and G. J. Langford. 2007. New and emended descriptions of gregarines from flour beetles (*Tribolium* spp. and *Palorus subdepressus*: Coleoptera, Tenebrionidae). *Journal of Parasitology* **93**: 1155–1170.
- Jensen, J. B. 2005. William Trager, 1910–2005. *Journal of Parasitology* **91**: 728.
- \_\_\_\_\_, and W. Trager. 1977. *Plasmodium falciparum* in culture: Use of outdated erythrocytes and description of the candle jar method. *Journal of Parasitology* **63**: 883–886.
- Kamm, M. W. 1917a. The development of gregarines and their relation to the host tissues: (I) in *Stenophora lactaria* Watson. *Journal of Parasitology* **3**: 124–130.
- \_\_\_\_\_. 1917b. Notes on known gregarines. *Journal of Parasitology* **4**: 40–43.
- \_\_\_\_\_. 1918a. New gregarines from Coleoptera. *Journal of Parasitology* **4**: 159–163.
- \_\_\_\_\_. 1918b. The development of gregarines and their relation to the host tissues: (II) in *Cephaloidophora delphinia* (Watson). *Journal of Parasitology* **5**: 35–40.
- \_\_\_\_\_. 1922a. Studies on gregarines II: Synopsis of the polycystid gregarines of the world, excluding those from the Myriapoda, Orthoptera, and Coleoptera. *Illinois Biological Monographs* **7**: 1–104.
- \_\_\_\_\_. 1922b. A list of the new gregarines described from 1911 to 1920. *Transactions of the American Microscopical Society* **41**: 122–152.
- Logan, J. D., J. Janovy, Jr, and B. Bunker. 2012. The life cycle and fitness domain of gregarine (Apicomplexa) parasites. *Ecological Modeling* **233**: 31–40.
- McGhee, R. B., and W. Trager. 1950. The cultivation of *Plasmodium lophurae* in vitro in chicken erythrocyte suspensions and the effects of some constituents of the culture medium upon its growth and multiplication. *Journal of Parasitology* **36**: 123–127.
- Metcalf, M. M. 1920. Upon an important method of studying problems of relationship and of geographical distribution. *Proceedings of the National Academy of Sciences USA* **6**: 432–433.

\_\_\_\_\_. 1922. A source for material of protozoan and other parasites. *Journal of Parasitology* **8**: 148.

\_\_\_\_\_. 1923a. The opalinid ciliate infusorians. *Bulletin of the United States National Museum* **120**: 1–484.

\_\_\_\_\_. 1923b. The origin and distribution of the Anura. *American Naturalist* **57**: 385–411.

\_\_\_\_\_. 1928a. The bell-toads and their opalinid parasites. *American Naturalist* **62**: 5–21.

\_\_\_\_\_. 1928b. Trends in evolution: A discussion of data bearing upon “orthogenesis.” *Journal of Morphology and Physiology* **45**: 1–45.

\_\_\_\_\_. 1929. Parasites and the aid they give in problems of taxonomy, geographical distribution, and palaeogeography. *Smithsonian Miscellaneous Collections* **81**: 1–36.

Oransky, I. 2005. William Trager. *The Lancet* **365**: 748.

Percival, T. J., R. E. Clopton, and J. Janovy, Jr 1995. Two new menosporine gregarines, *Hoplorhynchus acanthatholius* n. sp. and *Steganorhynchus dunwoodyi* n. g., n. sp. (Apicomplexa: Eugregarinorida: Actinocephalidae) from coenagrionid damselflies (Odonata: Zygoptera). *Journal of Eukaryotic Microbiology* **42**: 406–410.

Pritchard, M. H. 1985. Meet Dr. Bob. *Transactions of the Nebraska Academy of Sciences* **13**: 1–3.

Richardson, S., and J. Janovy, Jr 1990. *Actinocephalus carrilynnae* n. sp. (Apicomplexa: Eugregarinorida) from the blue damselfly, *Enallagma civile* (Hagen). *Journal of Protozoology* **37**: 567–570.

Ruhnke, T. R. and J. Janovy, Jr 1989. The site specificity of two species of *Gregarina* in *Tenebrio molitor* larvae. *Journal of Protozoology* **36**: 428–430.

\_\_\_\_\_, and \_\_\_\_\_. 1990. Life history differences between two species of *Gregarina* in *Tenebrio molitor* larvae. *Journal of Parasitology* **76**: 519–522.

Schawang, J. E., and J. Janovy, Jr 2001. The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. *Journal of Parasitology* **87**: 600–605.

Schreurs, J. S., and J. Janovy, Jr 2008. Gregarines on a diet: The effects of host starvation on *Gregarina confusa* Janovy et al., 2007 (Apicomplexa: Eugregarinida) in *Tribolium destructor* Uyttenboogaart, 1933 (Coleoptera: Tenebrionidae) larvae. *Journal of Parasitology* **94**: 567–570.

- Siddiqui, W. A., and W. Trager. 1966. Folic and folinic acids in relation to the development of *Plasmodium lophurae*. *Journal of Parasitology* **52**: 556–558.
- Trager, W. 1941 Studies on conditions affecting the survival *in vitro* of a malarial parasite (*Plasmodium lophurae*). *Journal of Experimental Medicine* **74**: 441–462.
- \_\_\_\_\_. 1943 Further studies on the survival and development *in vitro* of a malarial parasite. *Journal of Experimental Medicine* **77**: 411–420.
- \_\_\_\_\_. 1947. The development of the malaria parasite *Plasmodium lophurae* in red blood cell suspensions. *Journal of Parasitology* **33**: 345–350.
- \_\_\_\_\_. 1970. Recent progress in some aspects of the physiology of parasitic protozoa. *Journal of Parasitology* **56**: 627–633.
- \_\_\_\_\_. 1975. On the cultivation of *Trypanosoma vivax*: a tale of two visits in Nigeria. *Journal of Parasitology* **61**: 2–11.
- \_\_\_\_\_. 1976. Prolonged cultivation of malarial parasites (*Plasmodium coatneyi* and *P. falciparum*). In *Biochemistry of parasites and host-parasite relationships*, H. Van den Bossche (ed.). Elsevier-North Holland Biomedical Press, Amsterdam, Netherlands, p. 427–434.
- \_\_\_\_\_, and J. Jensen. 1976. Human malaria parasites in continuous culture. *Science* **193**: 674–675.
- University of Illinois. 1918. *The semi-centennial alumni record of the University of Illinois*. Lakeside Press, Chicago, Illinois, 1147 p.
- Watson, M. E. 1915. Some new gregarine parasites from arthropoda. *Journal of Parasitology* **2**: 27–36.
- \_\_\_\_\_. 1916a. Studies on gregarines: Including descriptions of twenty-one new species and a synopsis of the eugregarine records from the Myriapoda, Coleoptera and Orthoptera of the world. *Illinois Biological Monographs* **2**: 3–258.
- \_\_\_\_\_. 1916b. Three new gregarines from marine crustacea. *Journal of Parasitology* **2**: 129–136.
- \_\_\_\_\_. 1916c. Observations on polycystid gregarines from Arthropoda. *Journal of Parasitology* **3**: 65–75.
- Watwood, S., J. Janovy, Jr, E. Peterson, and M. A. Addison. 1997. *Gregarina triboliorum* (Eugregarinida: Gregarinidae) n. sp. from *Tribolium confusum*, and resolution of the confused taxonomic history of *Gregarina minuta* Ishii 1914. *Journal of Parasitology* **83**: 502–507.

Wegener, A. 1912. Die Entstehung der Kontinente. *Petermanns Geographische Mitteilungen* **1912**: 185–195.

Wise, M. R., J. Janovy, Jr, and J. C. Wise. 1999. Host specificity in *Metamera sillasenorum*, n. sp. a gregarine parasite of the leech *Helobdella triserialis* with notes on transmission dynamics. *Journal of Parasitology* **86**: 602–606.



# Chapter 3

## Notes on Two Cestodes from the Spotted Sting-Ray

Edwin Linton

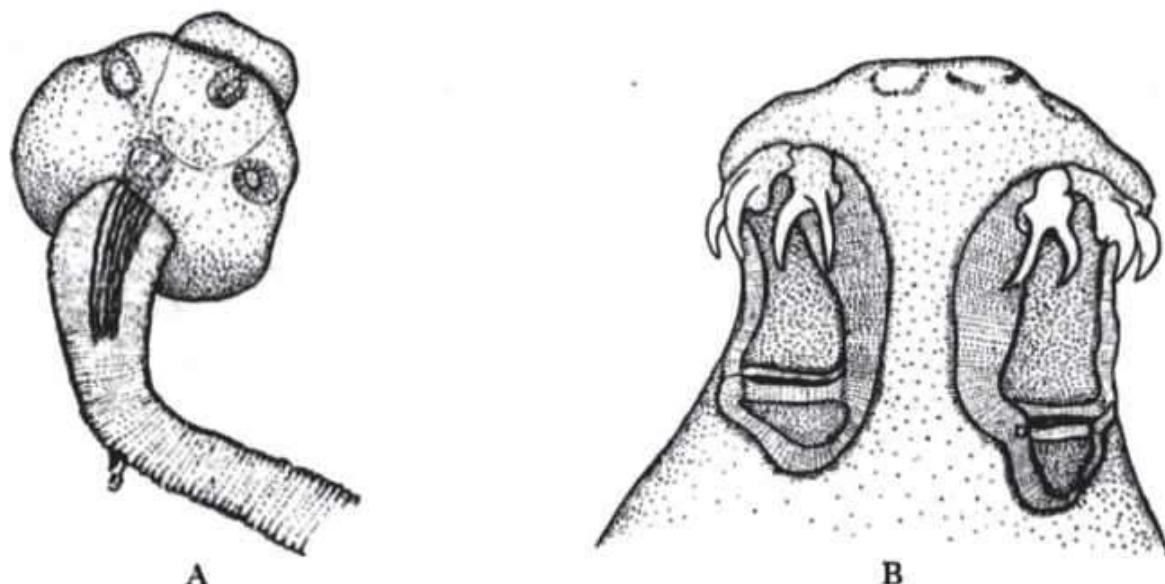
A single specimen of a species of cestode found in the spiral valve of a cow-nosed ray (*Rhinoptera bonasus*) at Woods Hole, July 29, 1887, was made the type of a new genus and species (*Tylocephalum pingue*). No other examples of this genus have been found at Woods Hole, but on June 30, 1908, at the Tortugas laboratory, I obtained two specimens of a cestode from the spotted sting-ray (*Aetobatis narinari*) which are to be referred to the genus *Tylocephalum*. The specimen from the cow-nosed ray was a less mature strobile than those from the spotted ray; a comparison of the genitalia, therefore, cannot be made. There appears to be enough difference, however, in other particulars to justify referring the Tortugas specimens to a new species. While both hosts belong to the family of eagle rays, there is enough difference between them in the way of geographical range and generic features to make it unlikely that the same species of cestodes should be found in each.

### TYLOCEPHALUM MARSUPIUM nov. spec.

Scolex: The relatively large, muscular portion (*myzorhynchus*) is subglobular, its length in a living specimen 0.16 and breadth 0.21 mm.; bothria united into a subglobular disc with four auxiliary acetabula, length of disc 0.30, breadth 0.69 mm. The constriction noted in the Woods Hole specimen not present. As in the case of the specimen from the cow-nosed ray, the scoleces were rather firmly fastened to the mucous membrane of the spiral valve. One of the worms was fixed without detaching it, and was sectioned together with a small piece of the intestinal wall. The sections show that the *myzorhynchus* alone had entered the mucous membrane.

Strobile: The segments begin nearer the scolex than they do in *T. pingue*. Just behind the scolex, where the breadth was 0.16, the strobile was crossed by crowded lines. One-half millimeter back of the scolex the well-defined segments were 0.014 mm. in length and 0.18 mm. in breadth. Three millimeters back the length of the segments is about 0.05 and the breadth 0.24; ten millimeters back the length is 0.12, the breadth 0.28; twenty millimeters back the length is 0.28, the breadth 0.24; thirty millimeters back the length is 0.46, the breadth 0.28; forty millimeters back of the scolex the length is 0.56, the breadth 0.38 mm. The last segments are somewhat variable in their dimensions, but are about one millimeter in length and 0.5 mm.

in greatest breadth. They are vase-shaped, constricted at the anterior end, swelling out to the maximum breadth behind the middle, slightly constricted near the posterior end with a moderately projecting posterior margin. One proglottis had the following dimensions: Length 0.84; breadth, anterior 0.21, maximum 0.56, posterior 0.39 mm. The strobile is especially distinguished by the strongly developed longitudinal muscles. The longitudinal muscles are disposed in radial bundles near the scolex (Fig. 1), but farther back lie in a well-defined zone (Fig. 2). In segments in which the genitalia have become differentiated this zone of muscle bundles coincides in position with the vitellaria (Fig. 4).



Text Fig. A.—*Tylocephalum marsupium*. View of scolex in life, somewhat flattened and seen from behind. Breadth of scolex 0.7 mm.

Text Fig. B.—*Onchobothrium tortum*. Side view of scolex; balsam. Diameter at base of hooks 0.64 mm.

**Genitalia:** The general plan of arrangement of the genitalia is shown in Figure 7. The vitellaria are peripheral and consist of rather finely granular masses lying between and also centrally to the muscle bundles. The testes are in the median region. In the younger proglottids they occupy most of the interior, but as the proglottids mature they give way to the seminal receptacle and ovary. The cirrus-pouch is relatively small and oval, opening near the margin not far from the middle of the length. The vagina opens into the genital cloaca, passes along one side of the cirrus pouch, becomes more or less convoluted and expands into a capacious seminal receptacle. This was filled with spermatozoa in all the later proglottids. The ovary is lobed and is situated at the posterior end of the proglottis.

The uterus was still rudimentary even in the mature proglottids. In a section a small cluster of minute bodies was seen. They lay in the lumen of the uterus, were yellowish brown, and about 0.010 by 0.007 mm. in the two principal diameters.

ONCHOBOTHRIUM TORTUM nov. spec.

Ten specimens of this form were obtained from a spotted sting-ray (*Aetobatis narinari*), June 30, 1908. The scolices were imbedded in the intestinal wall and had caused some ulceration. One of the worms, straightened out on a glass plate in sea water, measured 220 mm. in length. Anterior end sub-cylindrical, with a tendency to coil spirally; color dark ashy-gray. Scolex long-clavate, armed with four pairs of short, sharp, two-pronged hooks. Each pair of hooks situated at the anterior end of one of the four bothria. The latter are oblong, trough-shaped, with two costæ near the posterior end. Behind the scolex the body is at first sub-cylindrical and crossed by fine, closely crowded lines for a considerable distance. The segments outlined by these transverse lines remain closely crowded, while the adult proglottids begin rather abruptly. The average length of the first 12 adult proglottids was 0.8 mm., the breadth being about the same or slightly greater. The diameter of the sub-cylindrical portion of the strobile was about 1.5 mm. The scolex and anterior portion of the strobile are much thicker than the adult proglottids. Diameter of scolex, in alcohol, anterior 0.85, middle 0.77; diameter of neck, a short distance back of the scolex, 1.4 mm. Dimensions of one of the posterior proglottids: life, length 1.47; breadth, anterior 0.5; middle 0.8, posterior 0.6 mm. Dimensions of scolex mounted in balsam: length 0.97; breadth, at base of hooks, 0.97, behind hooks, 0.81; breadth of neck, a short distance behind the scolex, 1.27 mm. In the mounted specimen the neck is seen to be traversed by strong longitudinal muscle bundles which are closely crowded together, each bundle about 0.06 mm. in diameter. About 16 bundles were counted near the head; farther back they are divided into a larger number of smaller bundles. Two spiral vessels show distinctly in the mounted specimen. The strobile narrows as the proglottids become distinct. In the specimen which measured 220 mm. there were distinct and well-formed segments on the last 150 mm. The maturing segments were at first much broader than long, then squarish, then longer than broad, the last ones three times as long as broad. The posterior margins of the proglottids project slightly and have crenulate borders. One of the posterior proglottids of a mounted strobile has the following dimensions: length 1.86; breadth, anterior 0.36, constriction near anterior end 0.25, middle 0.40, posterior margin 0.54 mm. The genital apertures are marginal at about the middle of the length. They are irregularly alternate. No ova were seen.

The general plan of arrangement of the genitalia is shown in Figure 8. The cirrus is armed with slender, bristle-like spines; a few folds of the vas deferens are included in the oval cirrus-pouch at its

medial end. The voluminous folds of the vas deferens form the seminal vesicle and occupy the median third of the anterior half of the proglottis. The testes are situated in the anterior half of the proglottis, and occupy the median region on each side of the vas deferens. On the marginal sides of the testes are the vitelline glands which extend along each marginal border of the entire length of the proglottis, being interrupted only at the point where the cirrus pouch and the accompanying vagina approach the genital aperture. The uterus was represented by a tubular structure lying along the median line near one of the lateral faces of the proglottis, and extending from nearly one end of the proglottis to the other. The ovary is a lobed organ and fills all the space between the marginal vitellaria behind the cirrus pouch. The vagina opens at the genital pore immediately in front of the cirrus and lies alongside the anterior border of the cirrus pouch. At this point it is thick-walled and glandular. It becomes tubular at about the level of the median end of the pouch and passes along the median line beneath the uterus to about the middle of the ovary. The relative positions of vagina and uterus are shown in Figure 9, which is sketched from a transverse section of a maturing segment at a level which passes very near the genital aperture, shows a portion of the vagina near the margin, cuts into some folds of the vas deferens, and passes thru the vagina again near the middle of the segment, where it lies on the medial side of the uterus. The section also catches a few of the anterior lobes of the ovary. In this section the characteristic longitudinal muscles are seen as an inner circle of larger and an outer circle of smaller bundles. The lateral vitellaria and the median testes flanking the folds of the seminal vesicle are also shown.

#### SUMMARY

Two new species of cestodes, of the genera *Tylocephalum* and *Onchobothrium*, respectively, are described in this paper. One of them, *T. marsupium*, is the first cestode of this genus to be recorded since the genus was established in 1887. Thus far representatives of this genus have been found only in the eagle rays.

Altho the two genera belong to quite different families, they possess an interesting feature in common in the strongly fasciculated longitudinal muscle layers. Both species were fastened to the mucous membrane of the spiral valve which, at the point of attachment of the onchobothria, was somewhat ulcerated.

## EXPLANATION OF PLATE

Fig. 1.—*Tylocephalum marsupium*. Transverse section of neck. Diameter 0.22 mm.

Fig. 2.—*Tylocephalum marsupium*. Transverse section of early proglottis, showing rudiment of genitalia and peripherally arranged longitudinal muscle bundles. Greater diameter 0.65 mm.

Fig. 3.—*Onchobothrium tortum*. Transverse section of neck, showing longitudinal muscle bundles and vessels of the vascular system. Longer diameter of section 1.12 mm.

Fig. 4.—*Tylocephalum marsupium*. Transverse section of mature proglottis in front of cirrus bulb; longer diameter 0.45 mm.

Fig. 5.—*Onchobothrium tortum*. Longitudinal view of neck showing muscle bundles. Breadth 1.17 mm.

Fig. 6.—*Onchobothrium tortum*. View of retracted cirrus, and vagina; from longitudinal section.

Fig. 7.—*Tylocephalum marsupium*. Posterior proglottis; outline from life; genitalia partly diagrammatic. Length 0.8 mm.

Fig. 8.—*Onchobothrium tortum*. Posterior proglottis; balsam. Length 1.6 mm.

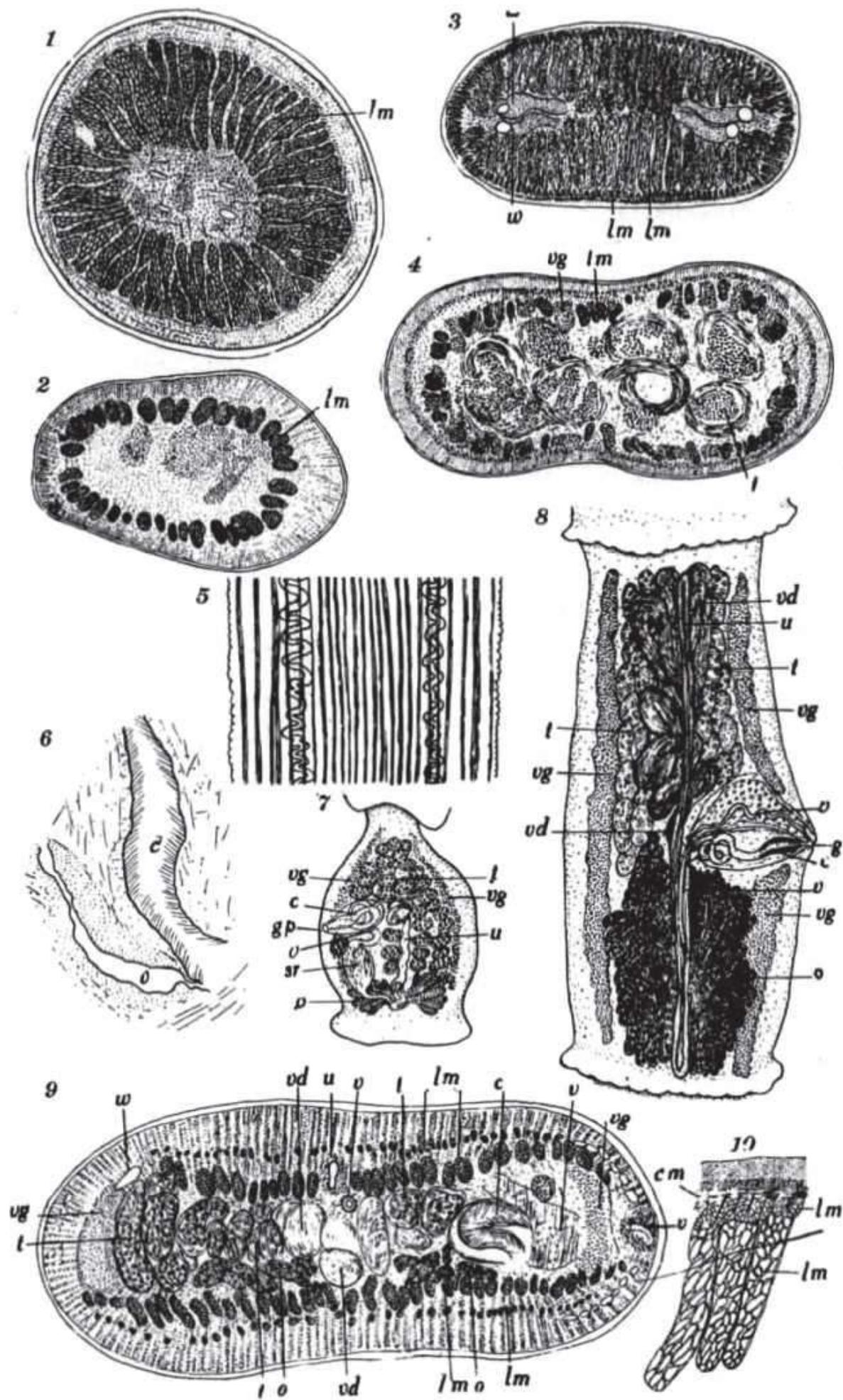
Fig. 9.—*Onchobothrium tortum*. Transverse section of a somewhat younger proglottis than that shown in Figure 8. Longer diameter of section 1.12 mm.

Fig. 10.—*Onchobothrium tortum*. Details of musculature.

### ABBREVIATIONS USED

<i>c</i> , retracted cirrus	<i>t</i> , testes
<i>cm</i> , circular muscle layer	<i>u</i> , uterus
<i>gp</i> , genital pore	<i>v</i> , vagina
<i>lm</i> , longitudinal muscle bundles	<i>vd</i> , vas deferens
<i>o</i> , ovary	<i>vg</i> , vitellaria
<i>sr</i> , seminal receptacle	<i>w</i> , longitudinal vessel

PLATE







# **Helminth biodiversity research transformed by a century of evolutionary thought**

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What if, during his voyage on the Beagle (1831–1836), Darwin had conducted as detailed an inventory of the parasites of the wondrous creatures he discovered as he did of those creatures themselves? Not only is it interesting to ponder what his formidable mind would have made of those parasitic organisms, but it is also intriguing to consider how differently our understanding of parasite biodiversity might have advanced had we *begun* with a global perspective. As it turns out (barnacles aside!), Darwin did, in fact, collect a few parasites over the course of his travels but from only a few vertebrates (i.e., “the *Rhea*”, “a Porcupine”, “a Snake”, “*Cavia Cobyaya*”, and “three sets from fish”; Cobbold, 1885, p. 174), all from South America. These specimens remained unexamined in his possession until 1869, almost 40 years later, when he offered them to T. Spencer Cobbold. Sixteen years after that, Cobbold (1885) reported these specimens to consist of filarial, oxyurid, and ascarid nematodes, as well as an unidentified digenean and several lernaeid copepods—a list that seems trivial in the larger scheme of Darwin's discoveries. In reflecting on Darwin's uncharacteristic lack of attention to parasite diversity, it seems plausible that the relatively small size of most parasites, combined with the labor-intensive and time-consuming methods of preparation required to properly identify such taxa, might have contributed to his “disinterest” in these animals at the time of their collection.

In fact, the history of the discovery and description of parasite biodiversity and all of its interconnected disciplines has unfolded with a markedly local focus, albeit simultaneously in multiple locations across the globe. As a consequence, one of the greatest challenges in the advancement of the field has been the accurate integration of geographically distant regional discoveries into a single unified framework. Because a historical treatment of all host–parasite systems in this context is beyond the scope of this chapter, we will focus here on helminths and specifically the cestodes (tapeworms) of elasmobranchs (sharks and rays), not only because it is our area of expertise, but also because this host–parasite system is ideal for illustrating many elements of the transformation in the field of parasite biodiversity research that has occurred over the past century. Again, largely because of our own interests, we will consider biodiversity in its broadest sense, encompassing the disciplines of taxonomy, systematics, phylogenetics and to some extent also host associations, coevolution, and biogeography. We will however, also draw on relevant work in other biological systems to illustrate

methodological and empirical advancements that have had a major impact on the field overall. As is our charge, we will highlight works published in *The Journal of Parasitology (JP)*.

The springboard for our chapter is the first paper in *JP* describing elasmobranch tapeworms. That paper, titled “Notes on two cestodes from the spotted sting-ray”, appeared in 1916, in Volume 3, and was authored by Edwin Linton. In fact, Linton figures prominently in the history of marine fish helminth biodiversity studies in North America overall. In 1882, he began his long and prodigious career working at the United States Fish Commission's Fisheries Laboratory at Woods Hole investigating the then uncharted field of North American marine fish parasites (see Moore, 1939, for a detailed account). At that time, essentially the only other parasitologist actively working in North America was Joseph Leidy whose interests were more focused on the parasites of humans and freshwater hosts (Osborn, 1913) than on those of marine vertebrates.

Globally, however, Linton was not alone. In the second half of the nineteenth century, a plethora of European investigators, influenced by the Linnaean School (see Sattmann, 2002) had already moved away from medical parasitology to work on the discovery and description of helminths of non-medical importance. Their home countries included Sweden (e.g., Rudolphi, 1819), Germany (e.g., Carus, 1863), Belgium (e.g., Van Beneden, 1849; 1850), Austria (e.g., Diesing, 1850; Wedl, 1855), and Switzerland (e.g., Zschokke, 1884), to name a few. In a large part because of the economic potential of the pearl oyster industry in the then British Raj of India and the British Crown Colony of Ceylon (now Sri Lanka), British workers also became actively involved relatively early on in this type of research (e.g., Shipley and Hornell, 1904, 1905, 1906; Hornell, 1912; Southwell, 1911, 1912, 1925, 1927, 1929).

From 1887 to 1908, Linton discovered and described the endoparasites of elasmobranchs (and also of some teleosts) throughout the western Atlantic seaboard, reporting his results largely in federal government publications (1889a, 1889b, 1892, 1894, 1897a, b, 1900, 1901, 1905, 1907a, b, 1908). Linton's paper in *JP*, in 1916, brought the description of North American marine helminths out of the obscurity of government publications and into a more visible outlet. The only other descriptions of novel helminths to appear in *JP* prior to 1916 came from Horace Stunkard (1915). Working at the University of Illinois under *JP*'s then managing editor Henry Baldwin Ward, Stunkard described four new species of digeneans from freshwater turtles in 1915. Despite explicitly self-identifying as “A Quarterly Devoted to Medical Zoology” until 1932 (Vol. 18), since its inception, *JP* has served as one of the primary outlets for the dissemination of parasite biodiversity discoveries from around the world.

In terms of content and approach, Linton's (1916) descriptions of *Tylocephalum marsupium* Linton, 1916, and *Onchobothrium tortum* Linton, 1916, were state-of-the art for that time. They were based on whole mounts and histological sections.

Live material and specimens *in situ* were also studied using light microscopy. Descriptions of both species were thorough and well-illustrated. However, as was typical of such works at that time, other aspects of his discoveries were less fully addressed. The locality was given only as “Tortugas Laboratory” (p. 34). The host was given as “spotted sting-ray (*Aetobatis* [sic] *narinari*)” (p. 34). There was no indication of the fate of the specimens on which the descriptions were based. Most conspicuous was the lack of a Literature Cited section. Linton made no comparisons between *O. tortum* and any of its seven congeners, and compared *Tylocephalum marsupium* only to the type species of the genus, *T. pingue*, which he himself had described in 1890, from “*Rhinoptera quadriloba*” at Woods Hole.

The following statement by Linton (1916, p. 34) attests to the fact that, after spending over 30 years documenting and describing novel taxa, he had a firm grasp on the remarkable degree of host specificity typical of so many cestodes from elasmobranchs, at least in a local context:

While both hosts belong to the family of eagle rays, there is enough difference between them in the way of geographical range and generic features to make it unlikely that the same species of cestodes should be found in each.

However, he made no mention of earlier descriptions of cestodes from hosts also identified as *A. narinari* by Shipley and Hornell (1905, 1906) and Southwell (1911) in Sri Lanka. Whether Linton was unaware of work on-going elsewhere or considered such comparisons to be unnecessary because he viewed cestodes and their hosts to be limited in geographic distribution is unclear.

What is clear is that the latter view was not universally held. Southwell, for example, considered species to be globally distributed. He routinely assigned names of species described by Linton and others from North America or Europe (e.g., Rudolphi, 1819, Van Beneden, 1849; 1850) to cestodes collected from hosts off India and Sri Lanka (e.g., Southwell, 1911, 1912, 1922, 1924a, 1924b, 1925, 1927, 1930). He regularly synonymized species collected from disparate geographic locations and in many cases, synonymized species collected from relatively unrelated hosts (e.g., Southwell, 1925). On several occasions Southwell mentioned having borrowed specimens from Linton (e.g., Southwell, 1925, p. 108) suggesting that his identifications were based on relaxed concepts of species boundaries and host specificity rather than a lack of knowledge of work ongoing elsewhere. Nonetheless, one can imagine that the challenges imposed on conducting comparative work on a global scale, given the limitations of the mechanisms for communication and dissemination of knowledge at that time, were immense.

To place Linton's paper in broader historical context—it appeared 158 years after publication of Linnaeus' *Systema Naturae* in 1758, 57 years after Darwin's (1859) *Origin of Species*, 50 years after Haeckel (1866) published the first “phylogenetic” tree, and only 16 years after the rediscovery of Mendel's formulation of genetic principles in 1900. Although work at the turn of the nineteenth century was

conducted in the framework of explicit classifications, those classifications were subjective and were based on features visible with the light microscope alone. If, following Wiley and Lieberman (2011), *Taxonomy* is describing, naming, and ordering groups of organisms in a classification, *Systematics* is the study of diversity as it is relevant to the evolutionary relationships thought to exist among organisms, and *Phylogenetics* is the recovering of phylogenetic (i.e., evolutionary) relationships among organisms and development of classification schemes consistent with those relationships, then in 1916, studies of parasite biodiversity consisted solely of *Taxonomy* in its strictest sense (see Mayr et al., 1953).

Overall, the field of parasite biodiversity has changed substantially since 1916. Not only has it expanded in scope to include *Systematics* and *Phylogenetics*, but the discipline of *Taxonomy* itself has become more formalized and comprehensive. Today parasite biodiversity represents a rich and multi-dimensional area of investigation. The challenge of determining the extent to which parasites and hosts are locally or globally distributed remains, but assessing degree of host specificity, estimating the magnitude of parasite and host diversity globally, understanding phylogenetic relationships, and co-evolutionary and biogeographic patterns and their underlying mechanisms are all now elements of parasite biodiversity research. Approaches for addressing these questions have also changed considerably. Next, we reflect on the roles advances in four interconnected areas have played in this transformation. We also explore the impact these advances have had on studies of helminth biodiversity in particular, and provide some thoughts on the future of the field.

## Rules of nomenclature

The advantages of the taxonomic stability resulting from the formalization of a mandated set of rules governing the structure and establishment of scientific names of animals, set forth by the International Code of Zoological Nomenclature (ICZN), cannot be over-emphasized, particularly given the globally-distributed nature of work describing novel taxa. In fact, those rules came relatively late to the science of animal taxonomy. There have been only four editions of the ICZN (for an account of the early history see Melville, 1995). The first edition was crafted at the direction of the International Commission of Zoological Nomenclature following discussions at the Fifteenth International Congress of Zoology in London in 1958, by a committee chaired by American Society of Parasitologists' past-president Norman R. Stoll. That edition appeared in print in 1961 (Stoll et al., 1961) and was followed closely by a slightly modified second edition in 1964 (Stoll et al., 1964). Several decades later, the third edition (Ride et al., 1985) appeared, followed by the fourth, and current, edition at the turn of the twenty-first century (Ride et al., 1999).

One point is of particular note—while recommended beginning with the first edition, it was not until the most recent edition of the Code that the explicit designation and deposition of name-bearing type specimens for species-group names was actually mandated. Important amendments to the Code, since publication of the fourth edition, include expanding and refining allowed methods of publication as they pertain to electronic publication after 2011 (see ICZN, 2012). The recent establishment of ZooBank as the official registry of Zoological Nomenclature (see Pyle and Michel, 2008) provides a crucial mechanism for streamlining the tracking of nomenclatural acts, nomenclatural publications, authors of scientific names, and type specimens in an electronic era. Perhaps most importantly, although it is not yet the case, ZooBank has the potential to serve as a “master” list of all names in zoology!

# Technology

Studies of parasite biodiversity have benefited from the same vast assortment of technological advancements that have radically transformed essentially all other areas of science over the past 100 years (*National Geographic*, 2012). Although these advances seem almost too obvious to list, some are interesting to consider briefly in the context of Linton's effort in 1916. The microcomputer, procurable by individual consumers to serve as a personal computer as early as 1977 (e.g., Commodore PET), greatly facilitated the electronic generation of descriptions and all of their associated illustrations, as well as the development of methods for phylogenetic analysis of large data sets. The digital camera (e.g., Fuji DS-1P; MegaVision Tessera), which was available commercially by the late 1980s and ubiquitous by the early 1990s, made it feasible and cost effective to capture images from microscopes, and also to document individual hosts in the field. The launching of the U.S. Department of Defense's network of satellites comprising the Global Positioning System (GPS) in 1978, followed by the mass marketing of GPS devices to civilians in the early 1980s, made georeferencing of collecting localities possible.

The global system of interconnected computer networks constituting the Internet became largely available to academia in the early 1980s. This innovation paved the way for development of the first webpages and web browsers in 1990, and ultimately for development of the remarkable resource for sharing information globally represented by the World Wide Web. The combined result is the current unprecedented capacity for communication and electronic dissemination of information globally that we enjoy today—of course these endeavors are made even easier by the development of e-mail and advanced search engines such as Google in 1997. As a consequence, at this point in time, taxonomic literature (e.g., Biodiversity Heritage Library), museum specimen data and in many cases compilations of museum specimen data (e.g., Global Biodiversity Information Facility [GBIF], Arctos), images of parasites and hosts (e.g., Global Cestode Database [GCD]), and even maps of parasite (e.g., Australian Faunal Directory) and host (e.g., FishBase) distributions, are all only a click of a mouse away. Overall, the time lag for obtaining taxonomic information has been substantially reduced.

Although more limited in application across disciplines, advances allowing assessment of previously unobservable characters such as ultrastructural and molecular features have revolutionized the types of data included in parasite biodiversity work (and biology overall). Electron beam imaging technology heralded the advent of high-resolution microscopy. The first commercially available transmission electron microscope (TEM), providing insight on internal structure in ultra-thin sections, was installed in 1939 in the German company IG Farben. TEM methods ultimately revealed that chloroplasts possess DNA (Ris and Singh, 1961) and then, more relevant to molecular phylogenetic work on animals

(see next section), so do mitochondria (Sagan, 1967). The commercial availability of the scanning electron microscope (SEM) in 1965 (McMullan, 1995) led to its serving as a source of important morphological insights beginning in the late 1960s.

It is difficult to separate the history of the development of molecular technologies and their use in phylogenetics from the history of the concepts that underlie them. The discovery of the basic structure of DNA (Watson and Crick, 1953), determination of the nature of the codon (Nirenberg and Matthaei, 1961), and sequencing of the first complete gene and genome (Jou et al., 1972) were key historical events. Of particular note was refinement of polymerase chain reaction (PCR) methods by Mullis in 1983 (see Bartlett and Stirling, 2003), which, given the small amount of template DNA required, made it possible to sequence DNA from extremely small organisms, such as many parasite taxa. Also of note was the development of chain termination methods (e.g., Sanger and Coulson, 1975) and commercial availability of high-throughput (i.e., next-generation) sequencing technologies (e.g., Roche 454 see Margulies et al., 2005).

# Phylogenetic (analytical) methods

Following Haeckel's introduction of the concept of representing phylogenies as branching diagrams in 1866, the search for facts to improve such representations dominated the second half of the nineteenth century (Mayr et al., 1953). While there was some notion that taxonomic categories should reflect natural affinities, the trees presented for any taxon were subjective assessments because methods for objectively inferring evolutionary relationships were not yet available.

Publication of the book *Grundzüge einer Theorie der Phylogenetischen Systematik* by the German entomologist Willi Hennig in 1950, and in a revised version in English in 1966, followed closely by the works of Brundin (1966) and Nelson (1969), changed the approach for generating phylogenetic trees globally forever. Although other strategies for inferring evolutionary relationships, including evolutionary taxonomy (Mayr, 1969; Mayr and Ashlock, 1991) and distance methods such as numerical taxonomy (i.e., phenetics) (Sokal and Sneath, 1963) were proposed, the fundamental principles of cladistic methods, and in particular the explicitly character-based grouping by shared derived traits (i.e., synapomorphies) as determined by outgroup comparison largely as outlined by Hennig, took hold and would be employed to infer the phylogenetic relationships among all manner of plant and animal taxa for the next two decades (Wiley, 1981).

The unwieldy nature of endeavoring to infer phylogenetic trees by hand from datasets that included greater and greater numbers of taxa and characters, exacerbated by the advent of molecular sequence data, led to the development of computer packages for performing phylogenetic analyses. These resources were ultimately expanded to incorporate explicit models of molecular evolution and their accompanying statistical tests. Initially, the most widely used approaches employed parsimony as an optimality criterion (e.g., Hennig86—Farris, 1988; PAUP—Swofford, 1991), although likelihood approaches (Felsenstein, 1973) were also available (e.g., PHYLIP—Felsenstein, 1979). Development of Markov chain Monte Carlo (MCMC) algorithms for calculating posterior probabilities (Li, 1996; Mau, 1996) subsequently led to development of Bayesian inference methods of phylogenetic analysis (e.g., MrBayes—Huelsenbeck and Ronquist, 2001). A wide array of programs for phylogenetic inference and managing and examining characters is now available (see

<http://evolution.genetics.washington.edu/phylip/software.html>.

We have reached the point at which it is possible to generate phylogenetic hypotheses using explicit methods. These inferences can be used to explore all sorts of interesting questions regarding the evolution of morphological characters and various adaptations to parasitism. Because it is now routine to generate trees of hosts independently from those of their parasites, questions of coevolution can be explored in a more formalized manner. The development of conceptual frameworks aimed at explaining such historical associations (e.g., Brooks, 1981, 1987; Page, 1993) led to major advancements in this area and the generation of a

range of computer packages for formally assessing cophylogeny. These include event-based methods such as Jungles (Charleston, 1998), BPA (Brooks et al., 2001), TreeMap (Charleston and Robertson, 2002), Tarzan (Merkle and Middendorf, 2005), Jane (Conow et al., 2010), as well as global-fit methods such as ParaFit (Legendre et al., 2002), CopyCat (Meier-Kolthoff et al., 2007), MRCAlink (Schardl et al., 2008), HCT (Hommola et al., 2009), and PACo (Balbuena et al., 2013).

## Synthesis and integration of knowledge and ideas

The literature is replete with discussions of the impact of the modern synthesis (i.e., the union of ideas across biological disciplines) on evolutionary biology (Huxley, 1942)—it certainly revolutionized the science of taxonomy. Up until the 1940s most taxonomists embraced a typological species concept (Mayr et al., 1953) and thus species were recognized and treated, both in principle and in practice, based on morphological criteria alone. The modern synthesis led to abandonment of the notion that species were stable, discrete entities. It heralded the development of alternate notions of species such as the biological species concept (Mayr, 1942), which ultimately led to the formulation of a diversity of other species concepts (e.g., see Wheeler and Meier, 2000). The modern synthesis also provided unifying principles for systematics (i.e., a new systematics) and the detection of evolutionary signal (Huxley, 1940), and, perhaps most importantly, prompted investigators to think more broadly about evolution and mechanisms of speciation.

Historically, the challenge of maintaining, let alone breeding, parasites “in captivity” in their hosts has caused parasitological practitioners to shy away from a biological species concept for it is simply impractical to apply in parasite systems—instead a morphological species concept has traditionally been employed in parasite biodiversity studies. Of course, many authors (e.g., Sites and Marshall, 2004; de Queiroz, 2007) caution against using a single criterion for delimiting such entities. But, the advent of molecular methods has provided a whole new suite of characters that can be brought to bear on questions of species boundaries, and species concepts. In some instances molecular characters have challenged morphology-based species determinations (e.g., Reyda and Marques, 2011; Nakao et al., 2013). In many other cases molecular data have bolstered support for morphology-based species determinations (e.g., Blasco-Costa et al., 2010; Caira et al., 2013). Of course, the most robust and plausible determinations are those supported by a combination of multiple types of data (Hansen et al., 2007; Caira, 2011). Nonetheless, the nature and extent of molecular criteria appropriate for recognizing species remain controversial and it seems likely they will be found to vary among taxonomic groups.

In principle, the integration of knowledge between host and parasite systems, in the form of coevolutionary studies, have their origin in helminth systems and specifically in the work of von Ihering (1891; 1902). As evolutionary thought has expanded, so too has coevolutionary thought (see Klassen, 1992 for a detailed history). By their very nature parasite systems invoke questions about the extent to which parasite evolution is tied to that of their hosts. The assumption that cospeciation is a primary determinant of parasite speciation and faunal radiation was a foundational principle of much of the work conducted over the twentieth century (Price, 1980; Hoberg et al., 1997). The series of “Rules” formulated to explain the apparent correspondence between host and parasite phylogenies

(and/or geographic distributions), which include Fahrenholz' Rule (Stammer, 1957; Cameron, 1964), Szidat's Rule (Szidat, 1956), Manter's Rules (Manter, 1955, 1963, 1966), and Eichler's Rule (Eichler, 1940a, 1940b, 1948) (see Brooks, 1979; Hoberg et al., 1997), all assume cospeciation as a primary (if not sole) determinant.

# Applications to helminth biodiversity studies

## Rules of nomenclature

As it turns out, authors publishing in *JP* have always held themselves to very high standards with respect to following protocols that were ultimately mandated by the ICZN. For example, Weidman (1916), Jewell (1916), and Van Cleave (1918a, 1918b), all deposited type specimens of their new taxa in museum collections that were specifically identified at the time of publication. Perhaps one of the greatest testaments to *JP*'s conviction in the importance of taxonomic stability is the fact that authority citations *that include dates* are still required for the first use of any scientific name in all papers. One wonders if this tradition perhaps has its origin with the active role in the development of the ICZN played by ASP past-president Norman Stoll.

## Technology

Hockley's (1968) images of the cercariae of *Schistosoma mansoni* were the first SEM images to appear in *JP*, which is an impressive feat given that his paper appeared only three years after this technology was commercially available. The first SEMs of an elasmobranch tapeworm published in *JP* were those of Whittaker et al. (1982). Caira and Pritchard (1986) were the first to include features visible with SEM in the descriptions of novel species of elasmobranch cestodes. Over time, SEM has been used to greater and greater advantage in characterizing novel taxa (e.g., Ivanov and Caira, 2013; Cielocha et al., 2014). For example, electron microscopy led to the discovery of unique ultrastructural surface features of cestodes referred to as microtriches, that have been found to exist in a spectacular variety of forms across cestode taxa and to be of enormous taxonomic value in many groups (Chervy, 2009).

The exact points in time at which microcomputers and other electronic advances such as GPS began to be employed in helminth biodiversity work are more elusive to determine. The first paper in *JP* presenting geographic coordinates for a collecting locality appears to be that of Neiland et al. (1970), but as the GPS satellite network system was not launched until several years later, it is likely these data were obtained from a gazetteer or other non-electronic resource. The first images to appear in *JP* that were captured with digital technology are similarly difficult to identify. Certainly their use increased substantially after 2002 when *JP* began accommodating the on-line submission of manuscripts.

Nadler (1990) provided an excellent review of the early history of the application of molecular data to questions of helminth taxonomy, systematics, and phylogenetics. In brief, the earliest molecular work on helminths employed protein-electrophoretic data (e.g., Capron et al., 1972; Bullini et al., 1978; Woodruff et al., 1987; Bristow and Berland, 1988). Even this type of rudimentary data hinted at the existence of greater species diversity than suggested by

morphology in some cases (e.g., Baverstock et al., 1985). Restriction fragment length polymorphism (RFLP) mapping was employed by some workers in the identification of helminth species (e.g., Curran et al., 1985). But helminthologists, unlike their colleagues in many other fields (see Templeton, 1983), avoided the RFLP bandwagon and instead moved immediately to embrace nucleic acid sequencing methods (sensu Hillis et al., 1996) as a source of molecular data for phylogenetic inferences.

The first paper applying molecular data to an evolutionary question to appear in *JP* was by Nadler (1987) who used protein electrophoresis to explore times of divergence and cospeciation in ascaridoid nematodes. Johnson et al. (1988) published the first paper in *JP* in which sequence data were used to explore phylogenetic relationships, in this case 18S rRNA of apicomplexan taxa. That focus expanded to include other nuclear ribosomal RNA genes such as 28S rRNA (e.g., Waeschenbach et al. 2007), and in some instances EF1 $\alpha$ , ITS1, and ITS2 (e.g., Qu et al., 1986; Gill et al., 1988; Barker et al., 1993; Barker and Blair, 1996; Olson and Caira, 1999; Zehnder et al., 2000). Mitochondrial ribosomal RNA molecules such as 16S rRNA and NADH1 were also among the first loci sequenced in helminths (e.g., Despres et al., 1992; Bowles and McManus, 1993, 1995). Prompted largely by the barcoding initiative (Hebert et al., 2003), sequence data for the mitochondrial DNA subunit cytochrome c oxidase subunit 1 (CO1) was also generated for a number of taxa (e.g., Prosser et al., 2013).

As methodologies have been refined, sequencing efficiency has increased, cost has decreased and the amount of data it is now feasible to generate is truly remarkable. Data for large portions of mitochondrial genomes (e.g., Waeschenbach et al., 2012) and even complete mitochondrial genomes (e.g., Park et al., 2007; Liu et al., 2012) are now available for some helminth taxa. Entire nuclear genomes are available for others, and although at present these come mostly from taxa of medical importance, genomes of selected other taxa are also beginning to be available (e.g., Berriman et al., 2009; Young et al., 2012; Tsai et al., 2013). At this point, the lack of bioinformatic tools capable of manipulating and analyzing large volumes of data are much more of a limitation than the generation of the data themselves (e.g., Lee et al., 2012).

That having been said, it is somewhat disappointing (and surprising) that the repertoire of genes employed in exploring helminth, and certainly cestode, phylogenetic relationships remains almost entirely limited to the nuclear and mitochondrial markers listed here (e.g., Healy et al., 2009; Palm et al., 2009; Olson et al., 2010; Caira et al., 2013, 2014). There is hope that, as the number and diversity of fully annotated genomes expands, so too will their utility as a source of other viable target genes for use in systematics and phylogenetic studies. But this expectation has yet to be realized.

## Phylogenetic methods

The application of cladistic methods to studies of helminth evolution was pioneered by Dan Brooks. In addition to expanding the parasitology community's general understanding of the method (e.g., Brooks, 1983; Brooks et al., 1984), Brooks generated many of the first explicit trees of helminth relationships (e.g., Brooks, 1978a, 1978b, 1979a, 1979b; Brooks and Overstreet, 1978; Brooks et al., 1981). The first phylogenetic tree in *JP* appeared in a paper describing a new nematode of the genus *Echinocephalus* by Deardorff et al. in 1981. That same year, Brooks and colleagues offered a workshop at the annual meeting of the American Society of Parasitologists in Montreal to introduce its members to these methods. That effort led to generation of a cladistics workbook (Brooks et al., 1984), which ultimately was published in its new incarnation as the *Compleat Cladist* (Wiley et al., 1991). In an invited review in *JP* on the status and future of helminth systematics, Brooks (1985) noted that over 20 formal phylogenetic analyses of helminths had been conducted up to that time. All 20 of these studies, admittedly few of which were published in *JP*, were based on morphological data and all, as was customary at the time, presented trees that had been generated by hand.

This situation has changed dramatically as molecular data have increasingly come to be relied upon as a source of characters for phylogenetic analyses. The Johnson et al. (1988) apicomplexan paper cited earlier was the first in *JP* to present a phylogenetic tree generated from sequence data (i.e., 18S rRNA). The authors employed parsimony, likelihood, and distance methods. The first phylogeny of a helminth taxon based on sequence data to be published in *JP* was by Barker and Blair, in 1996, examining interrelationships of *Schistosoma* species; they too used parsimony, likelihood, and distance methods. The first molecular phylogenies in *JP* to focus on cestodes were those of Mariaux (1998) and Olson and Caira (1999). Both papers explored cestode inter-ordinal relationships; both also employed cladistic methodology. The first paper employing Bayesian methods for phylogenetic analysis in *JP* (Xu et al., 2003) appeared only two years after those methods were formalized by Huelsenbeck and Ronquist in 2001. The first paper to appear in *JP* employing these methods on helminths was that of Bott and Cribb (2005), which focused on gorgoderid cercaria. The digest provided by Caira and Jensen (2014) includes a treatment of phylogenetic relationships among elasmobranch cestodes as they are understood today.

## Synthesis of knowledge and ideas

There can be no question that our understanding of parasite biodiversity is far richer when placed in the context of an understanding of the evolutionary factors that drive the process of diversification. The advent of formalized methods for phylogenetic inference revitalized interest in exploring such factors. Among other things, it fostered development of methods for assessing coevolutionary relationships between parasites and their hosts, such as Hennig's *Parasitological Method* (Brooks, 1981), and their derivatives, for exploring the historical biogeography of such associations, such as Brooks' *Parsimony Method* (Wiley,

1988) (see Page and Charleston, 1998 for a review).

Until very recently, it was assumed that cospeciation was a primary determinant of parasite speciation and faunal radiation, and thus, constituted a foundational principle of most coevolutionary work and also of biodiversity research (e.g., Price, 1980; Hafner and Nadler, 1988; Hoberg et al., 1997). This is nicely illustrated by the excellent series of papers providing insights from a diversity of empirical studies across a wide array of host–parasite systems (Brooks, 1992b; Gardner and Campbell, 1992; Hoberg, 1992; Platt, 1992; Janovy et al., 1992) resulting from a symposium held at the ASP meetings in 1991 celebrating von Ihering's role as the father of coevolutionary thought (working largely on helminths systems) on the occasion of the 100th anniversary of his seminal work published in 1901 (Brooks, 1992a). It is of note that all of these studies were conducted using early versions of phylogenetic methodologies and all were based on morphological data. Like other earlier studies (e.g., Brooks and Glen, 1982; Klassen and Beverley-Burton, 1987) these works presented substantial evidence of cospeciation between parasites and hosts. However, a somewhat different picture is now emerging from more recent analyses across host–parasite systems.

# Present day

## Linton's two species

Returning for a moment to the two species described by Linton in 1916—both are now considered to belong to genera other than those in which they were originally described, and both arrived in their current taxonomic homes via somewhat circuitous paths. *Onchobothrium tortum* was synonymized with *A. coronatum* (Rudolphi, 1819) Van Beneden, 1850 by Southwell (1925), transferred to *Pinguicollum* Riser, 1955 by Riser (1955), and then to *Acanthobothrium* Van Beneden, 1850 by Baer and Euzet (1962). The species was ultimately redescribed and wonderfully illustrated by Campbell (1970) who also provided the data required to convincingly justify its current placement in *Acanthobothrium* as *Acanthobothrium tortum* (Linton, 1916) Baer and Euzet, 1962. Linton's second species, *Tylocephalum marsupium*, was synonymized with *Adelobothrium aetiobatidis* Shipley, 1900 by Southwell (1925) and then resurrected as *T. marsupium* by Campbell and Williams (1984) who also provided a detailed and redescription based in part on Linton's original material. However, recent work comparing *Tylocephalum* Linton, 1890 and *Adelobothrium* Shipley, 1900 (see Cielocha, 2013) suggests that, although not a synonym of *A. aetiobatidis*, the species does in fact belong in *Adelobothrium*. Although both species have yet to be investigated using SEM or molecular methods, our understanding of their evolutionary relationships has moved well beyond Linton's statement that the two species "belong to quite different families". In fact, molecular data suggest that these genera belong to different orders, and that these orders are only distantly related. Whereas *Tylocephalum* is a member of the Lecanicephalidea (see Jensen, 2005), *Acanthobothrium* is a member of the newly established order Onchoproteocephalidea (see Caira et al., 2014).

Significant advances have also been made in our understanding of "*Aetobatis* [sic] *narinari*", the host from which Linton's two species were originally collected. Although until very recently (e.g., Last and Stevens, 2009) this eagle ray was considered to be cosmopolitan in distribution, a combination of morphological, molecular, and parasite work has revealed that it represents a complex of at least five species. *Aetobatus narinari sensu stricto* occurs only in Atlantic waters. Each of its congeners is restricted to other distinct geographic regions (White et al., 2010). This revelation means that, regardless of Linton's rationale for failing to compare his new taxa to those described previously from Southeast Asia by Southwell and colleagues, the worms Linton described came from a different species of host and thus, given the substantial degree of oioxeny exhibited by members of both orders of cestodes, such comparisons were perhaps not so critical after all.

## Elasmobranch-cestode biodiversity

What have we learned about elasmobranch-cestode diversity over the last

century? Or, more specifically, what progress has been made in determining the extent to which cestode and elasmobranch species are locally or globally distributed, the degree of specificity cestodes exhibit for their elasmobranch hosts, the full magnitude of global diversity of both cestodes and their elasmobranch hosts, and the coevolutionary and biogeographic patterns, and the mechanisms that underlie them?

As a result of the much richer suite of data that is now being brought to bear on characterizations of species, modern descriptions are more thorough and are illustrated in more detail; as a consequence they are more accurate and species are easier to recognize with confidence. Descriptions of elasmobranch cestodes are routinely based on a series of specimens from multiple host specimens, and include characterizations from whole mounts, histological sections, and scanning electron microscopical data. Molecular data are also now beginning to be employed with some regularity and while they are not, nor should they ever be, required elements of descriptions, such data can be informative. Collection localities are specified using GPS coordinates. Host identities are grounded using NADH<sub>2</sub> sequence data in the context of a library of similar data for a diversity of species (see Naylor et al., 2012a), host voucher specimens, and/or digital images; additional host data are deposited in on-line databases such as the Global Cestode Database ([tapeworms.uconn.edu](http://tapeworms.uconn.edu)). In the cases of particularly problematic host taxa, photos are included in publications (e.g., Caira et al., 2007; Ivanov, 2009; Koch et al., 2012; Caira et al., 2013).

This more thorough taxonomic approach, in combination with the collection of new material, is largely responsible for the fact that the number of cestode species known to parasitize elasmobranchs has grown from approximately 165 in 1916, to 977 today (Caira and Jensen, 2014)! Based on the small proportion of elasmobranch species for which cestode faunas have been fully characterized, and the relatively large number of elasmobranchs species whose faunas have yet to be characterized, this number is likely to represent a gross underestimate of the global fauna.

The case of the spotted eagle ray (“*Aetobatus narinari*”) serves to highlight the much larger issue of uncertainty that exists with respect to host identifications in general. In fact, most elasmobranch species are now known to be much more geographically restricted than previously thought (White and Last, 2012). The global count of elasmobranch species has increased substantially from the approximately 500 species recognized in 1916 to the current tally of close to 1,300 species worldwide. Furthermore, with the exception of trypanorhynchs (see Palm and Caira, 2008), most elasmobranch cestodes appear to exhibit a much higher degree of specificity for their definitive hosts (Caira and Jensen, 2001, 2014) than thought by earlier workers. As a consequence, most historically documented cestode-elasmobranch associations require verification in the context of modern taxonomic frameworks. In the end, Linton's “local” concept of cestode-elasmobranch systems turns out to be much closer to reality than Southwell's

“global” concept. It would not be surprising to discover that the parasite faunas of many, if not all, other host groups are similarly underestimated globally as geographic distributions and host specificities in general are determined to be much more restricted than originally thought.

The number of elasmobranch groups for which reasonably robust phylogenetic hypotheses are available is growing. Hypotheses are now available for a diversity of genera (e.g., Last et al., 2010; Stelbrink et al., 2010; White et al., 2010) and families (e.g., Lopez et al., 2006; Straube et al., 2010). More comprehensive analyses, some of which include a large proportion of known taxa are also beginning to appear (Vélez-Zuazo and Agnarsson, 2011; Naylor et al., 2012b).

Our understanding of the higher-level phylogenetic relationships among elasmobranch cestodes has markedly improved and classifications are being brought into line with these phylogenetic hypotheses. For example, four novel genera of diphylloideans were established to align generic level classification with the results of phylogenetic analyses of the order (Caira et al., 2013; Abbott and Caira, 2014). Phylogenetic work has also led to the erection of the new orders Rhinebothriidea (see Healy et al., 2009), Phyllobothriidea, and Onchoproteocephalidea (see Caira et al., 2014), bringing the total number of elasmobranch-hosted cestode orders to nine (out of the 19 cestode orders now recognized). Dismantling of the wildly polyphyletic “Tetraphyllidea” continues (Caira et al., 2014). Relatively comprehensive phylogenetic hypotheses are now available for the Trypanorhyncha (see Palm et al., 2009; Olson et al., 2010) and phylogenetic analysis of the Lecanicephalidea is well underway. The key role elasmobranch cestodes have played in the evolution of cestodes overall is now also apparent (Caira and Jensen, 2014).

## Host–parasite associations

Somewhat unexpectedly, recent studies are yielding a relatively high degree of incongruence between phylogenetic hypotheses of parasites and their hosts—this is the case across host–parasite systems (e.g., Weckstein, 2004; Gustafsson and Olsson, 2012; Harbison and Clayton, 2011). Even in instances of strict host specificity, the degree of correspondence between parasite and host phylogenies is remarkably low (e.g., Desdevises et al., 2002; Desdevises, 2007). As a result, the paradigm of host–parasite coevolution is beginning to shift away from an expectation of co-speciation. We believe three aspects of modern systematic work are contributing to this shift. First, phylogenetic hypotheses generated using modern methods are arguably more robust for they are based on greater amounts of data using more efficient and powerful analytical methods. Second, phylogenetic hypotheses are being generated *independently* for hosts and their parasites, which allows “the context and extent of co-evolution” (*sensu* Brooks, 1979a) to be assessed much more rigorously than allowed by earlier methods, some of which generated host trees from parasite data (e.g., Brooks, 1981). Third, the methods (be they event-based or global fit) now available for formally

assessing congruence between phylogenies allow quantifiable determinations of degree of cophylogeny to be made.

## The future

This is a crucial and potentially transformative time in the history of parasite biodiversity research. Taxonomic assignments and classifications have begun to stabilize. Morphological and molecular strategies for reliably assessing host and parasite identities are available. We have at our disposal a diversity of methods for generating objective assessments of parasite and host phylogenetic relationships. Global communication has become routine. Molecular technologies have been refined such that they are efficient and relatively inexpensive to employ and this technology continues to improve at an extremely rapid pace. The assembly of a truly global picture of parasite diversity, distributions, host associations, phylogenetic relationships, and coevolutionary scenarios is now finally possible. We lack only the person-power and funding to achieve this planet-wide goal!

Unfortunately, just as we have reached this pivotal point in time the world is experiencing an unprecedented climate of anthropocentric attitudes from funding agencies, or more accurately from the political bodies that control the budgets of funding agencies, regarding the types of science “worthy” of support. Early in 2014, Canada's National Science Research Council's (NSRC) announcement of what constitutes a shift in priority from funding basic research to funding “research areas that create long-term economic advantages for Canada” shocked global the scientific community. United States Senators questioning the merits of projects supported by the National Science Foundation (NSF) have become more commonplace—outcries arguing that taxpayer funds would be better spent on projects aimed at improving the human condition (e.g., Coburn, 2011) have become more commonplace. Given human nature, it is not unexpected that areas of applied research (be they in technology, engineering, or medicine) are much easier to justify and thus much less controversial to fund than areas of basic research. The direct potential benefits of applied research to humankind, either economically or in terms of quality of life, are much more obvious and immediate than the benefits of basic research. In fact, by definition basic research has no direct connection to humankind. But, ironically, it represents the foundation on which applied research is based.

In truth, in the almost 65 years since the Act of Congress that brought the National Science Foundation (NSF) into being was passed, society has lost sight of the original intent of establishing infrastructure to support and encourage basic research and education in the sciences, and the pivotal role basic research plays in fostering advances in applied research. Perhaps it is time to revisit some of the points made by Vannevar Bush and the four committees of distinguished scientists who, in 1945, prepared a report to President Roosevelt, entitled “Science, the endless frontier”, which ultimately led to establishment of the NSF (reprinted in 1960, with an excellent introduction by Alan Waterman, then Director of the NSF). In a section entitled *The Importance of Basic Research*, these outstanding scientists wrote (p. 18):

Basic research is performed without thought of practical ends. It results in general knowledge and an understanding of nature and its laws. This general knowledge provides the means for answering a large number of important practical problems, though it may not give a complete answer to any one of them. The function of applied research is to provide such complete answers. The scientist doing basic research may not be at all interested in the practical applications of his work, yet the further progress of industrial development would eventually stagnate if basic scientific research were long neglected... One of the peculiarities of basic science is the variety of paths, which lead to productive advance. Many of the most important discoveries have come as a result of experiments undertaken with very different purposes in mind. Statistically it is certain that important and highly useful discoveries will result from some fraction of the undertakings in basic science; but the results of any one particular investigation cannot be predicted with accuracy... Basic research leads to new knowledge. It provides scientific capital. It creates the funds from which the practical application of knowledge must be drawn. New products and new processes do not appear full-grown. They are founded on new principles and new conceptions, which in turn are painstakingly developed by research in the purest realms of science.

Nonetheless, as science becomes more complex, the challenge of establishing a connection between basic and applied research becomes even greater. It is key that the importance of basic research, not only for its own sake, but also for the sake of humankind, not be forgotten. Of course as researchers each of us must continue to do our part in sharing our discoveries with the public. NSF's mandate to include consideration of the broader impacts of our work has helped encourage and guide the efforts of basic researchers along this front. Perhaps it is time to begin thinking about establishing a more formalized mechanism for making such connections more achievable, more commonplace, and more evident to the general public. The recently developed International Cooperative Biodiversity Groups program is an example of one such initiative. A cooperation between federal agencies such as the NSF and the National Institutes of Health (NIH), it is aimed at investigating potential human health applications from biodiversity exploration. But perhaps other mechanisms for connecting basic and applied research must also be established. As a more explicit example, we note that this past year we published data (Caira and Jensen, 2014) on the composition of hooks of a diversity of orders of tapeworms noting that the protein comprising the hooks of members of one particular order, the elasmobranch-hosted Trypanorhyncha, is particularly intriguing for it includes an unusually high concentration (i.e., 60–70%) of the amino acid histidine. Preliminary searches of protein databases suggest this protein may be new to science, but how do we proceed from here? Our expertise is in discovering novelty, and *not* exploring potential applications of that novelty!

Finally, as nicely summarized by Lindenmayer and Likens (2011), the pursuit of

research involving generation of empirical data, in this case required for completing the global picture of helminth biodiversity, is being challenged by a cultural shift away from fundamental disciplines such as taxonomy and natural history towards more synthetic approaches involving modeling, data-mining and meta-analysis. Exacerbating the issue, as noted by Grimaldi and Engel (2007), is the declining appreciation of the significance of descriptive science overall. While “synthetic” approaches rely on empirical data, they do nothing to support or facilitate the generation of such data. They can be conducted without the expense and time-consuming logistical tribulations of fieldwork, and are generally more highly cited than the empirical work on which they are based. We note that NSF's Biotic Surveys and Inventories (BS&I), Partnership for Enhancing Expertise in Taxonomy (PEET), and Planetary Biodiversity and Inventory (PBI) programs, focused on the discovery and description of novel taxa globally, have been replaced by programs with a much greater emphasis on analysis than discovery. But here too the irony is clear—for the testing of models and predictions, and the validation of simulations, require the availability of additional empirical data.

## Lessons learned

What key lessons have we learned over the past century to help guide future research on helminth biodiversity? Four things come to mind: (1) Regardless of whether hosts and/or parasites are locally or globally distributed, the nature of their distributions can only be definitively determined if considered in a global context. (2) The importance of accurately determining and documenting host and parasite identities cannot be overemphasized. (3) The reciprocal illumination provided by using morphological and molecular data *in combination* is compelling. There is no question that as molecular methods continue to be refined, the generation of greater amounts of sequence data will become more affordable. In fact, projects to sequence genomes for helminths species (e.g., Wellcome Trust Sanger Institute's 50 Helminth Genomes Initiative) are already underway. One could imagine the day when description of new species would routinely include characterizations of full genomes to augment complete morphological characterizations, thus rendering the question of marker choice for phylogenetic analyses moot. However, if future generations are to remember us with gratitude rather than contempt we heartily caution against yielding to the obvious temptation to focus solely on the generation of molecular data because of the immediate gratification they provide. (4) Against all odds we must persist in our efforts to discover and describe novelty on the planet through field-based studies, despite the many challenges they entail. Full documentation of the global fauna is within our grasp. We must *not* be dissuaded from achieving this goal for any reason.

## Literature cited

- Abbott, L. M., and J. N. Caira. 2014. Morphology meets molecules: a new genus and two new species of diphylloidean cestodes from the yellowspotted skate, *Leucoraja wallacei*, from South Africa. *Journal of Parasitology* **100**: 323–330.
- Baer, J. G., and L. Euzet. 1962. Revision critique des cestodes tetraphyllides decrits par T. Southwell (1re partie). *Bulletin de la Societe Neuchateloise des Sciences Naturelles* **85**: 143–172.
- Balbuena, J. A., R. Míguez-Lozano, and I. Blasco-Costa. 2013. PACo: A novel Procrustes application to cophylogenetic analysis. *PLoS ONE* **8**: e61048.
- Barker, S. C., and D. Blair. 1996. Molecular phylogeny of *Schistosoma* species supports traditional groupings within the genus. *Journal of Parasitology* **82**: 292–298.
- \_\_\_\_\_, D. Blair, A. R. Garrett, and T. H. Cribb. 1993. Utility of the D1 domain of nuclear 28S rRNA for phylogenetic inference in the Digenea. *Systematic Parasitology* **26**: 181–188.
- Bartlett, J. S., and D. Stirling. 2003. A short history of the polymerase chain reaction. In *PCR Protocols*, J. S. Bartlett, and D. Stirling (eds.). Humana Press, New York, NY, p. 3–6.
- Baverstock, P. R., M. Adams, and I. Beveridge. 1985. Biochemical differentiation in bile duct cestodes and their marsupial hosts. *Molecular Biology and Evolution* **2**: 321–337.
- Berriman, M., B. J. Haas, P. T. LoVerde, R. A. Wilson, G. P. Dillon, G. C. Cerqueira, S. T. Mashiyama, B. Al-Lazikani, L. F. Andrade, P. D. Ashton, M. A. Aslett, D. C. Bartholomeu, G. Blandin, C. R. Caffrey, A. Coghlan, R. Coulson, T. A. Day, A. Delcher, R. DeMarco, A. Djikeng, T. Eyre, J. A. Gamble, E. Ghedin, Y. Gu, C. Hertz-Fowler, H. Hirai, Y. Hirai, R. Houston, A. Ivens, D. A. Johnston, D. Lacerda, C. D. Macedo, P. McVeigh, Z. Ning, G. Oliveira, J. P. Overington, J. Parkhill, M. Pertea, R. J. Pierce, A. V. Protasio, M. A. Quail, M.-A. Rajandream, J. Rogers, M. Sajid, S. L. Salzberg, M. Stanke, A. R. Tivey, O. White, D. L. Williams, J. Wortman, W. Wu, M. Zamanian, A. Zerlotini, C. M. Fraser-Liggett, B. G. Barrell, and N. M. El-Sayed. 2009. The genome of the blood fluke *Schistosoma mansoni*. *Nature* **460**: 352–358.
- Blasco-Costa, I., J. A. Balbuena, J. A. Raga, A. Kostadinova, and P. D. Olson. 2010. Molecules and morphology reveal cryptic variation among digeneans infecting sympatric mullets in the Mediterranean. *Parasitology* **137**: 287–302.
- Bott, N. J., and T. H. Cribb. 2005. First report of intramolluscan stages of a gorgoderid digenean from a marine bivalve. *Journal of Parasitology* **91**: 838–842.

- Bowles, J., and D. P. McManus. 1993. NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *International Journal for Parasitology* **23**: 969–972.
- \_\_\_\_\_, D. Blair, and D. P. McManus. 1995. A molecular phylogeny of the genus *Echinococcus*. *Parasitology* **110**: 317–328.
- Bristow, G. A., and B. Berland. 1988. A preliminary electrophoretic investigation of the gyrocotylid parasites of *Chimaera monstrosa* L. *Sarsia* **73**: 75–77.
- \_\_\_\_\_. 1978a. Evolutionary history of the cestode order Proteocephalidea. *Systematic Zoology* **27**: 312–323.
- \_\_\_\_\_. 1978b. Systematic status of proteocephalid cestodes from reptiles and amphibians in North America with descriptions of three new species. *Proceedings of the Helminthological Society of Washington* **45**: 1–28.
- \_\_\_\_\_. 1979a. Testing hypotheses of evolutionary relationships among parasites: the digeneans of crocodilians. *American Zoologist* **19**: 1225–1238.
- \_\_\_\_\_. 1979b. Testing the context and extent of host-parasite coevolution. *Systematic Zoology* **28**: 299–307.
- \_\_\_\_\_. 1981. Hennig's parasitological method: a proposed solution. *Systematic Zoology* **30**: 229–249.
- \_\_\_\_\_. 1983. What's going on in evolution? A brief guide to some new ideas in evolutionary theory. *Canadian Journal of Zoology* **61**: 2637–2645.
- \_\_\_\_\_. 1985. Phylogenetics and the future of helminth systematics. *Journal of Parasitology* **71**: 719–727.
- \_\_\_\_\_. 1987. Analysis of host-parasite coevolution. *International Journal for Parasitology* **17**: 291–297.
- \_\_\_\_\_. 1992a. Introduction to the von Ihering Centenary Symposium. *Journal of Parasitology* **78**: 571–572.
- \_\_\_\_\_. 1992b. Origins, diversification, and historical structure of the helminth fauna inhabiting neotropical freshwater stingrays (Potamotrygonidae). *Journal of Parasitology* **78**: 588–595.
- \_\_\_\_\_, and D. R. Glen. 1982. Pinworms and primates: A case study in coevolution. *Proceedings of the Helminthological Society of Washington* **49**: 76–85.
- \_\_\_\_\_, and R. M. Overstreet. 1978. The family Liolopidae (Digenea) including a new genus and two new species from crocodilians. *International Journal for Parasitology* **8**: 267–273.

\_\_\_\_\_, J. N. Caira, T. R. Platt, and M. H. Pritchard. 1984. *Principles and methods of phylogenetic systematics: a cladistics workbook*. University of Kansas Publications of the Museum of Natural History i–v, 1–92.

\_\_\_\_\_, M. G. P. van Veller, and D. A. McLennan. 2001. How to do BPA, really. *Journal of Biogeography* **28**: 345–358.

\_\_\_\_\_, T. B. Thorson, and M. A. Mayes. 1981. Fresh-water stingrays (Potamotrygonidae) and their helminth parasites: testing hypotheses of evolution and coevolution. *Advances in Cladistics* **1**: 147–175.

Brundin, L. 1966. *Transantarctic relationships and their significance, as evidenced by chironomid midges: with a monograph of the subfamilies Podonominae and Aphroteniinae and the Austral Heptagyiae*. Almqvist & Wiksell, Stockholm, 472 p.

Bullini, L., G. Nascetti, S. Ciafre, F. Rumore, and E. Biocca. 1978. Ricerche cariologiche ed elettroforetiche su *Parascaris univalens* e *Parascaris equorum*. *Atti della Accademia Nazionale dei Lincei Rendiconti Classe di Scienze Fisiche Matematiche e Naturali* **65**: 151–156.

Bush, V. 1945. *Science, the Endless Frontier. A Report to the President*. (Reissued 1960, with foreword by A. T. Waterman). National Science Foundation, Washington, D.C., 220 p.

Caira, J. N. 2011. Synergy advances parasite taxonomy and systematics: an example from elasmobranch tapeworms. *Parasitology* **138**: 1675–1687.

\_\_\_\_\_, and K. Jensen. 2001. An investigation of the co-evolutionary relationships between onchobothriid tapeworms and their elasmobranch hosts. *International Journal for Parasitology* **31**: 960–975.

\_\_\_\_\_, and K. Jensen. 2014. A digest of elasmobranch tapeworms. *Journal of Parasitology* **100**: 373–391.

\_\_\_\_\_, and M. H. Pritchard. 1986. A review of the genus *Pedibothrium* Linton, 1909 (Tetraphyllidea: Onchobothriidae) with description of two new species and comments on the related genera *Pachybothrium* Baer and Euzet, 1962 and *Balanobothrium* Hornell, 1912. *Journal of Parasitology* **72**: 62–70.

\_\_\_\_\_, F. P. L. Marques, K. Jensen, R. Kuchta, and V. Ivanov. 2013. Phylogenetic analysis and reconfiguration of genera in the cestode order Diphyllidea. *International Journal for Parasitology* **43**: 621–639.

\_\_\_\_\_, K. Jensen, A. Waeschenbach, P. D. Olson, and D. T. J. Littlewood. 2014. Orders out of chaos—molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *International Journal for Parasitology* **44**: 55–73.

- \_\_\_\_\_, \_\_\_\_\_, and C. Rajan. 2007. Seven new *Yorkeria* species (Cestoda: Tetraphyllidea) from Borneo and Australia and their implications for identification of *Chiloscyllium* (Elasmobranchii: Orectolobiformes) species. *Journal of Parasitology* **93**: 357–376.
- \_\_\_\_\_, N. Rodriguez, and M. Pickering. 2013. New African species of *Echinobothrium* (Cestoda: Diphylidae) and implications for the identities of their skate hosts. *Journal of Parasitology* **99**: 781–788.
- Cameron, T. W. M. 1964. Host specificity and the evolution of helminthic parasites. In *Advances in Parasitology*, D. Ben (ed.). Academic Press, Waltham, MA, p. 1–34.
- Campbell, R. A. 1970. Notes on tetraphyllidean cestodes from the Atlantic coast of North America, with descriptions of two new species. *Journal of Parasitology* **56**: 498–508.
- \_\_\_\_\_, and A. D. Williams. 1984. *Tylocephalum* Linton, 1890 (Cestoda: Lecanicephalidae) from the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815) with a discussion of its validity and systematic relationships. *Proceedings of the Helminthological Society of Washington* **51**: 121–134.
- Capron, A., E. R. Brygoo, and D. Afchain. 1972. Apport de l'étude de la structure antigenique à la phylogénie des helminthes. *Bulletin de Muséum National d'Histoire Naturelle (Zoologie)* **55**: 877–885.
- Carus, J. V. 1863. Vermes. In *Handbuch der Zoologie*, J. V. Carus, and C. E. A. Gerstaecker (eds.). Verlag von Wilhelm Engelmann, Leipzig, Germany, p. 422–484.
- Charleston, M. 1998. Jungles: A new solution to the host/parasite phylogeny reconciliation problem. *Mathematical Biosciences* **149**: 191–223.
- \_\_\_\_\_, and D. Robertson. 2002. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *Systematic Biology* **51**: 528–535.
- Chervy, L. 2009. Unified terminology for cestode microtriches: a proposal from the international workshops on cestode systematics in 2002–2008. *Folia Parasitologica* **56**: 199–230.
- Cielocha, J. J. 2013. *Contributions to the systematics, comparative morphology, and interrelationships of selected lecanicephalidean tapeworms (Platyhelminthes: Cestoda: Lecanicephalidae)*. Ph.D. University of Kansas, 244 p.
- \_\_\_\_\_, K. Jensen, and J. N. Caira. 2014. *Floriparicapitus*, a new genus of lecanicephalidean tapeworm (Cestoda) from sawfishes (Pristidae) and guitarfishes (Rhinobatidae) in the Indo-West Pacific. *Journal of Parasitology* **100**: 485–499.

Cobbold, T. S. 1885. Notes on Parasites collected by the late Charles Darwin, Esq. *Journal of the Linnaean Society* xix: 174–178.

Coburn, T. A. 2011. The National Science Foundation: Under the microscope. [Online] Available at: rsc.flores.house.gov/uploadedfiles/nsf-april2011.pdf (accessed July 24, 2015).

Conow, C., D. Fielder, Y. Ovadia, and R. Libeskind-Hadas. 2010. Jane: a new tool for the cophylogeny reconstruction problem. *Algorithms for Molecular Biology* 5: 16–26.

Curran, J., D. L. Baillie, and J. M. Webster. 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. *Parasitology* 90: 137–144.

Darwin, C. S. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London, U.K., 502 p.

de Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886.

Deardorff, T. L., D. R. Brooks, and T. B. Thorson. 1981. A new species of *Echinocephalus* (Nematoda: Gnathostomidae) from Neotropical stingrays with comments on *E. diazi*. *Journal of Parasitology* 67: 433–439.

Desdevises, Y. 2007. Cophylogeny: insights from fish-parasite systems. *Parassitologia* 49: 125–128.

\_\_\_\_\_, S. Morand, O. Jousson, and P. Legendre. 2002. Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): The study of a complex host-parasite system. *Evolution* 56: 2459–2471.

Despres, L., D. Imbert-Establet, C. Combes, and F. Bonhomme. 1992. Molecular evidence linking hominid evolution to recent radiation of schistosomes (Platyhelminthes: Trematoda). *Molecular Phylogenetics and Evolution* 1: 295–304.

Diesing, K. M. 1850. *Systema helminthum*. Vol. I. Vindobonae, 679 p.

Eichler, W. 1940a. Korrelationen in der Stammesentwicklung von Wirten und Parasiten. *Zeitschrift für Parasitenkunde* 12: 94.

\_\_\_\_\_. 1940b. Wirtsspezifität und stammesgeschichtliche Gleichläufigkeit (Fahrenholzsche Regel) bei Parasiten im allgemeinen und bei Mallophagen im besonderen. *Zoologischer Anzeiger* 132: 254–292.

\_\_\_\_\_. 1948. Some rules in ectoparasitism. *Annals and Magazine of Natural History (Series 12)* 1: 588–598.

Farris, J. S. 1988. Hennig86, version 1.5. Distributed by the author. Port Jefferson Station, NY.

Felsenstein, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Biology* **22**: 240–249.

\_\_\_\_\_. 1979. Alternative methods of phylogenetic inference and their interrelationship. *Systematic Biology* **28**: 49–62.

Gardner, S. L., and M. L. Campbell. 1992. Parasites as probes for biodiversity. *Journal of Parasitology* **78**: 596–600.

Gill, L. L., N. Hardman, L. Chappell, L. H. Qu, M. Nicoloso, and J. P. Bachellerie. 1988. Phylogeny of *Onchocerca volvulus* and related species deduced from rRNA sequence comparisons. *Molecular and Biochemical Parasitology* **28**: 69–76.

Grimaldi, D. A., and M. S. Engel. 2007. Why descriptive science still matters. *Bioscience* **57**: 646–647.

Gustafsson, D. R., and U. Olsson. 2012. Flyway homogenization or differentiation? Insights from the phylogeny of the sandpiper (Charadriiformes: Scolopacidae: Calidrinae) wing louse genus *Lunaceps* (Phthiraptera: Ischnocera). *International Journal for Parasitology* **42**: 93–102.

Haeckel, E. 1866. Generelle Morphologie der Organismen. Allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformirte Descendenz-Theorie. Zweiter Band: *Allgemeine Entwicklungsgeschichte der Organismen*. Georg Reimer, Berlin, 462 p.

Hafner, M. S., and S. A. Nadler. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* **332**: 258–259.

Hansen, H., T. A. Bakke, and L. Bachmann. 2007. DNA taxonomy and barcoding of monogenean parasites: lessons from *Gyrodactylus*. *Trends in Parasitology* **23**: 363–367.

Harbison, C. W., and D. H. Clayton. 2011. Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proceedings of the National Academy of Sciences* **108**: 9525–9529.

Healy, C. J., J. N. Caira, K. Jensen, B. L. Webster, and D. T. J. Littlewood. 2009. Proposal for a new tapeworm order, Rhinebothriidea. *International Journal for Parasitology* **39**: 497–511.

Hebert, P. D., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* **270**: 313–321.

- Hennig, W. 1950. *Grundzüge einer Theorie der phylogenetischen Systematik*. Deutscher Zentralverlag, Berlin, 370 p.
- \_\_\_\_\_. 1966. *Phylogenetic systematics*. University of Illinois Press, Urbana, IL, 263 p.
- Hillis, D. M., C. Moritz, and B. K. Mable. 1996. *Molecular systematics*. Sinauer Associates, Sunderland, MA, 655 p.
- Hoberg, E. P. 1992. Congruent and synchronic patterns in biogeography and speciation among seabirds, pinnipeds, and cestodes. *Journal of Parasitology* **78**: 601–615.
- \_\_\_\_\_. 1997. Phylogeny and historical reconstruction: host-parasite systems as keystones in biogeography and ecology. In *Biodiversity 2: Understanding and Protecting our Biological Resources*, M. L. Reaka-Kudla, D. E. Wilson, and E. O. Wilson (eds.). Joseph Henry Press, Washington, D.C., p. 243–261.
- \_\_\_\_\_, J. Mariaux, J. L. Justine, D. R. Brooks, and P. J. Weekes. 1997. Phylogeny of the orders of the Eucestoda (Cercozoa) based on comparative morphology: historical perspectives and a new working hypothesis. *Journal of Parasitology* **83**: 1128–1147.
- Hockley, D. J. 1968. Scanning electron microscopy of *Schistosoma mansoni* cercariae. *Journal of Parasitology* **54**: 1241–1243.
- Hommola, K., J. E. Smith, Y. Qiu, and W. R. Gilks. 2009. A permutation test of host-parasite cospeciation. *Molecular Biology and Evolution* **26**: 1457–1468.
- Hornell, J. 1912. New cestodes from Indian fishes. *Records of the Indian Museum* **7**: 197–204.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- Huxley, J. 1940. *The new systematics*. The Clarendon Press, Oxford, UK, 583 p.
- \_\_\_\_\_. 1942. *Evolution: The Modern Synthesis*. Allen & Unwin, London, U.K., 645 p.
- ICZN. 2012. Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Bulletin of Zoological Nomenclature* **69**: 161–169.
- Ivanov, V. A. 2009. New species of *Crossobothrium* (Cestoda: Tetraphyllidea) from the broadnose sevengill shark, *Notorynchus cepedianus*, in Argentina. *Journal of Parasitology* **95**: 1479–1488.

Ivanov, V. A., and J. N. Caira. 2013. Two new species of *Halysioncum* Caira, Marques, Jensen, Kuchta et Ivanov, 2013 (Cestoda, Diphylloidea) from Indo-Pacific rays of the genus *Aetomylaeus* Garman (Myliobatiformes, Myliobatidae). *Folia Parasitologica* **60**: 321–330.

Janovy, J., Jr, R. E. Clopton, and T. J. Percival. 1992. The roles of ecological and evolutionary influences in providing structure to parasite species assemblages. *Journal of Parasitology* **78**: 630–640.

Jensen, K. 2005. A monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). *Bulletin of the University of Nebraska State Museum* **18**: 1–241.

Jewell, M. F. 1916. *Cylindrotaenia americana* nov. spec, from the cricket frog. *Journal of Parasitology* **2**: 181–192.

Johnson, A. M., S. Illana, P. Hakendorf, and P. R. Baverstock. 1988. Phylogenetic relationships of the apicomplexan protist *Sarcocystis* as determined by small subunit ribosomal RNA comparison. *Journal of Parasitology* **74**: 847–860.

Jou, W. M., G. Haegeman, M. Ysebaert, and W. Fiers. 1972. Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. *Nature* **237**: 82–88.

Klassen, G. J. 1992. Coevolution: a history of the macroevolutionary approach to studying host-parasite associations. *Journal of Parasitology* **78**: 573–587.

\_\_\_\_\_, and M. Beverley-Burton. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their ictalurid (Siluriformes) hosts: an hypothesis. *Proceedings of the Helminthological Society of Washington* **54**: 84–90.

Koch, K., K. Jensen, and J. N. Caira. 2012. Three new genera and six new species of lecanicephalideans (Cestoda) from eagle rays of the genus *Aetomylaeus* (Myliobatiformes: Myliobatidae) from Northern Australia and Borneo. *Journal of Parasitology* **98**: 175–198.

Last, P. R., and J. D. Stevens. 2009. *Sharks and Rays of Australia*. Second edition. CSIRO Publishing, Collingwood, VIC, i–ix, 1–644 p.

\_\_\_\_\_, Fahmi, and G. J. P. Naylor. 2010. *Pastinachus stellurostris* sp. nov., a new stingray (Elasmobranchii: Myliobatiformes) from Indonesian Borneo. *CSIRO Marine and Atmospheric Research Paper* **032**: 129–139.

Lee, H. C., K. Lai, M. T. Lorenc, M. Imelfort, C. Duran, and D. Edwards. 2012. Bioinformatics tools and databases for analysis of next-generation sequence data. *Briefings in Functional Genomics* **11**: 12–24.

Legendre, P., Y. Desdevises, and E. Bazin. 2002. A statistical test for host–parasite coevolution. *Systematic Biology* **51**: 217–234.

Li, S. S. 1996. *Phylogenetic tree construction using Markov chain Monte Carlo*. Ph.D. The Ohio State University, 91 p.

Lindenmayer, D. B., and G. E. Likens. 2011. Losing the culture of ecology. *Bulletin of the Ecological Society of America* **92**: 245–246.

Linnæus, C. 1758. *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Impensis Direct. Laurentii Salvii, Holmiæ*, 824 p.

Linton, E. 1889a. Notes on cestoid Entozoa of marine fishes. (Abstract). *American Journal of Science and Arts*. 3rd Series. **37**: 239–240.

\_\_\_\_\_. 1889b. Notes on Entozoa of marine fishes of New England, with descriptions of several new species. Annual Report of the Commissioner of Fish and Fisheries (1886) **14**: 453–511.

\_\_\_\_\_. 1890. *Notes on Entozoa of marine fishes of New England, with descriptions of several new species*. Part II. Annual Report of the Commissioner of Fish and Fisheries (1887) 719–900.

\_\_\_\_\_. 1892. On the anatomy of *Thysanocephalum crispum*, Linton, a parasite of the tiger shark. Report of the United States Commissioner of Fisheries (1888): 543–556.

\_\_\_\_\_. 1894. Some observations concerning fish-parasites. *Bulletin of the United States Fish Commission* **13**: 101–112.

\_\_\_\_\_. 1897a. Notes on cestode parasites of fishes. *Proceedings of the United States National Museum* **20**: 423–456.

\_\_\_\_\_. 1897b. Notes on larval cestode parasites of fishes. *Proceedings of the United States National Museum* **19**: 787–824.

\_\_\_\_\_. 1900. Fish parasites collected at Woods Hole in 1898. U.S. Fish Commission Bulletin for 1899267–304.

\_\_\_\_\_. 1901. Parasites of fishes of the Woods Hole region. *Bulletin of the United States Fish Commission (1899)* **19**: 405–492.

\_\_\_\_\_. 1905. Parasites of fishes of Beaufort, North Carolina. *Bulletin of the United States Bureau of Fisheries (1904)* **24**: 321–428.

\_\_\_\_\_. 1907a. Notes on *Calyptrobothrium*, a cestode genus found in the torpedo. *Proceedings of the United States National Museum* **32**: 275–284.

\_\_\_\_\_. 1907b. Preliminary report on animal parasites collected at Tortugas, Florida, June 30 to July 18, 1906. *Carnegie Institute of Washington* **5**: 112–117.

\_\_\_\_\_. 1908. Helminth fauna of the Dry Tortugas. I. Cestodes. Publication No. 102, *Papers from the Tortugas Laboratory of the Carnegie Institution of Washington* **1**: 157–190.

\_\_\_\_\_. 1916. Notes on two cestodes from the spotted sting-ray. *Journal of Parasitology* **3**: 34–37.

Liu, G. H., C. Li, J. Y. Li, D. H. Zhou, R. C. Xiong, R. Q. Lin, F. C. Zou, and X. Q. Zhu. 2012. Characterization of the complete mitochondrial genome sequence of *Spirometra erinaceieuropaei* (Cestoda: Diphyllobothriidae) from China. *International Journal of Biological Sciences* **8**: 640–649.

Lopez, J. A., J. A. Ryburn, O. Fedrigo, and G. J. P. Naylor. 2006. Phylogeny of sharks of the family Triakidae (Carcharhiniformes) and its implications for the evolution of carcharhiniform placental viviparity. *Molecular Phylogenetics and Evolution* **40**: 50–60.

Manter, H. W. 1955. The zoogeography of trematodes of marine fishes. *Experimental Parasitology* **4**: 62–86.

\_\_\_\_\_. 1963. The zoogeographical affinities of trematodes of South American freshwater fishes. *Systematic Zoology* **12**: 45–70.

\_\_\_\_\_. 1966. Parasites of fishes as biological indicators of recent and ancient conditions. Pp. 59–71. Proceedings of the 26th Annual Biology Colloquium on Host-Parasite Relationships, Oregon State University Biology Colloquium.

Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bemben, J. Berka, M. S. Braverman, Y.-J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. I. Alenquer, T. P. Jarvie, K. B. Jirage, J.-B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**: 376–380.

Mariaux, J. 1998. A molecular phylogeny of the Eucestoda. *Journal of Parasitology* **84**: 114–124.

Mau, R., Jr. 1996. *Bayesian phylogenetic inference via Markov chain Monte Carlo methods*. Ph.D. The University of Wisconsin–Madison, 107 p.

Mayr, E. 1942. *Systematics and the origin of species, from the viewpoint of a zoologist*. Columbia University Press, New York, NY, 334 p.

- \_\_\_\_\_. 1969. *Principles of systematic zoology*. McGraw-Hill, New York, NY.
- \_\_\_\_\_, and P. D. Ashlock. 1991. *Principles of systematic zoology*, 2nd ed. McGraw-Hill, New York, NY.
- \_\_\_\_\_, E. G. Linsley, and R. L. Usinger. 1953. *Methods and principles of systematic zoology*. McGraw-Hill, New York, NY, 328 p.
- McMullan, D. 1995. Scanning electron microscopy 1928–1965. *Scanning* **17**: 175–185.
- Meier-Kolthoff, J. P., A. F. Auch, D. H. Huson, and M. Göker. 2007. CopyCat: cophylogenetic analysis tool. *Bioinformatics* **23**: 898–900.
- Melville, R. V. 1995. *Towards stability in the names of animals—a History of the International Commission on Zoological Nomenclature 1895–1995*, International Trust for Zoological Nomenclature, Natural History Museum, London.
- Merkle, D., and M. Middendorf. 2005. Reconstruction of the cophylogenetic history of related phylogenetic trees with divergence timing information. *Theory in Biosciences* **123**: 277–299.
- Moore, J. P. 1939. In memoriam. Edwin Linton (1855–1939). *Journal of Parasitology* **25**: 450–453.
- Nadler, S. A. 1987. Biochemical and immunological systematics of some ascaridoid nematodes: genetic divergence between congeners. *Journal of Parasitology* **73**: 811–816.
- \_\_\_\_\_. 1990. Molecular approaches to studying helminth population genetics and phylogeny. *International Journal for Parasitology* **20**: 11–29.
- Nakao, M., A. Lavikainen, T. Yanagida, and A. Ito. 2013. Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). *International Journal for Parasitology* **43**: 1017–1029.
- National Geographic. 2012. *100 scientific discoveries that changed the world*. National Geographic Society, Washington, D.C., 128 p.
- Naylor, G. J. P., J. N. Caira, K. Jensen, K. A. M. Rosana, N. Straube, and C. Lakner. 2012a. Elasmobranch phylogeny: A mitochondrial estimate based on 595 species. In *The biology of sharks and their relatives*, J. C. Carrier, J. A. Musick, and M. R. Heithaus (eds.). CRC Press, Taylor & Francis Group, Boca Raton, p. 31–56.
- \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, W. T. White, and P. R. Last. 2012b. A DNA sequence-based approached to the identification of shark

and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* **367**: 1–262.

Neiland, K. A., B. L. Holden, and D. W. Rice. 1970. Helminths of marine mammals. 1. The genus *Nasitrema*, air sinus flukes of delphinid Cetacea. *Journal of Parasitology* **56**: 305–316.

Nelson, G. J. 1969. Origin and diversification of teleostean fishes. *Annals of the New York Academy of Sciences* **167**: 18–30.

Nirenberg, M. W., and J. H. Matthaei. 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. *Proceedings of the National Academy of Sciences* **47**: 1588–1602.

Olson, P. D., and J. N. Caira. 1999. Evolution of the major lineages of tapeworms (Platyhelminthes: Cestoidea) inferred from 18S ribosomal DNA and elongation factor-1 alpha. *Journal of Parasitology* **85**: 1134–1159.

\_\_\_\_\_, \_\_\_\_\_, K. Jensen, R. M. Overstreet, H. W. Palm, and I. Beveridge. 2010. Evolution of the trypanorhynch tapeworms: Parasite phylogeny supports independent lineages of sharks and rays. *International Journal for Parasitology* **40**: 223–242.

Osborn, H. F. 1913. The biographical memoir of Joseph Leidy 1823–1891. *National Academy of Sciences Biographical Memoirs* **7**: 335–396.

Page, R. D. M. 1993. Parasites, phylogeny and cospeciation. *International Journal for Parasitology* **23**: 499–506.

\_\_\_\_\_, and M. A. Charleston. 1998. Trees within trees: phylogeny and historical associations. *Trends in Ecology & Evolution* **13**: 356–359.

Palm, H. W., A. Waeschenbach, P. D. Olson, and D. T. J. Littlewood. 2009. Molecular phylogeny and evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution* **52**: 351–367.

\_\_\_\_\_, and J. N. Caira. 2008. Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. *International Journal for Parasitology* **38**: 381–388.

Park, J. K., K. H. Kim, S. Kang, H. K. Jeon, J. H. Kim, D. T. Littlewood, and K. S. Eom. 2007. Characterization of the mitochondrial genome of *Diphyllobothrium latum* (Cestoda: Pseudophyllidea) - implications for the phylogeny of eucestodes. *Parasitology* **134**: 749–759.

Platt, T. R. 1992. A phylogenetic and biogeographic analysis of the genera of Spirorchinae (Digenea: Spirorchidae) parasitic in freshwater turtles. *Journal of Parasitology* **78**: 616–629.

Price, P. W. 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton, NJ, 237 p.

Prosser, S. W. J., M. G. Velarde-Aguilar, V. Leon-Regagnon, and P. D. N. Hebert. 2013. Advancing nematode barcoding: A primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. *Molecular Ecology Resources* **13**: 1108–1115.

Pyle, R. L., and E. Michel. 2008. ZooBank: developing a nomenclatural tool for unifying 250 years of biological information. *Zootaxa* **1950**: 39–50.

Qu, L. H., N. Hardman, L. Gill, L. Chappell, M. Nicoloso, and J.-P. Bachellerie. 1986. Phylogeny of helminths determined by rRNA sequence comparison. *Molecular and Biochemical Parasitology* **20**: 93–99.

Reyda, F. B., and F. P. L. Marques. 2011. Diversification and species boundaries of *Rhinebothrium* (Cestoda; Rhinebothriidea) in South American freshwater stingrays (Batoidea; Potamotrygonidae). *PLoS One* **6**: e22604, 22601–22626.

Ride, W. D. L., C. W. Sabrosky, G. Bernardi, and R. V. Melville. 1985. *International Commission on Zoological Nomenclature*. 3rd ed. International Trust for Zoological Nomenclature, London, U.K., 338 p.

Ride, W. D. L., H. G. Cogger, C. Dupuis, O. Kraus, A. Minelli, F. C. Thompson, and P. K. Tubbs. 1999. *International Commission on Zoological Nomenclature*. 4th ed. International Trust for Zoological Nomenclature, London, U.K., 338 p.

Ris, H., and R. N. Singh. 1961. Electron microscope studies on blue-green algae. *Journal of Biophysical and Biochemical Cytology* **9**: 63–80.

Riser, N. W. 1955. Studies on cestode parasites of sharks and skates. *Journal of the Tennessee Academy of Sciences* **30**: 265–312.

Rudolphi, C. A. 1819. *Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi*. Sumtibus Augusti Rücker, Berolini, 811 p.

Sagan, L. 1967. On the origin of mitosing cells. *Journal of Theoretical Biology* **14**: 225–274, IN221–IN226.

Sanger, F., and A. R. Coulson. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology* **94**: 441–448.

Sattmann, H. 2002. Anfänge der systematischen Helminthologie in Österreich. *Denisia* **6**: 271–290.

Schardl, C. L., K. D. Craven, S. Speakman, A. Stromberg, A. Lindstrom, and R. Yoshida. 2008. A novel test for host-symbiont codivergence indicates ancient origin of fungal endophytes in grasses. *Systematic Biology* **57**: 483–498.

Shipley, A. E., and J. Hornell. 1904. The parasites of the pearl oyster. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part II: 77–106.

\_\_\_\_\_, and \_\_\_\_\_. 1905. Further report on parasites found in connection with the pearl oyster fishery at Ceylon. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part III: 49–56.

\_\_\_\_\_, and \_\_\_\_\_. 1906. Report on the cestode and nematode parasites from the marine fishes of Ceylon. Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar (Herdman), Part V: 43–96.

Sokal, R. R., and P. H. Sneath. 1963. *Principles of numerical taxonomy*. W. H. Freeman & Co., San Francisco, CA, 359 p.

Southwell, T. 1911. Description of nine new species of cestode parasites, including two new genera from marine fishes of Ceylon. *Ceylon Marine Biological Reports* **1**: 216–225.

\_\_\_\_\_. 1912. A description of ten new species of cestode parasites from marine fishes of Ceylon, with notes on other cestodes from the same region. *Ceylon Marine Biological Report* **1**: 259–278.

\_\_\_\_\_. 1922. Cestodes in the collection of the Indian Museum. *Annals of Tropical Medicine and Parasitology* **16**: 127–152.

\_\_\_\_\_. 1924. Notes on some tetrarhynchid parasites from Ceylon marine fishes. *Annals of Tropical Medicine and Parasitology* **18**: 459–491.

\_\_\_\_\_. 1924. The pearl-inducing worm in the Ceylon pearl oyster. *Annals of Tropical Medicine and Parasitology* **18**: 37–53.

\_\_\_\_\_. 1925. A monograph on the Tetraphyllidea with notes on related cestodes. *Memoirs of the Liverpool School of Tropical Medicine (New Series)* **2**: 1–368.

\_\_\_\_\_. 1927. On a collection of cestodes from marine fishes of Ceylon and India. *Annals of Tropical Medicine and Parasitology* **21**: 351–373.

\_\_\_\_\_. 1929. A monograph on cestodes of the order Trypanorhyncha from Ceylon and India. Part I. Ceylon Journal of Science. *Section B. Zoology and Geology* **15**: 169–312.

\_\_\_\_\_. 1930. *Cestoda*. Vol. **I**. Taylor and Francis, London, 391 p.

Stammer, H. J. 1957. Gedanken zu den parasitophyletischen Regeln und zur Evolution der Parasiten. *Zoologischer Anzeiger* **159**: 255–267.

Stelbrink, B., T. von Rintelen, G. Cliff, and J. Kriwet. 2010. Molecular systematics and global phylogeography of angel sharks (genus *Squatina*). *Molecular Phylogenetics and Evolution* **54**: 395–404.

Sites, J. W., Jr., and J. C. Marshall. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics* **35**: 199–227.

Stoll, N. R., R. P. Dollfus, J. Forest, N. D. Riley, C. W. Sabrosky, C. W. Wright, and R. V. Melville. 1961. *International Commission on Zoological Nomenclature*. 1st ed. International Trust for Zoological Nomenclature, London, UK, 176 p.

, , , , , ,  
, and . 1964. *International Commission on  
Zoological Nomenclature*. Second Edition. International Trust for Zoological  
Nomenclature, London, U.K., 176 p.

Straube, N., S. P. Iglesias, D. Y. Sellos, J. Kriwet, and U. K. Schliewen. 2010. Molecular phylogeny and node time estimation of bioluminescent lantern sharks (Elasmobranchii: Etmopteridae). *Molecular Phylogenetics and Evolution* **56**: 905–917.

Stunkard, H. W. 1915. Notes on the trematode genus *Telorchis*, with descriptions of new species. *Journal of Parasitology* **2**: 57–66.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1 Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Szidat, L. 1956. Der marine Charakter der Parasitenfauna der Süßwasserfische des Stromsystems des Rio de la Plata und ihre Deutung als Reliktaufauna des Tertiären Tethys Meeres. *Proceedings International Congress of Zoology* **14**: 128–138.

Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**: 221–244.

Tsai, I. J., M. Zarowiecki, N. Holroyd, A. Garcíarrubio, A. Sanchez-Flores, K. L. Brooks, A. Tracey, R. J. Bobes, G. Fragoso, E. Sciutto, M. Aslett, H. Beasley, H. M. Bennett, J. Cai, F. Camicia, R. Clark, M. Cucher, N. De Silva, T. A. Day, P. Deplazes, K. Estrada, C. Fernandez, P. W. H. Holland, J. Hou, S. Hu, T. Huckvale, S. S. Hung, L. Kamenetzky, J. A. Keane, F. Kiss, U. Koziol, O. Lambert, K. Liu, X. Luo, Y. Luo, N. Macchiaroli, S. Nichol, J. Paps, J. Parkinson, N. Pouchkina-Stantcheva, N. Riddiford, M. Rosenzvit, G. Salinas, J. D. Wasmuth, M. Zamanian, Y. Zheng, X. Cai, X. Soberon, P. D. Olson, J. P. Laclette, K. Brehm, and M. Berriman. 2013. The genomes of four tapeworm species reveal adaptations to parasitism. *Nature (London)* **496**: 57–63.

Van Beneden, P. J. 1849. Les helminthes cestoïdes, considérés sous le rapport de leurs métamorphoses, de leur composition anatomique et de leur classification, et mention de quelques espèces nouvelles de nos poissons plagiostomes. *Bulletins de l'Académie Royale des Sciences, des Lettres et Beaux-Arts de Belgique* **16**: 269–282.

\_\_\_\_\_. 1850. Recherches sur la faune littorale de Belgique. Les vers cestoides, considérés sous le rapport physiologique, embryogénique et zooclassique. *Mémoires de l'Academie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique* **25**: 1–199, 201–204 (Supplement).

Van Cleave, H. J. 1918a. Acanthocephala of the subfamily Rhadinorhynchinae from American fish. *Journal of Parasitology* **5**: 17–24.

\_\_\_\_\_. 1918b. *Centrorhynchus pinguis* n. sp. from China. *Journal of Parasitology* **4**: 164–167.

Vélez-Zuazo, X., and I. Agnarsson. 2011. Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Molecular Phylogenetics and Evolution* **58**: 207–217.

von Ihering, H. 1891. On the ancient relations between New Zealand and South America. *Transactions and Proceedings of the New Zealand Institute* **24**: 431–445.

\_\_\_\_\_. 1902. Die Helminthen als Hilfsmittel der zoogeographischen Forschung. *Zoologischer Anzeiger* xxvi: 42–51.

Waeschenbach, A., B. L. Webster, R. A. Bray, and D. T. J. Littlewood. 2007. Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. *Molecular Phylogenetics and Evolution* **45**: 311–325.

Waeschenbach, A., B. L. Webster, and D. T. J. Littlewood. 2012. Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. *Molecular Phylogenetics and Evolution* **63**: 834–847.

Watson, J. D., and F. H. C. Crick. 1953. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature* **171**: 737–738.

Weckstein, J. D. 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. *Systematic Biology* **53**: 154–164.

Wedl, K. 1855. Helminthologische Notizen. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Classe* **16**: 371–395.

Weidman, F. D. 1916. *Cytoleichus penrosei*, a new arachnoid parasite found in the diseased lungs of a prairie dog, *Cynomys ludovicianus*. *Journal of Parasitology*

- Wheeler, Q., and R. Meier. 2000. *Species concepts and phylogenetic theory: a debate*. Columbia University Press, New York, N.Y., 230 p.
- White, W. T., and P. R. Last. 2012. A review of the taxonomy of chondrichthyan fishes: a modern perspective. *Journal of Fish Biology* **80**: 901–917.
- White, W. T., P. R. Last, G. J. P. Naylor, K. Jensen, and J. N. Caira. 2010. Clarification of *Aetobatus ocellatus* (Kuhl, 1823) as a valid species, and a comparison with *Aetobatus narinari* (Euphrasen, 1790) (Rajiformes: Myliobatidae). *CSIRO Marine and Atmospheric Research Paper* **032**: 141–164.
- Whittaker, F. H., J. Carvajal, and R. Apkarian. 1982. Scanning electron microscopy of the scolex of *Grillotia dollfusi* Carvajal 1971 (Cestoda, Trypnorhyncha). *Journal of Parasitology* **68**: 1173–1175.
- Wiley, E. O. 1981. *Phylogenetics: The theory and practice of phylogenetic systematics*. John Wiley & Sons, Inc., New York, N.Y., 439 p.
- \_\_\_\_\_. 1988. Parsimony analysis and vicariance biogeography. *Systematic Biology* **37**: 271–290.
- \_\_\_\_\_, and B. S. Lieberman. 2011. *Phylogenetics: theory and practice of phylogenetic systematics*. Wiley-Blackwell, Hoboken, N.J., 432 p.
- \_\_\_\_\_, D. Siegel-Causey, D. R. Brooks, and V. A. Funk. 1991. The Compleat Cladist: a primer of phylogenetic procedures. Special Publication. *Museum of Natural History, University of Kansas* **19**: 1–158.
- Woodruff, D. S., A. M. Merenlender, E. S. Upatham, and V. Viyanant. 1987. Genetic variation and differentiation of three *Schistosoma* species from the Philippines, Laos, and Peninsular Malaysia. *American Journal of Tropical Medicine and Hygiene* **36**: 345–354.
- Xu, G., Q. Q. Fang, J. E. Keirans, and L. A. Durden. 2003. Molecular phylogenetic analyses indicate that the *Ixodes ricinus* complex is a paraphyletic group. *Journal of Parasitology* **89**: 452–457.
- Young, N. D., A. R. Jex, B. Li, S. Liu, L. Yang, Z. Xiong, Y. Li, C. Cantacessi, R. S. Hall, X. Xu, F. Chen, X. Wu, A. Zerlotini, G. Oliveira, A. Hofmann, G. Zhang, X. Fang, Y. Kang, B. E. Campbell, A. Loukas, S. Ranganathan, D. Rollinson, G. Rinaldi, P. J. Brindley, H. Yang, J. Wang, J. Wang, and R. B. Gasser. 2012. Whole-genome sequence of *Schistosoma haematobium*. *Nature Genetics* **44**: 221–225.
- Zehnder, M. P., A. de Chambrier, C. Vaucher, and J. Mariaux. 2000. *Nomimoscolex suspectus* n. sp. (Eucestoda: Proteocephalidea: Zygobothriinae) with morphological and molecular phylogenetic analyses of the genus. *Systematic Parasitology* **47**: 157–172.

Zschokke, F. 1884. Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. *Archives de Biologie* **5**: 1–90 + Plates IX.



# Chapter 4

## Eorhynchus: A Proposed New Name for Neorhynchus Hamann Preoccupied\*

H. J. Van Cleave

The genus *Neorhynchus* was founded in 1892 by Hamann to include *Echinorhynchus rutili* Müller and *Echinorhynchus agilis* Rudolphi. Practically all investigators dealing with the Acanthocephala since that date have accepted this generic name. Recently attention has been called to the fact that the name *Neorhynchus* is preoccupied. Sclater, in 1869, and, again, Milne Edwards, in 1879, employed it for other groups. In accordance with the laws of nomenclature, it then becomes necessary to reject the name *Neorhynchus* as applied to Hamann's genus. I propose the name *Eorhynchus* to designate these forms. While all other investigators dealing with this genus have been limited to a study of the two original species, it has been my good fortune to include five additional species in a comparative study, the results of which have led me to a restatement of its essential characteristics. As pointed out in an earlier paper (1913), I consider the following points as diagnostic for this genus of *Acanthocephala*:

1. Six giant nuclei in the subcuticula arranged, normally, five in the middorsal line of the body and one in the midventral line.
2. Two giant nuclei in one lemniscus and only one in the other.
3. Proboscis receptacle with but a single muscle layer in its wall.

In the light of this analysis, the contentions of de Marval (1904: 582) and of Monticelli (1905: 217), that *Apororhynchus hemignathi* Shipley should be included in this genus are based on an inadequate understanding of its natural limits.

Shipley, in his description of *A. hemignathi* (1896: 210), wrote: "As in *Neorhynchus*, the number of nuclei is very small, some twelve to twenty seem to suffice for the whole subcuticle, and perhaps two to four for each lemniscus. The nuclei are scattered about in a most irregular fashion. . . ."

I have shown (1913) that not alone the *presence* of giant nuclei, but more strikingly their *number* and *arrangement* furnish a sure criterion for the determination of members of this genus. Shipley's genus *Apororhynchus*, because of its radical departure from the typical structure of the *Eorhynchi*, cannot be included within the genus *Eorhynchus*. The valid species of this genus are, then, *Eo. rutili* (Müller

1784), *Eo. agilis* (Rudolphi 1819), *Eo. emydis* (Leidy 1852), *Eo. gracilisentis* (Van Cleave 1913), *Eo. longirostris* (Van Cleave 1913), *Eo. cylindratus* (Van Cleave 1913) and *Eo. tenellus* (Van Cleave 1913).

Hamann (1892) also created the family Neorhynchidae for the single genus *Neorhynchus*. Porta (1907:409) accepted Hamann's revision of the Acanthocephala only in part, recognizing but two families, Echinorhynchidae and Gigantorhynchidae, and included *Neorhynchus* under the former. The characteristics already listed as diagnostic for the genus *Eorhynchus*, together with the complete fusion of the cement glands, are such essential features that the inclusion of *Eorhynchus* in the same family with *Echinorhynchus* would so distort our conception of the family Echinorhynchidae that it would cease to be a natural division of the Acanthocephala, and would become a purely artificial assemblage. In view of these facts I consider that the evidence fully justifies the retention of the family rank originally attributed to these forms for which the family name now becomes Eorhynchidae.

The writer's extensive studies on the cytology of the Eorhynchidae furnish conclusive evidence in support of the foregoing arguments. These studies in detail appear in the June number of the *Journal of Morphology*.

#### REFERENCES

- Hamann, O. 1892. Das System der Acanthocephalen. Zool. Anz., 15: 195.  
de Marval, L. 1904. Sur les acanthocéphales d'oiseaux. Note préliminaire, Rev. suisse de zool., 12: 573-583.  
Monticelli, F. S. 1905. Per una rettifica. A proposito di una proposta classificazione degli Acantocefali. Boll. Soc. Natural. Napoli, 19: 217-218.  
Porta, A. 1907. Contributo allo studio degli Acantocefali dei Pesci. Biologica Torino, 1:377-423.  
Shipley, A. E. 1896. On Arhynchus hemignathi, a new genus of Acanthocephala. Quart. Jour. Micr. Sci., 39: 207-218.  
Van Cleave, H. J. 1913. The Genus *Neorhynchus* in North America. Zool. Anz., 43: 177-190.

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# **Acanthocephala in *The Journal of Parasitology*, 1914–2014**

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The launch of *The Journal of Parasitology (JP)* by Henry Baldwin Ward in September 1914 was a historical landmark that witnessed the passage of the torch of acanthocephalan work, especially higher taxonomy, from Europe to the United States, with the exception of the works of Anton Meyer, Yves Golvan, and a few Russian workers in the early twentieth century. At that juncture, there were only two primary English-language parasitological journals, i.e., *The Journal of Parasitology* and *Parasitology*. Research then was optical microscopy-based taxonomy work. There are now more than 30 parasitology-based journals with multiple sub-specialties because of rapidly advancing technologies. Advances in molecular and gene sequencing techniques, especially over the last two decades, have helped resolve many taxonomic and evolutionary questions.

The present paper is thematic and will address the *JP* contributions to resolving taxonomic, evolutionary, biological, behavioral, and related issues posed by the acanthocephalan challenge as models for parasitological research. The most notable observation is that taxonomic studies of Acanthocephala, whether such work utilized classical or molecular approaches, has been one of the most solid and consistent fields of study over the years. Life cycle, behavioral, seasonal, developmental, and related studies, like fads, seem to last for a few decades before they fade away, and are replaced by new approaches. Changes of emphasis, as well as progressive developments in technology, especially in the areas of gene sequencing, molecular, and evolutionary biology, account for most of these trends that also reflect world-wide patterns of intellectual interest. The two taxonomy sections below incorporate *JP* as well as other journal sources. All subsequent sections address only *JP* articles.

## Classical taxonomy

Classical taxonomy articles were the most commonly published research on Acanthocephala in *JP* during its 100 years of existence. The first number of *JP* included Van Cleave's (1914) proposal of *Eorhynchus* (Hamann, 1892), now *Neoechinorhynchus* Stiles and Hassall, 1905, as a new name for *Neorhynchus* (Hamann, 1892).

Harley J. Van Cleave, the “father” of acanthocephalan taxonomy in the US, picked up where the European masters left off. Rudolphi (1802) was the first to name these worms Acanthocephala and gave them an ordinal rank, with one genus, *Echinorhynchus*. Most early taxonomic works lacked detailed morphological information until Lühe's (1904, 1905) critical reviews of the early descriptions. Hamann (1892) recognized the diversity of this group of worms and split the old genus *Echinorhynchus* into three families (Echinorhynchidae, Gigantorhynchidae, Neorhynchidae), an action that formed a basis for later classifications of Acanthocephala. These divisions were subsequently elevated to ordinal rank by Meyer (1931) and Van Cleave (1936), but only to subordinal rank by Southwell and MacFie (1925), among taxonomic variations proposed by other observers, including Travassos (1926), Thapar (1927), Witenberg (1932a, 1932b), and Meyer (1931–1933).

Uncertainty about the position of Acanthocephala among other animal groups was marked by Leuckart's (1848) speculation of lines of descent between the cestodes and acanthocephalans, groups he placed as two orders in his class Anenteraeti, whereas Meyer (1932, 1933) regarded Acanthocephala as a class of the Aschelminthes that included two orders, Palaeacanthocephala and Archiacanthocephala based on morphology and ontogeny. Van Cleave (1936) removed the inconsistencies of Meyer's system by establishing a third order, Eoacanthocephala. He also recognized Acanthocephala as a phylum (Van Cleave 1941, 1948), closely associated with the Cestoda.

More recently, Petrochenko (1956) devised a system based heavily on acanthor spination. Golvan (1959–1961, 1965, 1969, 1994) considered Eoacanthocephala (now regarded as the most ancient group), Palaeacanthocephala, and Archiacanthocephala to be classes, relying heavily on the number of cement glands and on trunk spination. Yamaguti (1963) recognized three orders, namely, Neoechinorhynchidea, Echinorhynchidea, and Gigantorhynchidea, corresponding to the Meyer–Van Cleave classes, as well as a new fourth order, Apororhynchidea. Golvan's (1994) nomenclature of the Acanthocephala was a culmination of his life-long contributions to the systematics of this phylum (Amin, 2013).

The standard classification of the Acanthocephala provided by Amin (1985), and an earlier synopsis (Amin, 1982), were updated to include hierachal changes and a considerable number of new taxa (Amin, 2013). This latest classification continued to retain its affiliation with the systems of Meyer (1931–1933) and Van Cleave (1936, 1941, 1947–1949, 1951, 1952), but incorporated new contributions of

molecular taxonomy and phylogenetic studies to the taxonomic system. The recognition of a new order, Neoacanthocephala (now Neoechinorhynchidae; Southwell and MacFie, 1925) (Van Cleave, 1936), the treatment of the Eoacanthocephala of North America (Van Cleave, 1947), and Van Cleave's (1948) expansions of the phylum Acanthocephala were landmark contributions that were originally published in *JP*. These quoted workers did exactly what modern molecular taxonomists are doing, but with a different set of tools. They considered individual species and lower taxa as models for higher patterns and trends to which these groups needed to be assigned in order to resolve the larger evolutionary picture.

Taxonomy in the highest sense is a science of relationships. Taxonomists dealing with higher taxa see their world in a gestalt perspective. Regrettably, modern systematists, the molecular taxonomists included, often see the leaves and, at best the small branches, but do not see the trunk and the roots of the classification system. Just because the roots are buried in the darkness of the earth does not mean that they are not there. Higher taxonomy has faded away with the passing of the masters who had great vision and were not encumbered by the limitations of the optical tools. Having a taxonomically based Ph.D. in parasitology or publishing a new species here and there is not the same as really practicing taxonomy. One can spend a lifetime classifying crabs based on differences in their neurological systems, but at the end of the day, one still does not really know the crab, or the shore, or the ocean. It is in these more inclusive relationships that higher taxonomy belongs.

There are many world-class taxonomists who have described a substantial number of species or genera of Acanthocephala over the years. Because of space limitations, it will not be possible to list all the authors and associated descriptions published over a 100-year period. However, such a list is available in Amin's (2013) classification, with an index to families and genera. Authors of descriptions of lower taxa published in *JP*, since 1914, are herein included in chronological order based on first appearance: H. J. Van Cleave, D. R. Lincicome, E. M. Pratt, A. C. Chandler, M. L. Perry, H. L. Ward, M. A. Tubangui, V. A. Masilungan, H. W. Manter, J. D. Webster, E. E. Byrd, J. F. Denton, E. C. Haderlie, R. B. Williams, H. F. Timmons, L Sarmiento, R. M. Cable, W. B. Hopp, L. Margolis, W. L. Bullock, E. N. Das, F. M. Fisher, Jr., K. A. Neiland, G. D. Schmidt, J. Linderoth, F. L. Dunn, R. M. Laurs, J. E. McCauley, R. E. Kuntz, D. R. Nelson, B. B. Nickol, L. M. Cordonnier, F. E. Kellogg, V. E. Thatcher, E. J. Huggins, A. M. Dunn, G. Samuel, O. M. Amin, E. H. Williams, D./ F. Oettinger, R. L. Buckner, S. C. Buckner, I. Paperna, D. G. Huffman, T. N. Padilha, S. H. Loetta, A. A. Kocan, B. Marchand, G. Vassiliades, H. L. Ching, W. A. Rogers, T. P. Deveaux, M. Krishnasamy, L. R. Smales, S. J. Edmonds, N. P. Boyce, R. A. Heckmann, N. V. Ha, A. M. El-Naggar, O. N. Bauer, E. G. Sidorov, S. Ortubay, C. Ubeda, L. Semenas, C. R. Kennedy, J. A. Ewald, D. W. T. Crompton, F. M. Nahhas, J. F. Munro, R. C. Stoddart, B. S. Dezfuli, S. Monks, D. Bolette, D. W. Searle, G. Munoz, M. George-Nascimento, G.

Salgado-Maldonado, A. Cruz-Reyes, S. Monks, G. Pulido-Flores, C. R. Bursey, S. Goildberg, F. Thielen, M. Muenderle, H. Tarascheeski, B. Sures, M. Garcia-Varela, G. Perez-Ponce- de Leon, F. J. Aznar, S. Nadler, A. L. Lanfranchi, O. L. Lisitsyna, V. V. Tkach, S. A. Bush, Z Gholami, M. Akhlaghi, A. Maria Santana-Pineros, Y. Cruz-Quintana, O. Arturo Centeno-Chale, V. M. Vidal-Martinez, D. Vrcibradic, F. H. Hatano, C. F. D. Rocha, V. L. Olmos, E. M. Habit, N. A. Radwan, J. S. Mantuano Anchundia, M. A. Zambrano Alcivar, F. M. Vieira, N. N. Felizardo, J. L. Luque, D. W. Duszynski, A. Halajian, A. Eslami, J. T. Timi, and C. A. Fuller.

(Editors' note: This list is a truly remarkable one that includes a very diverse group of scientists from around the world, not only people who have made an intellectual career studying Acanthocephala, but also those whose main research interests have been with other groups. A Google® or library database search on these names, for example using online Biological Abstracts, would be a major lesson in the history of parasitology, the way in which interest and opportunity drive investigations, and the impact that curiosity has on a scientist's life. We thank Dr. Amin for compiling this list!)

Contributors to the higher taxonomy of Acanthocephala who also dealt with generic and supra-generic taxa include G. D. Schmidt and collaborators, D. J. Richardson, L. R. Smales, and O. M. Amin. Schmidt and Neiland (1966) revised the helminth fauna of Nicaragua and described new centrorhynchid species, while Schmidt and Kuntz (1967a) revised the Porrorchinae and described new genera and species. Schmidt (1972) revised the class Archiacanthocephala Meyer, 1931. Schmidt and Huggins (1973a, 1973b) revised the Eoacanthocephala and the Palaeacanthocephala of South American fishes, respectively. Richardson and Nickol (1995) revised the genus *Centrorhynchus* in North America and Smales (2002) examined species of *Mediorhynchus* Van Cleave, 1916 in Australian birds.

Amin's contributions included the classification of the Acanthocephala (Amin, 1982, 1985, 2013), the erection of a new class, Polyacanthocephala, with keys to acanthocephalan families and subfamilies (Amin, 1987), a review of *Polymorphus*, including synonymization of previously described species from other genera (Amin, 1992), review of Acanthocephala of the Neotropical region (Amin, 2000), a revision of *Neoechinorhynchus*; Stiles and Hassall, 1905, with the erection of two new subgenera, *Neoechinorhynchus*; Hamann, 1892, and *Hebesoma*; Van Cleave, 1928 (Amin, 2002), and the erection of a new acanthocephalan order (Heteramorphida; Palaeacanthocephala) and family (Pyrirhynchidae) that is intermediate between Polymorphidae and Heteracanthocephalidae, and parasitic in birds in Vietnam (Amin and Ha, 2008). Amin (2000) listed and annotated the Acanthocephala in the Neotropical region. In an obscure publication, Bhattacharya (2007) listed 251 acanthocephalan species from India and described a few species and genera, but did not recognize the Neoechinorhynchida; he also included its families in Gyracanthocephala. Salgado-Maldonado (2006) listed and discussed all helminth parasites of freshwater fishes in Mexico. Salgado-Maldonado and Amin (2009) followed with the acanthocephalian species of the

Gulf of Mexico. Khatoon and Bilqees (2011) then published an expanded version of their conventional classification from 1991, but their work included a number of errors and misplacements of higher taxa.

## Molecular-evolutionary taxonomy

Advances in molecular and gene sequencing techniques, especially during the last two decades, have helped resolve many taxonomic and evolutionary questions. This work dealt with a number of questions of long standing and provided a new set of answers from different perspectives. These studies include the following contributions as reviewed in Amin (2013). Meyer (1932, 1933) grouped the Acanthocephala with the Rotifera, Gastrotricha, Kinorhyncha, Priapuloidea, Nematomorpha, and Nematoda within the phylum Aschelminthes. Recent molecular studies by Garey et al. (1996), García-Varela et al. (2000), Welch (2000), and Near (2002), among others, even suggest that Rotifera and Acanthocephala are phylogenetically related sister groups. Garey et al. (1996) asserted that the Acanthocephala represent a taxon within phylum Rotifera. Several workers have since demonstrated the sister group relationship of Acanthocephala with Rotifera, forming a new phylum, Syndermata Ahlrichs, 1997. In an extension of these findings, Garey et al. (1998), Zrzavý (2001), Kristensen (2002), García-Varela and Nadler (2006), Witek et al. (2008), Fontaneto and Jondelius (2011) linked Gnathostomulida with Micrognathozoa and moved Syndermata into a larger clade, Gnathifera. The Gnathifera was first proposed by Rieger and Tyler (1995) and has been established as a monophyletic clade (Syndermata + Gnathostomulida) by Witek et al. (2009).

Phylogeny within the Syndermata subtaxon Acanthocephala was studied by sequencing the mitochondrial genomes of species from Palaeacanthocephala, Eoacanthocephala, Archiacanthocephala, and Bdelloidea, as well as of other syndermatans, 18 lophotrochozoan (spiralian) taxa, and one outgroup representative (Weber et al., 2013). Phylogenetic analyses have shown that the monophyletic Archiacanthocephala represented the sister taxon of a clade comprising Eoacanthocephala and the monophyletic Palaeacanthocephala. This topology suggests the secondary loss of lateral sensory organs (sensory pores) in Palaeacanthocephala and is in further agreement with the emergence of apical sensory organs in the stem lineage of Archiacanthocephala as defined by Weber et al. (2013).

Because acanthocephalans and tapeworms are without an intestine, the two groups have been considered as being related. Cholodkovsky (1897) was the first to propose such a relationship after Leuckart's (1848) early accounts; this view has been supported by Skrjabin and Shults (1931), Petrochenko (1952), Van Cleave (1941), and Amin et al. (2009). The latter authors identified what appear to be microtriches on the trunk epidermis of *Rhadinorhynchus ornatus* Van Cleave, 1918 (Rhadinorhynchidae) from skipjack tuna, *Katsuwonus pelamis* (Linnaeus), in the Pacific Ocean off South America. Another marine rhadinorhynchid acanthocephalan, *Leptorhynchoides polycristatus*, from sturgeons in the Caspian Sea, appears to have similar structures (Amin et al., 2013). However, these structures may not be homologous with microtriches of cestodes; see Chervy

(2009) for details regarding microtriches in cestodes.

According to Garey et al. (1998), combining molecular and morphological analyses of Bilateria leads to a tree with Platyhelminthes, Rotifera, Acanthocephala, and Gnathostomulida (and probably Gastrotricha) as a sister group to the annelid-mollusk lineage of the Spiralia (Lophotrochozoa). Steinauer et al. (2005), using mitochondrial (mt) genome sequences, suggested that Acanthocephala, as inferred from the mt genome of *Leptorhynchoides thecatus* (Linton, 1891), are closer to Platyhelminthes than was previously supposed. Their data are consistent with the data contained in numerous related studies based on RNA analysis. For instance, Min and Park (2009) linked Syndermata with Platyhelminthes as the Platyzoa. Under all proposals, monophyly of the major taxonomic groups of the Acanthocephala has been established (Near et al., 1998; Monks, 2001; Near, 2002), suggesting that the present classification of higher taxa is natural.

Many examples of molecular taxonomy of lower taxa leading to reassessments or to creation of higher taxa are included in Amin (2013). Ten instructive examples follow:

- 1.** Distinguishing *Mediorhynchus africanus* Amin, Evans, Heckmann, El-Naggar, 2013 from the Asian *M. gallinarum* (Bhalerao, 1937) Van Cleave, 1947, was based on morphological evidence, SEM, and gene sequence analysis using DNA sequence from one mitochondrial gene (cytochrome oxidase subunit 1) and one nuclear gene, 18S rRNA, to infer the phylogenetic relationships of *M. africanus* and *M. gallinarum* and selected Acanthocephala. *Mediorhynchus* was shown to be monophyletic and *M. africanus* and *M. gallinarum* to be allopatric sister species with 9.7% sequence divergence.
- 2.** Rosas-Valdez et al. (2012) presented phylogenetic trees for two known species of *Floridosentis* Ward, 1953. These authors showed that *Floridosentis* is monophyletic, and is comprised of two major, well-supported clades that correspond with the two noted species and their geographical distribution.
- 3.** Salgado-Maldonado (2006) suggested the existence of two cryptic species within *Neoechinorhynchus* (*Neoechinorhynchus*) *golvani*, one associated with cichlids and the other with eleotrids in Mexico. Monks et al. (2011) subsequently described *N. (N.) brentnickoli* from eleotrid fishes. Martinez-Aquino et al. (2009) revealed a complex of three cryptic species within *N. golvani* using two nuclear gene sequences that were associated with eleotrid and cichlid fish lineages in waters of different salinities.
- 4.** The criteria for the classification of families of Palaeacanthocephala based on morphological characteristics may need to be re-evaluated using gene sequencing methods to establish phylogenetic relationships; for example, see García-Varela and Nadler (2005). Verwegen et al. (2011) analyzed 39 species from all four classes of Acanthocephala using nuclear 18S rDNA sequences. They found that the resulting trees suggested a paraphyletic arrangement of

the Echinorhynchida and Polymorphida within the Palaearctocephala.

**5.** Väinölä et al. (1994) demonstrated strong allozyme divergence between the marine *Echinorhynchus gadi* (Zoega in Müller, 1776) Van Cleave, 1924, and the fresh-brackish-water *E. salmonis*, supporting the genetic distinction between these two taxa. Sobecka et al. (2012) compared populations of *E. gadi* from the Atlantic cod, *Gadus morhua* Linneaus, in the Baltic Sea and the North Atlantic morphometrically and genetically, using polymerase chain reaction restriction fragment length polymorphism and selected PCR products. Their analysis indicated that the nucleotide sequences of *E. gadi* rDNA from cod collected from all sites are identical. Morphometric analysis, however, demonstrated the separation of *E. gadi* into two groups, corresponding with the separation of cod into two subspecies, *G. m. morhua* in the Atlantic and *G. m. callarias* in the Baltic.

**6.** Tkach et al. (2013) used comparative analysis of nuclear ribosomal rRNA sequences encompassing the 3' end of 18S nuclear rDNA gene, internal transcribed spacer region (ITS 1+5.8S+ITS 2), and 5' end of the 28S gene to demonstrate significant differences between *Pseudoacanthocephalus nickoli* Tkach, Lisitsyna, Crossley, Binh, and Bush, 2013, and *P. smalesi* Tkach, Lisitsyna, Crossley, Binh, and Bush, 2013, as well as between these two species and closely related species from China and Vietnam.

**7.** Based on isoenzyme analysis, Dudiňák and Šnábel (2001) described the genetic differences between the *Pomphorhynchus laevis* (Zoega in Müller, 1776) Van Cleave, 1924, populations of the Slovak and Czech Republics. Geographic isolation has evidently produced distinct genetic forms irrespective of host species. Perrot-Minnot (2004) demonstrated a high level of sequence divergence at ITS 1, ITS 2, and cytochrome c oxidase between smooth and wrinkled cystacanths of *P. laevis*, molecular differences that corresponded with phototactile behavioral differences in gammarid hosts. She speculated that the smooth type corresponds to *P. laevis* and the wrinkled type to *P. tereticollis*, a former synonym of *P. laevis*. Špakulová et al. (2011) distinguished between *P. laevis* and *P. tereticollis* based on differences in proboscis armature and gene sequences using ITS 1, ITS 2, and COI.

**8.** Aznar et al. (2006) split *Corynosoma* Lühe, 1904 (*fide* Van Cleave 1945) into two genera, *Corynosoma* for marine species and *Pseudocorynosoma* for freshwater species, based on anatomical, ecological, and phylogenetic divergences. García-Varela et al. (2013) further demonstrated that *Pseudocorynosoma* species form an independent lineage that does not share a common ancestor with species of *Corynosoma* or *Andracantha*.

**9.** Amin's (1992) revision of *Polymorphus* Lühe, 1911, recognized two subgenera, *Polymorphus* Lühe, 1911, and *Profilicollis* Meyer, 1931. The elevation of these two subgenera to the generic level has been controversial, e.g., Nickol et al. (1999) supported it based on intermediate host affinities, but

García-Varela and Pérez-Ponce de León (2008) disagreed based on sequences of the *cox 1* gene.

**10.** The validity of class Polyacanthocephala; Amin, 1987, was supported by ribosomal RNA gene sequence studies by García-Varela et al. (2002).

Parts of this discussion on taxonomy were adapted from Amin (1985, 2013).

## **Lessons learned from the history of acanthocephalan taxonomy**

Molecular and gene sequencing techniques are elegant tools that help to distinguish the identity of newly described taxa from related ones, confirm the lineages and assignment of higher taxa using character states of evolutionary significance, and establish relationships among higher taxa that may be in agreement or disagreement with previously proposed ones based only on morphological grounds. It is to be expected that genotypic distinctions may not be sufficient alone and that phenotypic assignments can, and do, still carry considerable weight.

## Life cycles

Life cycle studies were the second most commonly reported in *JP*, especially during the 1960s. Like fads, they gradually declined until they came to a virtual stop by the end of the 1980s. Van Cleave's studies of the life cycles of *Echinorhynchus coregoni* Linkins in Van Cleave, 1919, and *Leptorhynchoides thecatus* (Linton, 1891) Kostylew, 1924, inaugurated this field of study in the 1920s. De Giusti (1939, 1949) provided more detailed life cycle studies of *L. thecatus* in the 1930s and 1940s. Moore (1946) added similar information regarding *Macracanthorhynchus ingens* Meyer, 1933. Reish (1950) later reported on the life cycle of *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947 (= *Polymorphus kenti* Van Cleave, 1947); Hopp (1954) for *Neoechinorhynchus emydis* (Leody, 1851) Van Cleave, 1919, (*nec* 1916); Moore (1962) for *Mediorhynchus grandis* Van Cleave, 1916; Merriott and Pratt (1964) for *Neoechinorhynchus rutili* (Müller, 1780); Schmidt and Olson (1964) for *Plagiorhynchus (Prosthorhynchus) cylindraceus* (Goeze, 1782) Schmidt and Kuntz, 1966 (= *Prosthorhynchus formosus* (Van Cleave, 1918) Travassos, 1926); and Harms (1965) for *Octospinifer macilentis* Van Cleave, 1919.

The life cycle of *Prosthenorchis elegans* (Diesing, 1851) Travassos, 1915, was reported by Stunkard (1965; Stoddart, 1965), that of *Profilicollis formosus* by Schmidt and Kuntz (1967a), and that of *Paulisentis fractus* Van Cleave and Bangham, 1949, by Cable and Dill (1967); and Uglem and Larson (1969) for *Neoechinorhynchus saginatus* Van Cleave and Bangham, 1949. The life cycles of three polymorphids, *Corynosoma constrictum* Van Cleave, 1918, *Polymorphus contortus* (Bremser, 1821) Travassos, 1926, and *P. trochus* Van Cleave, 1945, and their development in *Hyalella azteca* Saussure, 1858, were reported by Podesta and Holmes (1970). Olson and Pratt (1971) reported the life cycle and larval development of *Echinorhynchus lageniformis* Ekbaum, 1938. Uglem (1972) studied the life cycle of *Neoechinorhynchus cristatus* Lynch, 1936, and provided information on the hatching of eggs. Nickol (1977) discussed the life history and host specificity of *Mediorhynchus centurorum* Nickol, 1969; Samuel and Bullock (1981) for *Paratenuisentis ambiguus* (Van Cleave, 1921) Bullock and Samuel, 1975; De Mont and Corkum (1982) for *Octospiniferoides chandleri* Bullock, 1957; and Brattey (1988) and by Benesh and Valtonen (2007) for *Acanthocephalus lucii* (Müller, 1776) Lühe 1911.

## Lessons learned from the study of acanthocephalan life cycles

Life cycle studies are valuable not only for the understanding of the metamorphosis of specimens of studied species and their host and environmental relationships (if not studied only under laboratory conditions), but also for other reasons. Most species of acanthocephalans are described from the adult stages and some are known only from immature specimens. In both situations, the relationship between the taxonomic characters in specimens of each of the two

stages remains unknown. For example, trunk, proboscis or lemniscus form, position of the gonopore, patterns of trunk spination, hook, and hook root development in mature and immature forms often vary. Life cycle studies, including good morphometrics, would resolve questions of the assignment of mature and immature stages to the same or other species.

## Behavioral studies

Behavioral studies reported in *JP*, especially of crustacean intermediate hosts infected with larval acanthocephalans, were also popular for a while, after a late start. These studies appear to have been a fashion that lasted about 20 years between the 1970s and the 1990s, and have since faded away. Bowen (1967) observed defense reactions in millipedes infected by larval *M. ingens*. Bethel and Holmes (1973, 1974) correlated the evasive behavior and responses to light of *Gammarus lacustris* Sars, 1864, infected with *Polymorphus paradoxus* Connell and Corner, 1957, with promoting infection in the definitive host. Altered color, behavior, and predation susceptibility of the isopod, *Asellus intermedius* Forbes, 1876, infected with *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936, were reported by Camp and Huizinga (1979). Oettinger and Nickol (1982a, 1982b) examined the spectrophotometric characteristics of the integument pigments of *A. intermedius* and the developmental relationships with *A. dirus*.

Moore (1983) and Moore and Gottelli (1992) examined the altered behavior in two species of cockroaches infected with *Moniliformis moniliformis* (Bremser, 1811) Travassos, 1915. Later, Carmichael and Moore (1991) compared the altered behavior in the brown cockroach and the American cockroach infected with *M. moniliformis*, but Allely et al. (1992) showed that infections with *M. Moniliformis* had no behavioral effects on the viviparous pacific cockroach *Diploptera punctata* (Eschscholtz). Zhao and Wang (1992) examined the defense reaction against the larvae of *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos, 1917, in laboratory-infected beetles. Freehling and Moore (1993) established the susceptibility of 13 species of cockroaches to infections with *M. moniliformis*. Moore et al. (1994) described the altered behavior of two species of blattid cockroaches infected with *M. moniliformis*. Moore and Gotelli (1996) explored the evolutionary patterns of altered behavior and susceptibility in parasitized hosts.

Maynard et al. (1998) studied the altered behavior of the amphipod *Echinogammarus stammeri* Karaman, 1931, infected with *Pomphorhynchus laevis* (Zoega in Müller, 1776) Van Cleave, 1924. Benesh et al. (2005) studied behavioral response to light by amphipods infected with *Corynosoma constrictum* Van Cleave, 1918. Benesh and Valtonen (2007) studied the effect of *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911, infections on intermediate host growth and survival. Benesh et al. (2008) observed the effect of *Echinorhynchus cinctulus* (Porta, 1905) Amin, 2013 (= *Echinorhynchus borealis* von Linstow, 1901) on anti-predator behavior of the amphipod intermediate host. The behavior of some vertebrate animals is also affected by acanthocephalans. Thus, McLennan and Shires (1995) were able to correlate the intensity of brook stickleback behavior with level of infection with *Neoechinorhynchus rutili* (Müller, 1780).

## Lessons learned from the behavioral studies

Altered behavior involving color changes, behavioral alteration, and increased predation susceptibility of larval stages of acanthocephalans are rather commonplace in both aquatic and terrestrial systems. Accordingly, worms reach definitive hosts more effectively and in larger numbers than they would be able to otherwise, evidently leading to parasite survival at a low, or no, energy cost. These cases are excellent examples of strategies by which these parasites are assumed to insure their evolutionary success.

# **Ecological, seasonal and geographical distribution, and host-parasite relationships**

This section has a wider, but related, coverage in *The Journal of Parasitology*. The first of these sorts of studies was by Van Cleave (1916), who reported the seasonal distribution of *Neoechinorhynchus emydis* (Leidy, 1851) Van Cleave, 1919 (= *neoechinorhynchus gracilisentis* Van Cleave, 1913) Van Cleave, 1919 (= *Neoechinorhynchus gracilisentis* Van Cleave, 1913), and *Tanaorhampus longirostris* (Van Cleave, 1913) Ward, 1918 (= *Neoechinorhynchus longirostris* (Van Cleave, 1913) Van Cleave, 1916). Thirty five years later, Fischthal (1950) reported on the geographic and host distribution of *Leptorhynchoides thecatus*. It took another 17 years for the ecology of *N. rutili* to be published by Walkey (1967).

The 1970s and 1980s appear to have been a reasonably good period for ecological/seasonal studies, but those have dwindled considerably in the twenty-first century. Seidenberg (1973) studied the ecology of *Acanthocephalus dirus* in its isopod intermediate host, *Asellus intermedius*. Amin (1975, 1986, 1987) examined the host and seasonal distribution of *A. dirus* (= *Acanthocephalus parksidei* Amin, 1975), of species of *Neoechinorhynchus* Stiles and Hassall, 1905, and of *Pomphorhynchus bulbocollis* Linkins in Van Cleave, 1919, respectively, in Wisconsin lakes. Muzzall and Bullock (1978) reported the seasonal and host-parasite relationships of *Neoechinorhynchus saginatus* in *Semotilus corporalis* (Mitchell). The seasonal occurrence and host specificity of *Gracilisentis gracilisentis* and *Tanaorhampus longirostris* in an Illinois lake were reported by Jilek (1978). Buckner and Nickol (1979) studied the geographic and host-related variation among species of *Fessisentis* Van Cleave, 1931. Muzzall (1980) explored the ecology and seasonal abundance of three acanthocephalan species infecting white suckers in New Hampshire. Camp and Huizinga (1980) studied the seasonal population interactions of *A. dirus* in *Semotilus atromaculatus* Mitchell, 1818 and *A. intermedius*. Elkins and Nickol (1983) studied the epizootiology of *M. ingens* in Louisiana. Gleason (1987) studied the population dynamics of *P. bulbocollis* in *Gammarus pseudolimnaeus* Bousfield, 1958. Ashley and Nickol (1989) examined the dynamics of *L. thecatus* suprapopulation in a Great Plains reservoir. Stoddart et al. (1991) examined the influence of host strain and helminth isolate on the relationship between rats and *M. moniliformis*.

Moser and Hsieh (1992) investigated biological tags for stock separation in Pacific herring. Trejo (1992) examined the host–parasite relationship of *Pomphorhynchus patagonicus* Ortubay, Ubeda, Semenas and Kennedy, 1991, in two species of fish from Argentina. Olson and Nickol (1996) compared recruitment of *L. thecatus* in green sunfish and largemouth bass. Steinauer et al. (2006) examined the geographic and host use of *L. thecatus* in the U.S. Rauque et al. (2006) observed the seasonal recruitment and reproduction of *A. tumescens* in fishes from Argentina. Balboa et al. (2009) examined the distribution of cystacanths of two *Profilcollis* species in sympatric crustacean hosts in Chile.

Rauque and Semanas (2011) used parasite volume as an indicator of competition between *Acanthocephalus tumescens* (von Linstow, 1896) Porta, 1905, and *Pseudocorynosoma* sp. in their intermediate host. Kopp et al. (2011) and Wahl and Sparkes (2012) reported on the dispersal of *A. dirus* in the U.S.

## **Lessons learned from ecological studies on acanthocephalans**

Ecological, geographical, and host-parasite specificity and relationships are currently being recognized as important variables embodying character states critical for the creation of phylogenetic trees addressing taxonomic issues. It is no small feat that these variables can, and do, affect the taxonomy and evolution of the Acanthocephala directly or indirectly. As distinct as these areas of study may seem, they are actually connected in an integrated way. Throughout the last 50 years, life history and behavioral studies have been effectively used to answer ecological questions. They are not as expensive to execute as molecular work, and yield academic recognition and frequent publications. It is not surprising that the chronology of the high activity and decline of these studies followed the same pattern, fading away at the same time in the 1980s and 1990s. This decline also corresponded with the advent of new technology and molecular techniques that are regarded by some as better ones for answering some of the age-old questions. It is likely that in the near future, editors will require gene sequencing for new species descriptions.

It is inescapable to conclude that behavioral, life history and ecological questions can sometimes be addressed using molecular techniques. Molecular ecology is an emerging science addressing the role of genetic constitutions in the ecological and host parasite distribution. While sequencing may not provide the complete answer to the larger ecological or behavioral questions, it can shed light on some of these larger questions especially those involving evolutionary components.

## Anatomy and ultrastructure

Anatomical studies published in *JP* also had a late start in the 1950s, blossomed in the 1970s and 1980s, and dwindled to a virtual end by the early 1990s.

Ultrastructural studies became popular with the advent of technological advances. Electron microscopy is not regarded as particularly sophisticated nowadays, especially when compared to molecular approaches and related emphasis preferred by many journals and editors. Nonetheless, ultrastructural studies have made major contributions to our understanding of parasite biology. Examples of such research follow. Chromosomes of *M. hirudinaceus* were described by Jones and Ward (1950) and Robinson (1964), and those of *M. moniliformis* by Robinson (1965). West (1964) demonstrated the presence of acanthor membranes in two species of Acanthocephala. Wright and Lumsden (1968, 1969) described the ultrastructural and histochemical properties of the acanthocephalan epicuticle, and of the pore canal system of *M. moniliformis*. Robinson (1973) described the growth and differentiation of giant nuclei in *M. Monilifomis*. Bone (1974) studied the chromosomes of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) Van Cleave, 1919, and *L. thecatus*.

Dunagan and Miller (1976, 1978) described the cerebral ganglion and the genital ganglion of *M. moniliformis*, respectively. Schmidt (1977) described the praesomal musculature of acanthocephalan genus *Mediorhynchus* (Van Cleave, 1916). Miller and Dunagan (1978) and Dunagan and Miller (1981) described the lacunar system and the cerebral ganglion of *Oligacanthorhynchus tortuosa* (Leidy, 1850, Schmidt, 1972, respectively). Hutton and Oettinger (1980) demonstrated the morphogenesis of proboscis hooks of *M. moniliformis* Miller and Dunagan (1983) and Dunagan and Miller (1983) described a support cell to the sensory organs and the apical sense organs of *M. hirudinaceus*, respectively. Marchand (1984) studied the ultrastructure of acanthor shells of 13 species of acanthocephalans. Budziakowski and Mettrick (1985) described the neuropile of the cerebral ganglion of *M. moniliformis*.

Dunagan and Miller (1985) also described the reproductive apparatus of *N. cylindratus* and the protonephridia in male *M. hirudinaceaus*. These same authors (Dunagan and Miller, 1986, 1987) described the sense organs of *M. moniliformis* using SEM, reviewed the protonephridia in Acanthocephala, and provided a model of the cerebral ganglion in *M. hirudinaceus*. Krapf and Dunagan (1987) and Dunagan and Bozzola (1989, 1992) described the structure of the protonephridia of female *M. hirudinaceaus* and the apical sense organ of the same species. Dunagan and Rasheed (1988) described the urogenital system of *Oligacanthorhynchus atratus* (Meyer, 1931) Schmidt 1972 (= *Echinopardalis atrata* Meyer, 1931). Holloway and Gee (1990) described a process in the proboscis of *Corynosoma hamanni* (von Linstow, 1892) Railliet et Henry, 1907, extending from the inner wall of the receptacle, which has been described by Amin in a number of other acanthocephalan species. Marchand and Grita-Timoulaliz

(1992) compared the ultrastructure of larval and adult cuticle of *Centrorhynchus milvus* Ward, 1956. Oettinger and Buckner (1993) described the genital vestibule of *Neoechinorhynchus carinatus* Buckner and Buckner, 1993. Foata et al. (2005) described the ultrastructure of spermiogenesis of *M. hirudinaceus*.

## **Lessons learned from the study of acanthocephalan anatomy**

Ultrastructural studies are another way of looking at anatomical structures at a different level of perception. Ultrastructural observations often provide answers to questions of function that gross anatomical questions cannot. For instance, to know the frog by observing its external anatomy is a far cry from knowing the ultrastructure of its muscle cells and nerve insertions that tell us more about how the frog leaps. Similarly, the ultrastructure and the anatomy of nerve cells of the cephalic ganglion or apical organ of acanthocephalans provide a better understanding of the function of connected organs as well as the evolutionary history of higher taxa to which studied specimens are assigned.

## **Experimental studies**

The sub-specialty of experimental parasitology appears to have had reasonable exposure in *JP* despite the presence of other specialty journals that address this need. Van Cleave and Ross (1944) examined the physiological responses of *N. emydis* to various solutions, and Ward (1951) studied the use of antibiotics in artificial media for *in vitro* experiments with Acanthocephala. Kilejan (1963) and Horvath (1971) and Horvath and Fisher (1971) experimented on glycogenesis in *M. moniliformis*. Graff (1964) described the metabolism of  $^{14}\text{C}$ -glucose by *M. moniliformis*. Fisher (1964) and McAlister and Fisher (1972) demonstrated the synthesis of trehalose in *M. moniliformis*. Graff (1965) showed the utilization of  $^{14}\text{CO}_2$  in the production of acid metabolites by *M. moniliformis*. Hibbard and Cable (1968) demonstrated the uptake and metabolism of various chemicals by adult *Paulisentis fractus* Van Cleave and Bangham, 1949. Horvath and Fisher (1971) and Körting and Fairbairn (1972) reported on the enzymes of  $\text{CO}_2$  fixation and on anaerobic energy metabolism in *M. moniliformis*, respectively. Uglem and Beck (1972) showed that habitat specificity was correlated with aminopeptidase activity in *Neoechinorhynchus cristatus* Lynch, 1936 (*nec cristatum*) and *N. crassus* Van Cleave, 1919 (*nec crassum*). Starling and Fisher (1975, 1979) described the kinetics and specificity of hexose absorption and carbohydrate transport in *M. moniliformis*. Farland and MacInnis (1978) demonstrated thymidine kinase activity in *M. moniliformis*. Donahue et al. (1981) reported on the carbohydrate regulatory enzymes of *M. hirudinaceus*.

Tokeson and Holmes (1982) studied the effect of temperature and oxygen on the development of *Polymorphus marilis* Van Cleave, 1939, in its amphipod host. Wilkes et al. (1982) studied fumarase activity in *M. moniliformis*. Sangster and Mettrick (1987) showed the effect of cholinergic drugs on muscle contraction in *M. moniliformis*. Richardson and Nickol (2000) experimented with the physiological factors influencing site selection of *L. thecatus* in green sunfish. Reyda and Nickol (2001) compared the biological performances of laboratory raised and wild populations of *M. moniliformis*. Alibert et al. (2002) examined developmental stability in *Gammarus pulex* Linnaeus, 1758, infected with two species of acanthocephalans. Guinnee and Moore (2004) studied temperature related cockroach fecundity affected by acanthocephalan infections.

## **Lessons learned from experimental studies on acanthocephalans**

Efforts to link physiological and biochemical properties of acanthocephalans with other aspects of host-parasite relationships have been only partially successful. The studies are not always easy to perform, and as is the case with other parasite groups, the focus has been on those acanthocephalan species amenable to laboratory maintenance with *M. moniliformis* being a favorite, likely because its life cycle can be maintained in cockroaches and rats. Species using aquatic hosts such as small crustaceans and fish are sometimes difficult to deal with in the lab,

primarily because the intermediate hosts are not always very cooperative in culture. Nevertheless, a rich realm of inquiry into host–parasite relationships awaits a young scientist with the patience and dedication to work with acanthocephalans in the lab.

## **Surveys, other endeavors, and conclusions**

Surveys of parasitic groups, including acanthocephalans, did not make the headlines on the pages of *JP* until 1992 (one survey). There were a few others in 1996 (one), 1997 (five), 1998 (two), 2000 (one), 2001 (one), and 2011 (one). Clearly, surveys were not a favored topic to submit, or accept, in *JP*. Several other sub-specialties in acanthocephalan research in *JP* were even less favored over the years, for example, areas such as toxicity, chemistry, development, metabolism, histology, and pathology. Notes on genetic research are selectively incorporated within the molecular taxonomy section, previously. It is clear that acanthocephalan research and dissemination of knowledge could never be the same without the contributions of authors who have published in *The Journal of Parasitology*.

## Literature cited

- Alibert, P., L. Bollache, D. Corberant, V. Guesdon, and F. Cézilly. 2002. Parasitic infection and developmental stability: fluctuating asymmetry in *Gammarus pulex* infected with two acanthocephalan species. *Journal of Parasitology* **88**: 47–54.
- Allely, Z., J. Moore, and N. J. Gotelli. 1992. *Moniliformis moniliformis* infection has no effect on some behaviors of the cockroach *Diploptera punctata*. *Journal of Parasitology* **78**: 524–526.
- Amin, O. M. 1975. *Acanthocephalus parksidei* sp. n. (Acanthocephala: Echinorhynchidae) from Wisconsin Fishes. *Journal of Parasitology* **61**: 301–306.
- \_\_\_\_\_. 1982. Acanthocephala. In *Synopsis and classification of living organisms*, S. P. Parker (ed.). McGraw-Hill Book Co., New York, NY, p. 933–941.
- \_\_\_\_\_. 1985. Classification. In *Biology of the Acanthocephala*, D. W. T. Crompton and B. B. Nickol (eds.). Cambridge University Press, London and New York, p. 27–72.
- \_\_\_\_\_. 1986. Acanthocephala from lake fishes in Wisconsin: Host and seasonal distribution of species of the genus *Neoechinorhynchus* Hamann, 1892. *Journal of Parasitology* **72**: 111–118.
- \_\_\_\_\_. 1987. Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). *Journal of Parasitology* **73**: 1216–1219.
- \_\_\_\_\_. 1987a. Acanthocephala from lake fishes in Wisconsin: Ecology and host relationships of *Pomphorhynchus bulbocolli* (Pomphorhynchidae). *Journal of Parasitology* **73**: 278–289.
- \_\_\_\_\_. 1987b. Acanthocephala from lake fishes in Wisconsin: Morphometric growth of *Pomphorhynchus bulbocolli* (Pomphorhynchidae). *Journal of Parasitology* **73**: 806–810.
- \_\_\_\_\_. 1992. Review of the genus *Polymorphus* Lühe, 1911 (Acanthocephala: Polymorphidae) with the synonymization of *Hexaglandula* Petrochenko, 1950, and *Subcorynosoma* Khokhlova, 1967, and a key to the species. *Qatar University Science Journal* **12**: 115–123.
- \_\_\_\_\_. 2000. Acanthocephala in the Neotropical region. In *Metazoan parasites in the Neotropics: A systematic and ecological approach*, G. Salgado-Maldonado, A. N. García Aldrete and V. M. Vidal-Martínez (eds.). Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico, p. 167–174.
- \_\_\_\_\_. 2013. Classification of the Acanthocephala. *Folia Parasitologica*

\_\_\_\_\_, and Amin, O. M. 2002. Revision of *Neoechinorhynchus* Stiles & Hassall, 1905 (Acanthocephala: Neoechinorhynchidae) with keys to 88 species in two subgenera. *Systematic Parasitology* **53**: 1–18.

\_\_\_\_\_, and N. V. Ha. 2008. On a new acanthocephalan family and a new order, from birds in Vietnam. *Journal of Parasitology* **94**: 1305–1310.

\_\_\_\_\_, R. A. Heckmann, N. A. E. Radwan, J. S. Mantuano Anchundia, and M. A. Zambrano Alcivar. 2009. Redescription of *Rhadinorhynchus ornatus* (Acanthocephala: Rhadinorhynchidae) from skipjack tuna, *Katsuwonus pelamis*, collected in the Pacific Ocean off South America, with special reference to new morphological features. *Journal of Parasitology* **95**: 656–664.

Ashley, D. C., and B. B. Nickol. 1989. Dynamics of the *Leptorhynchoides thecatus* (Acanthocephala) suprapopulation in a Great Plains reservoir. *Journal of Parasitology* **75**: 46–54.

Aznar, F. J., G. Pérez-Ponce de Léon, and J. A. Raga. 2006. Status of *Corynosoma* (Acanthocephala: Polymorphidae) based on anatomical, ecological, and phylogenetic evidence, with the erection of *Pseudocorynosoma*, n. gen. *Journal of Parasitology* **92**: 548–564.

Balboa, L., A. Hinojosa, C. Riquelme, S. Rodriguez, J. Bustos, and M. George-Nascimento. 2009. Allo xenic distribution of cystacanths of two *Profilicollis* species in sympatric crustacean hosts in Chile. *Journal of Parasitology* **95**: 1205–1208.

Benesh, D. P., L. M. Duclos, and B. B. Nickol. 2005. The behavioral response of amphipods harboring *Corynosoma constrictum* (Acanthocephala) to various components of light. *Journal of Parasitology* **91**: 731–736.

\_\_\_\_\_, and E. T. Valtonen. 2007. Effects of *Acanthocephalus lucii* (Acanthocephala) on intermediate host survival and growth: Implications for exploitation strategies. *Journal of Parasitology* **93**: 735–741.

\_\_\_\_\_, J. Kitchen, K. Pulkkinen, I. Hakala, and E. T. Valtonen. 2008. The effect of *Echinorhynchus borealis* (Acanthocephala) infection on the anti-predator behavior of a benthic amphipod. *Journal of Parasitology* **94**: 542–545.

Bethel, W. M., and J. C. Holmes. 1973. Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. *Journal of Parasitology* **59**: 945–956.

\_\_\_\_\_, and \_\_\_\_\_. 1974. Correlation of development of altered evasive behavior in *Gammarus lacustris* (Amphipoda) harboring cystacanths of *Polymorphus paradoxus* (Acanthocephala) with the infectivity to the definitive

- host. *Journal of Parasitology* **60**: 272–274.
- Bhattacharya, S. B. 2007. *Handbook on Indian Acanthocephala*. Zoological Survey, Kolkata, India, 255 p.
- Bone, L. W. 1974. The chromosomes of *Neoechinorhynchus cylindratus* (Acanthocephala). *Journal of Parasitology* **60**: 731–732.
- Bowen, R. C. 1967. Defense reactions of certain spirobolid millipedes to larval *Macracanthorhynchus ingens*. *Journal of Parasitology* **53**: 1092–1095.
- Brattey, J. 1988. Life history and population biology of adult *Acanthocephalus lucii* (Acanthocephala: Echinorhynchidae). *Journal of Parasitology* **74**: 72–80.
- Buckner, R. L., and B. B. Nickol. 1979. Geographic and host-related variation among species of *Fessisentis* (Acanthocephala) and confirmation of the *Fessisentis fessus* life cycle. *Journal of Parasitology* **65**: 161–166.
- Budziakowski, M. E., and D. F. Mettrick. 1985. Ultrastructural morphology of the neuropile of the cerebral ganglion of *Moniliformis moniliformis* (Acanthocephala). *Journal of Parasitology* **71**: 75–85.
- Cable, R. M., and W. T. Dill. 1967. The morphology and life history of *Paulisentis fractus* Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **53**: 810–817.
- Camp, J. W., and H. W. Huizinga. 1979. Altered color, behavior and predation susceptibility of the isopod *Asellus intermedius* infected with *Acanthocephalus dirus*. *Journal of Parasitology* **65**: 667–669.
- \_\_\_\_\_, and \_\_\_\_\_. 1980. Seasonal population interactions of *Acanthocephalus dirus* (Van Cleave 1931) in the creek chub, *Semotilus atromaculatus*, and isopod, *Asellus intermedius*. *Journal of Parasitology* **66**: 299–304.
- Carmichael, L. M., and J. Moore 1991. A comparison of behavioral alterations in the brown cockroach *Periplaneta brunnea* and the American cockroach *Periplaneta americana* infected with the acanthocephalan *Moniliformis moniliformis*. *Journal of Parasitology* **77**: 931–936.
- Chervy, L. 2009. Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002–2008. *Folia Parasitologica* **56**: 199–230.
- Cholodkovsky, N. A. 1897. Classification of the Acanthocephala. Trudy San Petersburg. *Obshchestva Estestvoispytatelei* **28**:14–20.
- De Giusti, D. L. 1939. Preliminary note on the life cycle of *Leptorhynchoides thecatus*, an acanthocephalan parasite of fish. *Journal of Parasitology* **25**: 180.

- \_\_\_\_\_. 1949. The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. *Journal of Parasitology* **35**: 437–460.
- De Mont, D. J., and K. C. Corkum. 1982. The life cycle of *Octospiniferooides chandleri* Bullock, 1957 (Acanthocephala: Neoechinorhynchidae) with some observations on parasite-induced, photophilic behavior in ostracods. *Journal of Parasitology* **68**: 125–130.
- Donahue, M. J., N. J. Yacoub, M. R. Kaeini, S. Tu, R. A. Hodzi, and B. G. Harris. 1981. Studies on potential carbohydrate regulatory enzymes and metabolite levels in *Macracanthorhynchus hirudinaceus*. *Journal of Parasitology* **67**: 756–758.
- Dunagan, T. T., and J. J. Bozzola. 1989. Fine structure of anterior terminus of apical sense organ in *Macracanthorhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **75**: 297–302.
- \_\_\_\_\_, and \_\_\_\_\_. 1992. Morphology of the apical organ in *Macracanthorhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **78**: 899–903.
- \_\_\_\_\_, and D. M. Miller. 1976. Nerves originating from the cerebral ganglion of *Moniliformis moniliformis* (Acanthocephala). *Journal of Parasitology* **62**: 442–450.
- \_\_\_\_\_, and \_\_\_\_\_. 1978. Anatomy of the genital ganglion of the male acanthocephalan, *Moniliformis moniliformis*. *Journal of Parasitology* **64**: 431–435.
- \_\_\_\_\_, and \_\_\_\_\_. 1981. Anatomy of the cerebral ganglion in *Oligacanthorhynchus tortuosa* (Acanthocephala) from the opossum (*Didelphis virginiana*). *Journal of Parasitology* **67**: 881–885.
- \_\_\_\_\_, and \_\_\_\_\_. 1983. Apical sense organ of *Macracanthorhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **69**: 897–902.
- \_\_\_\_\_, and \_\_\_\_\_. 1985. Microscopic anatomy of the reproductive apparatus of male *Neoechinorhynchus cylindratus* (Acanthocephala). *Journal of Parasitology* **71**: 329–354.
- \_\_\_\_\_, and \_\_\_\_\_. 1986. Apical and lateral sense organs in *Moniliformis moniliformis* (Acanthocephala): An SEM view. *Journal of Parasitology* **72**: 176–178.
- \_\_\_\_\_, and \_\_\_\_\_. 1987. A model of the cerebral ganglion in *Macracanthorhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **73**: 853–855.
- \_\_\_\_\_, and R. M. A. Rashed. 1988. Capsular protonephridia in male

*Oligacanthorhynchus atrata* (Acanthocephala). *Journal of Parasitology* **74**: 180–185.

Dudiňák, V., and V. Šnábel. 2001. Comparative analysis of Slovak and Czech populations of *Pomphorhynchus laevis* (Acanthocephala) using morphological and isoenzyme analysis. *Acta Zoologica. Universitatis Comenianae* **44**: 41–50.

Elkins, C. A., and B. B. Nickol. 1983. The Epizootiology of *Macracanthorhynchus ingens* in Louisiana. *Journal of Parasitology* **69**: 951–956.

Farland, W. H., and A. J. MacInnis. 1978. In vitro thymidine kinase activity: Present in *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala), but apparently lacking in *Ascaris lumbricoides* (Nematoda). *Journal of Parasitology* **64**: 564–565.

Foata, J., J.-L. Culioni, and B. Marchand. 2005. Ultrastructure of spermiogenesis and the spermatozoon of *Macracanthorhynchus hirudinaceus* (Pallas, 1781) (Acanthocephala: Archiacanthocephala), a parasite of the wild boar *Sus scrofa*. *Journal of Parasitology* **91**: 499–506.

Fontaneto, D., and U. Jondelius. 2011. Broad taxonomic sampling of mitochondrial cytochrome c oxidase subunit 1 does not solve the relationship between Rotifera and Acanthocephala. *Zoologischer Anzeiger* **250**: 80–85.

Fischthal, J. H. 1950. Additional hosts and geographical distribution records for the common fish acanthocephalan, *Leptorhynchoides thecatus*. *Journal of Parasitology* **36**: 88.

Fisher, Jr., F. M. 1964. Synthesis of trehalose in Acanthocephala. *Journal of Parasitology* **50**: 803–804.

Freehling, M., and J. Moore. 1993. Susceptibility of 13 cockroach species to *Moniliformis moniliformis*. *Journal of Parasitology* **79**: 442–444.

García-Varela, M., and S. A. Nadler. 2005. Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rRNA gene sequences. *Journal of Parasitology* **91**: 1401–1409.

, and . 2006. Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogeny and Evolution* **40**: 61–72.

, and G. Pérez-Ponce de Léon. 2008. Validating the systematic position of *Profilicollis* Meyer, 1931 and *Hexaglandula* Petrochenko, 1950 (Acanthocephala: Polymorphidae) using cytochrome C oxidase (COX 1). *Journal of Parasitology* **94**: 212–217.

, M. P. Cummings, G. Pérez-Ponce de Léon, S. L. Gardner, and J. P. Laclette. 2002. Phylogenetic analysis based on 18S ribosomal RNA gene

sequences supports the existence of class Polyacanthocephala (Acanthocephala). *Molecular Phylogenetics and Evolution* **23**: 288–292.

\_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, P. de la Torre, M. P. Cummings, S. S. S. Sarma, and J. P. Laclette. 2000. Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution* **50**: 532–540.

\_\_\_\_\_, G. Perez-Ponce de Leon, G. F. J. Aznar, and S. A. Nadler. 2013. Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution* **68**: 176–184.

Garey, J. R., T. J. Near, M. R. Nonnemacher, and S. A. Nadler. 1996. Molecular evidence for Acanthocephala as a subtaxon of Rotifera. *Journal of Molecular Evolution* **43**: 287–292.

\_\_\_\_\_, A. Schmidt-Rhaesa, T. J. Near, and S. A. Nadler. 1998. The evolutionary relationships of rotifers and acanthocephalans. *Hydrobiologia* **387**: 83–89.

Gleason, L. N. 1987. Population dynamics of *Pomphorhynchus bulbocolli* in *Gammarus pseudolimnaeus*. *Journal of Parasitology* **73**: 1099–1101.

Golvan, Y. J. 1959. Le phylum des Acanthocephala. Deuxième note. La classe de Eoacanthocephala (Van Cleave, 1936). *Annales de Parasitologie Humaine et Comparée* **34**: 5–52.

\_\_\_\_\_. 1960. Le phylum des Acanthocephala. Troisième note. La classe des Palaeacanthocephala (Meyer, 1931). *Annales de Parasitologie Humaine et Comparée* **35**: 76–91, 138–165, 350–386, 573–593, 713–723.

\_\_\_\_\_. 1961. Le phylum des Acanthocephala. Troisième note. La classe des Palaeacanthocephala (Meyer, 1931). *Annales de Parasitologie Humaine et Comparée* **36**: 76–91, 612–647, 717–738.

\_\_\_\_\_. 1965. Acanthocéphales de Madagascar récoltés par E. R. Brygoo. *Annales de Parasitologie Humaine et Comparée* **40**: 303–316.

\_\_\_\_\_. 1969. Systematique des Acanthocephales (Acanthocephala Rudolphi, 1801), l'ordre des Palaeacanthocephala Meyer, 1931. La superfamille des Echinorhynchidea (Cobbold, 1876) Golvan et Houin 1963. Mémoires du Muséum National d' Histoire Naturelle, nouvelle ser. A. *Zoologie*, **57**: 1–373.

\_\_\_\_\_. 1994. Nomenclature of the Acanthocephala. *Research and Reviews in Parasitology* **54**: 135–205.

Graff, D. J. 1964. Metabolism of C<sup>14</sup>-Glucose by *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **50**: 230–234.

- \_\_\_\_\_. 1965. The utilization of C<sup>14</sup> O<sub>2</sub> in the production of acid metabolites by *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **51**: 72–75.
- Guinnee, M. A., and J. Moore. 2004. The effect of parasitism on host fecundity is dependent on temperature in a cockroach-acanthocephalan system. *Journal of Parasitology* **90**: 673–377.
- Hamann, O. 1892. Das system der Acanthocephalen. *Zoologischer Anzieger* **15**: 195–197.
- Harms, C. E. 1965. The life cycle and larval development of *Octospinifer macilentis* (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **51**: 286–293.
- Hibbard, K. M., and R. M. Cable. 1968. The uptake and metabolism of tritiated glucose, tyrosine, and thymidine by adult *Paulisentis fractus* Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **54**: 517–523.
- Holloway, H. L., and R. J. Gee. 1990. Vesicles in the proboscis of Acanthocephala. *Journal of Parasitology* **76**: 585–587.
- Hopp, W. B. 1954. Studies on the morphology and life cycle of *Neoechinorhynchus emydis* (Leidy), an acanthocephalan parasite of the map turtle, *Graptemys geographica* (Le Sueur). *Journal of Parasitology* **40**: 284–299.
- Horvath, K. 1971. Glycogen metabolism in larval *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **57**: 132–136.
- \_\_\_\_\_, and F. M. Fisher, Jr. 1971. Enzymes of CO<sub>2</sub> fixation in larval and adult *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **57**: 440–442.
- Hutton, T. L., and D. F. Oettinger 1980. Morphogenesis of the proboscis hooks of an archiacanthocephalan, *Moniliformis moniliformis* (Bremser 1811) Travassos 1915. *Journal of Parasitology* **66**: 965–972.
- Jilek, R. 1978. Seasonal occurrence and host specificity of *Gracilisentis gracilisentis* and *Tanaorhamphus longirostris* (Acanthocephala: Neoechinorhynchidae) in Crab Orchard Lake, Illinois. *Journal of Parasitology* **64**: 951–952.
- Jones, A. W., and H. L. Ward 1950. The chromosomes of *Macracanthorhynchus hirudinaceus* (Pallas). *Journal of Parasitology* **36**: 86.
- Khatoon, N., and F. M. Bilqees. 2011. *Acanthocephala of vertebrates*. A world record. Dr. Müller GmbH and Co., VDM Verlag, Saarbrücken, Germany, 566 p.

- Kilejan, A. 1963. The effect of carbon dioxide on glycogenesis in *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **49**: 862–863.
- Kopp, D. A., D. A. Elke, S. C. Caddigan, A. Raj, L. Rodriguez, M. K. Young, and T. C. Sparkes. 2011. Dispersal in the acanthocephalan *Acanthocephalus dirus*. *Journal of Parasitology* **97**: 1101–1105.
- Körting, W., and D. Fairbairn. 1972. Anaerobic energy metabolism in *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **58**: 45–50.
- Krapf, K., and T. T. Dunagan 1987. Structural features of the protonephridia in female *Macracanthorhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **73**: 1176–1181.
- Kristensen, R. M. 2002. An introduction to Loricifera, Cycliophora, and Micrognathozoa. *Integrative and Comparative Biology* **42**: 641–652
- Leuckart, R. 1848. *Über die Morphologie und Verwandtschaftsverhältnisse der wirbellosen Thiere. Ein Beitrag zur Charakteristik und Classification der Thierischen Formen*. F. Vieweg und Sohn, Braunschweig, Germany, 180 p.
- Lühe, M. 1904–1905. Geschichte und Ergebnisse der Echinorhynchen-Forschung bis auf Westrumb (1821) (Mit Bemerkungen über alte und neue Gattungen der Acanthocephalen). *Zoologische Annalen, Zeitschrift Geschichte Zoologie Herausgegeben von B. Braun. Würzburg, Germany*, **1**: 139–353.
- Marchand, B. 1984. A comparative ultrastructural study of the shell surrounding the mature acanthor larvae of 13 acanthocephalan species. *Journal of Parasitology* **70**: 886–901.
- \_\_\_\_\_, and Grita-Timoulaliz. 1992. Comparative ultrastructural study of the cuticle of larvae and adults of *Centrorhynchus milvus* Ward 1956, (Acanthocephala, Centrorhynchidae). *Journal of Parasitology* **78**: 355–359.
- Martinez-Aquino, A., M. E. Reyna-Fabián, Z. R. Rosas-Valde, U. Razo-Mendivil, G. Pérez-Ponce de León, and M. García-Valera. 2009. Detecting a complex of cryptic species within *Neoechinorhynchus golvani* (Acanthocephala: Neoechinorhynchidae) inferred from ITSs and LSU rDNA gene sequences. *Journal of Parasitology* **95**: 1040–1047.
- Maynard, B. J., T. A. Wellnitz, N. Zanini, W. G. Wright, and B. S. Dezfull. 1998. Parasite-altered behavior in a crustacean intermediate host: Field and laboratory studies. *Journal of Parasitology* **84**: 1102–1106.
- McAlister, R. O., and F. M. Fisher, Jr. 1972. The biosynthesis of trehalose in *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **58**: 51–62.
- McLennan, D. A., and V. L. Shires. 1995. Correlation between the level of infection with *Bunodera inconstans* and *Neoechinorhynchus rutili* and behavioral intensity

in female brook sticklebacks. *Journal of Parasitology* **81**: 675–682.

Merriott, S. V., and I. Pratt. 1964. The life history of *Neoechinorhynchus rutili* and its development in the intermediate host (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **50**: 394–400.

Meyer, A. 1931. Neue Acanthocephalen aus dem Berliner Museum. Burgründung eines neuen Acanthocephalen Systems auf Grund einer Untersuchung der Berliner Sammlung. Zoologische Jahrbücher, Abteilung für Systematik, Ökologie Geographie Tiere **62**: 53–108.

\_\_\_\_\_. 1932. Acanthocephala. In Dr. H.G. Bronn's *Klassen und Ordnungen des Tier-Reichs*. Akademische Verlagsgesellschaft MBH, Leipzig, Germany **4**: 1–332.

\_\_\_\_\_. 1933. Acanthocephala. In Dr. H.G. Bronn's *Klassen und Ordnungen des Tier-Reichs*. Akademische Verlagsgesellschaft MBH, Leipzig, Germany **4**: 333–582.

Miller, D. M., and T. T. Dunagan. 1978. Organization of the lacunar system in the acanthocephalan, *Oligacanthonrhynchus tortuosa*. *Journal of Parasitology* **64**: 436–439.

\_\_\_\_\_, and \_\_\_\_\_. 1983. A support cell to the apical and lateral sensory organs in *Macracanthonrhynchus hirudinaceus* (Acanthocephala). *Journal of Parasitology* **69**: 534–538.

Min, G. S., and J. K. Park. 2009. Eurotatorian paraphyly: Revisiting phylogenetic relationships based on the complete mitochondrial genome sequence of *Rotaria rotatoria* (Bdelloidea: Rotifera: Syndermata). *BMC Genomics* **10**: 533.

Monks, S. 2001. Phylogeny of the Acanthocephala based on morphological characters. *Systematic Parasitology* **48**: 81–116.

\_\_\_\_\_, G. Pulido-Flores, and J. Violante-González. 2011. A new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) in *Dormitator latifrons* (Perciformes: Eleotridae) from the Pacific coast of Mexico. *Comparative Parasitology* **78**: 21–28.

Moore, D. V. 1946. Studies on the life history and development of *Macracanthonrhynchus ingens* Meyer, 1933, with a redescription of the adult worm. *Journal of Parasitology* **32**: 387–399.

\_\_\_\_\_. 1962. Morphology, life history, and development of the acanthocephalan *Mediorhynchus grandis* Van Cleave, 1916. *Journal of Parasitology* **48**: 76–86.

Moore, J. 1983. Altered behavior in cockroaches (*Periplaneta americana*) infected with an archiacanthocephalan, *Moniliformis moniliformis*. *Journal of*

*Parasitology* **69**: 1174–1176.

\_\_\_\_\_, and N. J. Gottelli. 1992. *Moniliformis moniliformis* increases cryptic behaviors in the cockroach *Supella longipalpa*. *Journal of Parasitology* **78**: 49–53.

\_\_\_\_\_, and \_\_\_\_\_. 1996. Evolutionary patterns of altered behavior and susceptibility in parasitized hosts. *Evolution* **50**: 807–819.

\_\_\_\_\_, M. Freehling, and N. J. Gottelli. 1994. Altered behavior in two species of blattid cockroaches infected with *Moniliformis moniliformis* (Acanthocephala). *Journal of Parasitology* **80**: 220–223.

Moser, M., and J. Hsieh. 1992. Biological tags for stock separation in Pacific herring *Clupea harengus pallasi* in California. *Journal of Parasitology* **78**: 54–60.

Muzzall, P. M. 1980. Ecology and seasonal abundance of three acanthocephalan species infecting white suckers in SE New Hampshire. *Journal of Parasitology* **66**: 127–133.

\_\_\_\_\_, and W. L. Bullock. 1978. Seasonal occurrence and host-parasite relationships of *Neoechinorhynchus saginatus* Van Cleave and Bangham 1949 in the fallfish, *Semotilus corporalis* (Mitchill). *Journal of Parasitology* **64**: 860–865.

Near, T. J. 2002. Acanthocephalan phylogeny and the evolution of parasitism. *Integrative and Comparative Biology* **42**: 668–677.

\_\_\_\_\_, J. R. Garey, and S. A. Nadler. 1998. Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* **10**: 287–298.

Nickol, B. B. 1977. Life history and host specificity of *Mediorhynchus centurorum* Nickol 1969 (Acanthocephala: Gigantorhynchidae). *Journal of Parasitology* **63**: 104–111.

\_\_\_\_\_, D. W. T. Crompton, and D. W. Searle. 1999. Reintroduction of *Profilicollis* Meyer, 1931, as a genus in Acanthocephala: Significance of the intermediate host. *Journal of Parasitology* **85**: 716–718.

Oettinger, D. F., and R. L. Buckner. 1993. Morphology of the genital vestibule of *Neoechinorhynchus carinatus* (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **79**: 930–934.

\_\_\_\_\_, and B. B. Nickol. 1982. Spectrophotometric characterization of integumental pigments from uninfected and *Acanthocephalus dirus*-infected *Asellus intermedius*. *Journal of Parasitology* **68**: 270–275.

- \_\_\_\_\_, and \_\_\_\_\_. 1982. Developmental relationships between acanthocephalans and altered pigmentation in freshwater isopods. *Journal of Parasitology* **68**: 463–469.
- Olson, P. D., and B. B. Nickol. 1996. Comparison of *Leptorhynchoides thecatus* (Acanthocephala) recruitment into green sunfish and largemouth bass populations. *Journal of Parasitology* **82**: 702–706.
- Olson, R. E., and I. Pratt 1971. The life cycle and larval development of *Echinorhynchus lageniformis* Ekbaum, 1938 (Acanthocephala: Echinorhynchidae). *Journal of Parasitology* **57**: 143–149.
- Perrot-Minnot, M.-J. 2004. Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of *Pomphorhynchus laevis* (Acanthocephala). *International Journal for Parasitology* **34**: 45–54.
- Petrochenko, V. I. 1952. On the position of the Acanthocephala in the zoological system. (Phylogenetic connections of the Acanthocephala with other groups of invertebrates). *Zoologicheskii Zhurnal* **31**: 288–327.
- \_\_\_\_\_. 1956. Acanthocephala of domestic and wild Animals. Vol. **1**. *Izdatel'stvo Akademii Nauk SSSR*. English translation by Israel Program for Scientific Translations, Ltd., Jerusalem, Israel (1971), 465 p.
- Podesta, R. B., and J. C. Holmes. 1970. The life cycles of three polymorphids (Acanthocephala) occurring as juveniles in *Hyalella azteca* (Amphipoda) at Cooking Lake, Alberta. *Journal of Parasitology* **56**: 1118–1123.
- Rauque, C. A., L. G. Semenas, and G. P. Viozzi. 2006. Seasonality of recruitment and reproduction of *Acanthocephalus tumescens* (Acanthocephala) in fishes from Lake Moreno (Patagonia, Argentina). *Journal of Parasitology* **92**: 1265–1269.
- \_\_\_\_\_, and L. Semenas. 2011. Parasite volume as an indicator of competition: The case of *Acanthocephalus tumescens* and *Pseudocorynosoma* sp. (Acanthocephala) in their intermediate host. *Journal of Parasitology* **97**: 999–1002.
- Reish, D. J. 1950. Preliminary note on the life cycle of the acanthocephalan, *Polymorphus kenti* Van Cleave, 1947. *Journal of Parasitology* **36**: 496.
- Reyda, F. B., and B. B. Nickol. 2001. A comparison of biological performances among a laboratory-isolated population and two wild populations of *Moniliformis moniliformis*. *Journal of Parasitology* **87**: 330–338.
- Richardson, D. J., and B. B. Nickol. 1995. The genus *Centrorhynchus* (Acanthocephala) in North America with description of *Centrorhynchus robustus* n. sp., redescription of *Centrorhynchus conspectus*, and a key to species. *Journal of Parasitology* **81**: 767–772.

- \_\_\_\_\_, and \_\_\_\_\_. 2000. Experimental investigation of physiological factors that may influence microhabitat specificity exhibited by *Leptorhynchoides thecatus* (Acanthocephala) in green sunfish (*Lepomis cyanellus*). *Journal of Parasitology* **86**: 685–690.
- Rieger, R. M., and S. Tyler. 1995. Sister-group relationship of Gnathostomulida and Rotifera – Acanthocephala. *Invertebrate Biology* **114**: 186–188.
- Robinson, E. S. 1964. Chromosome morphology and behavior in *Macracanthorhynchus hirudinaceus*. *Journal of Parasitology* **50**: 694–697.
- \_\_\_\_\_. 1965. The chromosomes of *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **51**: 430–432.
- \_\_\_\_\_. 1973. Growth and differentiation of giant nuclei in *Moniliformis* (Acanthocephala). *Journal of Parasitology* **59**: 678–684.
- Rosas-Valdez, R., J. J. Morrone, and M. García-Varela. 2012. Molecular phylogenetics of *Floridosentis* Ward, 1953 (Acanthocephala: Neoechinorhynchidae) parasites of mullets (Osteichthyes) from Mexico, using 28S rDNA sequences. *Journal of Parasitology* **98**: 855–862.
- Rudolphi, C. A. 1802. Fortsetzung der Beobachtungen über die Eingeweidewurmer. *Wiedmann's Archiv Zoologie Braunschweig* **2**: 1–67.
- Salgado-Maldonado, G. 2006. Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa* **1324**: 1–357.
- \_\_\_\_\_, and O. M. Amin. 2009. Acanthocephala of the Gulf of Mexico. In *Origin, waters, and biota*. Vol. **1**, Biodiversity, D. L. Felder and D.K. Camp (eds.). Texas University Press, Corpus Christi, Texas, p. 539–552.
- Samuel, G., and W. L. Bullock. 1981. Life cycle of *Paratenuisentis ambiguus* (Van Cleave, 1921) Bullock and Samuel, 1975 (Acanthocephala: Tenuisentidae). *Journal of Parasitology* **67**: 214–217.
- Sangster, N. C., and D. F. Mettrick. 1987. Effects of cholinergic drugs on muscle contraction in *Moniliformis moniliformis* (Acanthocephala). *Journal of Parasitology* **73**: 998–1004.
- Schmidt, G. D. 1972. Revision of class Archiacanthocephala Meyer, 1931 (Phylum Acanthocephala), with emphasis on Oligacanthorhynchidae Southwell and MacFie, 1925. *Journal of Parasitology* **58**: 290–297.
- \_\_\_\_\_. 1977. Praesomal musculature of the acanthocephalan genus *Mediorhynchus*. *Journal of Parasitology* **63**: 112–116.
- \_\_\_\_\_, and E. J. Huggins. 1973a. Acanthocephala of South American fishes. Part 1. Eoacanthocephala. *Journal of Parasitology* **59**: 829–835.

- \_\_\_\_\_, and \_\_\_\_\_. 1973b. Acanthocephala of South American fishes. Part 2. Palaeacanthocephala. *Journal of Parasitology* **59**: 836–838.
- \_\_\_\_\_, and K. A. Neiland. 1966. Helminth fauna of Nicaragua. III. Some Acanthocephala of birds, including three new species of *Centrorhynchus*. *Journal of Parasitology* **52**: 739–745.
- \_\_\_\_\_, and R. E. Kuntz. 1967a. Notes on the life cycle of *Polymorphus (Profilicollis) formosus* sp. n., and records of *Arhythmorhynchus hispidus* Van Cleave, 1925 (Acanthocephala) from Taiwan. *Journal of Parasitology* **53**: 805–809.
- \_\_\_\_\_, and \_\_\_\_\_. 1967b. Revision of Porrorchinae (Acanthocephala: Plagiorhynchidae) with descriptions of two new genera and three new species. *Journal of Parasitology* **53**: 130–141.
- \_\_\_\_\_, and O. W. Olsen. 1964. Life cycle and development of *Prosthorhynchus formosus* (Van Cleave, 1918) Travassos, 1926, an acanthocephalan parasite of birds. *Journal of Parasitology* **50**: 721–730.
- Seidenberg, A. J. 1973. Ecology of the acanthocephalan, *Acanthocephalus dirus* (Van Cleave, 1931), in its intermediate host, *Asellus intermedius* Forbes (Crustacea: Isopoda). *Journal of Parasitology* **59**: 957–962.
- Skrjabin, K. I., and R. E. S. Shults. 1931. *Helminthoses of man (Foundations of Medical Helminthology) for medical doctors and veterinarians, biologists and students*. Part II. State Medical Publisher, Moscow, Russia, 250 p.
- Smales, L. R. 2002. Species of *Mediorhynchus* (Acanthocephala: Gigantorhynchidae) in Australian birds with the description of *Mediorhynchus colluricincla* n. sp. *Journal of Parasitology* **88**: 375–381.
- Sobecka, E., B. Szostakowska, K. Mackenzie, W. Hemmingsen, S. Prajsnar, and M. Eydal. 2012. Genetic and morphological variation in *Echinorhynchus gadi* Zoega in Müller, 1776 (Acanthocephala: Echinorhynchidae) from Atlantic cod *Gadus morhua* L. *Journal of Helminthology* **86**: 16–25.
- Southwell, T., and J. W. S. MacFie. 1925. On a collection of Acanthocephala in the Liverpool School of Tropical Medicine. *Annals of Tropical Medicine and Parasitology* **19**: 141–184.
- ŠpakulováM. M. Perrot-Minnot, and B. Neuhaus. 2011 Resurrection of *Pomphorhynchus tereticollis* (Rudolphi, 1809) (Acanthocephala: Pomphorhynchidae) based on new morphological and molecular data. *Helminthologia* **48**: 268–277.
- Starling, J. A., and F. M. Fisher, Jr. 1975. Carbohydrate transport in *Moniliformis dubius* (Acanthocephala). I. The kinetics and specificity of hexose absorption.

*Journal of Parasitology* **61**: 977–990.

\_\_\_\_\_, and \_\_\_\_\_. 1979. Carbohydrate transport in *Moniliformis dubius* (Acanthocephala). III. Post-absorptive fate of fructose, mannose, and galactose. *Journal of Parasitology* **65**: 8–13.

Steinauer, M. L., B. B. Nickol, R. Broughton, and G. Orti. 2005. First sequenced mitochondrial genome from the phylum Acanthocephala (*Leptorhynchoides thecatus*) and its phylogenetic position within Metazoa. *Journal of Molecular Evolution* **60**: 706–715.

Stoddart, R. C. 1965. New intermediate hosts in the life cycle of *Prosthenorchis elegans* (Diesing, 1851), an acanthocephalan parasite of primates. *Journal of Parasitology* **51**: 645–649.

\_\_\_\_\_, D. W. T. Crompton, and D. E. Walters. 1991. Influence of host strain and helminth isolate on the first phase of the relationship between rats and *Moniliformis moniliformis* (Acanthocephala). *Journal of Parasitology* **77**: 372–377.

Stunkard, H. W. 1965. New intermediate host in the life cycle of *Prosthenorchis elegans* (Diesing, 1851), an acanthocephalan parasite of primates. *Journal of Parasitology* **51**: 645–649.

Thapar, G. S. 1927. On *Acanthogyrus* n. gen. from the intestine of the Indian fish *Labeo rohita*, with a note on the classification of the Acanthocephala. *Journal of Helminthology* **5**: 109.

Tkach, V. V., O. I. Lisitsyna, J. L. Crossley, T. T. Binh, and S. F. Bush. 2013. Morphological and molecular differentiation of two new species of *Pseudoacanthocephalus* Petrochenko, 1958 (Acanthocephala: Echinorhynchidae) from amphibians and reptiles in the Philippines, with identification key for the genus. *Systematic Parasitology* **85**: 11–26.

Tokeson, J. P. E., and J. C. Holmes 1982. The effects of temperature and oxygen on the development of *Polymorphus marilis* (Acanthocephala) in *Gammarus lacustris* (Amphipoda). *Journal of Parasitology* **68**: 112–119.

Travassos, L. 1926. Contribuições para o conhecimento da fauna helminthológica brasileira. XX. Revisão dos acanthocefalos brasileiros. Part II. Fam. Echinorhynchidae. Sub-fam. Centrorhynchinae Travassos, 1919. *Memorias do Instituto Oswaldo Cruz* **19**: 31–125.

Trejo, A. 1992. A comparative study of the host-parasite relationship of *Pomphorhynchus patagonicus* (Acanthocephala) in two species of fish from Lake Rosario (Chubut, Argentina). *Journal of Parasitology* **78**: 711–715.

Uglem, G. L. 1972. The life cycle of *Neoechinorhynchus cristatus* Lynch, 1936

(Acanthocephala) with notes on the hatching of eggs. *Journal of Parasitology* **58**: 1071–1074.

\_\_\_\_\_, and S. M. Beck. 1972. Habitat specificity and correlated aminopeptidase activity in the acanthocephalans *Neoechinorhynchus cristatus* and *N. crassus*. *Journal of Parasitology* **58**: 911–920.

\_\_\_\_\_, and O. R. Larson. 1969. The life history and larval development of *Neoechinorhynchus saginatus* Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). *Journal of Parasitology* **55**: 1212–1217.

Väinölä, R., E. T. Valtonen, and D. I. Gibson. 1994. Molecular systematic in the acanthocephalan genus *Echinorhynchus* (sensu lato) in northern Europe. *Parasitology* **108**: 105–114.

Van Cleave, H. J. 1914. *Eorhynchus*: a proposed new name for *Neorhynchus* Hamann Preoccupied. *Journal of Parasitology* **1**: 50–51.

\_\_\_\_\_. 1936. The recognition of a new order in the Acanthocephala. *Journal of Parasitology* **22**: 202–206.

\_\_\_\_\_. 1941. Relationships of the Acanthocephala. *American Naturalist* **75**: 31–47.

\_\_\_\_\_. 1947. The Eoacanthocephala of North America, including the description of *Eocollis arcanus*, new genus and new species, superficially resembling the genus *Pomphorhynchus*. *Journal of Parasitology* **33**: 285–296.

\_\_\_\_\_. 1948: Expanding horizons in the recognition of a phylum. *Journal of Parasitology* **34**: 1–20.

\_\_\_\_\_. 1949: Morphological and phylogenetic interpretation of cement glands in the Acanthocephala. *Journal of Morphology* **84**: 427–457.

\_\_\_\_\_. 1951. Speciation and formation of genera in the Acanthocephala. *Anatomical Record* **111**: 525–526.

\_\_\_\_\_. 1952. Acanthocephalan nomenclature introduced by Lauro Travassos. *Proceedings of the Helminthological Society of Washington* **19**: 1–8.

\_\_\_\_\_, H. J., and E. L. Ross. 1944. Physiological responses of *Neoechinorhynchus emydis* (Acanthocephala) to various solutions. *Journal of Parasitology* **30**: 369–372.

Verweyen, L., L. S. Klimpe, and H. W. Palm. 2011. Molecular phylogeny of the Acanthocephala (class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. *PLoS ONE* **6**: e28285.

Wahl, G. M., and T. C. Sparkes. 2012. Egg dispersal in the acanthocephalian

- Acanthocephalus dims*: Field data. *Journal of Parasitology* **98**: 894–896.
- Walkey, M. 1967. The ecology of *Neoechinorhynchus rutili* (Müller). *Journal of Parasitology* **53**: 795–804.
- Ward, H. L. 1951. The use of antibiotics in artificial media for *in vitro* experiments with Acanthocephala. *Journal of Parasitology* **37**: 319.
- Weber, M., A. R. Wey-Fabrizius, L. P. Lowski, A. Witek, R. O. Schill, R. L. Suga, H. Herlyn, and T. Hankeln. 2013. Phylogenetic analyses of endoparasitic Acanthocephala based on mitochondrial genomes suggest secondary loss of sensory organs. *Molecular Phylogenetics and Evolution* **66**: 182–189.
- Welch, D. B. M. 2000. Evidence from a protein-coding gene that acanthocephalans are rotifers. *Invertebrate Biology* **119**: 17–26.
- West, A. J. 1964. The acanthon membranes of two species of Acanthocephala. *Journal of Parasitology* **50**: 731–734.
- Wilkes, J., R. A. Cornish, and D. F. Mettrick. 1982. Fumarase activity in *Moniliformis dubius* (Acanthocephala). *Journal of Parasitology* **68**: 162–163.
- Witek, A., H. Herlyn, I. Ebersberger, D. B. M. Welch, and T. Hankeln. 2009. Support for the monophyletic origin of Gnathifera from phylogenomics. *Molecular Phylogenetics and Evolution* **53**: 1037–1041.
- \_\_\_\_\_, \_\_\_\_\_, A. Meyer, L. Boell, G. Bucher, and T. Hankeln. 2008. EST based phylogenomics of Syndermata questions monophyly of Eurotatoria. *BMC Evolutionary Biology* **8**: 345.
- Witenberg, G. 1932a. Akanthocephalen Studien. I. Über einige für Systematik der Akanthocephalen wichtige anatomisch Merkmale. *Bulletino di Zoologia, Publicato della Unione Zoologica Italiana* **3**: 243–252.
- Witenberg, G. 1932b. Akanthocephalen Studien. II. Über das System der Akanthocephalen. *Bulletino di Zoologia, Publicato della Unione Zoologica Italiana* **3**: 253–256.
- Wright, R. D., and R. D. Lumsden. 1968. Ultrastructural and histochemical properties of the acanthocephalan epicuticle. *Journal of Parasitology* **54**: 1111–1123.
- \_\_\_\_\_, and \_\_\_\_\_. 1969. Ultrastructure of the tegumentary pore-canal system of the acanthocephalan *Moniliformis dubius*. *Journal of Parasitology* **55**: 993–1003.
- Yamaguti, S. 1963. Acanthocephala. *Systema helminthum*. John Wiley & Sons, Ltd, London, U.K., 423 p.

Zhao, B., and M. X. Wang. 1992. Ultrastructural study of the defense reaction against the larvae of *Macracanthorhynchus hirudinaceus* in laboratory-infected beetles. *Journal of Parasitology* **78**: 1098–1101.

Zrzavý, J. 2001. The interrelationships of metazoan parasites: a review of phylum- and higher- level hypotheses from recent morphological and molecular phylogenetic analyses. *Folia Parasitologica* **48**: 81–103.



# **Chapter 5**

## **Tocotrema Lingua (Creplin) the Adult Stage of a Skin Parasite of the Cunner and other Fishes of the Woods Hole Region\***

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In 1884 John A. Ryder published an account of a skin parasite of the cunner (*Tautogolabrus adspersus*). The diseased fish which furnished the material for Ryder's paper were from two localities, Woods Hole, Mass., and Cape Breton, N. S., and had attracted the attention of collectors on account of a peculiar spotted and rough appearance due to the presence of cysts in the skin. While Ryder did not determine the exact nature of the animal inhabiting these cysts he concluded that it was most probably a flat worm belonging to the Trematoda.

In 1889 I noted the occurrence of similar cysts in the skin of a cunner (Linton, 1890). The appearance of the infected fish agreed with Ryder's description, and each cyst opened contained an immature trematode. Again I recorded (1901) the finding of this parasite in the tautog (*Tautoga onitis*). Since that date I have found this parasite on a number of the species of fish of the Woods Hole region. They are of frequent occurrence on the winter flounder, tomcod and eel, less so on other fishes, while cunners and tautog are seldom wholly free from them.

It was not until July 24, 1911, that an adult trematode was recognized as the final stage of the skin parasite. On that date I obtained from the intestine of the loon (*Gavia imber*) a large number of small distomes, 4,789 by actual count. Another loon was examined on September 1 of the same year with similar results. It was at once noticed that these trematodes bore a close resemblance to the immature forms occurring in the skin cysts of the cunner and other fishes of the region. A re-examination of some cysts from the skin of fishes was at once made, and the young forms liberated from the cysts compared with adults and immature from the intestine of the loon. Details of this examination are given later in this paper. It is sufficient to state in this place that enough points of resemblance were made out to warrant the conclusion that the trematodes found in the intestine of the loon are the adult stage of some, at least, of the immature encysted forms found in the skin of various fishes.

These small distomes from the loon appear to be identical with the species originally named *Distomum lingua* by Creplin, as that species is figured by Olsson, and the anatomy of the peculiar genital sucker agrees with that shown for this species in the excellent description and figures of Jaegerskiöld. Following is a description of the species based on my material.

*Tocotrema lingua* (Creplin) Looss.—Small, body depressed, margins, especially in the vicinity of the neck, with a tendency to fold ventrally; outline varying from linear to pyriform but mostly oval, tapering toward anterior end and bluntly rounded posteriorly; greatest breadth, in adults with eggs, at the level of the folds of the uterus, which is usually at about the posterior two-thirds of the length. The neck is from one-half to two-thirds of the entire length of the worm, depending on the state of contraction, and the outline varies accordingly. Young forms without ova may be nearly linear in outline or broader in front of the genital aperture than behind that structure. Very minute scale-like spines cover the anterior part of the body densely. These are plainly shown on the surface and margins in front of the genital aperture. Behind that point they are seen with difficulty. The acetabulum is minute, and forms an anterior, internal muscular portion of the genital sucker. The genital aperture is situated on the median line, from the middle of the length to the posterior two-thirds, according to the condition of contraction of the neck.

The oral sucker is subterminal and its aperture is circular. There is a very short prepharynx followed by the pharynx which is longer than broad, oval-elliptical, its length about equaling the diameter of the oral sucker. The slender esophagus is longer than the pharynx and may be more than twice as long. The forks of the intestine are slender, simple, and extend to the posterior end of the body.

Near the posterior end of the body are the two testes which are contiguous, diagonally placed, and, as seen in compressed specimens, distinctly lobed. In uncompressed individuals the lobes of the testes are often indistinct. The vas deferens is obscured by the folds of the uterus so that its course is difficult to trace. In a few specimens, which had a smaller number of ova than are found in most cases, a tubular seminal vesicle could be made out lying in a few loose folds dorsally behind the genital sucker. It terminates in an ejaculatory duct which lies in the dorsal portion of a short, papillary cirrus-like body (kegel-förmiges körper of Jaegerskiöld) and empties into a genital sinus on the anterior margin of the cirrus-like body. The latter is surrounded by the muscles of the genital sucker. At the anterior margin of the right testis lies the relatively large seminal receptacle. In some cases this is nearly circular in outline; in others it is oval with the longer

diameter transverse. In sections it is seen to extend from the dorsal to the ventral wall of the body, but in the whole mounts it is perhaps more clearly seen in dorsal than in ventral view.

Along the anterior margin of the seminal receptacle is the ovary, which may be in part obscured by the folds of the uterus. When the worm is flattened and the ova are not too numerous the ovary is seen to be distinctly lobed. The ovary, seminal receptacle and testes therefore are massed together near the posterior end of the body. The folds

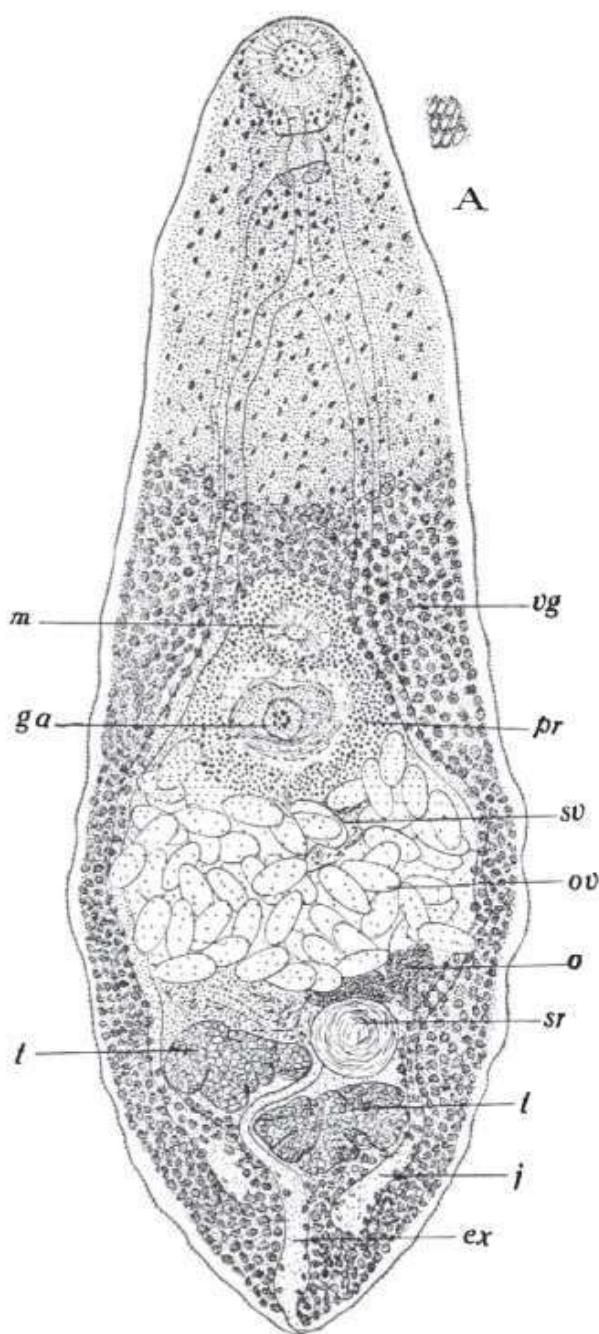


Fig. 1.—Adult specimen of *Tocotrema lingua* from Loon; dorsal view, in balsam. Length 0.84 mm. *a*, surface spines, 0.001 mm. long.

Abbreviations Used in Figures: *de*, ductus ejaculatorius; *ex*, excretory vessel; *ga*, genital aperture; *gp*, genital papilla; *gs*, genital sinus; *i*, intestine; *m*, ventral sucker; *o*, ovary; *ov*, ovum, ova in uterus; *pr*, prostate; *sr*, seminal receptacle; *sv*, seminal vesicle; *t*, testis; *v*, outlet of uterus; *vd*, vas deferens and seminal vesicle; *vg*, vitelline glands.



of the uterus are crowded between them and the genital aperture, the ova appearing as a golden yellow mass which extends nearly from margin to margin. The ova are oval-elliptical and, compared with the small size of the worm, are of good size. The terminal portion of the uterus opens into a cleft or sinus at a point adjacent to and dorsal to the opening of the ejaculatory duct. The genital sinus lies in front of the organ, called in the explanation of figures the genital papilla, (Figs. 4, 5, 6, *gp*), communicates anteriorly with the ventral sucker, and ventrally with the genital aperture. The genital sucker is surrounded

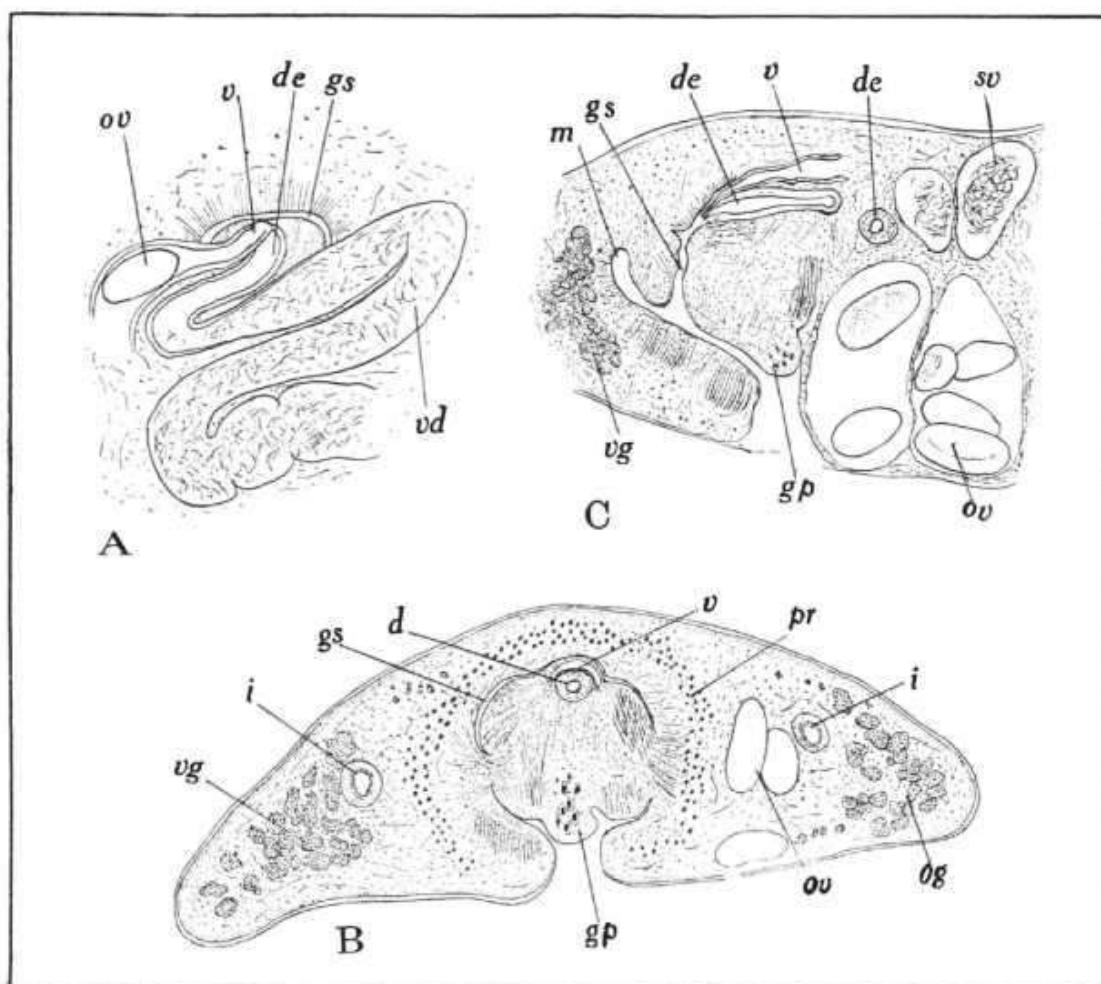


Fig. 2.—Sections thru dorsal portion of genital sucker; *A*, horizontal; *B*, transverse; *C*, sagittal.

by a somewhat triangular area of cells which are probably prostatic. The vitellaria are diffuse and fill the posterior and lateral margins of the body to a point in front of the genital aperture where they meet on the median line.

The excretory vessels were not completely made out. An excretory pore was distinguished situated dorsally at the posterior end of the body. From it a vessel was traced which passed between the testes. Transverse sections show a small lateral vessel on each side of the neck



region, but not distinctly enough to admit of satisfactory reconstruction.

A nerve mass lying dorsal to the pharynx, with short anterior branches, and lateral nerves traceable posteriorly to the anterior borders of the vitellaria are visible in many of the stained and mounted specimens.

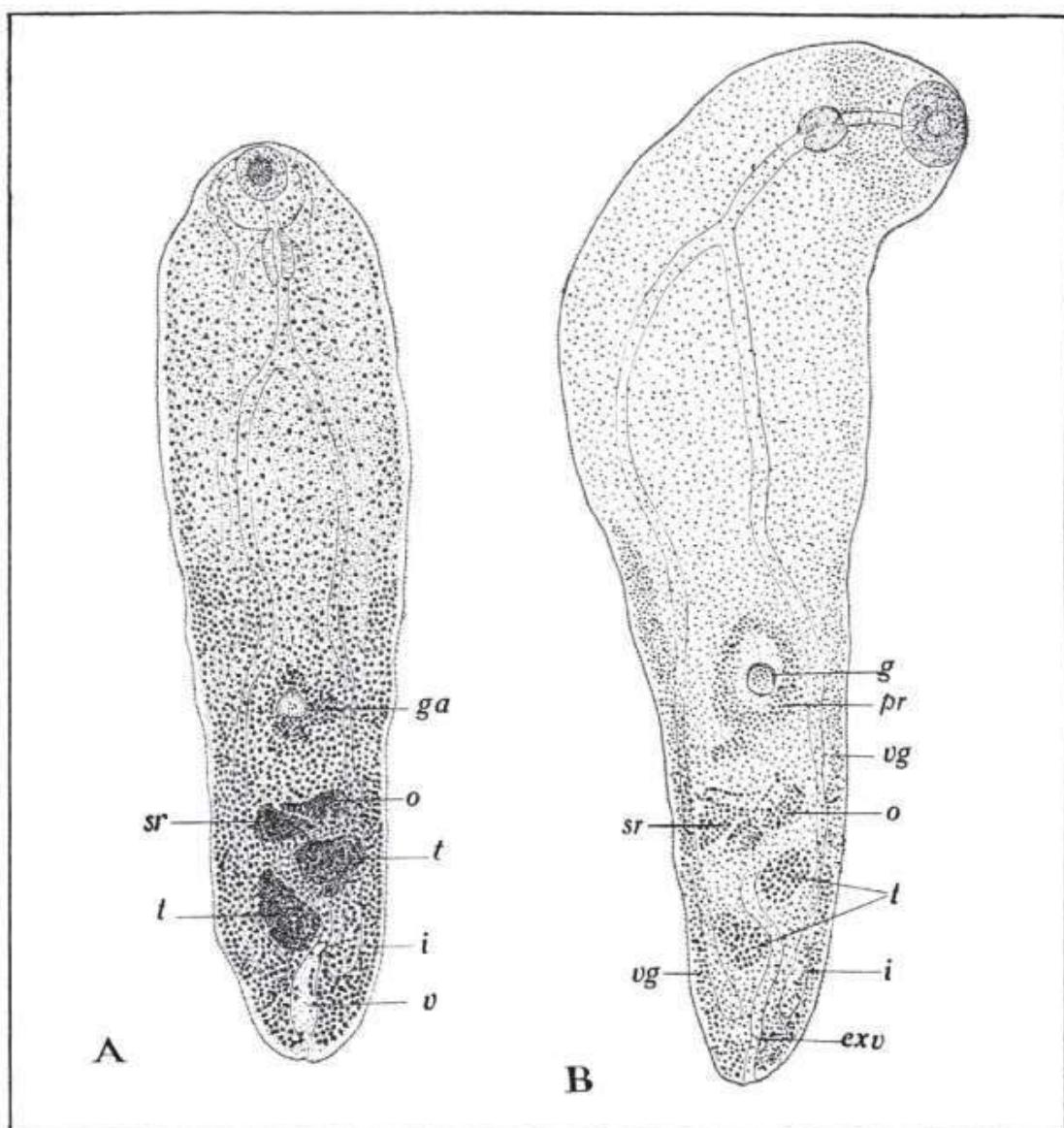


Fig. 3.—A. Young specimen from Loon; dorsal view, in balsam. Length 0.55 mm. B. Young distome from cyst in skin of cunner, in balsam. Length, 0.7 mm.

Dimensions of a living specimen in millimeters: Length 0.75; maximum breadth, 0.35; diameter of oral sucker, 0.07; ova, 0.04 by 0.02.

A specimen flattened under the cover-glass had the following dimensions: Length, 1.40; maximum breadth 0.70; diameter of oral sucker, 0.09; pharynx, length 0.04, breadth 0.03; ova, 0.047 by 0.023; genital sucker, 0.56 from the anterior end; bifurcation of the intestine



midway between anterior end and genital sucker. Following are lengths and breadths of ten specimens taken at random from a large number mounted in balsam:

Length,	0.70	0.97	0.76	0.80	0.55	0.80	0.76	0.67	0.72	0.64
Breadth	0.32	0.20	0.35	0.28	0.22	0.30	0.27	0.22	0.28	0.32

The above measurements were made of specimens from the loon. Following are dimensions of four specimens taken at random from a considerable number mounted in balsam, from the herring-gull:

Length	1.32	0.84	1.52	0.91
Breadth	0.40	0.33	0.48	0.33

Following is a record of the finds of *Tocotrema lingua* at Woods Hole. Some of these were made in 1904, but were not given special attention at the time of collecting.

*Colymbus auritus*: Feb. 8, 1912; 1.

*Gavia imber*: July 24, Sept. 1, 1911; very numerous on each date. Feb. 21, 1914, 2, immature.

*Larus argentatus*: Feb. 16, 17, 1912, numerous on each date. July 22, 1912, 252; Sept. 4, 1912, few. Jan. 22, 1914, 150; April 29, 1914, 1; Sept. 28, 1914, 1.

*Larus atricilla*: Aug. 12, 1904, 27 from one gull, 86 from another. Aug. 15, 1913, 18 from one gull, 6 from another.

*Nyctocorax nictocorax*: July 15, 1913; few, immature.

*Sterna dougalli*: Aug. 3, 1904, 1; Aug. 12, 1904, 21.

*Sterna hirundo*: Aug. 5, 1904, 1.

The specimens recorded for January and February are from the material collected by Vinal N. Edwards.

Young trematodes (Fig. 3B) from cysts taken from the skin of cunners, tautog, and other species of fish, were compared with specimens of *Tocotrema lingua* from the loon and other fish-eating birds with the following results: The body in each case was covered with a dense coat of minute scale-like spines of similar appearance; oral sucker, prepharynx, esophagus and intestinal rami agree; rudiments of genital sucker, genital papilla, testes, ovary, sperm receptacle and vitellaria agree in relative positions to the finished structures in the adult. Furthermore nothing was seen in the one that contradicted any point in the other.

A few immature specimens were found among the adults in the final hosts (Fig. 3A). These agree very closely in form with those removed from cysts in that the neck is wider than the posterior third of the body. With the maturing of the testes and ovary and the accumulation of ova in the uterus the posterior third becomes normally wider than the neck.

## SUMMARY

Certain trematodes encysted in the skin of the fishes of the Woods Hole region are the young of an adult which lives in the intestine of the loon and other fish-eating birds.

The identification of these encysted distomes with *Tocotrema lingua* renders Stafford's name (*Dermocystis ctenolabri*) for these encysted forms inapplicable.

## LITERATURE CITED

Linton, E. 1900. Fish Parasites Collected at Woods Hole in 1898. Bull. U. S. Fish Com. for 1899, 281-296; pl. 40. 1901. Parasites of Fishes of the Woods Hole Region. Bull. U. S. Fish Com. for 1899, 462-463.

Ryder, J. A. 1884. On a Skin Parasite of the Cunner (*Ctenolabrus adspersus*). Bull. U. S. Fish Com. for 1884, 37-42.

Stafford, J. 1905. Trematodes from Canadian Vertebrates. Zool. Anz., 28: 682.

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# A century (1914–2014) of studies on marine fish parasites published in *The Journal of Parasitology*

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During its first 100 years of existence, *The Journal of Parasitology* (*JP*) became the main source of peer-reviewed information on parasites of marine fishes at a continental level, and a reference at the global level. As argued in the following paragraphs, the variety of subjects and contributions in this field in *JP* is enormous and range from taxonomic and life cycles of parasites infecting marine fishes, to ecology, marine fish kills (e.g., Solangi and Overstreet, 1980; Ogawa et al., 1995; Jones and Hargreaves 2007), parasites as biological tags (e.g., Baldwin et al., 2011; Khan et al., 2011), and parasites as bioindicators of environmental impact (e.g., Khan and Hooper, 2007, Sánchez-Ramírez et al., 2007). Something that all these investigations share is that they rely on the massive body of taxonomical knowledge developed by many parasitologists throughout time in *JP*. Without a doubt, the accumulation of all this peer-reviewed knowledge in an organized fashion to make it publicly available has been one of the many contributions of *JP* authors to the field of marine fish parasites during the last 100 years. As will be shown in this chapter, the history of parasites in marine fishes in *JP* can be naturally divided into two periods. The first 80 years (1914–1994) include a large number of papers on taxonomy, life cycles, and applied subjects such as fish kills or the use of parasites as biological tags. The second period (1995–2014), has been marked by the arrival of the Internet, and an even larger number of papers dealing with taxonomy, phylogenetics, molecular biology, and the use of parasites as bioindicators of environmental quality.

In addition to its main core of taxonomic papers, some of the most influential publications on parasites of marine fishes in *JP* in the period 1914–1994 included those of Hunninen and Cable (1943; 43 citations), Durio and Manter (1968; 41), Deardorff and Overstreet (1980; 33), Solangi and Overstreet (1980; 52). Hunninen and Cable (1943) described the life cycle of the hemiurid trematode, *Lecithaster confusus*, and provided an exacting example on how to study a parasite life cycle. It details the experimental infection of *Acartia* sp. copepods with cercariae obtained from the mollusc, *Odostomia trifida*. The infected copepods were fed to sticklebacks, *Apeltes quadratus*, which successfully developed adult worms, completing the life cycle. This kind of information on parasite life cycles is fundamental when those in the aquaculture industry, for example, must face problems related to fish mortality, and for tropical regions where few parasite life cycles have been studied (see Scholz and Aguirre-Macedo, 2000). Other examples of importance in this field include sanguinicolid

metacercariae in marine floating cages (Bullard and Overstreet, 2002), or the aesthetic problems produced by *Cryptocotyle lingua* as shown by Woo et al. (2002).

Durio and Manter (1968) described nine species of trematodes of Opeocoliidae and four species of Lepocreadiidae from fishes of the South Pacific. This descriptive paper is a classic, very useful for people working in one of the most diverse regions of the planet; it has been frequently cited by Australian (e.g. Cribb et al., 1994), French (Justine, 2010), and British parasitologists (Bray, 1985). It is perhaps not easily perceived, but when one is working in those wild and isolated places, this is the kind of taxonomic papers that encourage rapid advance on a robust basis. The work of Deardorff and Overstreet (1980) on zoonotic species like *Contracaecum* spp. has been very useful for study of the biology and distribution these parasites in both intermediate and definitive hosts (Mattiucci and Nascetti, 2008). Similarly, research by Solangi and Overstreet (1980) on applied grounds relative to fish kills has focused on the massive mortality of killifish (*Fundulus* spp.) in Alabama, Mississippi, and Virginia. They examined many aspects of this massive mortality, e.g., the life cycle of the parasite, the pathology, and its treatment; also emphasized were the potential implications for aquaculture because several *Fundulus* species are raised as baitfish in those states and others nearby.

The last 20 years (1995–2014) of publication on marine fish parasites by *JP* have brought an increase in the number of papers dealing with phylogeny using molecular and genetic tools, fish kills, parasites as tags, and parasites as indicators of environmental quality. In this context, the most influential papers during the period 1995–2014 on parasites of marine fishes included Mattiuci et al. (1997; 141 citations), Whipps et al. (2004; 69), Olson and Caira (1999; 66), and Ogawa et al. (1995; 56). The Mattiuci et al. (1997) work was a worldwide study on genetics of the *Anisakis* spp. complex, the reproductive isolation of the gene pools of three species of *Anisakis*, their intermediate and definite hosts, and the geographic distribution of these parasites. Many of the papers citing Mattiucci et al. (1997) deal with the seafood industry and the risk of anisakid nematodes for human health (e.g., Chai et al., 2005). The paper by Ogawa et al. (1995) dealt with a report of *Neobenedenia girellae*, a monogenean, infecting and killing Japanese flounder, *Paralichthys olivaceus*, and tiger puffer, *Takifugu rubripes*. In addition to a detailed parasite description, the authors discussed the possible source of infection, in this case amberjacks imported into Japan without proper sanitary inspection.

During 1995–2014, *JP* saw the arrival of the phylogenetics. A good example of phylogeny was the influential research of Olson and Caira (1999), who studied the interrelationships of tapeworms (Platyhelminthes: Cestoidea), based on an analysis of complete and partial gene DNA sequences. This paper was a very important one because, based on molecular tools, it was possible to clarify many of the phylogenetic relationships in Cestoidea. Also using molecular tools, Whipps

et al. (2004), dealt with the phylogenetic relationships of the Myxosporidia. Using small-subunit (SSU) and long-subunit (LSU) ribosomal DNA sequences and morphological attribute, they proposed an evolutionarily consistent taxonomic scheme for extant myxozoan genera. Myxosporidia belong to the “dirty dozen” group of micro and macroparasites currently affecting the seafood industry (Llarena-Reino et al., 2015).

During the last 20 years, among many other research subjects *JP* has received papers on the use of parasites as bioindicators of environmental quality (e.g., Marcogliese and Cone, 1996; 65 citations; Keas and Blankespoor, 1997; 21; Koprivnikar et al., 2006; 22). These contributions have examined the effect of different environmental insults on the hosts of various parasites as models, e.g., eel parasites and pH, frog parasites and pesticides, and flounder parasites and pulp mill effluents. The common outcome has been that parasites are affected by environmental factors. For example, Marcogliese and Cone (1996) demonstrated the effect of acidification on the species richness of the parasite communities of eels, while Keas and Blankespoor (1997) conducted a 50-year comparison of the deleterious effect of anthropogenic disturbance on the species richness of larval trematodes in freshwater snails (*Stagnicola* spp.) Without doubt, these papers and many more published in *JP* in this field (e.g., Khan, 2004; Koprivnikar et al., 2006), although not restricted to marine environments, have been a source of inspiration for those working on parasites of marine fishes as bioindicators of environmental quality.

Analyzing the trends of study on parasites of marine fishes in *JP* has been a very ambitious enterprise. To approach this task, we identified the papers published in the sub-disciplines in which the journal is currently divided, and addressed three questions: Where have we come from? Where we are now? Where do we go from here? For the first two questions, we compiled and analyzed a database including all the papers on marine fish parasites published in *JP* that we were able to identify as such from 1914 to 2014. Then, for the first two questions, our objective was to determine potential statistical associations between time (in decades) and the number of citations for the sub-disciplines in which *JP* is divided, and an analysis of the publication trends of *JP* on parasites of marine fishes in comparison with similar peer-reviewed journals. For the third question, in addition to determining the relationship between time (in decades) and the number of citations as per *JP* sub-disciplines, we presented a summary of our studies on the use of parasites as bioindicators of environmental quality in recognition to the inspiration provided by the research published on this subject in *JP*.

# The database on parasites of marine fishes in *JP* and the trends of its sub-disciplines divided into 10-year periods

From JSTOR and the Web of Science, we identified 940 papers dealing with parasites of marine fishes, published between 1914 and 2014, using the following as keywords: parasites, helminths, Protozoa, Microsporidia, Myxosporidia, Trematoda, Gyrocotylidea, Monogenea, Cestoda, Cestodaria, Nematoda, Acanthocephala, Hirudinea, Crustacea, Copepoda, Isopoda, communities, populations, ecology, phylogeny, and physiology. The collected information (name of the paper, authors, year of publication, volume, number of pages, and number of citations per paper) was compiled in Excel and is available on request from VMVM ([vvidal@mda.cinvestav.mx](mailto:vvidal@mda.cinvestav.mx)). In the case of the number of citations for papers older than 1950, there was no information in JSTOR or the Web of Science, and the only way to track them was by using Google Academic.

To determine the potential temporal trends on each one of the *JP* sub-disciplines, we used meta-analysis and model fitting. We grouped each sub-discipline by decade. Altogether, there were enough number of papers and citations per sub-discipline per decade to consider the historical analysis of 10 sub-disciplines, namely Systematics–Phylogenetics, Ecology, Epidemiology and Behavior, Life Cycles-Survey, Pathology, Therapeutics-Diagnostics, Molecular-Cell Biology, Biochemistry-Physiology, Functional Morphology, Genetics-Evolution, and Invertebrate-Parasite Relationships. The temporal patterns of the number of papers and citations were similar. Therefore, it was assumed that the number of citations would be a good proxy for the impact of the papers published on each sub-discipline. Consequently, we decided to present the results based on the number of citations. Thus, the effect size was defined as a measure of the strength of the relationship between the number of citations per sub-discipline and time, and the effect size estimator was the Pearson' correlation coefficient ( $r_p$ ).

Therefore, a positive association between time (in decades) and the number of citations meant a successful trend in the increase in the number of citations. A negative association meant that the number of citations for that sub-discipline was decreasing through time, and the lack of relationships between time and the number of citations meant that the number of citations has not changed in time.

We used the 95% confidence intervals (CI) as a measure of the precision of the mean. Outcomes were estimates of the overall effect (= correlation) of time on the number of citations for each sub-discipline. The null hypothesis was that there was no correlation between the number of citations by sub-discipline and time. See Vidal-Martínez et al. (2010) for a more detailed description for the use of meta-analysis. Additionally, we determined the best model describing the relationship between time and the number of citations per sub-discipline per decade. For the meta-analysis, we used the Comprehensive Meta-analysis (CME) software and Infostat to produce the models between time and the number of

citations (linear, exponential, polynomials, etc.) and the lowest value of the Akaike's Information Criterion (AIC) to choose the best model describing these relationships.

# Methods for using parasites as bioindicators of environmental quality

For our research on parasites as bioindicators of environmental quality, we used data from 162 sampling sites (sites henceforth) in the southern Gulf of México with depths between 1 and 3,571 m. Bottom sediments, water, and flatfishes, *Syacium gunteri* (n = 165), *Cyclopsetta chittendeni* (240), and *Syphurus plagiusa* (158) were collected by shrimp boats and oceanographic vessel from each site.

We obtained 48 physicochemical parameters from the water and sediments, including oxygen (mg/L), salinity (UPS), and pH, among others, and performed standard parasitological procedures to obtain the larval cestode, *Oncomegas wageneri*, and the adult acanthocephalan, *Acanthocephaloidea plagiussae*. The data on prevalence and mean abundance of the parasites and morphometric measurements of the fish have been published elsewhere (see Vidal-Martínez et al., 2014 for details). The hydrocarbon, metals, and physicochemical sampling procedures have been described elsewhere (Gold-Bouchot et al., 1997; Vidal-Martínez et al., 2006). Even when the fish sampling procedures were standardized, the number of flatfishes collected was highly uneven among sites, making it difficult to compare the infection parameters of the parasite species. We took two radical strategies to overcome these problems. First, we pooled the data for the flatfishes (*S. gunteri*, *C. chittendeni*, and *S. plagiusa*) infected with *O. wageneri* and *A. plagiussae*, assuming that the individuals of these fish species were used by the parasites as replicated habitats (*sensu* Holmes and Price, 1986).

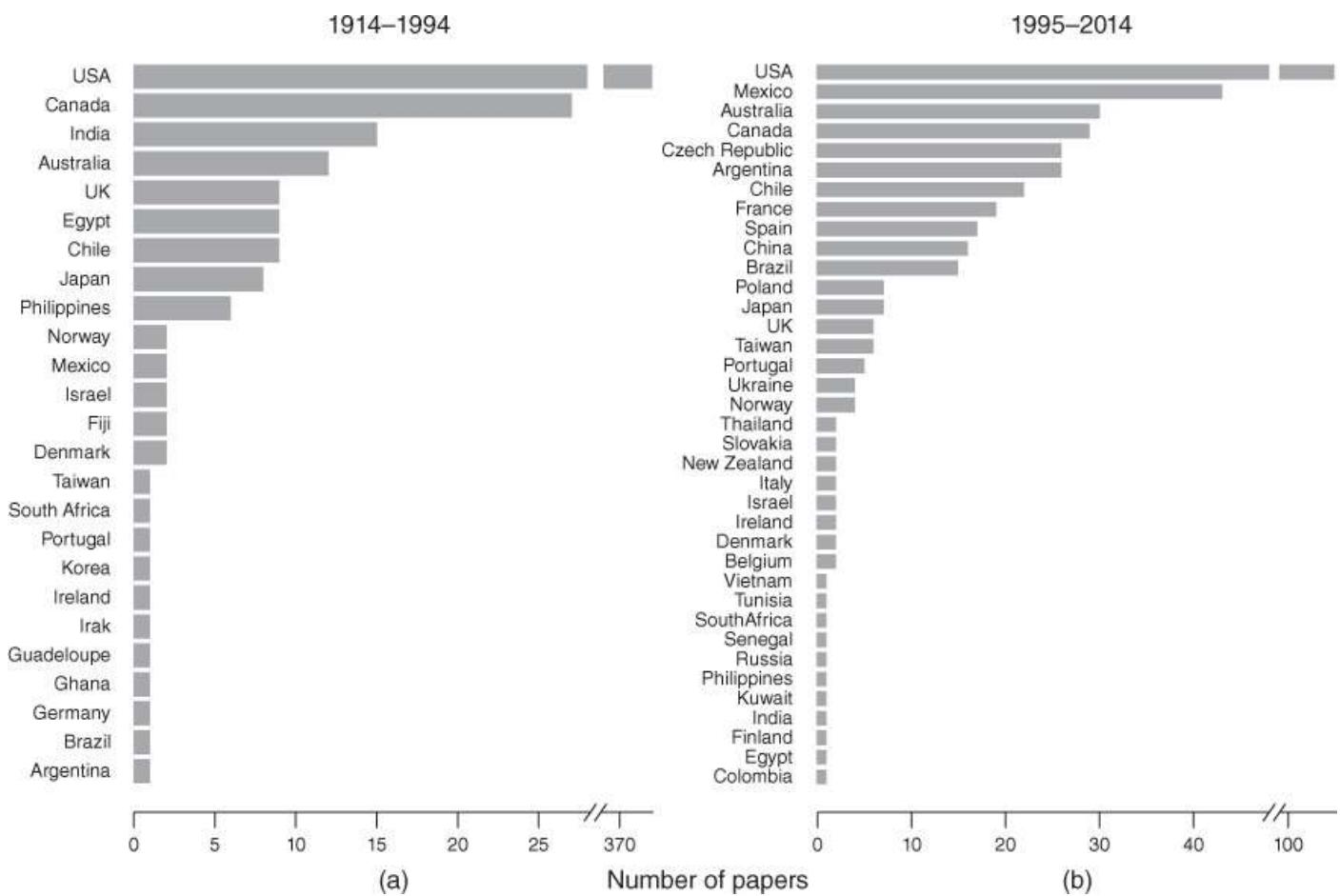
For sites from where enough fishes were collected, 10 were examined for the parasites, but, in several sites, 1–5 fish individuals were collected. Thus, we transformed mean intensity values of the parasites to presence or absence values. For example, if we caught five shoal flounders at a one site infected with 5, 2, 3, 0, and 0 *O. wageneri*, the mean number of parasites per fish was 3.33, and we had three presences and two absences at that site. The same procedure was applied for *A. plagiussae*. This method diminished the problem for the dependence of the number of *O. wageneri* and *A. plagiussae* on the number of fish collected and allowed us to represent the number of parasites as the number of presences per site (adapted from Torres-Irineo et al., 2014). This method also allowed us to estimate the probability of occurrence of the parasites based on a binomial distribution. We fitted boosted generalized additive models (boosted GAMs) using the R package “mboost” (Hofner et al., 2012) to examine whether the environmental variables statistically related to the probability of occurrence of *O. wageneri* and *A. plagiussae*. We also used the maximum entropy algorithm (MaxEnt) to estimate the probability of habitat suitability for the establishment of populations of the parasites (Phillips et al., 2006). The package “dismo” in R was used to compare the performance of the boosted GAMs and MaxEnt (<http://cran.r-project.org/web/packages/dismo/dismo.pdf>). To evaluate the performance of both the boosted GAM and MaxEnt models, we used Cohen's

kappa, ROC (receiver operating characteristic curves) and pROC curves (partial receiver operating characteristic curves) for both methods. All of the sampling points with, and without, flatfish and parasite occurrences were used in the boosted GAMs and MaxEnt models.

# Where have we come from? The first 80 years of knowledge on parasites of marine fishes in *JP*

The first issue of *JP* was published on September, 1914, and the first paper on parasites of marine fishes was written by Edwin Linton in the March, 1915 issue, i.e., only 6 months after inception of the journal. The paper was about the presence of the metacercarial stages of *Cryptocotyle lingua* (originally identified by Linton as *Tocotrema lingua*) infecting the cunner, *Tautogolabrus adspersus*, and other marine fishes (e.g., winter flounder, tomcod, eel, and tautog) in the Woods Hole region (Linton, 1915). Since the paper by Linton, there have been very few issues in which there were no papers on marine fish parasites. In fact by now, 940 papers have been published by authors from 41 countries, which in total have accumulated 5,520 citations. For the first 100 years of *JP*, the main countries contributing with papers on parasites of marine fishes have been the United States with 513, followed by Canada (56), México (45), Australia (43), Chile (31), the Czech Republic (26), and Argentina (20).

If we look at the first 80 years of *JP* (1914–1994), 498 papers were published by scientists from 25 countries, producing 2,351 citations. In this period, 382 out of 498 papers (77%) published on parasites of marine fishes in *JP* were authored by USA, followed by Canada (27 papers; 5%), and Australia (12; 2%) ([Fig. 1a](#)). The remaining 16% of the papers were produced from the rest of the countries in [Fig. 1a](#).



**Figure 1** Number of papers on parasites of marine fishes contributed by country. (a) Number of papers on parasites of marine fishes published by country during the first 80 years (1914–1994) of *JP*. (b) Number of papers on parasites of marine fishes published by country during the last 20 years (1995–2014) of *JP*.

The reason the number of papers mentioned before (498) does not coincide with the sum of the total number of papers analyzed in our Excel file (539) was that there were individual papers dealing with different subjects, and then considered as separate papers. During the period 1914–1994 for parasites of marine fishes, there were 369 (68%) papers published on Systematics-Phylogenetics, 50 (10%) in Life Cycles-Survey, 39 (7%) in Ecology, Epidemiology and Behavior, 32 (6%) in Pathology, 23 (4%) in Functional Morphology, 11 (2%) in Biochemistry-Physiology, 7 (1%) in Therapeutics-Diagnostics, 4 (1%) in Genetics-Evolution, and 3 (1%) in Invertebrate-Parasite Relationships. We did not detect papers in Molecular-Cell Biology for this period.

The mean number of citations during this period was  $10.81 \pm 10.18$  citations per paper. It was evident that the largest contribution in terms of papers and citations was made by developed countries with USA leading the list. During this period, famous American parasitologists such as Edwin Linton, Harley J. Van Cleave, Horace W. Stunkard, Elmer R. Noble, Asa Chandler, and Harold W. Manter together with many others established the foundation for research on the parasites of marine fishes that we enjoy today.

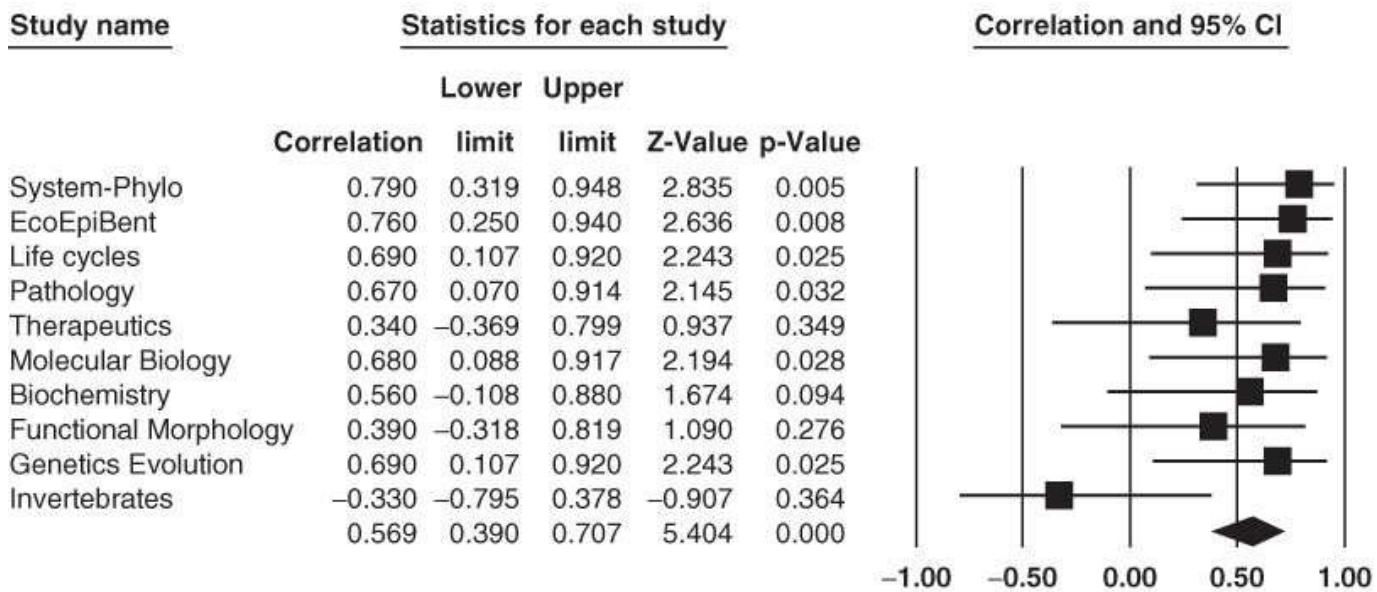
## Where are we now? Modern times and the arrival of the Internet

A huge contrast was evident with respect to the number of papers published on marine fish parasites in *JP* during the last 20 years (1995–2014) as compared to the previous 80 years period (1914–1994). In this recent period, 442 papers were published by authors from 37 countries, yielding 3,169 citations. The number of countries contributing to *JP* in marine parasites also changed from 25 in the period 1914–1994 to 37 for the period 1995–2014 and 131 of 442 papers (30%) were authored by US researchers. The countries contributing papers on marine parasites to *JP* changed with respect to the previous period, with countries like México (43 papers), Australia (31), Canada (29), and the Czech Republic and Argentina (26 each) making a substantial contribution ([Fig. 1b](#)). These numbers evidently show a trend in *JP* to the internationalization of the discipline, possibly related to the arrival of the Internet. Definitively, this international network is functioning to overcome geographical barriers, allowing the developing countries to more easily submit papers to *JP*, while also providing access to published papers in electronic format and enhancing interaction with peers from developed countries. During this period (1995–2014) for parasites of marine fishes in *JP*, there were 312 (59%) papers published on Systematics-Phylogenetics, 99 (19%) in Ecology, Epidemiology and Behavior, 45 (8%) in Molecular-Cell Biology, 32 (6%) in Life Cycles-Survey, 17 (3%) in Pathology, 10 (1.90%) in Functional Morphology, 10 (1.90%) in Genetics-Evolution, 6 (1%) in Biochemistry-Physiology, and 1 (0.2%) in Invertebrate-Parasite Relationships. We did not detect papers in Therapeutics-Diagnostics for this period. The number of papers mentioned at the beginning of this section (442) was not the same the sum of the total number of papers for this paragraph (532), and the reason was that there were many individual papers dealing with different subjects, and they were considered as separated papers.

Interestingly, the mean number of citations for this new period was lower ( $9.08 \pm 17.03$  citations per paper) but with a higher standard deviation in comparison with the period 1914–1994. Evidently, this pattern is related to the fact that with time, old papers are still accumulating citations, while the papers of the period 1995–2014 are still relatively new and still adding citations. Furthermore, we are obviously speaking of two periods of different sizes (80 vs. 20 years), which in turn, may influence the number of citations.

## The meta-analysis

In Volume 65 (4), 1979, *JP* was divided into sub-disciplines by the acting Editor Prof. Austin J. McInnis (Esch, et al., 2014). Using these sub-disciplines as groups, we pursued the historical trends of citation in these sub-disciplines throughout time using a meta-analysis. [Figure 2](#) shows the result of the meta-analysis for the sub-disciplines in which there was enough information to undertake the analysis. The overall value of effect was  $r_p = 0.57$ , a positive one and highly significant ( $p < 0.0001$ ). From [Fig. 2](#) it was evident that, six out of 10 sub-disciplines had positive effect values, meaning that with time the number of citations on those sub-disciplines has been increasing. There were some sub-disciplines with stronger values of correlation than others. This was the case of Systematics-Phylogenetics ( $r_p = 0.79; p < 0.005$ ) followed by Ecology-Epidemiology-Behavior with ( $r_p = 0.76; p < 0.008$ ), Life Cycles-Survey ( $r_p = 0.69; p < 0.03$ ), Genetics-Evolution ( $r_p = 0.69; p < 0.03$ ), and Pathology ( $r_p = 0.67; p < 0.001$ ). There were some weak correlation values such as that of Molecular-Cell Biology ( $0.68; p < 0.03$ ), attributable to the fact that only recently (the last two or three decades) there has been an increase in the number of citations to the papers published in *JP* in this sub-discipline.



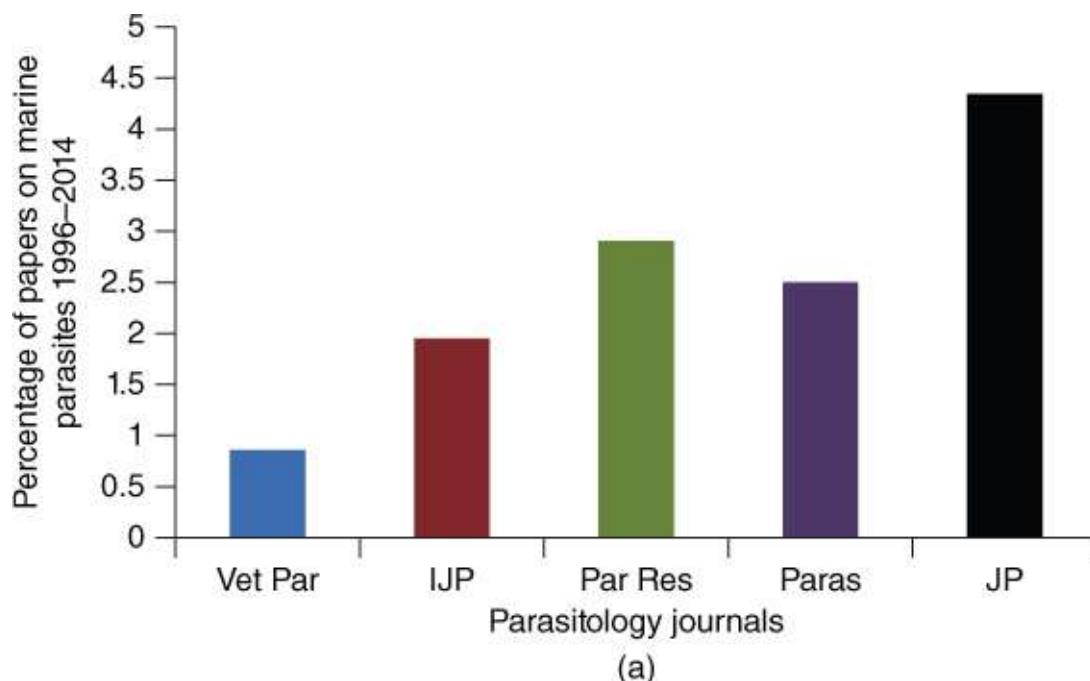
**Figure 2** Average effect sizes ( $\pm 95\%$  confidence intervals) of time on the number of citations for the sub-disciplines in which the *Journal of Parasitology* is divided. The effect size estimator was the Pearson's correlation coefficient ( $r_p$ ). The  $r_p$  values for overall effects are significantly different from zero if the confidence interval (CI) does not overlap zero. For each abbreviation (e.g., System-Phylo), the value of  $r_p$  is presented, followed by the lower and upper limits of confidence intervals at 95%, the Z-value and the P-value for  $r_p$ . The symbol  $\blacktriangleleft$  is the overall effect of time on the number of citations for all the sub-disciplines, that in this case was positive and significant ( $r_p = 0.57$ ;  $p < 0.001$ ). Abbreviations: System-Phylo, Systematics-Phylogenetics; EcoEpiBent, Ecology, Epidemiology and Behavior; Life cycles, Life Cycle-Survey; Pathology; Therapeutics, Therapeutics-Diagnostics; Molecular Biology, Molecular-Cell Biology; Biochemistry, Biochemistry-Physiology; Functional Morphology; Genetics-Evolution; Invertebrates, Invertebrate-Parasite Relationships.

Non-significant values were obtained for Biochemistry-Physiology, Functional Morphology, Therapeutics-Diagnostics, and Invertebrate-Parasite Relationships (Fig. 2), suggesting that there has not been an increase in the number of citations to the papers published in this sub-discipline in *JP* during the last 100 years. The lack of an increase in the number of citations seems to be related to the low number of papers published in these sub-disciplines. In the case of Biochemistry-Physiology, the decrease in the number of citations seems to be related to the fusion of this sub-discipline with Molecular-Cell Biology. However, the information in Therapeutics-Diagnostics is badly needed in applied fields such as marine aquaculture (e.g., Bullard and Overstreet, 2002; Woo et al., 2002) and papers on Functional Morphology and Invertebrate-Parasite Relationships are necessary to understand marine parasites biology in general. Certainly, to study small parasites such as the intramolluscan larval stages of digenleans or coracidia in copepods is not an easy target. However, there are modern alternatives such as the use of molecular tools or scanning and transmission electron microscopy, and

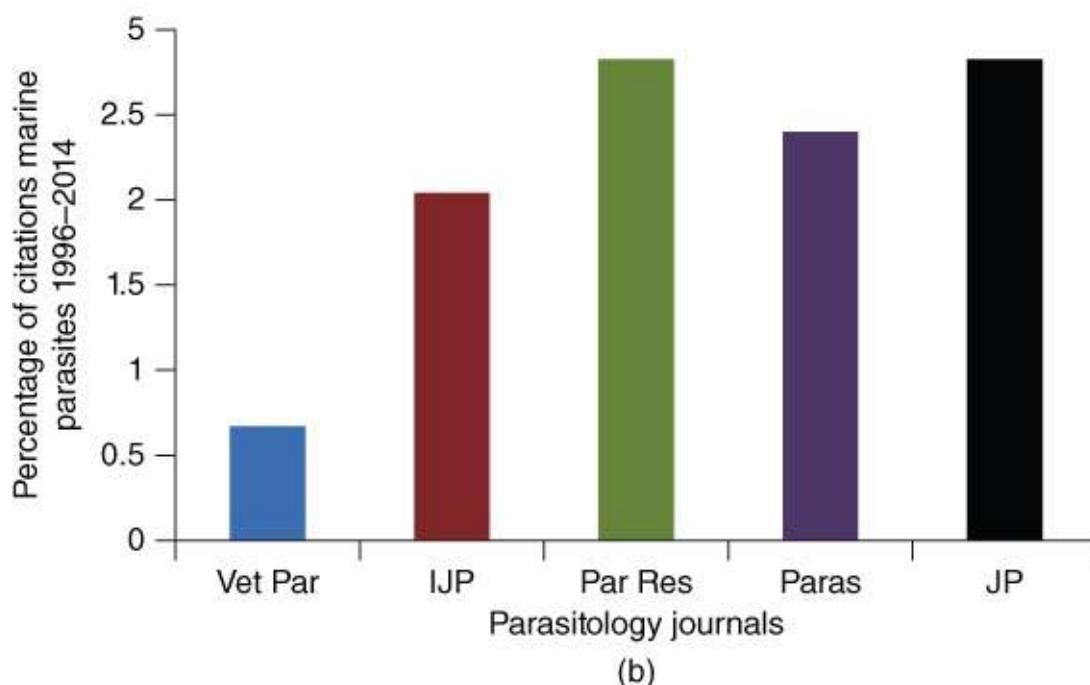
there are few, but excellent, papers still being published on this sub-discipline in *JP* (e.g., Miquel et al., 2007; Levron, et al., 2008).

## The performance of *JP* in comparison with other journals

We made a comparison of journals similar to *JP* in scope and impact factor in terms of the number of papers and citations on parasites of marine fishes, with the restriction that the information available in the web of science was only for the period 1996–2014. [Figures 3a](#) and [3b](#) show the results of this comparison among *Veterinary Parasitology*, *International Journal of Parasitology*, *Parasitology Research*, *Parasitology*, and *JP* in terms of the number of papers published and citations received by each journal on parasites of marine fishes. From [Fig. 3a](#) it was evident that the papers published in this subject in the five journals represent between 1% for *Veterinary Parasitology* to 4.5% for *JP*, and that *JP* is the journal publishing the largest number of papers on marine fish parasites, followed by *Parasitology Research* and *Parasitology*. However, when we come to the number of citations ([Fig. 3b](#)), *JP* and *Parasitology Research* had the highest but very similar values. A possible explanation for this fact is the relative size of the parasitologist communities among different regions of the world, if we take as a proxy the global production of papers in parasitology, assuming that similar percentages of paper production for different regions of the world apply also for those on parasites of marine fishes. The European Union (EU) is currently producing the largest number of papers in parasitology on the planet (35%) (Falagas et al., 2006). Therefore, the probability of citation for European papers in parasites of marine fishes is higher in this region than in other parts of the world. In contrast, USA produces 19.9% of the parasitology papers, and Latin America and the Caribbean 17.2%. Summing the contributions of USA, Latin America, and the Caribbean, we have 37.1%, which produces a similar number of citations in comparison to those from the EU.



(a)



(b)

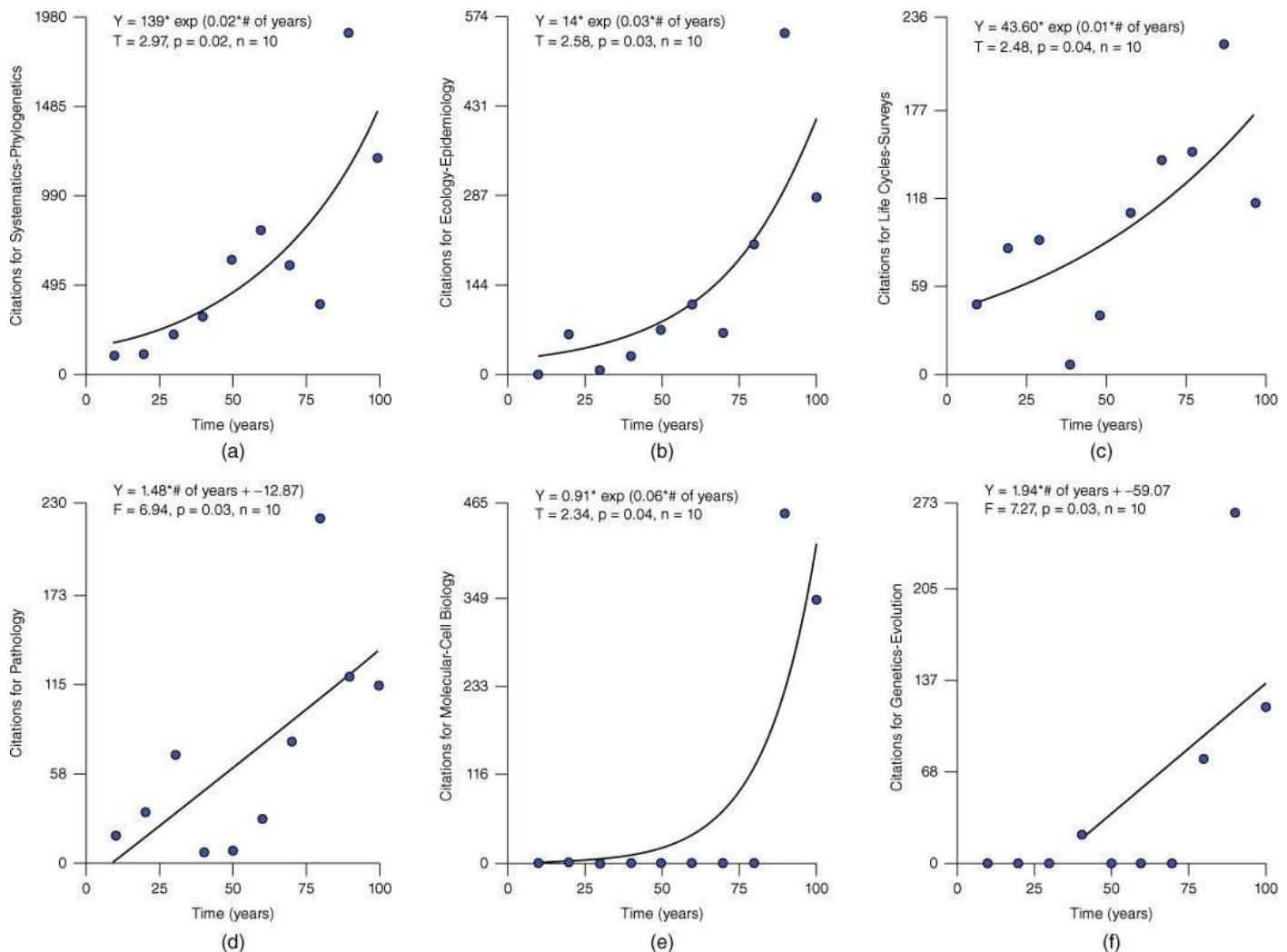
**Figure 3** Comparison of journals similar in scope and impact factor publishing on parasites of marine fishes for the period 1996–2014 (information obtained from the web of science). (a) Percentage of papers published on parasites of marine fishes by five scientific journals with respect to their whole production between 1996 and 2014. (b) Percentage of citations acquired by the papers from (a) for the same time period. Abbreviations: Vet Par, *Veterinary Parasitology*, IJP, *International Journal of Parasitology*, Par Res, *Parasitology Research*, Paras, *Parasitology*, and JP, *The Journal of Parasitology*.

Concluding this section, we can say that the number of citations acquired by the papers published on parasites of marine fishes in *JP* is very competitive at an international level. In fact, the results of both the meta-analysis and the comparison among journals suggested that for many of the sub-disciplines in which *JP* is divided, there is a positive trend over time, with the exception of the

Biochemistry-Physiology, Functional Morphology, Therapeutics-Diagnostics, and Invertebrate-Parasite Relationships. Thus, the results suggest that the arrival of the Internet and the fact that *JP* has become electronic appear to be the biggest positive technological changes that *JP* has experienced in this period (1995–2014).

## Where do we go from here?

To approach this question, we explored the relationship between time and the number of citations by sub-discipline for the whole data base (1914–2014). [Figure 4](#) shows the best fitting models for all the sub-disciplines in which we had enough information to undertake the analysis. The clearest pattern in [Fig. 4](#) was that most of the sub-disciplines (Systematics-Phylogenetics, Ecology, Epidemiology and Behavior, Life Cycle-Surveys, Molecular-Cell Biology) have experienced an exponential growth, especially during the last 20–30 years. Therefore, the expectation is that all these sub-disciplines will continue growing exponentially for the following decades. The exceptions were the Pathology and Genetics-Evolution sub-disciplines, in which we found weak but still significant linear positive relationships between time and the number of citations ([Fig. 4](#)). The explanation for these weak relationships was that, even when the number of citations for these sub-disciplines has been growing slowly throughout time, during the decade 1974–1983 they had an exceptionally high number of citations (i.e., an outlier). However, if we compare the number of citations for both sub-disciplines between periods (1914–1994 and 1995–2014), the most recent period accumulated more citations in the last 20 years than any other period of similar length for the 1914–1994 period.



**Figure 4** The relationship between time (independent variable) and the number of citations received by the sub-disciplines in which *JP* is divided (dependent variable) for the period 1914–2014. These models were chosen because they had the lowest values of the Akaike Information Criterion (AIC; data not shown).

Thus, with exception of the Biochemistry-Physiology, Functional Morphology, Therapeutics-Diagnostics, and Invertebrate-Parasite Relationships sub-disciplines, the publication trends on marine fish parasitology in *JP* suggest an increase in studies on Systematics-Phylogenetics, Ecology, Epidemiology and Behavior, Life Cycle-Surveys, and Molecular-Cell Biology. Hence, the publication trend in *JP* in marine fish parasites represents globalization with a faster publication rate, higher citation levels, and an expansion in the range of issues considered (e.g., molecular biology).

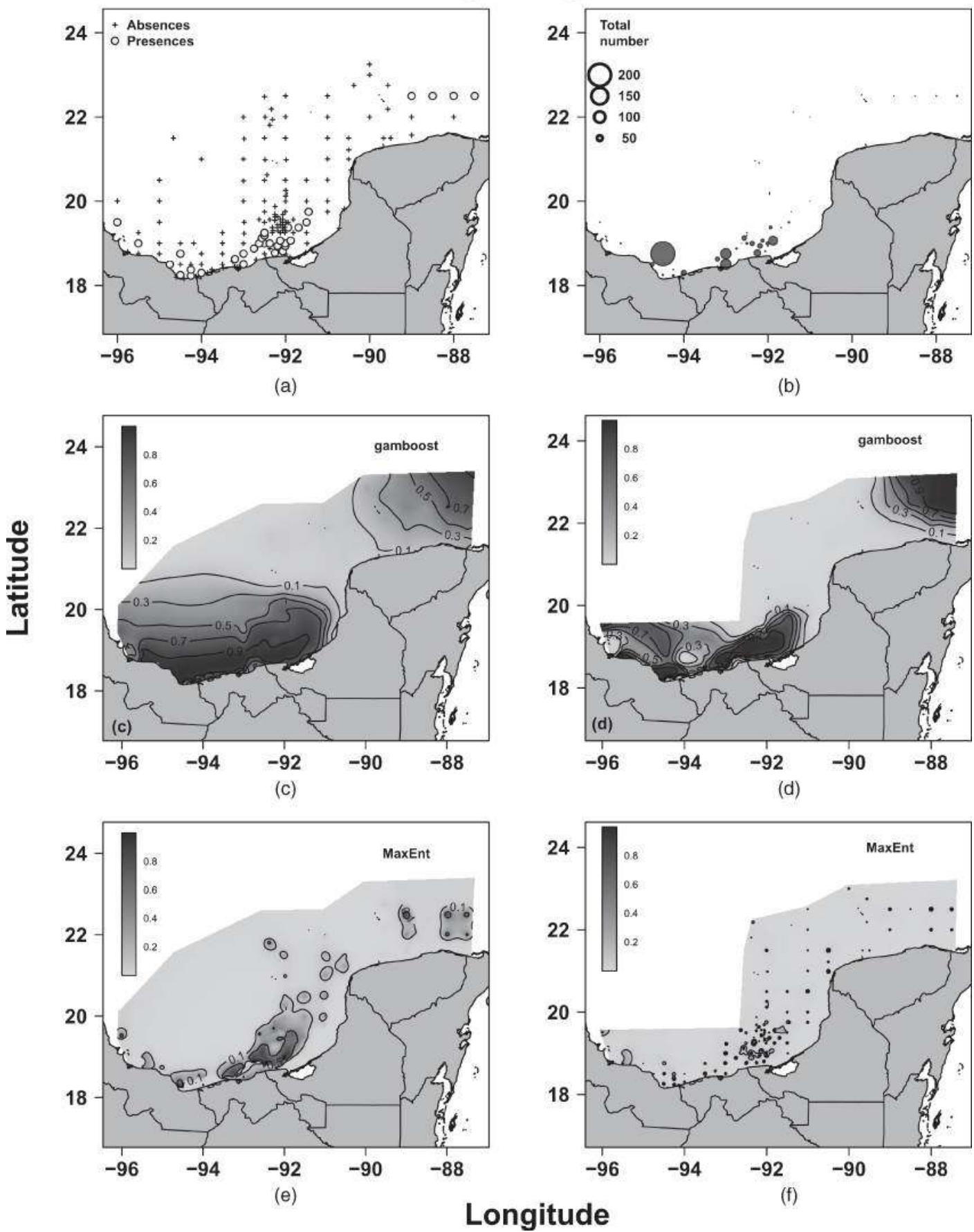
Living in an oil extraction region (The Campeche Sound in the southern Gulf of México), papers published in *JP* such as those of Marcogliese and Cone (1996), Keas and Blakespoor (1997), Koprivnikar et al. (2006), or Khan (2004) among many others on the use of parasites as bioindicators of environmental quality, have been a source of information and inspiration for us. In recognition of the inspiration provided by all these papers published in *JP*, in the next section we present the results of our research on parasites as bioindicators of environmental quality.

# **Environmental drivers of the probability of occurrence of the parasites of flatfishes in the southern Gulf of México**

During studies for more than a decade working in the southern Gulf of México to determine the environmental quality regarding sediments, water and organisms for the Mexican Oil Company (PEMEX henceforth), we have obtained data on the geographical distribution and abundance of the helminth parasites infecting flatfishes. These studies have been developed for PEMEX because inland and offshore oil extraction together with agriculture are significant economic activities in the region, and high concentrations of nutrients from river runoff, polycyclic aromatic hydrocarbons, pesticides, and other contaminants are released into the environment (e.g., García-Cuellar et al., 2004; Vidal-Martínez et al., 2006). As stated in the methods section, to study the potential effect of natural environmental factors (e.g., oxygen concentration, salinity, pH), nutrients, and chemical pollutants on the probability of occurrence of parasites, we selected the larval cestode *Oncomegas wageneri* and the adult acanthocephalan *Acanthocephaloïdes plagiuseae* infecting flatfishes. Because these parasites have transmission stages and intermediate hosts exposed to the environment, we hypothesized that the probability of occurrence of these parasites should reflect the environmental conditions experienced. Thus, the objective of our research was to determine whether the probability of occurrence of *O. wageneri* and *A. plagiuseae* present statistical associations with natural physicochemical environmental variables, nutrients, and polycyclic aromatic hydrocarbons in the southern Gulf of México.

The number of sampling sites where *O. wageneri* was present and the total number of individuals collected per sampling site are shown in Figs. 5a and 5b. Figure 5c shows the probability of *O. wageneri* occurrence, which had a strong statistical association with high molecular weight polycyclic aromatic hydrocarbons (PAHH) (selected by the boosting GAM with a 95% frequency, Table 1). All of the remaining spatial and nutrient variables showed a minor frequency of selection (5%) (Table 1). The boosted GAM for *O. wageneri* across the “full area” showed that the probability of occurrence for this parasite was high for the region between Cayo Arcas and the Coatzacoalcos River mouth (grey and black zones), and that probability strongly decreases with depth and distance from these areas. The continental shelf of the Yucatán Peninsula and the oceanic region in the middle of the Gulf of México had a very low probability of occurrence of *O. wageneri*. There was another area with a high probability of occurrence of this parasite: the northeast corner of the Yucatán Peninsula. Thus, the probability of *O. wageneri* occurrence based on the boosted GAM for the “full area” (Fig. 5c) closely resembles the actual spatial distribution of *O. wageneri* in the study area (Figs. 5a and 5b).

## *Oncomegas wageneri*



**Figure 5** Geographical distribution of *Oncomegas wageneri* (Cestoda: Trypanorhyncha) in the southern Gulf of Mexico. (a) Presence (+) and absences (o) of *O. wageneri* on the 162 sampling sites of the oceanographic expedition Xcambo 2 between September and October, 2005. (b). Total number of *O. wageneri* per sampling site. (c) Probability of occurrence of *O. wageneri* using a boosted General Additive Model for the “full area” (n = 162 sampling sites). (d) Probability of occurrence of *O. wageneri* using a boosted General Additive Model for the “polygon area” (n = 134 sampling sites 1500 m depth or above). (e) Probability of occurrence of *O. wageneri* using MaxEnt for the “full area.” (f) Probability of occurrence of *O. wageneri* using MaxEnt for the “polygon area.”

**Table 1** Environmental variables, nutrients and pollutants selected by the boosted general additive model for the “full area” and “polygon area” models for *Oncomegas wageneri* (*O. wageneri*) and *Acanthocephaloïdes plagiuseae* (*A. plagiuseae*). The mboost algorithm uses bootstrap estimates to undertake a cross-validation to prevent overfitting. This cross-validation generates a variable selection process that provides the frequency (Fr (%)) at which each variable is selected during a bootstrap process. These frequencies are a proxy of the importance (expressed in percentage) of each variable within the model. Abbreviations were as follows: bbs = a penalized regression spline base learner, bspatial = a bivariate tensor product P-spline base learner, DD = decimal degrees, PAHH = polyaromatic hydrocarbons of high molecular weight, PAHL = polyaromatic hydrocarbons of low molecular weight, S = sediment, W = water. The principal coordinates of neighbour matrices (PCNM) variables were used to factor the contribution of unknown environmental variables acting at three different spatial scales (see Santana-Piñeros et al., 2012 for a detailed explanation)

	Independent variables	Units	<i>O. wageneri</i> “full area” Fr (%)	<i>O. wageneri</i> “polygon area” Fr (%)	<i>A. plagiuseae</i> “full area” Fr (%)	<i>A. plagiuseae</i> “polygon area” Fr (%)
1	bbs (Aliphatic PAHs, S)	µg/g	—	0.60	—	—
2	bbs (Alkalinity, W)	meq/l	0.13	0.10	—	—
3	bbs (Clay, S)	%	0.04	—	0.40	4.80
4	bbs (CO <sub>2</sub> , W)	mmol/l	0.13	0.10	—	2.50
5	bbs (Depth, S)	m	—	4.80	—	—
6	bbs (Nitrate, W)	µMolar	0.21	3.00	—	0.50
7	bbs (Nitrogen, S)	micromol/g	—	0.30	1.20	6.00

8	bbs (Oxygen, W)	(mg/L)	0.17	5.00	—	—
9	bbs (PAHH, S)	µg/g	<b>94.66</b>	6.90	<b>90.32</b>	2.10
10	bbs (PAHL, S)	µg/g	0.47	5.70	—	3.70
11	bbs (PCNM2)	DD	0.30	<b>24.00</b>	2.30	2.30
12	bbs (PCNM21)	DD	0.47	3.70	—	6.60
13	bbs (PCNM58)	DD	0.68	11.90	—	2.00
14	bbs (Phosphate, W)	µMolar	0.09	0.70	—	5.30
15	bbs (Phosphorus, S)	micromol/g	0.04	0.20	—	0.20
16	bbs (Redox potential, S)	millivolts	—	—	—	<b>33.00</b>
17	bbs (Salinity, W)	UPS	0.13	0.60	0.40	1.10
18	bbs (Sand, S)	%	—	0.50	—	—
19	bbs (Sigma T, W)	Kg/m <sup>3</sup>	0.30	—	1.20	1.70
20	bbs (Silicate, W)	µMolar	—	1.10	—	5.80
21	bbs (Silt, S)	%	0.68	6.50	0.68	6.50
22	bbs (Temperature, W)	°C	0.09	5.40	—	—
23	bspatial (Lat, Long)	DD	1.45	18.90	3.50	17.10

For the “polygon” area ([Fig. 5d](#)), the spatial distribution of the probability of *O. wageneri* occurrence was similar to the one using the “full area” ([Fig. 5c](#)). However, the number of independent variables differed between the models, with 17 for the “full area” and 20 for the “polygon” model ([Table 1](#)). For the “polygon,” the most important component of the model was related to three spatial variables (55% frequency all together), followed by the PAHH (6.90%). The MaxEnt model for *O. wageneri* ([Figs. 5.5e](#) and [5f](#)) for the “full area” and for the “polygon” performed poorly in predicting the probability of occurrence of this parasite ([Fig.](#)

5a). The performance statistics (kappa, AUC, and pROC) for the boosted GAM for the “full area” and the “polygon” for *O. wageneri* were all above 0.8, which is considered a good value for the performance of these kind of models (Table 2). By contrast, all the performance statistics in the MaxEnt models for this parasite for the “full area” and the “polygon” area were below 0.8 (Table 2).

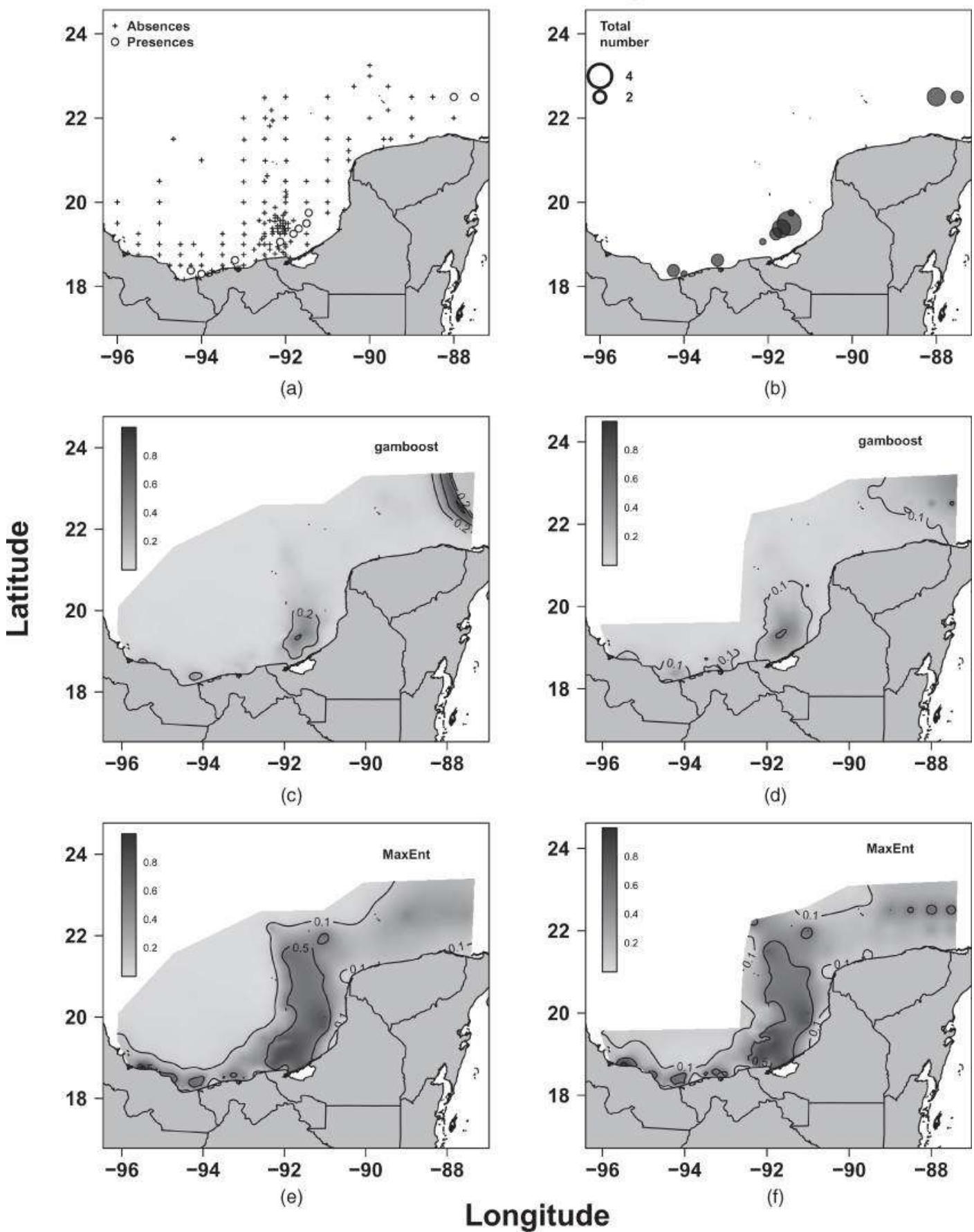
**Table 2** Performance statistics of the GAMmboost and MaxEnt models for *Oncomegas wageneri* (*O. wageneri*) and *Acanthocephaloïdes plagiuseae* (*A. plagiuseae*) for the “full area” (n = 162 sampling sites) and the “polygon area” (n = 134 sampling sites). Acronyms are as follows: Kappa = Cohen's Kappa, AUC = Area under the curve, pROC = Partial receiver operator curve. Kappa ranges from -1 (total disagreement) through 0 (random classification) to 1 (total agreement). For AUC and pROC curves, values below 0.5 indicate a performance no better than random, values between 0.7 and 0.8 are considered useful, and values >0.8 are excellent (Hosmer and Lemeshow, 2000)

	<i>O. wageneri</i> “full area”	<i>O. wageneri</i> “polygon area”	<i>A. plagiuseae</i> “full area”	<i>A. plagiuseae</i> “polygon area”
<b>GAMmboost</b>				
Kappa	0.889	0.958	0.912	0.968
AUC	0.997	0.998	0.985	0.997
pROC	0.994	0.991	0.908	0.998
<b>MaxEnt</b>				
Kappa	0.385	0.223	0.182	0.250
AUC	0.642	0.625	0.500	0.607
pROC	0.474	0.539	0.474	0.474

The number of sampling sites where *A. plagiuseae* was present and the total number of individuals collected per sampling site are shown in Figs. 6a and 6b. Figure 6c shows the probability of *A. plagiuseae* occurrence, which had a strong statistical association with high molecular weight polycyclic aromatic hydrocarbons (PAHH) (90% frequency of selection, Table 1). By contrast, all the remaining spatial and nutrient variables showed a minor frequency of selection (10%) (Table 1). The boosted GAM of *A. plagiuseae* for the “full area” shows that the probability of occurrence of this parasite is relatively high for the oceanic region just in front of Carmen Island. However, there was a substantial decrease in the probability of occurrence of this parasite to the west along the coast. Also, with exception of the northwest corner, there was a very low probability of occurrence of *A. plagiuseae* for most of the continental shelf of the Yucatán Peninsula (Fig. 6c). Thus, Fig. 6c resembles closely the actual spatial distribution of *A. plagiuseae* in the study area (Figs. 6a and 6b). All the performance statistics for the boosted GAM for the “full area” for *A. plagiuseae* were all above 0.9 (Table 2). For the “polygon” (Fig. 6d), the spatial distribution of the probability of occurrence of *A. plagiuseae* was similar to

[Fig. 6c](#), and even with higher probability values. However, the number of the independent variables in the model notably increased ( $n = 20$ ) in comparison with [Fig. 6c](#) ( $n = 8$  variables; [Table 1](#)). For the “polygon” ([Fig. 6d](#)), the Redox Potential presented a frequency of providing 33% of variability, followed by the spatial component (17%; [Table 1](#)). The MaxEnt models for *A. plagiuseae* ([Fig. 6e](#) and [6f](#)) for the “full area” and the “polygon” had a poor performance in predicting the probability of occurrence of this parasite. The performance statistics for the MaxEnt models for *A. plagiuseae* were near or below 0.5, the threshold to consider the model no different than random ([Table 2](#)).

# *Acanthocephalooides plagiustae*



**Figure 6** Geographical distribution of *Acanthocephaloïdes plagiuseae* (Acanthocephala: Arhythmacanthidae) in the southern Gulf of Mexico. (a) Presence (+) and absences (o) of *A. plagiuseae* on the 162 sampling sites of the oceanographic expedition Xcambo 2 between September and October, 2005. (b). Total number of *A. plagiuseae* per sampling site. (c) Probability of occurrence of *A. plagiuseae* using a boosted General Additive Model for the “full area” (n = 162 sampling sites). (d) Probability of occurrence of *A. plagiuseae* using a boosted General Additive Model for the “polygon area” (n = 134 sampling sites 1500 m depth or above). (e) Probability of occurrence of *A. plagiuseae* using MaxEnt for the “full area.” (f) Probability of occurrence of *A. plagiuseae* using MaxEnt for the “polygon area.”

The main hypothesis tested in this section was that because *O. wageneri* and *A. plagiuseae* have transmission stages and intermediate hosts exposed to a polluted environment, their probability of occurrence should reflect the environmental conditions experienced in the southern Gulf of México. Our results suggest that this pattern occurred. *Oncomegas wageneri* and *A. plagiuseae* (Figs. 5c and 6c) were evidently very much influenced not only by the distribution of the shoal flatfishes but also by the PAHH and nutrients ([Table 1](#)). With respect to the models two points were remarkable: first, the size of the background area (“full area” and “polygon”) was not relevant for the good performance of the boosted GAMs; and second, boosted GAMs would be a better choice for the analysis of parasitological data sets of the type presented here, in view of the surprisingly poor performance of MaxEnt. This result was a very unusual one because the MaxEnt method is normally very reliable even with few occurrences (Phillips et al., 2006). Thus, due to their poor performance, no further interpretation of the MaxEnt models was considered necessary. Applying the criterion of parsimony for choosing the best *O. wageneri* and *A. plagiuseae* models for interpretation, we selected the boosted GAMs with the smaller number of variables in [Table 1](#).

That the PAHH variable was the one with the highest frequency in the boosted GAM models for *O. wageneri* (95%) and *A. plagiuseae* (90%) for the “full area” ([Table 1](#)) suggests that both the flatfish hosts and these parasites have been chronically exposed to these pollutants. The low molecular weight polycyclic aromatic hydrocarbons (PAHL) were present in the *O. wageneri* and *A. plagiuseae* models but with a minor frequency ([Table 1](#)). The most common index to determine the main source of PAH is the PAHL/PAHH ratio. If this ratio is <1, the most likely origin is pyrolytic (transformation of a compound caused by heat, as in forest fires), whereas values >1 indicate a petrogenic origin (produced by the hydrocarbons extraction) (Tigănuş et al., 2013). In our data set, only 15 of the 162 sampling sites had PAHL/PAHH ratios >1 (data not shown). Thus, most of the PAH to which the flatfishes and their parasites had been exposed were pyrolytic; these hydrocarbons are related to the incomplete combustion of organic matter, combustion fossil fuels, vehicular engine combustion, smelting, waste incinerators, forest fires, and coal combustion (Tigănuş et al., 2013). For the

PAHL, it is difficult to associate their origin to extractive activities because there are natural oil seeps in the region (Soto et al., 2004). Regarding the potential toxicological effects of PAHL and PAHH, none of these compounds exceeded the probable effect level (PEL) established for marine and estuarine sediment quality (Canadian Council of Ministers of the Environment, 1999; Vidal-Martínez et al., 2014), but some of them (e.g., benzo[a]pyrene) are considered carcinogenic (Vidal-Martínez et al., 2006). Therefore, whether these compounds have a direct effect on *O. wageneri*, *A. plagiussae*, or on the flatfishes remains an open question, and direct experimental exposure to these compounds will be necessary to obtain an answer.

It is likely that the presence of the PAHHs together with the N and P carried from the continent through river discharge into the marine sediments enhances the growth of hydrocarbonoclastic bacteria; these are common, free-living bacteria in marine environments and include, for example, certain species of the genera *Bacillus*, *Pseudomonas*, and *Halomonas* that feed on these compounds (Singh et al., 2014). An increase in the number of colonies of these bacteria would in turn enhance primary and secondary productivity in the area, with a consequent increase in the number of intermediate hosts, as has been suggested for regions affected by oil spills, such as the Prestige spill (Pérez del Olmo et al., 2007). The previously mentioned provision of nutrients and PAHH from the continent into coastal zones (up to 200 m depth) has been widely documented (e.g., García-Cuellar et al., 2004; Soto et al., 2004) for the marine region from Terminos Lagoon to the Coatzacoalcos River zone.

Our conclusion was that the elevated concentrations of PAHH in our study area apparently enhanced the transmission of *O. wageneri* and *A. plagiussae*. The origin of these PAHH is most probably related to human activities, but the problem is evidently still not extreme because parasite transmission is high, judging by the overall percentage of hosts infected by *O. wageneri* (51%) in the area. Additionally, there is concurrence regarding the numerical dominance of larval parasites within the infracommunities of the flatfish species studied for parasites in the southern Gulf of México, namely, the larval cestode *O. wageneri* in *S. gunteri*, a juvenile nematode (Ascarididae) in *Ciclopsetta chittendeni*, and the larval digenean *Stephanostomum* sp. in *Sympurus plagiussa*, all as shown by Vidal-Martínez et al. (2014), Rodríguez-González and Vidal-Martínez (2008), and Centeno-Chalé (2012). Whether this pattern reflects a transition from contaminant-sensitive to contaminant-resistant parasites in the composition of species that infect these three flatfish species is difficult to assess. However, if this uncontrolled discharge of both PAHH and nutrients does not stop, a massive mortality of hosts (and their parasites), similar to that caused by the discharges of the Mississippi River (Rabelais, 2000), may occur in the near future in the southern Gulf of México.

## General conclusions

- 1.** The publication trends on marine fish parasitology in *JP* suggest an increase in studies on Systematics-Phylogenetics, Ecology, Epidemiology and Behavior, Life Cycles-Surveys, and Molecular-Cell Biology (including Biochemistry-Physiology). Hence, the publication trend in *JP* toward parasites of marine fishes represents globalization with a faster publication rate, and higher citation levels in these sub-disciplines.
- 2.** There has not been a significant increase in the number of citations to the papers published in Functional Morphology, Therapeutics-Diagnostics, and Invertebrate-Parasite Relationships in *JP* over the last 100 years. The lack of an increase in the number of citations seems to be related to the low number of papers published in these sub-disciplines in *JP*.
- 3.** There has been an increase in the number of countries publishing in *JP* on parasites of marine fishes in the last 20 years, most likely due to the arrival of the Internet. This trend brings us to consider whether *JP* should go electronic only.
- 4.** After the comparison of the performance of five international parasitology journals, the results suggested that *JP* publishes twice the number of papers on parasites of marine fishes in comparison with similar journals. Apparently, the reason for the similar number of citations between European journals (taking *Parasitology Research* as an example) and *JP* is related to the relative size of the parasitological community in the EU.
- 5.** The results of the study of the effect of chemical and sewage pollution on parasites of flatfishes in the southern Gulf of México, suggested that the elevated concentrations of PAHH enhanced the transmission of the cestode *O. wageneri* and the acanthocephalan *A. plagiuseae*. The origin of these PAHH is without a doubt related to human activities, and attention is brought to the fact that, if this uncontrolled discharge of both PAHH and nutrients does not stop, a massive mortality of economically important organisms (and their parasites), similar to that caused by the discharges of the Mississippi River (Rabelais, 2000), could be expected in the southern Gulf of México.
- 6.** Studies as the one developed on the southern Gulf of México in parasites of flatfishes, would not be possible without all the knowledge on parasites taxonomy, life cycles, distribution, etc. So, please continue providing this work because every little bit helps!

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## Literature cited

- Baldwin, R. E., Rew, M. B., Johansson, M. L., Banks, M. A. and Jacobson, K. C. 2011. Population Structure of Three Species of *Anisakis* Nematodes recovered from Pacific sardines (*Sardinops sagax*) distributed throughout the California current system. *Journal of Parasitology* **97**: 545–554.
- Bray, R. A. 1985. Some helminth parasites of marine fishes of South Africa: Families Gorgoderidae, Zoogonidae, Cephaloporidae, Acanthocolpidae and Lepocreadiidae (Digenea). *Journal of Natural History* **19**: 377–405.
- Bullard, S. A. and R. M. Overstreet. 2002. Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes. Proceedings of the 53rd Gulf and Caribbean Fisheries Institute: p. 10–25.
- Canadian Council of Ministers of the Environment. 1999. *Canadian sediment quality guidelines for the protection of aquatic life: Polycyclic aromatic hydrocarbons (PAHs)*. In *Canadian environmental quality guidelines*. Canadian Council of Ministers of the Environment, Winnipeg, Canada, 16 p.
- Centeno-Chalé, O. A. 2012. Efectos potenciales del impacto ambiental en la Sonda de Campeche sobre las comunidades de helmintos parásitos del “lenguado Mexicano” *Cyclopsetta chittendeni* Bean, 1985. MSc Thesis. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, Departamento de Recursos del Mar, Yucatán, México, 83 p.
- Chai, J-Y, K. D. Murrell, and A. J. Lymbery. 2005. Fish-borne parasitic zoonoses: Status and issues. *International Journal for Parasitology* **35**: 1233–1254.
- Cribb, T. H., R. A. Bray, S. C. Barker, R. D. Adlard, and G. R. Anderson. 1994. Ecology and diversity of digenetic trematodes of reef and inshore fishes of Queensland. *International Journal for Parasitology* **24**: 851–860.
- Deardorff, T. L., and R. M. Overstreet. 1980. *Contracaecum multipapillatum* (=C. robustum) from fishes and birds in the northern Gulf of Mexico. *Journal of Parasitology* **66**: 853–856.
- Durio, W. O., and H. W. Manter. 1968. Some digenetic trematodes of marine fishes of New Caledonia. Part II. *Opecoelidae and Lepocreadiidae*. *Journal of Parasitology* **54**: 747–756.
- Esch, G., S. Desser and B. Nickol. 2014. A history of *The Journal of Parasitology*. *Journal of Parasitology* **100**: 1–10.
- Falagas, M. E., P. A. Papastamataki, and I. A. Bliziotis. 2006. A bibliometric analysis of research productivity in parasitology by different world regions during a 9-year period (1995–2003). *BMC Infectious Diseases* **6**: 56.

García Cuellar, J. A., F. Arreguín Sanchez, S. Hernández Vázquez, and D. B. Lluch Cota. 2004. Impacto ecológico de la industria petrolera en la sonda de Campeche, México, tras tres décadas de actividad: una revisión. *Interciencia* **29**: 311–319.

Gold-Bouchot, G., M. Zavala-Coral, O. Zapata-Pérez, and V. Ceja-Moreno. 1997. Hydrocarbon concentrations in oysters (*Crassostrea virginica*) and recent sediments from three coastal lagoons in Tabasco, Mexico. *Bulletin of Environmental Contamination and Toxicology* **59**: 430–437.

Hofner, B., A. Mayr, N. Robinzonov, and M. Schmid. 2012. Model-based boosting in R – A hands-on tutorial using the R package mboost. *Computational Statistics*, DOI 10.1007/s00180–012–0382–5.

Holmes J. C., and P. W. Price. 1986. Communities of parasites. In *Community ecology: Patterns and processes*, Anderson D. J., and J. Kikkawa (eds.). Blackwell, Scientific Publications, Oxford, U.K., p. 187–213.

Hosmer, D. W., and S. Lemeshow. 2000. *Applied logistic regression*. John Wiley & Sons, Inc., New York, N.Y., 373 p.

Hunninen, A. V., and R. M. Cable. 1943. The life cycle of *Lecithaster confusus* Odhner (Trematoda: Hemiuridae). *Journal of Parasitology* **29**: 71–79.

Jones, S. R. M., and N. B. Hargreaves. 2007. The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon in coastal British Columbia. *Journal of Parasitology* **93**: 1324–1331.

Justine, J. L. 2010. Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia. *Belgium Journal of Zoology* **140** (Suppl.): 155–190.

Keas B. E., and H. D. Blankespoor. 1997. The prevalence of cercariae from *Stagnicola emarginata* (Lymnaeidae) over 50 years in northern Michigan. *Journal of Parasitology* **83**: 536–540.

Khan, R. A. 2004. Effect, distribution, and prevalence of *Glugea stephani* (microspora) in winter flounder (*Pleuronectes americanus*) living near two pulp and paper mills in Newfoundland. *Journal of Parasitology* **90**: 229–233.

\_\_\_\_\_, and R. G. Hooper. 2007. Influence of the termal discharge on parasites of a cold-water flatfish *Pleuronectes americanus*, as a bioindicator of subtle environmental change. *Journal of Parasitology* **93**: 1227–1230.

\_\_\_\_\_, C. V. Chandra and P. Earle. 2011. Comparison of metazoan parasites of Atlantic cod, *Gadus morhua*, from three geographical areas of coastal Newfoundland. *Journal of Parasitology*, **97**: 270–274.

Koprivnikar, J., R. L. Baker, and M. R. Forbes. 2006. Environmental factors

influencing trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles in Southern Ontario. *Journal of Parasitology* **92**: 997–1001.

Levron, C., L. G. Poddubnaya, R. Kuchta, M. Freeman, Y-H. Wang, and T. Scholz. 2008. Sem and Tem study of the armed male terminal genitalia of the tapeworm *Paraechinophallus japonicus* (Cestoda: Bothriocephalidea). *Journal of Parasitology*, **94**: 803–810.

Linton, E. 1915. *Tocotrema lingua* (Creplin): The adult stage of a skin parasite of the cunner and other fishes of the Woods Hole region. *Journal of Parasitology* **1**: 128–134.

Llarena-Reino, M. A., E. Abollo, M. Regueira, H. Rodríguez, and S. Pascual. 2015. Horizon scanning for management of emerging parasitic infections in fishery products. *Food Control* **49**: 49–58.

Marcogliese, D. J., and D. K. Cone. 1996. On the distribution and abundance of eel parasites in Nova Scotia: Influence of pH. *Journal of Parasitology* **82**: 389–399.

Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary. *Advances in Parasitology* **66**: 47–148.

\_\_\_\_\_, \_\_\_\_\_, R. Clanchi, L. Paggi, P. Arduino, L. Margolis, J. Brattey, S. Webb, S. D'Amelio, P. Orecchia, and L. Bullini. 1997. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *Journal of Parasitology* **83**: 401–416.

Miquel, J., Z. Świderski, L. Neifar, and C. Eira. 2007. Ultrastructure of the spermatozoon of *Parachristianella trygonis* dollfus, 1946 (Trypanorhyncha: Eutetrarhynchidae) *Journal of Parasitology*, **93**: 1296–1302.

Ogawa, K., M. G. Bondad-Reantaso, M. Fukudome, and H. Wakabayashi. 1995. *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology* **81**: 223–227.

Olson, P. D., and J. N. Caira. 1999. Evolution of the major lineages of tapeworms (Platyhelminthes: Cestoidea) inferred from 18s ribosomal DNA and elongation factor-1α. *Journal of Parasitology* **85**: 1134–1159.

Pérez-del Olmo, A., J. A. Raga, A. Kostadinova, and M. Fernández. 2007. Parasite communities in *Boops boops* (L.) (Sparidae) after the Prestige oil-spill: detectable alterations. *Marine Pollution Bulletin* **54**: 266–276.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**: 231–259.

- Rabelais, N. 2000. Nitrogen in aquatic ecosystems. *Ambio* **31**: 102–112.
- Rodríguez-Gonzales A., and V. M. Vidal-Martínez. 2008. Las comunidades de helmintos del lenguado (*Sympodus plagiusa*) en la costa de Campeche, México. *Revista Mexicana de Biodiversidad* **79**: 159–173.
- Santana-Piñeros, A. M., D. Pech, and V. M. Vidal-Martínez. 2012. Spatial structure of the helminth parasite communities of the tonguefish, *Sympodus plagiusa*, from the Campeche coast, southern Mexico. *International Journal for Parasitology*, **42**: 911–920.
- Sánchez-Ramírez, C., V. M. Vidal-Martínez, M. L. Aguirre-Macedo, R. Rodríguez-Canul, G. Gold-Bouchot, and B. Sures. 2007. *Cichlidogyrus sclerosus* (Monogenea: Ancyrocephalinae) and its host the Nile tilapia *Oreochromis niloticus* as bioindicators of chemical pollution. *Journal of Parasitology* **93**: 1097–1106.
- Scholz, T., and M. L. Aguirre-Macedo. 2000. Metacercariae of trematodes parasitizing freshwater fish in Mexico: a reappraisal and methods of study. In *Metazoan parasites in the neotropic: Ecological, taxonomic and evolutionary perspectives*. Commemorative Volume of the 70th Anniversary of the Instituto de Biología, Universidad Nacional Autónoma de México, A. N., G. García-Aldrete, G. Salgado-Maldonado, and V. M. Vidal-Martínez (eds.). Instituto de Biología, Universidad Nacional Autónoma de México, México D.F., México, p. 85–99.
- Singh, A. K., A. Sherry, N. D. Gray, D. M. Jones, B. F. J. Bowler, and I. M. Head. 2014. Kinetic parameters for nutrient enhanced crude oil biodegradation in intertidal marine sediments. *Frontiers in Microbiology* **5**: 1–13.
- Solangi, M. A. and R. M. Overstreet. 1980. Biology and pathogenesis of the coccidium *Eimeria funduli* infecting killifishes. *Journal of Parasitology* **66**: 513–526.
- Soto, L. A., S. Sánchez-García, and D. López-Veneroni. 2004. Environments influenced by natural sites of gas and fossil hydrocarbon seeps in the SW Gulf of Mexico. *Universidad y Ciencia, Número especial I*: **51–58**.
- Țigănuș, D., V. Coatu, L. Lazăr, A. Oros, and A. D. Spînu. 2013. Identification of the sources of polycyclic aromatic hydrocarbons in sediments from the Romanian Black Sea sector. *Cercetări Marine* **43**: 187–196.
- Torres-Irineo, E., J. Justin Amande, D. Gaertner, A. Delgado de Molina, H. Murua, P. Chavance, J. Ariz, J. Ruiz, and N. Lezama-Ochoa. 2014. By catch species composition over time by tuna purse seine fishery in the eastern tropical Atlantic Ocean. *Biodiversity and Conservation* **23**: 1157–1173.
- Vidal-Martínez V. M., M. L. Aguirre-Macedo, R. Del Rio Rodríguez, G. Gold-Bouchot, J. Rendón-von Osten, and G. A. Miranda-Rosas. 2006. The pink shrimp

*Farfantepenaeus duorarum*, its symbionts and helminths as bioindicators of chemical pollution in Campeche Sound, Mexico. *Journal of Helminthology* **80**: 159–174.

Vidal-Martínez, V. M., D. Pech, B. Sures, S. T. Purucker, and R. Poulin. 2010. Can parasites really reveal environmental impact? *Trends in Parasitology* **26**: 44–51.

\_\_\_\_\_, A. Centeno-Chalé, E. Torres-Irineo, G. Gold-Bouchot, J. I. Sánchez-Ávila, and M. L. Aguirre-Macedo. 2014. The metazoan parasite communities of the shoal flounder (*Syacium gunteri*) as bioindicators of chemical contamination in the southern Gulf of Mexico. *Parasites and Vectors* **27**: 541.

Whipps, C. M., G. Grossel, R. D. Adlard, H. Yokoyama, M. S. Bryant, B. L. Munday, and M. L. Kent. 2004. Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. *Journal of Parasitology* **90**: 618–622.

Woo, P. T. K., D. W. Bruno, and L. H. S. Lim. 2002. *Diseases and disorders of finfish in cage culture*. CABI Publishing, Wallingford, U.K., 354 p.



## **Part II**

# **Ecology and Life History**



# **Chapter 6**

## **Seasonal Fluctuation in the Infestation of *Planorbis* *Trivolvis* with Larval Trematodes\***

Oliver R. McCoy

Practically no attention has been given to the seasonal fluctuation in the degree of infestation of snails with larval trematodes. It has often been suggested that there is seasonal variation in the appearance of cercariae in their molluscan hosts but only a few extensive surveys have been made to determine the amount and nature of the variation.

The first extended observation of seasonal infestation with larval trematodes was made in the study of schistosomiasis in Egypt by Manson-Bahr and Fairley (1920) who kept a record of the cercariae found in the snails which they dissected over a period of one year. They found the cercariae of the human blood flukes, *Schistosoma haematobium* and *S. mansoni*, present in the snails at all seasons of the year. The highest percentage of infested snails occurred during the month of December. They note also that the infestations found in the autumn months were all mature, whereas in the spring months immature cercariae were the rule rather than the exception. Their observations on the other larval trematodes found in the snails indicate the same general trend, for the snails were infested throughout the year, with the highest percentage occurring in December. Their study was made in a part of the world in which drought has a great influence on the snail population and where there is not a marked fluctuation in temperature from season to season.

Soparkar (1921) published a two year record of the prevalence of the cercaria of *Schistosoma spindale* in the Indian fresh-water snail, *Planorbis exustis*, and found the percentage of infested snails highest in the autumn months and lowest during late winter and early spring. His observations were based on 25,000 individuals, but he recorded only the cercariae which emerged from the snails and did not dissect the snails to determine the total infestation.

Sewell (1922) reported a curve of the infestation with larval trematodes for the Indian fresh-water snail, *Melanoides tuberculatus*, in which the high points were in December and July. Sewell's curve, however, was based on only 139 snails. The most complete survey of the seasonal

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\* This investigation was conducted in the Zoological Laboratory of Washington University, St. Louis, from October, 1925, to October, 1927, under the direction of Dr. H. M. Miller, Jr., whom the writer takes great pleasure in thanking for his friendly interest in the work and his many valuable criticisms and suggestions.

fluctuation in the appearance of trematode cercariae was made by Miller and Northup (1926) over a 12 month period on the marine snail, *Nassa obsoleta*, from Woods Hole, Massachusetts. They examined over 8,000 specimens and found a definite semi-annual rise and fall in the infestation, the high points occurring in December and July. In their study the percentage of parasitized snails was quite low, ranging from 2.3 to 8.5%. Various isolated observations have been recorded on the seasonal occurrence of larval trematodes but records of no other extended surveys have been found.

A knowledge of the seasonal appearance of larval trematodes would not only suggest their probable life cycles but might also throw light on some general points upon which very little is known. Such problems, for example, as the length of time that the larvae parasitize the snails, or their possibility of surviving the winter in the molluscan host have never been definitely worked out. The purpose of this survey was to observe over a two-year period the seasonal infestation with larval trematodes of a fresh-water snail, *Planorbis trivolvis* Say, from a limited locality.

Snails were collected from Ramona Park Lake near St. Louis, Missouri, at intervals of from three to four weeks. The lake, which is about 150 yards long and 50 yards wide, is artificial but has been in existence for over 30 years. *Planorbis trivolvis* and *Physa integra* Haldeman are the only species of snail present. Four species of fish, and muskrats, turtles, snakes, frogs, and numerous migratory birds are possible vertebrate hosts known to live in or frequent the lake. The area is closely circumscribed, for no streams flow into the lake and no other bodies of water are near it.

Twenty-nine collections of *P. trivolvis* have been made over the two year period and a total of over 6,500 snails examined. *P. trivolvis* was abundant in the spring, summer, and fall and there was no difficulty in collecting several hundred specimens on each trip. The snails were always taken from only one side of the lake and as far as possible full-grown individuals were selected. In the winter, collections were made by breaking through the ice and it was sometimes difficult to obtain a large number. The snails were brought into the laboratory and isolated in glass vials. If no cercariae emerged in 48 hours, the snails were killed and dissected. Any infestation which did not give emerged cercariae in 48 hours was considered to be immature regardless of whether or not on dissection of the snail the cercariae appeared to be fully developed.

In the course of the study, six different species of cercariae were found in *P. trivolvis* but of these, only two were sufficiently abundant to furnish adequate data for observations on the seasonal infestation. The complete data of the occurrence of these two species, *Cercaria hamata*

Miller, 1923, and the cercaria of *Plagiorchis ameiurensis* McCoy, 1928, are given in the table.

The percentage of the snails in each collection which were infested with these two species are plotted in Figure 1, curves II and V. The percentage of the snails examined which harbored mature infestations, i. e., cercariae emerged in 48 hours, is also given in Figure 1, curves III and VI. The former curves show the prevalence of the cercariae at any certain season of the year, while the latter show the percentage of snails in which the cercariae were fully developed. A more definite indication of the seasonal cycle, however, is given by the consideration of the percentage of the infested snails in each collection in which the cercariae were mature. For instance, if in one collection, all of the infestations are immature, the indication is that the snails have recently been infected

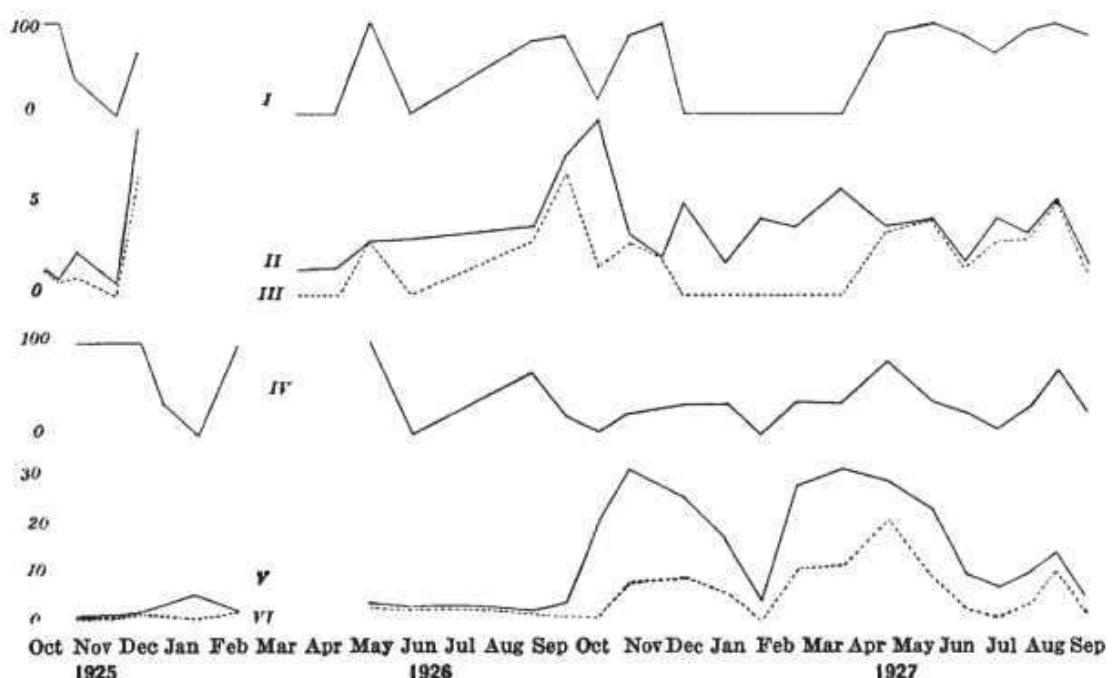


Figure 1.—Graphs showing the infestation with larval trematodes of *Planorbis trivolvis* from October, 1925, to October, 1927.

I. *Cercaria hamata*, percentage of the infested snails which gave emerged cercariae.

II. Percentage of snails infested with *C. hamata*.

III. Percentage of the snails harboring mature infestations of *C. hamata*.

IV. The cercaria of *Plagiorchis ameiurensis*, percentage of the infested snails which gave emerged cercariae.

V. Percentage of snails infested with the cercaria of *P. ameiurensis*.

VI. Percentage of the snails harboring mature infestations of the cercaria of *P. ameiurensis*.

and that sufficient time has not elapsed for cercariae to mature. On the other hand if all are mature, it is probable that no new infection has taken place. To illustrate this relationship curves have been plotted of the percentage of the infestations in each collection which were mature (Figure 1, curves I and IV).



The first of the two cercariae to be considered is *Cercaria hamata*, a furcocercous larva which was found experimentally to penetrate into the sunfish, where it developed into a larval holostome (McCoy, 1928). The definitive host is not known, but the cyclic fluctuations in the sea-

*Data of Infestation with Larval Trematodes of Planorbis trivolvus Collected from a Small Lake Near St. Louis, Mo.*

Date of Collection	No. of Snails	Species of Cercaria	No. of Infested Snails	Per Cent of Infested Snails	No. of Mature Infestations	Per Cent of Snails With Mature Infestations	Per Cent of Infestations Which Were Mature
10/ 5/25	68	<i>Cercaria hamata</i> .....	1	1.5	1	1.5	100
10/14/25	120	<i>C. hamata</i> .....	1	0.9	1	0.9	100
10/28/25	189	<i>C. hamata</i> .....	5	2.5	2	1.0	40
		<i>C. of Plagiocercis amelurensis</i> .....	1	0.5	1	0.5	100
11/25/25	149	<i>C. hamata</i> .....	1	0.7	0	0.0	0
		<i>C. of P. amelurensis</i> .....	1	0.7	1	0.7	100
12/ 9/25	76	<i>C. hamata</i> .....	7	9.2	5	6.6	71
		<i>C. of P. amelurensis</i> .....	1	1.3	1	1.3	100
1/18/26	17	<i>C. of P. amelurensis</i> .....	1	5.9	0	0.0	0
2/13/26	65	<i>C. of P. amelurensis</i> .....	1	1.5	1	1.5	100
3/17/26	133	.....	0	0.0	0	0.0	
3/24/26	143	<i>C. hamata</i> .....	2	1.4	0	0.0	0
4/19/26	207	<i>C. hamata</i> .....	3	1.5	0	0.0	0
5/13/26	234	<i>C. hamata</i> .....	7	3.0	7	3.0	100
		<i>C. of P. amelurensis</i> .....	3	1.3	1	0.4	33
6/10/26	321	<i>C. hamata</i> .....	10	3.1	0	0.0	0
		<i>C. of P. amelurensis</i> .....	1	0.3	0	0.0	100
8/30/26	275	<i>C. hamata</i> .....	10	3.7	8	2.9	90
		<i>C. of P. amelurensis</i> .....	3	1.1	2	0.7	67
9/21/26	290	<i>C. hamata</i> .....	22	7.6	19	6.6	86
		<i>C. of P. amelurensis</i> .....	10	3.5	2	0.7	20
10/13/26	197	<i>C. hamata</i> .....	19	9.6	3	1.5	16
		<i>C. of P. amelurensis</i> .....	42	21.8	1	0.5	2
11/ 2/26	212	<i>C. hamata</i> .....	7	3.3	6	2.8	86
		<i>C. of P. amelurensis</i> .....	69	32.5	16	7.5	23
11/26/26*	136	<i>C. hamata</i> .....	3	2.2	3	2.2	100
		<i>C. of P. amelurensis</i> .....	9	6.6	4	2.9	44
12/10/26	214	<i>C. hamata</i> .....	11	5.1	0	0.0	0
		<i>C. of P. amelurensis</i> .....	56	26.2	19	8.9	34
1/ 8/27	122	<i>C. hamata</i> .....	2	1.7	0	0.0	0
		<i>C. of P. amelurensis</i> .....	21	17.8	7	5.7	33
1/31/27	72	<i>C. hamata</i> .....	3	4.2	0	0.0	0
		<i>C. of P. amelurensis</i> .....	3	4.2	0	0.0	0
2/22/27	264	<i>C. hamata</i> .....	10	3.8	0	0.0	0
		<i>C. of P. amelurensis</i> .....	77	29.2	28	10.7	38
3/21/27	189	<i>C. hamata</i> .....	11	5.8	0	0.0	0
		<i>C. of P. amelurensis</i> .....	62	32.8	22	11.6	35
4/ 5/27	258	<i>C. hamata</i> .....	10	3.8	9	3.5	90
		<i>C. of P. amelurensis</i> .....	78	30.2	62	21.7	80
5/26/27	387	<i>C. hamata</i> .....	16	4.2	16	4.2	100
		<i>C. of P. amelurensis</i> .....	93	24.0	35	9.0	38
6/16/27	433	<i>C. hamata</i> .....	8	1.9	7	1.6	88
		<i>C. of P. amelurensis</i> .....	43	10.0	11	2.5	25
7/ 6/27	412	<i>C. hamata</i> .....	18	4.3	12	2.9	67
		<i>C. of P. amelurensis</i> .....	30	7.3	2	0.5	7
7/26/27	446	<i>C. hamata</i> .....	15	3.3	14	3.1	93
		<i>C. of P. amelurensis</i> .....	46	10.2	15	3.3	33
8/17/27	444	<i>C. hamata</i> .....	23	5.2	23	5.2	100
		<i>C. of P. amelurensis</i> .....	65	14.5	44	10.0	68
9/ 7/27	450	<i>C. hamata</i> .....	7	1.6	6	1.3	86
		<i>C. of P. amelurensis</i> .....	20	4.4	5	1.1	25

\* 75% of snails below average size; figures not included in curve.

sonal infestation of the snails with the cercariae indicate that it is most probably a water bird which is not present during the entire year.

Consideration of the seasonal cycle of this species will begin with the collection of June, 1926, in which none of the infestations were mature. During the summer and early fall, the percentage fluctuated but by December, 1926, all were mature (Figure 1, curve I). The figures for

the fall of 1925 are based on a smaller number of parasitized snails, but nevertheless, in general, verify this observation. None of the infestations found during the winter and early spring in 1926 and again in 1927 gave emerged cercariae, but they all had the appearance of being old, that is, the digestive gland, of the snail was completely permeated by sporocysts many of which were empty and partially collapsed. By the first of May practically all of the infestations found were mature.

The condition seems to indicate that ova are dropped into the water during the spring possibly by some aquatic bird. The coolness of the water probably retards the development of these ova and the first snails infected from them are not found until about the first of June, at which time the infestations are mostly immature. In no collection in the early summer of 1927 did all of the infested snails contain only immature cercariae as was the condition in June, 1926, but the distinct drop in the percentage of mature infestations in the collection of July 6 would indicate that a new infection had taken place in the late spring. The fact that the percentage of infested snails giving emerged cercariae did not drop very low in the early summer of 1927 might be explained by the spring infection occurring gradually. Infection probably occurs again during the late summer and early fall, but by the first of December nearly all infestations are mature. Apparently the sporocysts survive the winter in the snail, but no mature cercariae are given off again until about the first of May, when practically all infestations found are mature. These conclusions are based only on the evidence from the collection data, which may be influenced by such factors as the possible early death of parasitized snails, a low degree of infection of young snails, and so on. Complete understanding of the cycle of infestation can only be reached when the definitive host is discovered.

The second form to be considered is the cercaria of *Plagiorchis ameiurensis* which belongs to the group of Xiphidiocercariae and is the larva of a fluke parasitic in the intestine of the catfish (McCoy, 1928). Since the definitive host is present in the lake throughout the year, a continual occurrence of mature infestations might be expected in a temperate climate and such seems to be the case (Figure 1, curve IV). There are no clear-cut seasonal fluctuations in the infestation, but when the record as a whole is considered, great variation may be seen from time to time (Figure 1, curve V). Up until October, 1926, the percentage of infestation had been consistently very low, never exceeding 6%, but in the succeeding collections the percentage jumped to over 32%, falling irregularly during the course of the year, until in the fall of 1927 it was again below 10%. It is remarkable that in a seemingly balanced habitat, the occurrence of an established species should undergo such great variation, for reasons not apparent on the surface.

As a further observation on the instability of the larval trematode fauna of the lake, it may be noted that of the four other species of cercariae which were found in the study, but whose data of infestation are not included, one was present in only two collections during the first few months of the survey. Another species did not appear until the summer of 1927. The other two species were more or less irregular in their occurrence and always showed a very low percentage of infestation.

#### SUMMARY

The seasonal infestation with larval trematodes has been recorded for over 6,500 specimens of *Planorbis trivolvis* from a small lake near St. Louis, Missouri, over a period of two years. Although the data do not show any clearcut seasonal fluctuations, the degree of infestation varies widely from time to time.

From the curve of percentage of mature infestations of a furcocercous species, *Cercaria hamata* Miller, 1923, some inferences have been drawn as to the nature of the seasonal cycle. This larva is apparently able to survive the winter in the snail host.

Mature infestations of the cercaria of *Plagiorchis ameiurensis* McCoy, 1928, a fluke parasitic in the catfish, occur at all seasons of the year, a condition which, in a temperate climate, might be expected of a form whose definitive host is present in the lake throughout the year.

The exceedingly great variation in the percentage of infestation of the snails with the cercaria of *Plagiorchis ameiurensis* indicates that the larval trematode fauna of the lake is probably never in a very stable condition. Also the occasional appearance of new species and disappearance of old ones show a continual state of change, depending probably upon the visitations of the vertebrate hosts. The great variability in the degree of infestation makes isolated observations on this point of comparatively little value.

#### REFERENCES CITED

- Manson-Bahr, P. and Fairley, N. H. 1920.—Observations on Bilharziasis Amongst the Egyptian Expeditionary Force. *Parasit.*, 12:33-71.
- McCoy, O. R. 1928.—Life History Studies on Trematodes from Missouri. (In press.)
- Miller, H. M., Jr. 1923.—Notes on Some Furcocercous Larval Trematodes. *Jour. Parasit.*, 10:35-46.
- Miller, H. M., Jr. and Northup, Flora E. 1926.—The Seasonal Infestation of *Nassa obsoleta* (Say) with Larval Trematodes. *Biol. Bull.*, 50:490-506.
- Sewell, Seymour. 1922.—Cercariae Indicae. *Ind. Jour. Med. Res.*, 10:1-327.
- Soparkar, M. B. 1921.—The Cercaria of *Schistosoma spindale* (Montgomery). *Ind. Jour. Med. Res.*, 9:1-22.





# An overview of the history and advances in the population ecology of parasites

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During the past century, enormous advances have been made in numerous areas of parasitology, including diagnostics and therapeutics, immunology, biochemistry, physiology, and evolution, to name a few. However, not to be overlooked is the field of parasite population ecology, which has benefitted from theoretical advances, the adoption of ecological approaches, and the application of new technologies such as molecular biology, immunological techniques, remote sensing, and geographic information systems (GIS). Herein, we provide a brief overview of the history of parasite population ecology during the last 100 years, with a focus where possible on the role of *The Journal of Parasitology*, in commemoration of its centennial year.

Our overview focuses on the population ecology of the helminths, e.g., monogeneans, trematodes, cestodes, nematodes, acanthocephalans, and arthropod parasites (all of which are referred to as the so-called macroparasites). Although the scope of parasite population ecology is extremely broad, and not independent of host population ecology, our focus here is on the nature of parasite populations in non-model systems, their temporal and spatial variation, and the underlying factors leading to the loss and gain of parasites within individual hosts and within host populations. This focus reflects our research interests and it reflects the focus of much of the ecological work that has been published in *The Journal of Parasitology*.

## The early years

Early studies in parasite population ecology paralleled those in general ecology. Thus, early work was descriptive, concerned primarily with life-cycle descriptions and general host surveys. An initial pivotal study published in *The Journal of Parasitology* was published by McCoy (1928) involving an examination of over 6,500 specimens of the freshwater snail *Planorbis trivolvis* for trematode cercariae over 2 years in Ramona Park Lake near St. Louis, Missouri. Ten years later, Cort and colleagues (Cort et al., 1937, 1939) took a similar approach with samples of the freshwater snails, *Stagnicola emarginata angulata* and *Helisoma campanulatum smithii*, collected from Douglas Lake, Michigan. These studies were motivated by gaps in knowledge regarding the patency period and life span of infections in snails, the over-wintering capacity of the sporocysts/rediae, and the survival of infected hosts. In addition to establishing these key insights, their results were among the first to describe the magnitude of seasonal and annual variation in trematode recruitment into snails. Further, Cort et al. (1937) analyzed the frequency of multiple infections in samples of snails, and then speculated on mechanisms leading to the lower than expected co-occurrences of particular species pairs. Herein lay the roots of enquiry into the complexities of multi-species parasite transmission within natural host-parasite interactions, together with the complexities of interspecific interactions. It is interesting that the journal maintains a strong tradition covering the population ecology of freshwater and marine snail parasites.

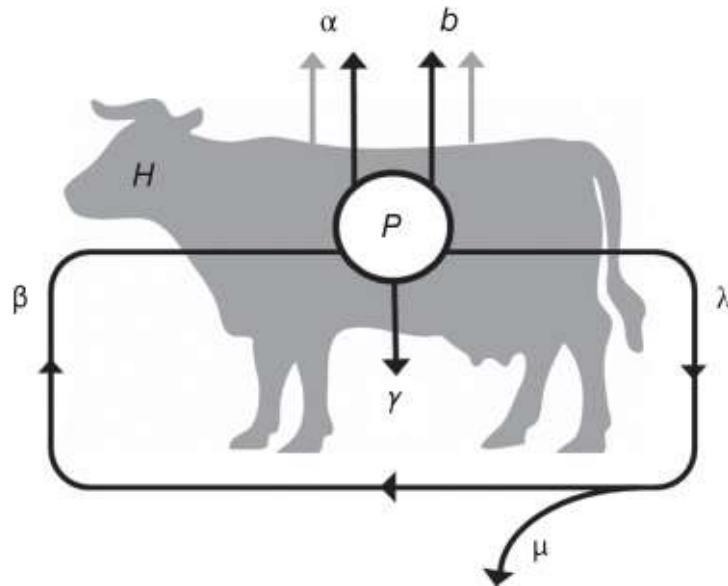
Population-level inquiry in the 1960s and 1970s tended to focus on host surveys that used “kill-and-count” approaches to quantify the numbers of parasites in individual hosts. In addition to providing early insight into the sheer magnitude of parasite biodiversity in natural systems, the results of these early surveys contributed two key pieces of information for parasite ecologists. First, individuals within a host population almost always varied in the numbers of parasites they harboured. Frequently, the magnitude of this variation was enormous. In a study involving a large sample of canvasback ducks, *Aythya valisineria*, collected from a slough in Western Canada, the range in worm (mostly gut-inhabiting cestodes) intensity spanned four orders of magnitude (Cornwell and Cowan, 1963). The results of countless host surveys, especially those involving water birds and small mammals, tended to show similar patterns (review in Kennedy, 1975). A second insight came from the results of field surveys that assessed parasite population sizes in samples of hosts collected over consecutive years. These early parasite-monitoring studies tended to show contrasting patterns. Thus, in some host-parasite interactions, parasite population sizes and prevalences were roughly consistent from year to year, whereas in others, they fluctuated wildly over time. These results inspired two key questions that would drive subsequent enquiry. Why were certain hosts in a population so heavily infected, and why did some parasite populations tend towards temporal stability, whereas others tend to wax and wane dramatically from year to year?

Extensions to these early field studies tended to focus on understanding the linkages between selected abiotic factors and estimates of parasite quantity within individual hosts (intensity, abundance, or rarely, density within host tissues) or within populations of hosts (mean intensity, mean abundance, or prevalence). Two influential studies involving freshwater fish infected with the cestode, *Caryophylleus laticeps*, showed that water temperature was the key factor that not only determined rates of transmission from intermediate hosts into fish, but also determined rates of adult worm development and rates of immunity-mediated parasite mortality (Anderson, 1974; Kennedy, 1975). Further examples of the role of temperature on the seasonality of transmission, growth, and reproduction of helminths were described by Chubb (1977, 1979, 1980, 1982) and Kennedy (1975) for helminths of fish, and by Wright (1972) for trematodes of snails. In follow-up studies involving aquatic host-parasite interactions, abiotic factors such as salinity, productivity, acidity, various contaminants, and eutrophication were also shown to directly or indirectly impact parasite population sizes, often via their action on free-living stages and/or intermediate hosts (Marcogliese, 2001, 2004, 2005).

Meanwhile, the results of laboratory-based studies that were completed at approximately the same time would also influence the future direction of population-level inquiries. Early demonstrations of the “crowding effect” in cestode and acanthocephalan populations (Read, 1951) were followed by Holmes' (1961) studies showing that competition determined the distribution, growth, and reproduction of gut helminths in laboratory rats. Thus, density dependence was a key feature determining the fitness of individual worms, with strong population-level implications. Similarly, the details of the complex vertebrate anti-parasite defense response were being described around this time, showing that patterns of worm recruitment, growth, survival, and reproduction could be strongly influenced by host immunity. These insights tended to arise from analyses of the responses of mice and other laboratory hosts to well-known parasites such as *Trichinella spiralis* and *Schistosoma mansoni*. Yet the results also provided key insight into the nature of between-host variation in parasite burdens. Thus, even under laboratory conditions when features such as exposure dose, parasite and host strain, host age, and host immune status could be carefully controlled, variation between individual hosts was still frequently observed (Sayles and Wassom, 1988). Taken together, the results of these early laboratory studies and those from field surveys foreshadowed the fascination, and the complexity, of population-level phenomena in parasites. But it was also clear that for this area of enquiry to move beyond description, to the discovery of underlying themes and ideas, a unifying synthesis was required.

## Theoretical advances

That synthesis was first provided by Crofton (1971a, 1971b). He was the first to provide a mathematical basis for the explosive development of epidemiological theory and parasite population biology and its applications to real host-parasite systems. Among his most important contributions, Crofton observed that parasites were aggregated within their host populations and could be modelled using a binomial distribution. That is, they had clumped distributions. Most hosts (about 80% in a host population) had a few parasites of a given species, while a few hosts harbored many. Indeed, Crompton considered this a general phenomenon and a defining criterion among parasite populations. Anderson and May (1979) and May and Anderson (1979) extended Crofton's approach, placing focus on the characterization of numerous rate parameters, some of which increased, and others that decreased, parasite population sizes. They argued that to fully understand the inherent variation characterizing real parasite populations in samples of hosts, including those in humans and in domestic livestock, researchers needed to understand variation in the rate that parasites were transmitted to and among hosts, the degree of immune system activation, the rate of natural parasite mortality, and so on. A schematic example of their general approach is shown in [Fig. 1](#). This example depicts the complex and interacting suite of rate parameters involved in determining gains and losses of adult and larval parasites in a simple, direct life-cycle macroparasite.



**Figure 1** Schematic representation of the gains and losses in numbers of individuals for a direct life-cycle macroparasite ( $P$ ) in a hypothetical host ( $H$ ). Gains and losses in a hypothetical parasite population are defined by parasite birth rate ( $\lambda$ ), parasite death rate ( $\mu$ ), transmission rate ( $\beta$ ) and by the death of hosts due to infection ( $\alpha$ ) and other causes ( $b$ ). (Figure courtesy of Lori Goater, from Goater, Goater & Esch 2014. Reproduced with permission of Cambridge University Press.)

The models developed by Anderson and May were also the first to emphasize

parameters such as Parasite Reproductive Rate ( $R_0$ ) and Transmission Thresholds. For macroparasites,  $R_0$  is the average number of offspring produced over the lifetime of a parasite that infect and reproduce in a new host. Thus, if the average individual in a parasite infrapopulation replaces itself with at least one successful offspring in the subsequent generation (i.e.,  $R_0 > 1$ ), the parasite population increases. If  $R_0 < 1$ , and the parasite does not replace itself, then population size decreases. A Transmission Threshold at  $R_0 = 1$  indicates a “tipping point” that must be crossed if a parasite is to spread within a host population. Because  $R_0$  provides an estimate of the reproductive potential of an individual macroparasite, and thus its potential to spread in a host population, it is a cornerstone of modern parasite population ecology and epidemiology.

Finally, output from the models developed by Anderson and May also emphasized the idea that the population sizes of hosts and parasites were inherently coupled. Thus, outputs of their models showed that the population size of a host could influence the population size of a parasite, and vice versa. This lead to the notion of mutual co-regulation of host and parasite population sizes, particularly the idea that host populations could be regulated by their parasites through parasite-induced host mortality. This assertion was pivotal because it demonstrated for the first time that host mortality due to parasites could act in addition to other sources of mortality such as malnutrition, resource competition, and predation.

Taken together, the synthetic approach offered first by Crofton and then by Anderson and May paved the way for a proliferation of mathematical theory to generate hypotheses explaining variation in parasite population sizes. They also provided the impetus for recognition that parasites were important organisms in their own right within ecosystems, potentially regulating populations of their hosts and affecting community structure, processes and dynamics. It should be no surprise that a key extension of their models lies in their use to predict future outcomes. Modern examples include predicting the impact of climate change on  $\beta$  and  $R_0$  for human and wildlife parasites (Lafferty, 2009), predicting the proportion of a host population required to be vaccinated for parasite control (Hudson et al., 1998), and forecasting the role of parasites in the regulation of host populations (Hudson et al., 1998).

# Key studies

A variety of host-parasite systems, both in the laboratory and in the field, have contributed significant advances in the study of population ecology of parasites. These include studies involving monogeneans, trematodes, cestodes and nematodes in fish, amphibians, birds and mammals. Here, we focus on a selection of those across different parasite taxa, while recognizing that this is not an exclusive set and others equally important could easily be included.

## The Asian fish tapeworm in Belews Lake

A series of studies on the invasive Asian fish tapeworm, *Bothriocephalus acheilognathi*, in Belews Lake, North Carolina provide good examples of the types of population studies undertaken in parasitology. Belews Lake is an artificial, thermally altered reservoir that provides cooling for a coal-fired power plant. During the first 10 years of operation, selenium leaching into the lake from the burning of coal bioaccumulated up the food chain and caused the elimination of the top piscivores, resulting in a simple fish community consisting primarily of two cyprinids and mosquitofish (*Gambusia affinis*). As such, the lake provided opportunities to examine effects of water temperature and pollution on the population of a parasite in fish, most of which were published in *The Journal of Parasitology*.

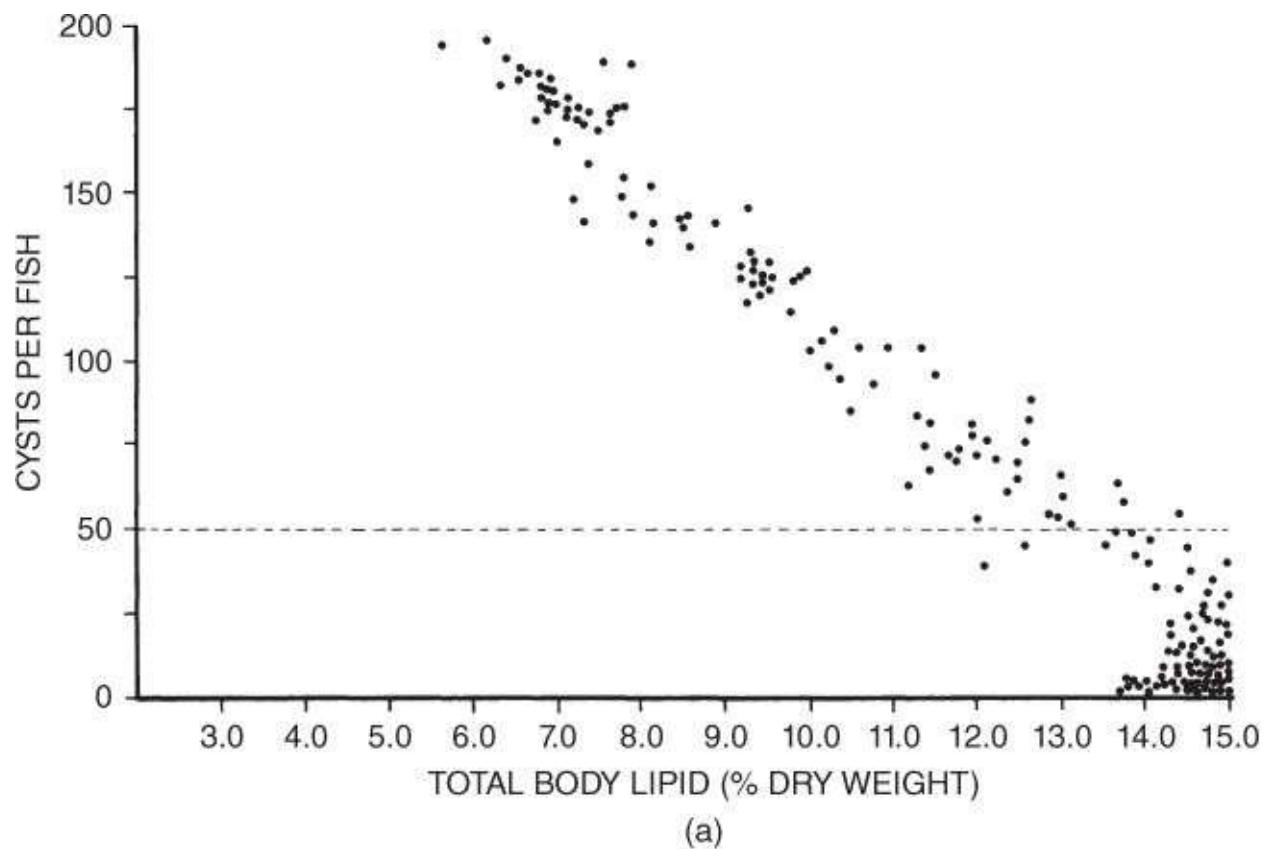
Granath and Esch (1983a) demonstrated that recruitment and abundance of the tapeworm differed between thermally altered and ambient portions of the lake. In the warmer section, recruitment began earlier, and was interrupted when water temperatures exceeded 35°C. They further showed experimentally that infections with the parasite could lead to mortality of mosquitofish at temperatures above 25°C (Granath and Esch 1983b). Abundance of the parasite differed among fathead minnows (*Pimephales promelas*), red shiners (*Cyprinella lutrensis*), and mosquitofish (Riggs and Esch, 1987). In addition, although the parasite was highly aggregated in all three hosts, the degree of aggregation was much higher in the red shiner than in the other two hosts. However, even though abundances were lowest in the fathead minnow, fecundity of the worms was highest in that host, demonstrating that parasite population size is not necessarily the best indicator of a host's role in the overall transmission of infective stages into the habitat (Riggs and Esch, 1987).

Fecundity of the parasite also was reduced in portions of the reservoir exposed to selenium (Riggs et al., 1987). Furthermore, population structure among three host fish species appeared to be related to food web structure, which differed between polluted and unpolluted parts of Belews Lake due to the absence of piscivores in the former, as well as the different foraging habits of the fish hosts (Riggs and Esch, 1987). The tapeworm was capable of infecting any of the cyclopoid copepod species found in the lake as intermediate host (Marcogliese and Esch, 1989a). Seasonal dynamics in the parasite changed over a seven-year period, fluctuations

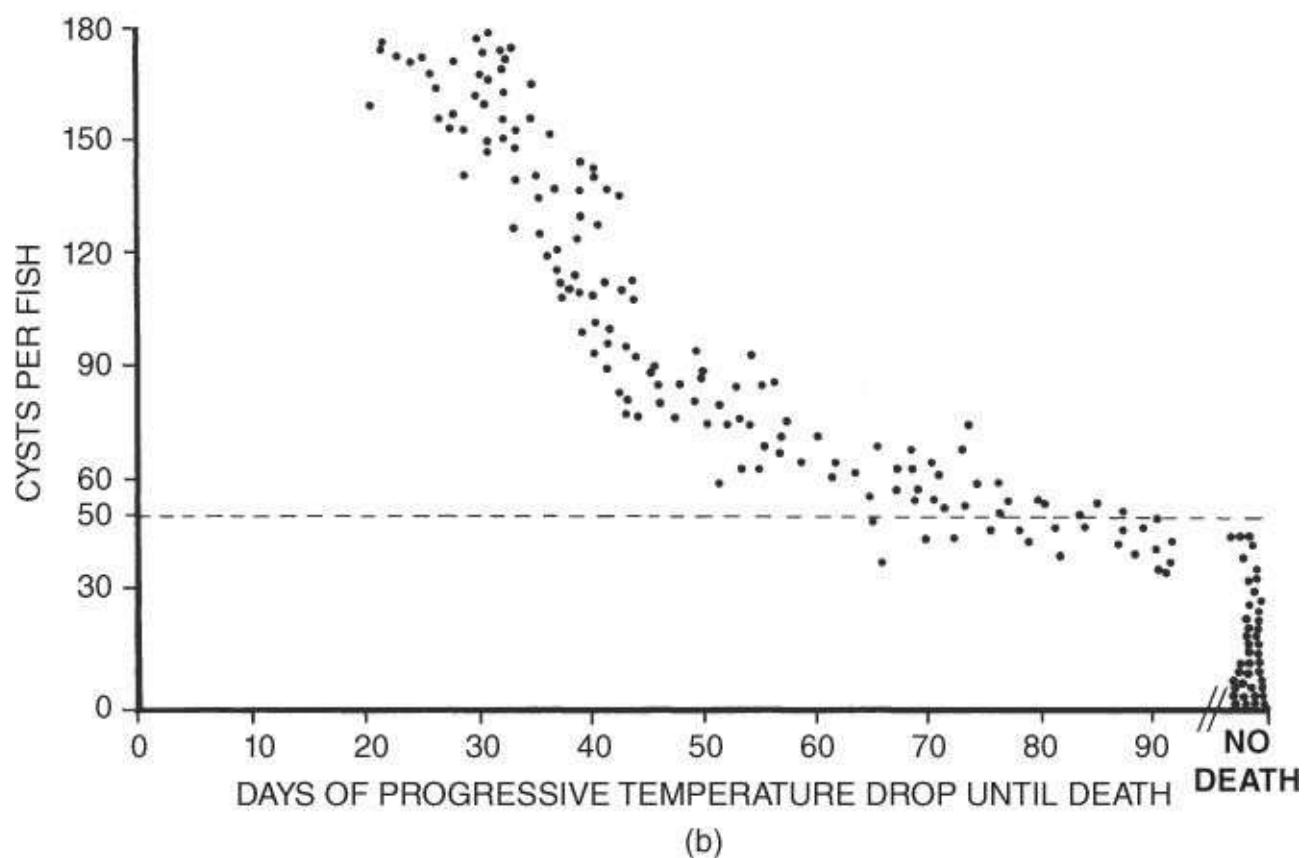
attributed to alterations in zooplankton community structure resulting from a trophic cascade following the elimination of piscivorous fish (Marcogliese and Esch, 1989b). Thus, the distribution and abundance of *B. acelognathi* among hosts are influenced by both biotic and abiotic factors in Belews Lake, involving direct and indirect interactions.

## Blackspot in bluegill sunfish

A key question coming out of the early theoretical studies was whether or not parasites can regulate populations of their hosts through parasite-induced host mortality. Lemly and Esch (1984) observed that a larval trematode causing blackspot (*Uvulifer ambloplitis*) evidently caused over-winter mortality of young-of-the-year (YOY) bluegill sunfish (*Lepomis macrochirus*) in Reed Pond's, North Carolina. They then performed field experiments using cages to show that heavily infected fish (>50 metacercarie) did not survive over winter ([Fig. 2](#)) (Lemly and Esch, 1984). They corroborated the field study with laboratory experiments showing not only that heavily infected fish could not survive, but that these fish had reduced lipid reserves, below a critical threshold of 5% dry weight. Thus, these authors provided unambiguous evidence of parasite-induced host mortality, accounting for 10–20% YOY mortality in the fish population. Whether or not this mortality serves to regulate the host population is another question. The authors also questioned the ubiquity of parasite-induced host mortality in this system. They surveyed centrarchid hosts in 43 other ponds, lakes and streams in the region, and never found blackspot infections approaching the levels in Reed's Pond (Lemly and Esch, 1985), concluding that parasite levels often are high enough to cause appreciable host mortality only under exceptional circumstances.



(a)



(b)

**Figure 2** Relationship between parasite intensity and total body lipid (a), and parasite intensity and survival following onset of dropping water temperature (b) for bluegill (*Lepomis macrochirus*) from Reed's Pond, November 1981 through October 1982. Data are for fish held in outdoor aquaria at ambient temperature or in live boxes in the littoral zone. All fish were naturally infected with *U. ambloplitis*. The dotted line indicates the maximum intensity observed for bluegill that overwintered successfully in Reed's Pond. (Reprinted from Lemly and Esch (1984); Reproduced with permission of Allen Press Publishing Services.)

## Polystomatid monogeneans in amphibians

Populations of polystomatid monogeneans, which are endoparasitic in amphibians, appear to be highly regulated at low intensities (Tinsley and Jackson, 2002). Through a combination of extensive long-term field and laboratory studies on two different species with different life cycle strategies, Richard Tinsley and colleagues have developed a comprehensive understanding of the intrinsic and extrinsic factors controlling transmission, intensity, and reproduction of these parasites in their hosts.

*Pseudodiplorchis americanus* infecting the spadefoot toad *Scaphiopus couchii* in the Arizona desert is a case in point. Breeding of the toads is restricted to a maximum of a few days a year in temporary ponds, the remainder of the time being spent buried in the ground. Transmission of the parasite is also confined to this brief window in time, and only at night. Following this transmission blitz, male toads may be infected with an average of about 100 worms per toad, indicating extremely high and efficient transmission success. Over the course of a year, the parasite experiences mortality attributed to various factors, including losses during migration and damage from a hyperparasite. In addition, there are likely losses due to intraspecific competition. These processes contribute only a limited portion of the losses to the populations of *P. americanus*, which decrease about 97% over the course of infection prior to parasite maturation each year. However, the relatively constant infection levels from year to year despite environmental variations, the lack of accumulation with age and decline in older age classes of host, an inverse relationship between parasite survival and temperature, and differences in susceptibility among individuals suggests that overall parasite burdens are regulated by an acquired immune response that overrides other ecological influences mentioned previously (Tinsley and Jackson, 2002).

*Protopolystoma xenopodis* is a parasite of the urinary bladder in the South African clawed frog, *Xenopus laevis*. Given that the host is fully aquatic, this parasite is transmitted continuously year-round, unlike *P. americanus*. Environmental factors, host ecology and behaviour, and intra- and inter-specific interactions all serve to modulate parasite population levels. Laboratory experiments show that temperature has an important effect on various stages of the parasite life cycle, including egg production and development, which are

mediated through temperature-dependent immunosuppression (Tinsley and Jackson, 2002). Final adult population intensities are only 12% of those that successfully invade the host, indicating extensive parasite mortality (Tinsley and Jackson, 2002). Experimental infections further demonstrate efficient host resistance to challenge infections, indicating effective acquired resistance (Tinsley and Jackson, 2002; Tinsley et al., 2012). A long-term mark-recapture field study on an introduced population of frogs in Wales further demonstrated the importance of acquired immunity in a wild population (Tinsley et al., 2002). Thus in this case, as with *P. americanus* in the spadefoot toad, host factors, in particular the immune response, also override the modulating influences of the various extrinsic factors mentioned previously. For *P. xenopodis*, persistence depends on host recruitment that contributes naive hosts to the population with each new cohort.

## The cestode, *Hymenolepis citelli*, in mice

Adults of the cestode *Hymenolepis citelli* are found within the intestines of deer mice, *Peromyscus maniculatus*, where they produce eggs that are shed in feces. When camel crickets, *Ceuthophilus utahensis*, ingest the eggs, a larva migrates to the intermediate host's haemocoel to develop into a resting cysticercoid. The cysticercoid develops into an adult following ingestion by a mouse.

The ease with which deer mice can be raised in the laboratory makes this model a suitable one for combined field and empirical approaches. Initial laboratory studies showed that young deer mice are 100% susceptible to *H. citelli*, but that most mice eliminate the worms before they mature (see Wassom et al., 1986). When these mice are subsequently challenged with *H. citelli*, most are resistant. Yet a few mice remained susceptible to both the stimulating and the challenge infection. This resistance was density-dependent, being directly related to the dose of cysticercoids presented to the mice in the challenge infection. It turns out that acquired resistance to *H. citelli* was controlled by a single autosomal dominant gene and that susceptible hosts were homozygous for the recessive gene. Moreover, the immunity could be transferred to uninfected hosts via cells originating from the lymph of resistant mice. The results of field surveys showed that the distribution of *H. citelli* was restricted to small foci of susceptible hosts that represent about 25% of the total population, reflecting an aggregated distribution. The authors concluded that host genetics, especially genetically based resistance/susceptibility, played a key role in determining population structure, transmission dynamics, and patterns of aggregation of this cestode in natural populations of mice (Wassom et al., 1986).

## Cecal nematodes in red grouse

One of the best known cases of parasite regulation of host populations is that of the nematode *Trichostrongylus tenuis* in red grouse (*Lagopus scoticus*).

Regulation of the host population by *T. tenuis* is supported by parasite removal experiments and correlations of parasite intensity with grouse mortality and susceptibility to predation (Hudson et al., 1992a, 1992b, 1998).

In the course of this work, Hudson and collaborators had to parameterize their models with key features of the life cycle and population biology of *T. tenuis* to determine under what conditions, if any, the nematode could influence population cycles in the grouse (Dobson and Hudson, 1992). Information was collected on many of the parameters indicated in [Fig. 1](#), including worm intensity and distribution among hosts, worm fecundity and egg output, adult worm mortality, and adult worm recruitment (Hudson et al., 1992a). Population cycles are generated by parasite-induced host mortality reducing the number of breeding birds, a low degree of parasite aggregation among birds, and arrested larval development (Hudson et al., 1992a). The latter, a feature of the *T. tenuis* life cycle, tends to decrease the parasite's intrinsic growth rate in grouse. Grouse populations fluctuated in the model when parasites display low levels of aggregation and reductions in host fecundity caused by the nematode are greater than parasite-induced host mortality. Parasite-related factors affecting grouse cycle periodicity include larval lifespan or duration of arrested development, as dictated in separate models. Prolonged larval development beyond 6 months dampens the grouse population cycles and results in stable parasite-host dynamics. The authors conclude that empirical parasitological data are consistent with parasite-induced cycling of the red grouse populations (Dobson and Hudson, 1992).

## “Sea lice,” *Lepeophtheirus salmonis*, in salmon

The complexities of parasite population dynamics are highlighted by studies that combine field surveys, experimental manipulations in the lab and field, and mathematical modeling. One recent example involves population assessments of the parasitic copepod, *Lepeophtheirus salmonis* on wild salmon on Canada's Pacific coast. This species has a direct life cycle involving at least 10 stages, four to five of which are parasitic, and an infective free-swimming copepodite. Motivation for a large, integrative research effort involving *L. salmonis* stems from concerns that high copepod intensities were responsible for a catastrophic decline in populations of pink salmon. The declines coincided with the first appearance of sea lice on pink salmon and also with the expansion of aquaculture operations that involved caged Atlantic salmon. Since these salmon are a suitable host for the development of *L. salmonis*, the concern was that copepodites derived from sea farms provided the source of pathogenic infections on native pink salmon—the only salmonid that is present along the coast during the period when copepodites

are most abundant in the water column.

Martin Krkošek and his colleagues initiated a series of empirical and modeling studies to answer the question of farm-to-wild salmon transmission and the question of lice-induced salmon mortality. Results from their field-based assessments of thousands of juvenile pinks showed that fish sampled after they passed regions of intensive salmon farming had much higher intensities of *L. salmonis* than those collected as they approached the farms. Further, the mortality of naturally infected juveniles raised within screened enclosures ranged between 9–95% over a 2-month period, whereas mortality of uninfected fish was near zero (Krkošek et al., 2006). In their application of the classical Anderson and May macroparasite models, the authors coupled a model of lice transmission dynamics with a model of lice-induced effects on salmon survival to estimate overall host mortality caused by farm-origin lice. The outcome of their combined model predicted a 99% collapse in Broughton pink salmon populations within 8 years (Krkošek et al., 2007). This dire prediction has led to one of the most scientifically and politically charged periods in Canada's coastal history (reviews in Brooks and Jones, 2008, Marty et al., 2010, and Krkošek et al., 2011).

One outcome of these intensive studies on the *Lepeophtheirus*/salmon interaction is that many of the key population rate parameters described in Fig. 1 have been determined. Thus, we have a good understanding of seasonal and annual variation in rates of transmission, and we know the extent to which these rates are determined by factors such as temperature, salinity, water currents, host community structure, and host population structure. Extensive laboratory studies have also provided estimates of *Ro* and have provided estimates of parasite loss due to sophisticated host immune responses. Yet on a more general level, research on this system has highlighted the key role of anthropogenic factors in determining rates of transmission. In this particular case study, it is the anthropogenic introduction and maintenance of high densities of introduced hosts that partly determines rates of transmission. Understanding the extent to which anthropogenic alterations to host habitats determines parasite population dynamics is a key area of current focus.

In addition to the results of these field-based studies, there is a large literature on the population dynamics of parasites of model and non-model hosts raised under laboratory and natural conditions. The general approach is to expose individual hosts to known numbers of infective larvae and then monitor as many of the rates described in Fig. 1 as possible. In this way, the contribution of host, parasite, and environmental factors to overall variation in targeted rate parameters can be assessed. A logical extension to this process is to expose hosts multiple times to known numbers of infective stages, a scenario that realistically parallels continuous exposure in nature. It is this general approach that allows for an assessment of the role of host immunity through various stages of an infection. Empirical approaches of this sort have sharply clarified the processes that underlie the patterns observed in the case studies described here: temperature-

dependent growth and survival of macro- and microparasites of fish and amphibians (e.g., Scott and Anderson, 1984), temperature- and density-dependent immunity in poikilotherms (e.g., Tinsley et al., 2012), density-dependent parasite growth and survival and the relative roles of parasite and host genetics (e.g., Sayles and Wassom, 1988).

# Methodological and conceptual advances

## Geographic information technology and spatial variation in parasite infections

The results from countless field monitoring studies, samples of which are covered previously, have clearly shown that the magnitude of  $\beta$ , i.e., parasite transmission rate ([Fig. 1](#)), varies with local conditions. Laboratory studies have confirmed the prominence of temperature in determining variation in the development and survival of free-living stages of parasites, of larval stages within intermediate hosts, and of vectors, although factors such as soil moisture, humidity, salinity, and pH are important within particular host/parasite combinations. For indirect life-cycle parasites that are transmitted through ingestion, variation in local conditions that affect host foraging decisions, e.g., activity, age, morphology, and social status, can also have profound effects on  $\beta$ . The pattern that consistently emerges is one of notoriously high variation in rates of transmission of parasites into individual hosts, leading to high variation in average rates of transmission within host populations. Characterizing variation in  $\beta$  at local, e.g., within a single pond, regional, and global scales, is an area of current research focus, with clear practical applications to the diagnosis and treatment of problem parasites. Studies designed to understand underlying factors leading to spatial variation in transmission rates are fundamental to queries in ecological epidemiology, host/parasite coevolution, and parasite-mediated natural selection.

The integration of modern geospatial tools such as geographical information systems (GIS) and satellite-based remote sensing (RS) techniques into parasitology has provided a powerful tool for characterizing and understanding spatial variation in transmission at a range of scales (reviewed in Hay et al., 2006). A paradigm of this overall approach is that the spatial distributions of many parasites are amenable to mapping based upon environmental data, especially temperature, that are readily available from satellite telemetry and other remote sensing sources. The procedure begins with estimates of parasite prevalence and abundance from hosts sampled at a defined spatial scale, typically ranging from tens of meters to hundreds of kilometers (Simoonga et al., 2009). Next, environmental data are collected at an appropriate scale, either from remote sensing and/or from ground-based field surveys and then imported as individual “layers” into GIS software. Various modelling tools are then used to evaluate statistical significance between parasitological parameters and independent environmental covariates. One advantage of this approach is that in addition to the incorporation of obvious covariates such as temperature and precipitation, derived data such as vegetation cover, habitat fragmentation, and various pollution indexes can be incorporated as layers into various GIS platforms. Ultimately, the general aim of the approach is to develop spatial models that can be used to map risk of transmission across a defined landscape. The accuracy of the models can then be tested with further sampling, including at sites that have

not previously been evaluated.

Pullan and Brooker (2012) used this approach to assess the role of habitat characteristics in determining patterns of prevalence and abundance of the major soil-transmitted nematodes of humans at almost 5,000 sites around the world. At this global scale, high and low temperature extremes and highly arid environments lead to limited rates of transmission. At regional scales, factors such as economic status and the rural versus urban nature of a particular site were important. A key outcome of their approach was their ability to characterize the locations where over five billion people were at risk of transmission of at least one of the nematodes. Similar approaches have been used, at smaller spatial scales, to develop risk maps for soil-transmitted helminths in sub-Saharan Africa (Brooker et al., 2000), for schistosomiasis in Africa (Simoonga et al., 2009), and for parasites that are vectored by arthropods, especially species of *Plasmodium*, *Trypanosoma*, and some filariid nematodes (Hay et al., 2006). Taken together, the recent integration of modern geospatial tools into parasitology provides a powerful tool for understanding spatial variation in parasite population dynamics over a range of scales.

The integration of traditional, population-level host surveys into the new subdisciplines of landscape ecology and environmental parasitology has also advanced our understanding of spatial patterns. This approach has been used extensively in the study of various wildlife diseases. More recently, it has been used to examine the distribution and abundance of helminth parasites in hosts evaluated from different environmental conditions, especially in frogs. For example, Schotthoefer et al. (2006) showed that woodland habitat, connectivity between waterbodies, and water cover were associated with abundance of larval trematodes in northern leopard frogs, *Lithobates pipiens*. Agricultural activity was associated with the prevalence of larval trematodes, while that of *Alaria* sp. was linked with the amount of forested areas around ponds in grey tree frogs, *Hyla versicolor*, as shown by Koprivnikar et al. (2006). In studies on leopard frogs and bullfrogs (*Lithobates catesbeianus*), parasites using bird and mammalian definitive hosts were more abundant in wetlands surrounded by forest cover, but rare in landscapes disturbed by agriculture and urban development (King et al., 2007, 2010).

## Molecular techniques and cryptic species

The development of molecular systematics has had a huge influence on all aspects of parasitology, including population ecology, in part because parasites being small and soft-bodied can be intrinsically difficult to identify, and larval stages are often morphologically simple and consequently impossible to identify to species or even higher taxa (Criscione et al., 2005). Indeed, cryptic species are common in numerous different parasite taxa (Criscione et al., 2005), rendering ecological studies problematic.

A case in point is the Diplostomoidea, a group of trematode parasites often infecting fish as metacercariae. They are among the most common and abundant parasites encountered in freshwater fish and include the eyeflukes (*Diplostomum* spp.). Locke et al. (2010a) were able to differentiate 12 species of *Diplostomum* in fishes from the St. Lawrence River, Quebec, Canada, using diagnostic molecular sequences. This local diversity was surprising given that previous work had only identified five to seven species from over 200 species of fish across Canada (Margolis and Arthur, 1979; MacDonald and Margolis, 1995; Gibson, 1996). Together with a second study on other genera including the widespread *Posthodiplostomum* and *Ornithodiplostomum* (Locke et al., 2011b), investigators also found that those parasites infecting fish lenses tended to be generalists, while those in other tissues tended to be specialists, restricted to species of fish from the same family. These studies have consequences for both past and future studies in parasite population ecology.

Although future studies need to account for this unprecedented diversity and occurrence of cryptic species, sometimes within the same host, results have implications for prior ecological studies on these parasites, especially those examining parasite populations and parasite exchange in phylogenetically diverse fishes. To illustrate this point, a previous study on *Diplostomum* spp. in the lenses of golden shiners (*Notemigonus crysoleucas*) and yellow perch (*Perca flavescens*) in the St. Lawrence River (Marcogliese et al., 2001), while recognizing they may have been dealing with more than one parasite species in each case, was actually dealing with a minimum of three and four species, respectively (Locke et al., 2010b; Désilets et al., 2013). An analogous situation, where both fishes and mammals may be infected with more than a single species of whaleworm (*Anisakis*) or sealworm (*Pseudoterranova*), exists in marine waters throughout the world (Mattiucci and Nascetti, 2008).

Molecular techniques also provide a powerful tool for linking life history stages of parasites (Locke et al., 2011). As stated previously, in many cases, larval stages cannot be identified morphologically. The use of molecular markers to discriminate among species and link stages within parasite life cycles provides a means not only to elucidate previously unknown parasite life cycles but to more fully comprehend the transmission and population ecology of parasites.

## Novel biotic interactions

Our discussion until to now has emphasized field and empirical evidence showing that direct extrinsic and intrinsic factors have profound, albeit variable, effects on parasite population dynamics at any given time, at any given place. Yet, the results of recent research points to the importance of numerous indirect factors in determining infection levels of various parasites in their hosts.

The use of mesocosms and other common-or-garden approaches to study species interactions and simplified biological communities has become widespread and

popular in ecology, especially for freshwater systems. Mesocosms have the advantage of allowing the examination of biotic interactions within somewhat complex systems while removing many of the artifacts and artificial conditions associated with laboratory experiments. These simplified ecosystems can be manipulated, replicated, and examined over time to test specific ecological hypotheses that may be difficult to examine in field experiments in natural habitats (Skelly and Kiesecker, 2001).

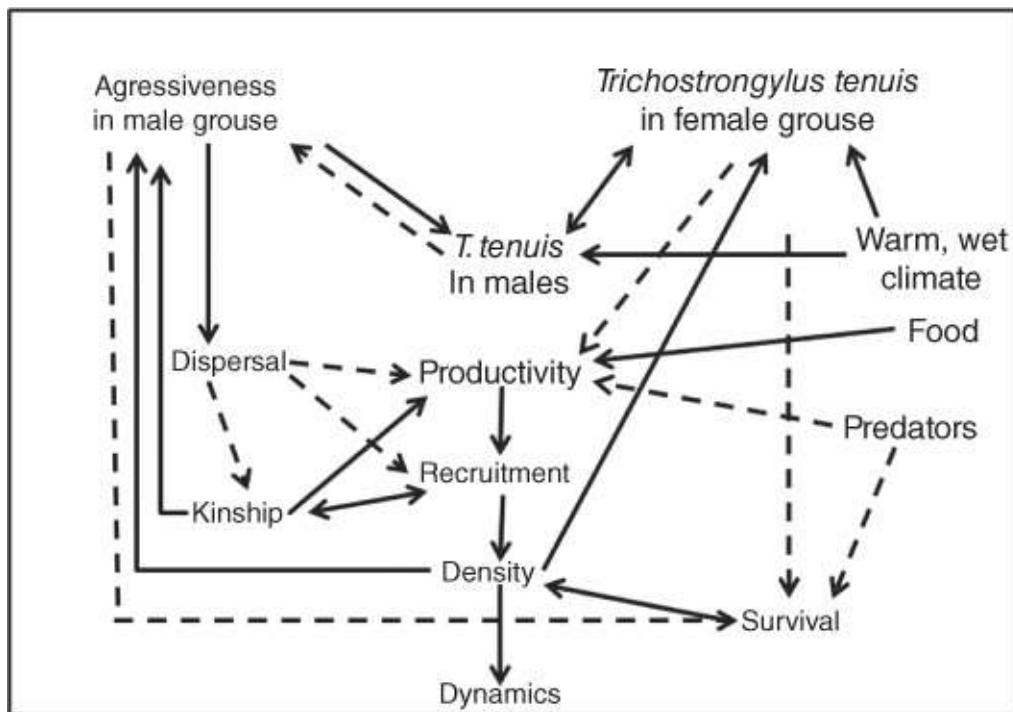
Novel insights into population processes have been derived from mesocosm experiments on larval trematodes in frog tadpoles. For example, using both mesocosm and laboratory venues, Johnson et al. (2008) showed that the species composition of the larval anuran community affected the abundance of metacercariae of *Ribeiroia ondatrae*, ultimately influencing parasite-induced malformation prevalence and mortality, as well as tadpole developmental rate. After running mesocosm experiments subjected to different temperature regimes for a full year, Paull and Johnson (2014) demonstrated that warming of 3 °C led to significantly earlier cercariae release from snails, and increased snail mortality, ultimately decreasing temporal overlap between the tadpoles and *R. ondatrae*. The reduction in transmission between snails and amphibians reduced parasite abundance as well as parasite-induced malformations in the amphibian hosts. These results would be unanticipated if one simply considered that increased temperature leads to enhanced cercarial productivity (Poulin, 2006), which would lead to the conclusion that climate change should increase trematode infection levels in hosts.

While it is well known that animals can reduce infection by parasites through behavioural avoidance or removal (Wisenden et al., 2009), the importance of behaviour for parasite epidemiology generally has not been appreciated until recently. Behavioural traits may affect susceptibility and indeed, avoidance capacity and susceptibility may co-vary, affecting parasite dynamics at a population level (Hawley et al., 2011).

Various species of larval anurans altered their activity levels to different degrees when exposed to cercariae of the trematode parasite *Echinoparyphium* spp. (Koprivnikar et al., 2014). Tadpoles of American toads (*Bufo americanus*), wood frogs (*Lithobates sylvaticus*), and bullfrogs displayed increased activity in the presence of the parasite's infective stages, unlike tree frogs (*Hyla versicolor*) and northern leopard frogs. Furthermore, activity was negatively correlated with parasite abundance in laboratory experiments (Koprivnikar et al., 2014). These experiments showed that the occurrence of anti-parasite behaviour varied among frog species but are not universal. In addition, anti-parasitic behaviours were as important if not more so than immune defenses in reducing infection of larval trematodes (*R. ondatrae* and *Echinostoma* sp.) in tadpoles of Pacific chorus frogs (*Pseudacris regilla*) (Daly and Johnson, 2011). Furthermore, overall activity levels and exploratory behaviour were shown to be successful in reducing infections of *Echinoparyphium* spp. in larval wood frogs (Koprivnikar et al., 2012).

In an elegant experimental study, Johnson and Hoverman (2014) compared transmission success of *R. ondotrae* in Pacific chorus frogs, which were immunologically compromised using corticosteroids and behaviourally constrained by anaesthetic. These treatments increased infection intensities 62–102% compared to controls. Furthermore, reductions in immune function or behaviour of individual frogs increased aggregation within their experimental group, although immune function only affected aggregation in the absence of behavioural manipulation. Thus, individual traits, in particular, behaviour, can affect parasite transmission success and distribution among hosts.

Recent evidence suggests that behaviour and infection with *T. tenuis* are interrelated in the red grouse system discussed previously, questioning the conclusions of Hudson and colleagues (Dobson and Hudson, 1992; Hudson et al., 1992a, 1998) that parasites directly regulate population cycles in the birds (reviewed in Martínez-Padilla et al., 2014). Indeed, these authors argue that population cycles result from density limitations imposed by territoriality due to aggressive behaviour of breeding males. However, susceptibility to *T. tenuis* increases with aggressiveness, due to testosterone-induced immune suppression, leading to destabilizing effects on the host population. Thus, parasitism and behaviour may interact to drive population cycles in red grouse ([Fig. 3](#)), and the authors call for studies that focus on interactions rather than single factors (Martínez-Padilla et al., 2014). It is important to note that these mechanisms are not mutually exclusive and both may be acting simultaneously, and their relative importance may be context-dependent.



**Figure 3** Schematic representation of how host behaviour and parasitism with the nematode *Trichostrongylus tenuis* have direct effects on population processes in red grouse (*Lagopus scoticus*), as well as interactions between them. Solid lines reflect positive interactions and dashed lines reflect negative interactions. Indirect interactions, such as selective predation on parasitized individuals are not shown. Modified from Martínez-Padilla et al. (2014).

In recent years, the existence of behavioural syndromes or personality traits, that is, consistent and repeatable individual differences in behaviour across time and space, has been posited to exist in numerous taxa (Barber and Dingemanse, 2010). While it has been suggested that parasites may influence the evolution of behavioural syndromes or “personalities” within host species (Barber and Dingemanse, 2010), it seems equally plausible that the existence of these personalities will result in differences in susceptibility and transmission of parasites among different groups of hosts within populations. We consider this a promising avenue of research and along with Barber and Dingemanse (2010) and Poulin (2010), advocate further research linking animal personalities with parasite population ecology.

## Interspecific interactions

Although studies of species interactions among parasites are usually the purview of community ecology, growing evidence suggests that species interactions between parasites affect the intensity and virulence of individual parasite species, especially in endothermic hosts. Parasite interactions may be positive or negative and result directly from direct competition for resources or indirectly from host immune responses (Pedersen and Fenton, 2007). For example, in wild rabbits (*Oryctolagus cuniculus*), the presence of the intestinal nematode *Trichostrongylus retortaeformis* not only was associated with a reduction in

intensity of the gastric nematode *Graphidium strigosum*, but also resulted in a delay of peak intensity as well (Lello et al., 2004). The authors suggested that this effect could not be due to direct interactions because of the worms' separate locations in the gastrointestinal tract, so must be the result of parasite-induced immune responses to one parasite on another, and reinforced this conclusion using a mathematical model (Lello et al., 2008). In additional studies, co-infections also affected egg production and shedding (Cattadori et al., 2014).

In a combined field and laboratory study, also on rabbits, infections with the respiratory bacterium, *Bordetella bronchiseptica* enhanced the intensity of *G. strigosum*. In contrast, the nematode did not affect pulmonary bacterial intensities, but did modulate nasal bacterial infections (Pathak et al., 2012). These patterns were associated with immune responses in co-infected individuals compared with monospecific infections. At the component population level, results suggest that the bacteria facilitate nematode infections in the intestine, while the nematode helps maintain the bacterial infection in the upper respiratory tract (Pathak et al. 2012). In a 5-year study of field voles, *Microtus agrestis*, Telfer et al. (2010) captured a total of 5,981 individual voles 14,075 times. Focussing on four blood parasites (the cowpox virus CPXV, the protozoan *Babesia microti*, the bacteria *Anaplasma phagocytophilum*, and *Bartonella* spp.) they demonstrated that infection of one parasite affected the susceptibility of infection with another as well as its longevity. These interactions were positive or negative, depending on the specific species involved resulting in a complex web of interactions (Telfer et al., 2010). In all of these studies, interactions among parasites moderated or were superimposed over effects of season and age. Thus, in evaluating the infection status of individual hosts in wild populations, these studies demonstrate that infections with other parasites should not be ignored.

## Conclusions and lessons

The results from countless host surveys show that the population sizes of parasites vary extensively between individual hosts and between populations of hosts. Studies designed to understand the myriad factors underlying this extensive variation have been a research focus since the development of the conceptual framework for parasite population ecology by pioneers such as David Crompton and Roy Anderson. The results of this 50-year research effort, much of it published in *The Journal of Parasitology*, have indicated that there are few host and parasite factors that do not play a role in determining population-level variation. On the one hand, laboratory studies involving selected strains of hosts and parasites have shown that variation persists even when both host and parasite genes are carefully controlled. On the other, studies by experimental and field ecologists have shown that subtle differences between hosts in age, behaviour, activity levels, and general condition can have enormous impact on their rates of exposure to parasites, and their subsequent response to them. We conclude that in most well-studied systems, population-level variation will arise from inherent host and parasite factors, but the degree to which those factors are manifested are influenced (and often masked) by a constellation of ecological factors that affect rate parameters (e.g., Fig. 1).

We further conclude that studies designed to characterize and understand variation in parasite population sizes will continue to dominate inquiry in parasite population ecology. From an applied perspective, the evaluation of spatial and temporal changes in variation will continue to be important so that we can diagnose and manage problem parasites. Likewise, characterizing population variation in more natural host/parasite systems will continue to have applied consequences for resource management and the conservation of biodiversity.

Our examples emphasize the central role of parasite transmission rate ( $\beta$ ) in determining parasite population dynamics at various temporal and spatial scales. One common theme that emerges is the manner in which anthropogenic factors influence  $\beta$ . At a whole-lake scale,  $\beta$  for a trophically transmitted cestode of fish is strongly influenced by artificially high water temperatures and metal pollution. Transmission rates of ectoparasitic copepods onto pink salmon in a large archipelago on Canada's west coast are determined by the artificially high population sizes of an alternative host. Transmission rates of the cecal nematode, *T. tenuis* within grouse populations in England and Scotland are undoubtedly influenced by management practices that emphasize high host population sizes to support a hunting economy. In the current era of unprecedented global change, we should expect that not just  $\beta$ , but other important rate parameters that affect the gain and loss of parasite populations (Fig. 1) will be influenced by countless anthropogenic factors. The manner in which these factors influence the already complex dynamics of parasite populations is a critical research direction (reviewed by Marcogliese, 2001, 2005).

Anthropogenic influences on rate parameters extend to the phenomenon of invasive and emerging parasites. Our poor understanding of population dynamics of new parasites in native host populations, or native parasites in new host populations is an important knowledge gap. We especially need to better understand dynamics of host generalists that transmit within complex multi-host systems. This particularly applies to the growing problem of invasive parasites. While their spread depends on the very parameters discussed throughout this review, the abundance, diversity, and competence of hosts in the invaded region are paramount to invasion success and subsequent ecosystem effects (Telfer and Brown, 2012).

It has long been recognized that the dynamics of parasite populations are complex. We have only scratched the surface of that complexity here. Although our review has emphasized the pivotal work set in motion by the pioneers of this sub-discipline, we emphasize that this is only a start. Indeed, we currently do not know the extent to which the highly simplified schematic in [Fig. 1](#) is relevant to the countless parasites that have indirect life cycles involving multiple hosts, or are vectored by arthropods. These and other important questions will be answered through a combination of innovative experimentation, insightful use of field systems, theoretical development, and technological progress. Undoubtedly *The Journal of Parasitology* will continue to publish important contributions in these areas to advance parasite population ecology in its next *100 years* of publication!

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## Literature cited

- Anderson, R. M. 1974. Population dynamics of the cestode *Caryophyllaeus laticeps* (Pallas, 1781) in bream (*Abramis brama* L.). *Journal of Animal Ecology* **43**: 305–321.
- \_\_\_\_\_, and R. M. May. 1979. Population biology of infectious diseases: Part I. *Nature* **280**: 361–367.
- Barber, I., and N. J. Dingemanse. 2010. Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society of London Series B* **365**: 4077–4088.
- Brooker, S., M. Rowlands, L. Haller, L. Savioli, and D. A. P. Bundy. 2000. Towards an atlas of human helminth infection in sub-Saharan Africa: The use of geographical information systems (GIS). *Parasitology Today* **16**: 303–307.
- Brooks, K. M., and S. R. M. Jones. 2008. Perspectives on pink salmon and sea lice: scientific evidence fails to support the extinction hypothesis. *Reviews in Fisheries Sciences* **16**: 403–412.
- Cattadori, I. M., B. R. Wagner, L. A. Wodzinski, A. K. Pathak, A. Poole, and B. Boag. 2014. Infections do not predict shedding in co-infections with two helminths from a natural system. *Ecology* **95**: 1684–1692.
- Chubb, J. C. 1977. Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. *Advances in Parasitology* **15**: 133–199.
- \_\_\_\_\_. 1979. Seasonal occurrences of helminths in freshwater fishes. Part II. Trematoda. *Advances in Parasitology* **17**: 141–313.
- \_\_\_\_\_. 1980. Seasonal occurrence of helminths in freshwater fishes. Part III. Larval Cestoda and Nematoda. *Advances in Parasitology* **18**: 1–120.
- \_\_\_\_\_. 1982. Seasonal occurrence of helminths in freshwater fishes. Part IV. Adult Cestoda, Nematoda and Acanthocephala. *Advances in Parasitology* **20**: 1–292.
- Cornwell, G. W., and A. B. Cowan 1963. Helminth populations of the canvasback (*Aythya valisineria*) and host-parasite environmental interrelationships. *Transactions of the North American Wildlife Conference* **28**: 172–199.
- Cort, W. W., D. B. McMullin, and S. Brackett. 1937. Ecological studies on the cercariae in *Stagnicola emarginata angulata* (Sowerby) in the Douglas Lake region, Michigan. *Journal of Parasitology* **24**: 504–532.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1939. A study of larval trematodes in *Helisoma complanatum smithii* (Baker) in the Douglas Lake region, Michigan. *Journal of Parasitology* **25**: 19–22.

Criscione, C. D., R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Molecular Ecology* **14**: 2247–2257.

Crofton, H. D. 1971a. A quantitative approach to parasitism. *Parasitology* **62**: 179–193.

\_\_\_\_\_. 1971b. A model for host-parasite relationships. *Parasitology* **63**: 343–364.

Daly, E. W. and P. T. J. Johnson. 2011. Beyond immunity: quantifying the effects of host anti-parasite behavior on parasite transmission. *Oecologia* **165**: 1043–1050.

Désilets, H. D., S. A. Locke, J. D. McLaughlin, and D. J. Marcogliese. 2013. Community structure of *Diplostomum* spp. (Digenea: Diplostomidae) in eyes of fish: main determinants and potential interspecific interactions. *International Journal for Parasitology* **43**: 929–939.

Dobson, A. P., and P. J. Hudson. 1992. Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. II. Population models. *Journal of Animal Ecology* **61**: 487–498.

Gibson, D. I. 1996. Trematoda. In *Guide to the parasites of fishes of Canada*, part IV. L. Margolis, and Z. Kabata (eds.). Canadian Special Publication of Fisheries and Aquatic Sciences **124**: 1–373.

Goater, T. M., C. P. Goater, and G. W. Esch. 2014. *Parasitism. The diversity and ecology of animal parasites*, 2nd ed. Cambridge University Press, Cambridge, U.K., 497 p.

Granath, Jr., W. O., and G. W. Esch. 1983a. Seasonal dynamics of *Bothriocephalus acheilognathi* in ambient and thermally altered areas of a North Carolina cooling reservoir. *Proceedings of the Helminthological Society of Washington* **50**: 205–218.

\_\_\_\_\_, and \_\_\_\_\_. 1983b. Survivorship and parasite-induced host mortality among mosquitofish in a predator-free, North Carolina cooling reservoir. *American Midland Naturalist* **110**: 314–323.

Hawley, D. M., R. S. Etienne, V. O. Ezenwa, and A. E. Jolles. 2011. Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integrative and Comparative Biology* **51**: 528–539.

Hay, S. I., C. J. Tucker, D. J. Rogers, and M. J. Packer. 1996. Remotely sensed surrogates of meteorological data for the study of the distribution and abundance of arthropod vectors of disease. *Annals of Tropical Medicine and Parasitology*

- Holmes, J. C. 1961. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). I. General effects and comparison with crowding. *Journal of Parasitology* **47**: 209–216.
- Hudson, P. J., D. Newborn, and A. P. Dobson. 1992a. Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. I. Monitoring and parasite reduction experiments. *Journal of Animal Ecology* **61**: 477–486.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1992b. Do parasites make prey vulnerable to predation: Red grouse and parasites. *Journal of Animal Ecology* **61**: 681–692.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1998. Prevention of population cycles by parasite removal. *Science* **282**: 2256–2258.
- Johnson, P. T. J., R. B. Hartson, D. J. Larson, and D. R. Sutherland. 2008. Diversity and disease: Community structure drives parasite transmission and host fitness. *Ecology Letters* **11**: 1017–1026.
- \_\_\_\_\_, and J. T. Hoverman. 2014. Heterogeneous hosts: how variation in host size, behaviour and immunity affects parasite aggregation. *Journal of Animal Ecology* **83**: 1103–1112.
- Kennedy, C. R. 1975. *Ecological animal parasitology*. Blackwell Scientific Publications, Oxford, U.K., 163 p.
- King, K. C., J. D. McLaughlin, M. Boily, and D. J. Marcogliese. 2010. Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. *Biological Conservation* **143**: 302–310.
- \_\_\_\_\_, \_\_\_\_\_, A. D. Gendron, B. D. Pauli, I. Giroux, B. Rondeau, M. Boily, P. Juneau, and D. J. Marcogliese. 2007. Impact of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. *Parasitology* **134**: 2063–2080.
- Koprivnikar , J. R. L. Baker, and M. R. Forbes. 2006. Environmental factors influencing trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles in southern Ontario. *Journal of Parasitology* **92**: 997–1001.
- \_\_\_\_\_, C. H. Gibson and J. C. Redfern. 2012. Infectious personalities: behavioural syndromes and disease risk in larval amphibians. *Proceedings of the Royal Society of London Series B* **279**: 1544–1550.
- \_\_\_\_\_, J. C. Redfern and H. L. Mazier. 2014. Variation in anti-parasite behaviour and infection among larval amphibian species. *Oecologia* **174**: 1179–1185.

- Krkošek M., M. A. Lewis, A. Morton, L. N. Frazer, and J. P. Volpe. 2006. Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences* **103**: 15506–15510.
- \_\_\_\_\_, J. Ford, A. Morton, S. Lele, R. A. Myers, and M. A. Lewis. 2007. Declining wild salmon populations in relation to parasites from farm salmon. *Science* **318**: 1772–1775.
- \_\_\_\_\_, B. M. Connors, A. Morton, M. A. Lewis, L. M. Dill, and R. Hilborn. 2011. Effects of parasites from salmon farms on productivity of wild salmon. *Proceedings of the National Academy of Sciences* **108**: 14700–14704.
- Lafferty, K. D. 2009. The ecology of climate change and infectious disease. *Ecology* **90**: 888–900.
- Lello, J., B. Boag, A. Fenton, I. R. Stevenson, and P. J. Hudson, 2004. Competition and mutualism among the gut helminths of a mammalian host. *Nature* **428**: 840–844.
- \_\_\_\_\_, R. A. Norman, B. Boag, P. J. Hudson, and A. Fenton. 2008. Pathogen interactions, population cycles, and phase shifts. *American Naturalist* **171**: 176–182.
- Lemly, A. D., and G. W. Esch. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: Ecological implications. *Journal of Parasitology*, **70**: 475–492.
- \_\_\_\_\_, and \_\_\_\_\_. 1985. Black-spot caused by *Uvulifer ambloplitis* (Trematoda) among juvenile centrarchids in the Piedmont area of North Carolina. *Proceedings of the Helminthological Society of Washington* **52**: 30–35.
- Locke, S. A., J. D. McLaughlin, S. Dayanandan, and D. J. Marcogliese. 2010a. Diversity, specificity and evidence of hybridization in *Diplostomum* spp. metacercariae in freshwater fishes is revealed by DNA barcodes and ITS sequences. *International Journal for Parasitology* **40**: 333–343.
- \_\_\_\_\_, \_\_\_\_\_, and D. J. Marcogliese. 2010b. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. *Molecular Ecology* **19**: 2813–2827.
- \_\_\_\_\_, J. D. McLaughlin, A.-R. Lapierre, P. T. J. Johnson, and D. J. Marcogliese. 2011. Linking larvae and adults of *Apharyngostrigaea cornu*, *Hysteromorpha triloba* and *Alaria mustelae* (Diplostomoidea: Digenea) using molecular data. *Journal of Parasitology* **97**: 846–851.
- Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology* **79**: 1331–1352.

- \_\_\_\_\_. 2004. Parasites: small players with crucial roles in the ecological theatre. *EcoHealth* **1**: 151–164.
- \_\_\_\_\_. 2005. Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology* **35**: 705–716.
- \_\_\_\_\_, S. Compagna, E. Bergeron, and J. D. McLaughlin. 2001. Population biology of eyeflukes in fish from a large fluvial ecosystem: the importance of gulls and habitat characteristics. *Canadian Journal of Zoology* **79**: 1102–1113.
- Marcogliese, D. J., and G. W. Esch. 1989a. Experimental and natural infection of planktonic and benthic copepods by the Asian tapeworm, *Bothriocephalus acheilognathi*. *Proceedings of the Helminthological Society of Washington* **56**: 151–155.
- \_\_\_\_\_, and \_\_\_\_\_. 1989b. Alterations in seasonal dynamics of *Bothriocephalus acheilognathi* in a North Carolina reservoir over a seven-year period. *Journal of Parasitology* **75**: 378–382.
- Margolis, L., and J. R. Arthur, 1979. Synopsis of the parasites of fishes of Canada. Bulletin of the Fisheries Research Board of Canada, No. 199, Ottawa, Canada, 269 p.
- Martínez-Padilla, J., S. M. Redpath, M. Zeineddine, and F. Mogeot. 2014. Insights into population ecology from long-term studies of red grouse *Lagopus lagopus scoticus*. *Journal of Animal Ecology* **83**: 85–98.
- Marty, G. D., Saksida, S. M., and T. J. Quinn. 2010. Relationship of farm salmon, sea lice, and wild salmon populations. *Proceedings of the National Academy of Sciences* **107**: 22599–22604.
- Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host–parasite co-evolutionary processes. *Advances in Parasitology* **66**: 47–148.
- May, R. M., and R. M. Anderson, 1979. Population biology of infectious diseases. II. *Nature* **280**: 455–461.
- McCoy, O. R. 1928. Fluctuation in the infestation of *Planorbis trivolvis* with larval trematodes. *Journal of Parasitology* **15**: 121–126.
- McDonald, T. E., and L. Margolis, 1995. Synopsis of the parasites of fishes of Canada: supplement (1978–1993). Canadian Special Publication of Fisheries and Aquatic Sciences, No. 122, Ottawa, Canada, 265 p.
- Pathak, A. K. C. Pelensky, B. Boag, and I. M. Cattadori. 2012. Immuno-epidemiology of chronic bacterial and helminth co-infections: Observations from the field and evidence from the laboratory. *International Journal for*

*Parasitology* **42**: 647–655.

Paull, S. H., and P. T. J. Johnson. 2014. Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. *Ecology Letters* **17**: 445–453.

Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. *Trends in Ecology and Evolution* **22**: 133–139.

Poulin, R. 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology* **132**: 143–151.

\_\_\_\_\_. 2010. Parasite manipulation of host behavior: An update and frequently asked questions. *Advances in the Study of Behaviour* **41**: 151–186.

Pullan, R. L., and S. J. Brooker. 2012. The global limits and population at risk of soil-transmitted helminth infections in 2010. *Parasites & Vectors* **5**, 81.

Read, C. P. 1951. The “crowding effect” in tapeworm infections. *Journal of Parasitology* **37**: 174–178.

Riggs, M. R., and G. W. Esch. 1987. The suprapopulation dynamics of *Bothriocephalus acheilognathi* in a North Carolina reservoir: abundance, dispersion, and prevalence. *Journal of Parasitology* **73**: 877–892.

\_\_\_\_\_, A. D. Lemly, and G. W. Esch. 1987. The growth, biomass, and fecundity of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir. *Journal of Parasitology* **73**: 893–900.

Sayles, P. C., and D. L Wassom. 1988. Immunoregulation in murine malaria: susceptibility of inbred mice to infection with *Plasmoidum yoelii* depends on the dynamic interplay of host and parasite genes. *The Journal of Immunology* **141**: 241–248.

Scott, M. E., and R. M. Anderson. 1984. The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology*, **89**: 159–194.

Schotthoefer, A. M., J. R. Rohr, R. A. Cole, A. V. Koehker, C. M. Johnson, L. B. Johnson, and V. R. Beasley. 2011. Effects of wetland vs. landscape variables on parasite communities of *Rana pipiens*: Links to anthropogenic factors. *Ecological Applications* **21**: 1257–1271.

Simoonga, C., J. Utzinger, S. Brooker, P. Vounatsou, C. C. Appleton, A. S. Stensgaard, A. Olsen, and T. K. Kristensen. 2009. Remote sensing, geographical information system and spatial analysis for schistosomiasis epidemiology and ecology in Africa. *Parasitology* **136**: 1683–1693.

Skelly, D. K., and J. M. Kiesecker. 2001. Venue and outcome in ecological

experiments: manipulations of larval anurans. *Oikos* **94**: 198–208.

Telfer, S., and K. Brown. 2012. The effects of invasion on parasite dynamics and communities. *Functional Ecology* **26**: 1288–1299.

Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010. Species interactions in a parasite community drive infection risk in a wildlife population. *Science* **330**: 243–246.

Tinsley R. C., and J. A. Jackson, 2002. Host factors limiting monogenean infections: A case study. *International Journal for Parasitology* **32**: 353–365.

\_\_\_\_\_, L. Stott, J. York, A. Everard, S. Chapple, J. Jackson, M. Viney, and M. C. Tinsley. 2012. Acquired immunity protects against helminth infection in a natural host population: long-term field and laboratory evidence. *International Journal for Parasitology* **42**: 931–938.

Wassom, D. L., T. A.. Dick, N. Arnason, D. Strickland, and A. W. Grundmann. 1986. Host genetics: a key factor in regulating the distribution of parasites in natural host populations. *Journal of Parasitology* **72**: 334–337.

Wisenden, B. D., C. P. Goater, and C. T. James. 2009. Behavioral defenses against parasites and pathogens. In *Fish defences*, Vol. 2, C. Zaccone, A. Perriere, A. Mathis and G. Kapoor (eds.). Science Publishers, Enfield State, p. 151–168.

Wright, C. A. 1971. *Flukes and snails*. Allen and Unwin, London, U.K., 176 p.



# Chapter 7

## Microevolution and the Genetic Structure of Parasite Populations

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**ABSTRACT:** The development of polymerase chain reaction-based methods for assessing the genotypes of small individual organisms will promote groundbreaking investigations of the genetic architecture of parasite populations. Both quantitative genetic models and general knowledge of parasite natural history are useful for making general predictions about the distribution of genetic variation over geographic space. However, designing experimental studies to assess relationships between specific life history variables and patterns of genetic structure in natural populations will be challenging. Traditional biochemical-genetic methods have already been used to study a limited number of parasite populations, and inferred patterns of genetic structure are distinctly different between certain species. Some of these differences in genetic architecture may be explained by parasite or host factors that either promote or retard the dissemination of life cycle stages over geographic space. Many additional empirical studies are needed to characterize basic features of parasite populations, including the spatial distribution and group size of random mating populations and levels of gene flow among parasite subpopulations.

Since the publication of *On the Origin of Species* (Darwin, 1859), the principal focus of studies in microevolution has been selection, although Darwin was aware that accident and interbreeding can oppose natural selection. Classical studies in ecological genetics, sensu Ford (1964), have documented and quantified the effects of natural selection on conspicuous phenotypic variation within populations. Subsequent studies of natural selection in wild populations of plants and animals (Endler, 1986) have proved invaluable for documenting additional examples and providing empirical evidence for theoretical predictions. Current studies in ecological genetics have, by necessity, become considerably more inclusive. This is because a thorough understanding of microevolution will occur only by considering, in an historical context, the effects of stochastic events, gene flow, natural selection, mutation, and mating systems on the metapopulation (the spatially separate, temporally extant, "interconnected" subpopulations of a species). Much of the theoretical groundwork for metapopulation studies was published prior to the advent of molecular genetic methods by the famous quantitative evolutionary geneticist Sewall Wright. It may be surprising to many parasitologists that Wright was a Master's student of Henry Baldwin Ward and published the first of his 211 scientific papers (Wright, 1912), on the morphology of the trematode *Microphallus opacus*. Wright's subsequent pioneering quantitative and theoretical studies in microevolution emphasized the importance of unpredictable changes in allelic frequencies due to stochastic events in finite populations (genetic drift), and modification of allele frequencies in populations due to the movement of gametes or individuals (gene flow). Wright's theoretical results have now received substantial empirical support from population-level studies of many free-living organisms. However, independent of any empirical corroboration, theoretical population genetic models are useful because of their predictive value regarding specific features of microevolution, such as the potentially complex interactions among demography, gene flow, genetic drift, natural selection, and other processes that influence genetic evolution.

Genetically based variation among individual parasites is a prerequisite for characterizing the genetic structure of their pop-

ulations. Using molecular methods to assess the genotypes of individual parasites, although relatively labor intensive, is generally more straightforward than using the resulting data to infer microevolutionary pattern and process. Although significant progress has been made in developing the theory and practice of inferring microevolutionary patterns, e.g., intraspecific phyleogeography (Avise et al., 1987), indirect quantification of gene flow (Wright, 1951; Slatkin, 1985, 1987; Weir, 1990), and coalescent theory (Hudson, 1990; Hudson et al., 1992), each of these inference methods is based on implicit or explicit assumptions that constitute the underlying models (Felsenstein, 1982). Interpretation of evolutionary process from the inferred pattern(s) adds another level of complexity because individual microevolutionary patterns may be compatible with multiple, nonexclusive processes. It must also be recognized that historical studies of micro- and macroevolutionary patterns are fundamentally different from investigations of processes that can be subjected to experimental manipulation. Finally, although historical studies may benefit from the sampling of additional genetic loci, nucleotide sites, or taxa, potentially intricate historical patterns may not be resolved in certain cases. Despite these limitations, microevolutionary studies have the potential to reveal basic information that is currently lacking on the population genetic structure of different parasite species, including parameters such as the average amount of gene flow among parasite "populations" contained within different individual hosts (each an infrapopulation, sensu Margolis et al. [1982]), and among metapopulations. Such studies are essential to understanding the probability for the dissemination of novel antihelminthic resistance mutations over geographic space and time.

Designing testable hypotheses concerning the relationship between genetic structure and specific ecological variables of parasites is likely to remain challenging. For free-living organisms, correlations between ecological variables and genetic structure often explain less than 50% of the overall variance (Avise, 1994). Obviously, the ecological genetics of parasites will be complicated not only by parasite-specific characteristics but also through their associations with hosts. For example, for most multicellular parasites, infrapopulations of definitive hosts are formed by recruitment (immigration) from the metapopulation and not as a result of natality within (or on) the host. Thus, both the vagility of the host and factors limiting infrapopulation density,

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for example, concomitant immunity, may serve to shape the genetic structure of recruited populations.

Although the paucity of available studies makes it premature to develop general conclusions about the genetic structure of parasite populations, it is likely that new technologies will soon promote groundbreaking research in this area. On the other hand, it is not premature to consider the types of questions that might be addressed in parasite population genetics. For example, what types of predictions about the genetic structure of parasite populations might result from examination of the basic features of microevolutionary theory? Given both specific evidence and untested doctrines on the population biology of parasites, what features of parasite natural history are likely to impact on their genetic structure? And finally, which, if any, of these predictions receives empirical support? These questions will be considered herein with a focus on multicellular parasites of animals.

#### GENETIC DRIFT, GENE FLOW, AND MICROEVOLUTION

Genetic drift is an important parameter influencing microevolution in populations of small size (Fisher, 1930; Wright, 1931). Wright demonstrated that it is the number of individuals contributing genes to the next generation ( $N_e$ , or effective population size) and not the population census size that determines the relative amount of genetic drift in a deme (Wright, 1931). The smaller the  $N_e$ , the greater the likelihood that the allelic frequencies and genetic variability characteristic of a particular population will not be transmitted to the next generation. With small  $N_e$ , the heterozygosity of the population will be reduced due to an inbreeding effect, and random changes in allele frequencies will occur as a result of sampling variance (genetic drift). Under certain conditions, inbreeding and genetic drift may respond differentially to small  $N_e$ , which caused Kimura and Crow (1963) to distinguish between the inbreeding effective number and the variance effective number, respectively. For the variance effective number, it is the number of offspring that is critical to estimating sample variance or random genetic drift. For the inbreeding effective number it is the quantity of reproducing individuals in the parental generation that determines the likelihood of identity by descent (autozygosity) for alleles at a locus. In many instances, these different approaches to calculating effective number will yield similar results; however, this illustrates just one of many potential difficulties of estimating the effective size, which is particularly complex for species with highly subdivided populations (Chesser et al., 1993). In addition, models for estimating  $N_e$  are not designed to incorporate the complications introduced by organisms with elaborate life cycles, for example, the clonal (agametic) reproductive strategies of larval digenous (Esch and Fernández, 1993).

One estimator of effective population size that employs several simplifying assumptions (Wright, 1931, 1938) can be calculated from the formula  $N_e = 4N_m N_f / N_m + N_f$ , where  $N_m$  and  $N_f$  are the number of males and females, respectively, that serve as parents in a particular generation. This formula is based, in part, on assumptions of random mating and the possibility of self-fertilization. The calculated  $N_e$  applies to a single generation; however, if  $N_e$  is variable over time, generations with smaller  $N_e$  will have the greatest influence on the average  $N_e$ .

over many generations. This is reflected by the observation that multiple-generation  $N_e$  ("long-term"  $N_e$ ) can be estimated by calculating the harmonic mean of  $N_e$  for individual generations (Crow and Kimura, 1970). Effective population size will also be reduced substantially in comparison to the total number of breeding adults by a skewed sex ratio because with biparental reproduction, half of the alleles in offspring must come from each sex. For example, a parasite infrapopulation consisting of 1 male and 12 reproducing females (a possibility for certain nematodes) has an  $N_e$  of only 3.7 by Wright's (1931) formula. Structured populations with an  $N_e$  of approximately 200 are likely to experience moderate amounts of genetic differentiation due to drift, whereas demes  $\geq 1,000$  will be only marginally affected, assuming average rates of mutation (Wright, 1978; Falconer, 1981). Microevolution in populations of  $N_e < 10$  will be characterized by nonadaptive differentiation because stochastic changes will overcome natural selection, regardless of intensity (Wright, 1978; Shields, 1993). Mean individual heterozygosity will also decrease at a relatively rapid rate when  $N_e < 1,000$  because average per generation mutation rates per locus are insufficient to replace variation lost by random genetic drift. Low long-term  $N_e$  or extreme population size bottlenecks may account for abnormally low levels of heterozygosity in certain helminth populations (Bullini et al., 1986; Nadler, 1990; Esch and Fernández, 1993). However, both the nature of recruitment of individuals comprising infrapopulations within definitive hosts and the potential longevity of certain other parasite infrapopulations within the environment, e.g., long-lived eggs or larvae within paratenic hosts, may complicate the interpretation of infrapopulation  $N_e$  on metapopulation genetic diversity. Wright (1940) noted that in species where local populations are subject to frequent extinction with repopulation from a few immigrants,  $N_e$  will be drastically reduced despite the large number of individuals that may comprise the suprapopulation. Maruyama and Kimura (1980) developed a model for estimating  $N_e$  based on estimates of the subpopulation extinction rate, migration rate between subpopulations, and mutation rate to neutral alleles. Although such estimates are not currently available for parasites, theoretical models (Maruyama and Kimura, 1980) have shown that if the rate of subpopulation extinction is much higher than the migration rate of individuals between subpopulations, the  $N_e$  of the suprapopulation (sensu Margolis et al., 1982) will be markedly reduced and genetic divergence of subpopulations is prevented.

To illustrate the possible impact of genetic drift on microevolution within a small deme of diploid parasites it is instructive to consider the probability of fixation (frequency of 1.0) for an allele, and the average time required to fixation for those alleles that are eventually fixed. The following examples are included to show that a simple deterministic model of allele frequency change, in which the allele of greatest fitness eventually replaces others, is not an appropriate model of microevolution for small natural populations of parasitic organisms. The variables related to estimating the probability of fixation for a particular allele include the  $N_e$  of the deme, the frequency of the allele, and whether the allele is selectively neutral, advantageous, or deleterious. Because of the influence of genetic drift at all loci irrespective of the relative fitness of alternative alleles, fixation of an allele is a probabilistic process (Kimura, 1962). For example, under an additive model of selection (het-

erozygote with mean fitness of the 2 homozygous genotypes), the probability of fixation ( $P$ ) for an advantageous allele introduced by mutation is,  $P = 1 - e^{-2Ns}/(1 + e^{-2Ns})$ , where  $s$  is the selection coefficient and  $p$  is the frequency of the advantageous allele (Hedrick, 1983). For a neutral allele, the probability of fixation is a function of its frequency; thus, for a neutral mutation,  $P = 1/2N$ . In a population where  $N = N_e = 500$ , the respective probabilities of fixation for an allele that is slightly advantageous ( $s = 0.01$ ), markedly advantageous ( $s = 0.10$ ), or neutral, are  $P \approx 0.009$ , 0.095, and 0.001, respectively. For a population where  $N = N_e = 50$ , the respective probabilities of fixation for the same selection values are  $P \approx 0.016$ , 0.095, and 0.010. In both populations, there is a high probability ( $\approx 90\%$ ) that a markedly advantageous allele introduced by mutation as a single copy will be lost by random genetic drift. Note also that the neutral and nearly neutral mutations have significantly greater probabilities of fixation in the smaller population (10-fold greater for the neutral mutation) due to genetic drift. Although counterintuitive, a slightly deleterious mutation also has a slight chance of being fixed in small natural populations. Most alleles introduced by mutation have a high probability of loss within large populations, and stochastic effects are responsible for the higher likelihood of fixation within very small populations.

The interaction of natural selection with random genetic drift in the microevolution of small populations can be illustrated by the average time required to reach fixation ( $t$ ) for an allele destined to reach a frequency of 1. For a selectively neutral allele introduced by mutation, the average conditional fixation time ( $t$ )  $\approx 4N$  generations (Kimura and Ohta, 1969). For a parasite population with  $N \approx N_e \approx 1,000$  and a generation time of 90 days, a neutral mutation will require approximately 1,000 yr for fixation; however, when  $N = 50$ ,  $t \approx 50$  yr. Time to fixation for a selectively advantageous allele is expressed by the formula  $t = (2/s)\ln(2N)$  generations (Maruyama and Kimura, 1974). Considering an advantageous mutation ( $s = 0.1$ ) that is destined to reach fixation (about 9.5% of such mutations when  $N_e = 50$ ),  $t \approx 37$  yr for  $N = 1,000$ , and  $t \approx 23$  yr for  $N = 50$ . This difference in the rate of fixation between selectively neutral versus non-neutral mutations demonstrates that selection can be particularly effective in rapidly changing the genetic composition of populations of small  $N_e$ . The results of this example are also consistent with expectations of neutral-mutation theory (Kimura, 1983), because it reveals that neutral mutations tend to have longer "transit" times within natural populations.

How will the genetic structure and evolution of parasite populations be shaped by the movement of parasite gametes, individuals, or groups of individuals among demes? Different perspectives concerning the evolutionary process have yielded contrasting viewpoints about the role of gene flow in evolution. The traditional viewpoint is that gene flow represents a constraining evolutionary force that, if sufficiently high, prevents marked genetic differentiation among subpopulations. From this same perspective, absence of gene flow is a prerequisite for classical allopatric speciation. In contrast, Wright (1932, 1982) and other population geneticists have developed models that show that gene flow may serve as a creative force in the evolution of populations. This viewpoint has been adopted by Mayr (1954) in developing the peripatric model of speciation. A key question is how much gene flow is necessary to prevent the genetic differentiation of subpopulations? The answer depends on the na-

ture of the variation under consideration. With selective neutrality, much less gene flow is required to prevent substantial genetic differentiation than for cases where one allele is of selective advantage in one subpopulation and a different allele is favored in another subpopulation.

The amount of gene flow among subpopulations in combination with other population parameters determines if evolution proceeds independently in different demes. In general, with very high levels of gene flow among demes, different subpopulations will evolve as a single evolutionary unit; with low levels of gene flow among demes, different subpopulations may evolve independently. Theoretical models are informative concerning how loci with neutral versus non-neutral variation respond to differing levels of gene flow. Wright's island model of population structure, in which each subpopulation has a fraction  $m$  of its residents replaced with individuals selected at random from the other subpopulations or islands, is instructive for the behavior of neutral or nearly neutral loci. If the quantity  $Nm$  (where  $N$  = the effective population size) is  $> 1$ , gene flow will overcome random genetic drift and prevent local differentiation at equilibrium. In many cases only a small amount of gene flow per generation, for example the successful reproduction of a single immigrant, is required to prevent genetic differentiation between 2 subpopulations. Conversely, if  $Nm < 1$ , genetic drift will act independently in the subpopulations to determine the allelic frequencies for "nearly neutral" loci (Slatkin, 1987). The amount of time required to reach genetic equilibrium for the subpopulations is dependent on the effective population size and the immigration rate (Takahata, 1983) and may be a large number of generations. This situation is predictably different in cases where  $N_e$  is moderate to large and the locus includes alleles that are strongly affected by selection (Haldane, 1930; Nagylaki, 1975). Even in the presence of relatively high levels of gene flow introducing a less favorable allele into a subpopulation, natural selection will tend to maintain a high frequency of the advantageous allele. Unlike the case for neutral loci, genetic equilibrium will tend to be established quickly under most rates of immigration, the amount of time depending on the relative fitness of the 2 alleles. Thus, moderate-to-high levels of gene flow may be relatively unimportant in preventing local differentiation when there is strong differential selection in different subpopulations.

Sewall Wright was instrumental in modeling how gene flow may serve as a creative, rather than a constraining force in the evolution of small populations, and his shifting-balance (Wright, 1932) and interdemic selection (Wright, 1956) models of microevolution both have relevance to certain parasitic organisms. In both cases, microevolutionary change is facilitated by small effective population size and genetic drift. In the shifting-balance model, the relative fitness of subpopulations may be visualized in terms of a 3-dimensional landscape. A local population may ascend the available adaptive surface and achieve a local optimal fitness, but remain isolated (by valleys of lower fitness) from adjacent peaks representing combinations of genes of greater relative fitness. With small  $N_e$  and low migration rates, random genetic drift may overcome selection and fix genes or groups of genes that constitute a peak of greater fitness, and gene flow could then spread this more fit genotype. The conditions that are conducive for this scenario are also likely to cause deme extinction in demographically stable species prior to gene flow.

and spread of the more fit genotype. However, for certain parasites and other species possessing a "weedy" population structure, colonization of new resource patches (hosts) is in itself a type of gene flow (Slatkin, 1987) that may counterbalance the local extirpation often associated with the demographic instability of metapopulations. Gene flow via local population extinction and recolonization of new hosts can be more effective in spreading new parasite genotypes than gene flow between resident metapopulations (Slatkin, 1987). Interdemic (group) selection should favor the evolution of traits that enhance dispersal ability, which for parasites is likely to include the utilization of paratenic (transfer) hosts, vagile intermediate hosts, or vectors for transmission.

### CHARACTERIZING GENE FLOW AND GENETIC STRUCTURE

Many recent studies have focused on the genetic variability of parasites, e.g., protein polymorphism and mean individual heterozygosity, in relation to natural selection from the panselectionist viewpoint, or to facets of population structure from a neutralist viewpoint (for review, see Nadler, 1990; Esch and Fernández, 1993). Some of these studies have included data on allelic frequencies from samples representing different geographic localities; however, observed frequency differences alone cannot be used to characterize amounts of gene flow among populations directly. Instead, indirect methods of estimating levels of gene flow from patterns of allelic frequency distributions are needed to distinguish among differences resulting from gene flow, genetic drift, and natural selection. At least 3 different general approaches have been used to estimate levels of gene flow from either allozyme allelic frequency or nucleotide sequence data. Traditionally, *F*-statistics as developed by Wright (1951) or modified by others (Weir and Cockerham, 1984) are used to quantify inbreeding due to population subdivision ( $F_{ST}$ ), and this measure is applied to a model for estimating the parameters  $Nm$ , where  $m$  is the proportion of alleles of migrant origin in a deme and  $N$  is the local population size. Other approaches for estimating  $Nm$  include methods based on the distribution of rare or unique alleles among subpopulations (Slatkin, 1985; Barton and Slatkin, 1986), or in the case of nucleotide sequence data, the nodes and branch lengths of a gene tree for a nonrecombining DNA region (Slatkin and Maddison, 1989; Hudson et al., 1992).

In addition to their application for estimating gene flow, *F*-statistics can be used to quantify levels of inbreeding with respect to different reference populations (Wright, 1922, 1965). Wright developed a numerical method to analyze systems of mating (ancestor-offspring relationships in pedigrees), such that a single numerical quantity (*F*) is sufficient to summarize the correlation of genetic state at a locus. *F* represents the correlation between uniting gametes such that under random mating  $F = 0$ , and with sustained inbreeding beyond that expected given the effective population size, *F* will be a positive number  $\leq 1$ . One effect of inbreeding is to reduce the individual heterozygosity within populations. The inbreeding coefficient ( $F_m$ ) describes the reduction in heterozygosity of an individual within its subpopulation compared to that expected in a randomly mating population with the same allelic frequencies. Inbreeding coefficients are calculated by determining the genotypes of individual or-

ganisms representing a population sample, and comparing the proportion of heterozygous genotypes observed ( $H$ ) to those expected in a randomly mating population ( $H_0$ , or  $2pq$  in the Hardy-Weinberg formula), where  $F = (H_0 - H)/H_0$ . The inbreeding coefficient may be interpreted as the probability that 2 alleles in a diploid individual are autozygous or identical by descent, that is, share common ancestry via replication from a single ancestral allele. Note that 2 alleles possessed by an individual can be identical in nucleotide sequence yet different with respect to replication or immediate common ancestry (the allozygous condition). The concept of inbreeding may be extended beyond a single subpopulation to estimate the probability of identity by descent for alleles selected at random from a sample representing more than 1 subpopulation. Subdivided populations may be characterized by nonrandom mating, and this type of inbreeding can be quantified at 2 additional levels, the subpopulation relative to the total population ( $F_{ST}$ ), and the individual with respect to the total population ( $F_{IT}$ ).  $F_{ST}$  measures the reduction in heterozygosity of a subpopulation due to random genetic drift; the overall inbreeding coefficient ( $F_{IT}$ ) reflects the reduction in heterozygosity due to nonrandom mating within subpopulations ( $F_{IS}$ ) plus that due to population subdivision ( $F_{ST}$ ). The interrelationship among these 3 inbreeding coefficients is  $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ . Calculations of *F*-statistics from allelic frequency data should be corrected for biases that may be introduced by sampling few individuals per subpopulation (Van Den Bussche et al., 1986), which is likely to be the case in many studies of natural parasite infrapopulations. The variances of *F*-statistics across loci (Nei and Chakravarti, 1977; Weir and Cockerham, 1984) are frequently significant and commonly unreported, therefore; small differences between mean *F*-statistics in empirical studies should be interpreted cautiously.

*F*-statistics calculated from genetic data are frequently used to estimate levels of gene flow among demes indirectly. Estimates of host gene flow, although of interest for comparative purposes, are unlikely to be informative about the range of possible gene flow levels for their parasites, with the possible exception of parasites that are either exclusively sexually transmitted or contact-transmitted at the time of host reproduction. Obviously, traditional direct estimates of gene flow, e.g., mark-recapture methods, are not appropriate for adult parasites that do not disperse to new hosts. By contrast, the direct approach of tracking the movement of unique genetic markers has been used to study gene flow over geographic space in ecological time (Handel, 1982), and this could be applied to parasitic organisms. However, one disadvantage of all direct approaches is the limited timescale of study because evolutionarily important yet sporadic increases in gene flow are likely to be missed by direct measurements. For parasites, episodes of high gene flow may be triggered by certain rare abiotic or biotic factors, including the establishment of novel parasite populations through the introduction of new definitive hosts to an ecosystem (Kennedy and Burrough, 1977; Kennedy, 1987).

Indirect estimates of gene flow are usually based on the current geographic distribution of allele frequencies. The advantage of indirect measurements is that they provide information on the average amount of gene flow over long spans of time (Slatkin, 1987). However, indirect measures depend, in part, on explicit assumptions of a specified model of inference to estimate how

TABLE I. Examples of ecological and natural history factors that may influence the population genetic structure of animal parasites. These predictions are of heuristic value, but may be confounded in certain taxa due to the influences of other interrelated variables.

Factors increasing genetic structure	Factors reducing genetic structure
Sedentary definitive host or extreme morbidity of all infected hosts	Highly vagile hosts (definitive, intermediate, paratenic) or vectors
Life cycle includes a large number of specific obligate hosts	Persistent (long-lived) life cycle stages in environment or definitive host
Suitable parasite niches patchily distributed in space or time	Low definitive host specificity/many reservoir hosts
Small effective size for parasite population	Underdispersed distribution of parasites among hosts
Parasite predominantly self-fertilizing	Life history with frequent metapopulation extinction followed by reestablishment
Physical contact between definitive hosts required for transmission	

much gene flow must have occurred to explain the observed geographic pattern of allele frequencies. For example, with respect to migration, Wright's island model for estimating gene flow assumes that all subpopulations are equally accessible to each other. For natural populations, this is equivalent to the assumption of extremely high dispersal ability for migrants. By contrast, a "stepping-stone" model of population structure (Slatkin and Barton, 1989) restricts migration to neighboring subpopulations and considers the geographic distance between subpopulations.

One assumption of all indirect methods is that the subpopulations have reached genetic equilibrium. Theoretical studies demonstrate that the time required for the fixation index ( $F_{ST}$ ) to reach equilibrium can be very long in populations of large size. Given an island model,  $F_{ST}$  will reach an equilibrium depending on either effective population size or  $1/m$  (where  $m$  = migration rate to subpopulations), depending on the time scale that is smaller (Takahata, 1983; Crow and Aoki, 1984). In systems with a relatively high migration rate, e.g.,  $m > 0.01$ ,  $F_{ST}$  will approach equilibrium in less than 100 generations. For stepping-stone models of gene flow, which would seem to be more appropriate for some parasitic organisms, time to equilibrium for  $F_{ST}$  is even greater than for island models (Slatkin, 1993). However, patterns of  $F_{ST}$  prior to the establishment of genetic equilibrium can also be informative, particularly for organisms such as parasites in which rapid colonization of previously uninfected hosts is followed by gene flow between adjacent infrapopulations or metapopulations (Slatkin, 1993). By focusing on pairwise comparisons of such subpopulations to estimate  $Nm$  from  $F_{ST}$ , a parameter denoted  $M$  may be calculated (Slatkin, 1991, 1993). This parameter is dependent on the geographic distance between pairs of locations in both 1- and 2-dimensional stepping-stone models of gene flow. Slatkin (1993) found that the isolation-by-distance pattern spreads over time such that there is a relationship between  $M$  and geographic distance only for a discrete geographic range, the size of which is dependent upon time since colonization. Thus, this model

relates isolation-by-distance to time since colonization and does not depend on the restrictive assumption of genetic equilibrium.

## BIOLOGICAL INSIGHTS AND EMPIRICAL EVIDENCE

From the preceding discussion it should be clear that the models underlying methods of inference in population genetics entail certain assumptions that may compromise the interpretation of data. However, the major current limitation for understanding helminth population genetic structure is the lack of available data. No doubt, this is primarily the result of technical limitations associated with obtaining biochemical-genetic data from small individual organisms. Approaches using the polymerase chain reaction (PCR) or other sensitive molecular methods (reviewed in Hillis and Moritz, 1990; Avise, 1994; Grant, 1994) offer new possibilities for studying numerous genetic loci from individual parasites at the DNA-level; however, these approaches have yet to be utilized fully.

Many basic population parameters remain to be defined for parasite species. For example, what group of individuals represents the deme or random mating population, and how does the corresponding neighborhood area and size differ among taxa? Although the infrapopulation represents the immediate group of interbreeding individuals, the genetic composition of this group may change by recruitment during the life span of the host. In addition, it seems likely that for certain parasites, individual hosts will differ dramatically in their histories of recruitment, and this may produce a chaotic pattern of parasite genetic structure among hosts. For parasites with geographically wide-ranging hosts, infrapopulations may be less inbred than if mating occurred at random among parasites within the metapopulation. By contrast, for a parasite species that may continually complete its life cycle within (or on) a single individual host, infrapopulations should primarily represent descendants of the colonizers, and such populations are likely to be highly inbred. For certain other heteroxenous species such as human schistosomes, premunition may have a similar effect, that is, restriction of the genetic composition of the deme to the colonizers.

The genetic structure of parasite populations will be shaped strongly by ecological factors of individual species, including their demography, life cycle, mechanisms of dispersal, and host specificity (Table I). Characteristics of species such as the mating system and population-level attributes such as the effective size will affect certain aspects of demes, including the likelihood of random genetic drift. For example, self-fertilization is a form of restricted genetic recombination that may result in local genetic differentiation in certain cestode species (Lymbery and Thompson, 1988). Likewise, haplodiploid reproduction among pinworms in combination with other life history features of the Oxyurida may promote extensive inbreeding within hosts (Adams, 1989). Perhaps more often than for free-living organisms, parasite species may experience considerable variation in the parameters influencing genetic structure due to variation in ecological factors among different definitive host species (in generalist parasites) or due to differences among parasite communities. Thus, special caution is advisable for studies of parasites because no single investigation is likely to be representative of conditions for populations of the entire species.

Price (1980) argued that from the parasite's "point of view," resources, i.e., parasite-host niche overlap, will have a patchy distribution in both space and time, and that host-parasite systems will experience considerable disruption due to their complexity, low probability of colonization, and high probability of extinction. Increased complexity of biotic associations, for example the number of obligate hosts in the life cycle of a parasite, should increase resource patchiness (Price, 1980). This is supported by theoretical models in which increasing the complexity of parasite systems decreases their stability (May, 1973). Price (1980) also characterized the "within-patch" population dynamics of parasites as a nonequilibrium system. This viewpoint is also consistent with limited evidence suggesting that numerous density-independent factors may regulate the infrapopulation size of certain species (Esch and Fernández, 1993). Many other factors may also contribute to resource patchiness, for example variation in genetic predisposition to infection among individual hosts, which can influence patterns of parasite aggregation (Schad and Anderson, 1985).

Although different levels of resource patchiness should have predictable effects on the genetic structure of parasite populations, assessing individual ecological factors promoting patchiness is likely to be difficult due to the large number of interactions in host-parasite systems and potential correlations among life history traits of parasites. In addition, although the demographic structure of parasites as assessed by censusing adult infrapopulations in definitive hosts may be unstable (Price, 1980; Esch and Fernández, 1993), the gene pool of parasite metapopulations may have greater stability for cases in which species produce large numbers of life cycle stages that persist in the environment for periods that exceed several parasite generations. This situation is analogous to the influence of seed dormancy and the seed pool on the evolution of plant populations. For many parasite species, colonization of uninfected definitive hosts is not analogous to a simple island model, with dispersal from adjacent colonized hosts. Clearly, long-lived life cycle stages are not produced by all helminths, and such differences may prove useful for predicting variation in population genetic architecture. For example, experimental human infections with *Ascaris lumbricoides* have demonstrated that ascaridoid eggs may remain infective for 10 yr or more in soil (Brudastov et al., 1971); in contrast, *Enterobius* spp. infecting humans are characterized by eggs that remain viable for approximately 1 wk. Thus, the life history pattern of human pinworms is more likely to promote genetic structuring among populations.

Given the range of ecological diversity that is characteristic of parasites, a broad spectrum of genetic architectures is likely to be revealed as more empirical studies are undertaken. Published studies have already revealed variation in genetic structure among different species and, as in free-living organisms (Avise, 1994), species with life histories conducive to the geographic movement of individuals or dissemination of life cycle stages tend to show less population structure than those with lower vagility. For example, Paggi et al. (1991) and Nascetti et al. (1993) have used multilocus protein-electrophoretic data to show that species of ascaridoid nematodes using seal definitive hosts (and fish/invertebrate intermediate and paratenic hosts) have low amounts of genetic structuring across broad geographic ranges of the Arctic-Atlantic Boreal region. Geographic population samples of the species referred to as *Contracaecum osc-*

*culatum* "A" (7 localities) and *C. osculatum* "B" (19 localities) had average  $F_{ST}$  values of 0.042 and 0.046, respectively, over distances spanning more than 5,000 km (Nascetti et al., 1993). Likewise, 3 species in the *Pseudoterranova decipiens* complex (referred to as "A, B, and C" by the authors), which tend to share the same definitive hosts as the *C. osculatum* species, showed average  $F_{ST}$  values of 0.059, 0.055, and 0.021, respectively, for the cryptic species "A," "B," and "C" (Paggi et al., 1991). These studies are particularly noteworthy in that species in these 2 complexes are known to have the same general life cycle patterns, and would seem to share many of the same intermediate, paratenic, and definitive hosts. The distribution of 94–98% of the total estimated gene diversity within all geographic localities is consistent with the hypothesis of Nascetti et al. (1993) that migration of the definitive hosts (seals) in combination with the transport of larvae in fish and invertebrates (intermediate/paratenic hosts) serve as highly effective mechanisms of gene flow over large geographic distances. Interestingly, studies of population structure in marine fishes also show high gene flow, with more than 98% of the total gene diversity found within all localities (Gyllensten, 1985).

Several other studies have suggested a relationship between high parasite dispersion or host vagility and low genetic structure for parasite populations. McManus (1985) reported on a helminth system that would appear to have the potential for high gene flow among localities. *Ligula intestinalis*, a pseudophyllidean from fish-eating birds, has an extremely wide intermediate host range (>20 cyprinid fish species may host plerocercoids). This life history would appear to be conducive to high gene flow due to properties intrinsic to both hosts and parasite. First, as a consequence of their high vagility, birds are characterized by the lowest levels of average population structure among vertebrates (Ward et al., 1992); this host property should serve to disperse parasite eggs effectively. Second, this parasite can use many different fish intermediate hosts, which means that many aquatic communities should be effective sources of infection. Although only 1 polymorphic enzyme locus was assessed among population samples of plerocercoids obtained from fish, samples (with  $n > 15$ ) from 4 geographic localities in southern England showed very similar frequencies for the 2 alleles at the phosphoglucomutase-2 locus, suggesting that avian definitive hosts may be responsible for high levels of gene flow via egg dispersal among localities. Similarly, the potential role of low host specificity and high host mobility for reducing genetic structure was shown by Hilburn and Sattler (1986a) in an electrophoretic study of the lone star tick, *Amblyomma americanum*. Analysis of 9 geographic population samples distributed across the widespread range of this species yielded an average  $F_{ST}$  value of 0.037, and no evidence for an isolation-by-distance effect. Because life cycle stages of this 3-host tick are relatively sedentary off the host, Hilburn and Sattler (1986a, 1986b) inferred that the observed lack of genetic structure must result from host mobility. *Amblyomma americanum* fits several of the general predictions for reduced genetic structure in Table I, including high vagility hosts (including deer and cattle), low host specificity, and the potential for a long life span for individual parasites.

Most recently, studies of mitochondrial-DNA (mtDNA) phylogeography in *Ostertagia ostertagi* (Blouin et al., 1992; Dame et al., 1993) have revealed very high genetic diversity within

geographic populations, but extremely low differentiation among localities. Using an estimate of mitochondrial genetic diversity that is analogous to  $F_{ST}$ , Bouin et al. (1992) reported that, on average, less than 1% of the total gene diversity was partitioned between geographic populations. For *O. ostertagi*, gene flow among localities appears to be high, and may be mediated by the transport of cattle throughout the U.S.A. Interestingly, despite this lack of observed differentiation in mtDNA (and with the inference of high levels of gene flow), genetically based geographic differences in the timing of developmental arrest (hypobiosis) have been demonstrated for temperate and subtropical populations of *O. ostertagi* (Smeal and Donald, 1981; Frank et al., 1986, 1988). Thus, Blouin et al. (1992) have hypothesized that the variation in timing of hypobiosis between the northern and southern U.S. populations may be maintained by strong selection in the presence of high gene flow, and that high intra-population diversity is a product of the large effective size of the populations (4–8 million individuals per geographic population based on the observed diversity of mtDNA).

Only a few published studies are available to demonstrate the potential effects of natural history traits that are expected to increase the genetic structure of parasite populations. Among studied parasites, pocket gopher chewing lice have shown levels of population genetic subdivision that are among the highest reported over limited geographic scales for any animal. For example, the mean  $F_{ST}$  value for microgeographic (within-locality) differentiation of *Geomysocetus actiosi* infrapopulations (5 independent comparisons) was 0.092, and ranged from 0.039 to 0.162 (Nadler et al., 1990). Macrogeographic differentiation of these infrapopulations over the 200-km range of the study area revealed an average  $F_{ST}$  of 0.24, which was virtually identical to that inferred for their hosts in a previous study (Hafner et al., 1983). The natural history of the pocket gopher-chewing louse assemblage would seem to include many factors promoting population subdivision. For example, the lice are autoxenous and exclusively contact-transmitted, pocket gophers are fossorial and actively avoid intraspecific contact, and louse populations appear to suffer seasonal bottlenecks in size that should reduce  $N_e$  (Nadler et al., 1990). Thus, gene flow among louse infrapopulations is dependent upon interhost contact, which in pocket gophers is primarily limited to mating encounters and the rearing of young. This unusual linkage between pocket gopher reproduction and louse transfer means that host gene flow is likely to represent the upper boundary for louse gene flow in this assemblage.

Recent research on *Ascaris suum* (Nadler et al., 1995), using protein electrophoretic and RAPD PCR-based markers, has also revealed significant patterns of genetic structure, with reported values of average  $F_{ST}$  for infrapopulations and localities indicative of moderate genetic differentiation. For example, values of  $F_{ST}$  among 7 infrapopulations were approximately 0.09 as inferred from both isoenzyme and RAPD markers. When infrapopulations from each collecting site were pooled,  $F_{ST}$  values averaged 0.078 (isoenzyme) and 0.062 (RAPD) for the 5 upper midwestern localities. Even infrapopulations representing different farms from a single geographic region showed moderate genetic differentiation, with isoenzyme and RAPD-based  $F_{ST}$  estimates of 0.080 and 0.093, respectively. *Ascaris suum* populations appear to be characterized by much more subdivision than described for *O. ostertagi* by Blouin et al. (1992), even

though both nematodes are parasites of livestock and are subjected to frequent transport. However, one potentially important difference between these species is that although susceptible cattle may each host 10,000–100,000 *O. ostertagi* (Armour et al., 1979; Williams et al., 1983), pigs infected with *A. suum* harbor much smaller infrapopulations. This difference in the size of breeding populations may cause genetic drift within geographic localities of *A. suum*, effectively promoting independent differentiation of populations.

Potential complicating effects of parasite genetic structure such as asexual amplification within intermediate hosts (digeneans), overdispersion, and considerations of geographical scale have rarely been investigated. One exception involves the studies of Lydeard et al. (1989) and Mulvey et al. (1991) on the genetic structure of liver flukes (*Fascioloides magna*) parasitizing white-tailed deer. Populations of *F. magna* representing Savannah River Site hunt units showed low average differentiation ( $F_{ST} = 0.016$ ), and patterns of genetic distance between populations of flukes were not related to patterns of host differentiation or to the geographic distance between hunt units. In contrast, populations of *F. magna* collected from different states (South Carolina and Tennessee) showed markedly greater levels of differentiation (mean  $F_{ST} = 0.176$ ) and an isolation-by-distance effect (Mulvey et al., 1991). As is characteristic of many host-parasite systems, the distribution of *F. magna* among deer hosts was highly aggregated, and this appeared to influence the genetic structure of fluke populations. Asexual reproduction of *F. magna* in snail intermediate hosts results in localized distributions of metacercariae of the same clone, and this clumping of identical metacercaria increases the likelihood that deer will be infected with adults representing 1 clone. In their study, Mulvey et al. (1991) found that deer tend to be infected with flukes of the same multilocus genotype, and simulation studies suggested that approximately one-half of the mean  $F_{ST}$  values may be explained by this nonrandom distribution of fluke genotypes among deer hosts.

#### FINAL COMMENTS

The development of PCR-based DNA markers has provided new opportunities for assessing the genotypes of small individual parasites and investigating population genetic structure. Published studies employing traditional biochemical genetic markers on relatively large individual parasites have revealed marked differences in population genetic structure among certain taxa, and some of these differences may be attributed to parasite or host factors that influence the geographic movement of individuals or dissemination of life cycle stages in the environment. Designing testable hypotheses to assess the relationships between life history variables of parasites and patterns of genetic structure is likely to be challenging. Due to the potentially confounding effects caused by host parameters, studies of genetic structure in parasites may benefit from a comparative approach in which several species of parasites that co-occur in populations of a single host species are investigated simultaneously. Investigating the genetic structure of the host populations may also prove beneficial, although few host-parasite systems are likely to be characterized by absolute linkage between host and parasite gene flow. Finally, a phylogenetic perspective may offer the greatest opportunity for investigating the contri-

butions of different parasite life history features to the genetic structure of their populations. For example, sister-taxa of parasites that coexist in the same host species and differ in one or few life history features would appear to represent the ideal case for assessing the influence of parasite attributes on population genetic structure.

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#### LITERATURE CITED

- ADAMSON, M. L. 1989. Evolutionary biology of the Oxyurida (Nematoda): Biofacies of a haplodiploid taxon. In *Advances in parasitology*, Vol. 28, J. R. Baker and R. Muller (eds.). Academic Press, London, U.K., p. 175-228.
- ARMOUR, J., K. BAIRDEN, J. L. DUNCAN, F. W. JENNINGS, AND J. J. PARKINS. 1979. Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. *Veterinary Record* **106**: 500-503.
- AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York; New York, 511 p.
- , J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, AND N. C. SAUNDERS. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**: 489-522.
- BARTON, N. H., AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**: 409-415.
- BLOUIN, M. S., J. B. DAME, C. A. TARRANT, AND C. H. COURTNEY. 1992. Unusual population genetics of a parasitic nematode: mtDNA variation within and among populations. *Evolution* **46**: 470-476.
- BRUDASTOV, A. N., V. R. LEMELEV, S. K. Kholnukhanev, AND L. N. KRASNOS. 1971. The clinical picture of the migration phase of ascariasis in self-infection. *Meditinskaya Parazitologiya i Parazitarnye Bolezni* **40**: 165-168.
- BULLINI, L., G. NASCETTI, L. PAGGI, P. ORECCHIA, S. MATTIUCI, AND B. BERLAND. 1986. Genetic variation of ascaridoid worms with different life cycles. *Evolution* **40**: 437-440.
- CHESSER, R. K., O. E. RHODES JR., D. W. SUOG, AND A. SCHNABEL. 1993. Effective sizes for subdivided populations. *Genetics* **135**: 1221-1232.
- CROW, J. F., AND K. AOKI. 1984. Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences USA* **81**: 6073-6077.
- , AND M. KIMURA. 1970. An introduction to population genetics theory, 1st ed. Harper and Row, New York, New York, 591 p.
- DAME, J. B., M. S. BLOUIN, AND C. H. COURTNEY. 1993. Genetic structure of populations of *Ostertagia ostertagi*. *Veterinary Parasitology* **46**: 55-62.
- DARWIN, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. Reprinted. E. A. Weeks and Company, Chicago, Illinois, 503 p.
- ENDLER, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, New Jersey, 336 p.
- ESCH, G. W., AND J. C. FERNANDEZ. 1993. A functional biology of parasitism. Chapman and Hall, London, U.K., 337 p.
- FALCONER, D. S. 1981. Introduction to quantitative genetics, 2nd ed. Longman, New York, New York, 340 p.
- FELSENSTEIN, J. 1982. How can we infer geography and history from gene frequencies? *Journal of Theoretical Biology* **96**: 9-20.
- FISHER, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford, U.K., 272 p.
- FORD, E. B. 1964. Ecological genetics. Methuen and Company, London, U.K., 335 p.
- FRANK, G. R., R. P. HERD, K. S. MARSHALL, AND J. C. WILLIAMS. 1986. Effects of transfer of *Ostertagia ostertagi* between northern and southern U.S.A. on the pattern and frequency of hypobiosis. *International Journal for Parasitology* **16**: 391-398.
- , —, —, —, AND E. R. WILLIS. 1988. Additional investigations on hypobiosis of *Ostertagia ostertagi* after transfer between northern and southern U.S.A. *International Journal for Parasitology* **18**: 171-177.
- GRANT, W. N. 1994. Genetic variation in parasitic nematodes and its implications. *International Journal for Parasitology* **24**: 821-830.
- GYLLENSTEN, U. B. 1985. The genetic structure of fish: Differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology* **26**: 691-699.
- HAFNER, J. C., D. J. HAFNER, J. L. PATTON, AND M. F. SMITH. 1983. Contact zones and the genetics of differentiation in the pocket gopher *Thomomys bottae* (Rodentia: Geomyidae). *Systematic Zoology* **32**: 1-20.
- HALDANE, J. B. S. 1930. A mathematical theory of natural and artificial selection. Part 4. Isolation. *Proceedings of the Cambridge Philosophical Society* **26**: 220-230.
- HANDEL, S. N. 1982. Dynamics of gene flow in an experimental population of *Cucumis melo* (Cucurbitaceae). *American Journal of Botany* **69**: 1538-1546.
- HEDRICK, P. W. 1983. Genetics of populations. Science Books International, Boston, Massachusetts, 629 p.
- HILBURN, L. R., AND P. W. SATTLER. 1986a. Electrophoretically detectable protein variation in natural populations of the lone star tick, *Amblyomma americanum* (Acar: Ixodidae). *Heredity* **56**: 67-74.
- , AND —. 1986b. Are tick populations really less variable and should they be? *Heredity* **57**: 113-117.
- HILLIS, D. M., AND C. MORITZ. 1990. Molecular systematics. Sinauer Associates, Sunderland, Massachusetts, 588 p.
- HUDSON, R. R. 1990. Gene genealogies and the coalescent process. In *Oxford surveys in evolutionary biology*, Vol. 7, D. J. Futuyma and J. Antonovics (eds.). Oxford University Press, Oxford, U.K., p. 1-44.
- , M. SLATKIN, AND W. P. MADDISON. 1992. Estimation of levels of gene flow from DNA sequence data using cladistic and pairwise methods. *Genetics* **132**: 583-589.
- KENNEDY, C. R. 1987. Long term stability in the population levels of the eye fluke *Tylocephalus podicipinus* (Digenea: Diplostomatidae) in perch. *Journal of Fish Biology* **31**: 571-581.
- , AND R. J. BURROUGH. 1977. The population biology of two species of eye fluke, *Diplostomum gasterostei* and *Tylocephalus clavata*, in perch. *Journal of Fish Biology* **11**: 619-633.
- KIMURA, M. 1962. On the probability of fixation of mutant genes in a population. *Genetics* **47**: 713-719.
- , 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, U.K., 367 p.
- , AND J. F. CROW. 1963. The measurement of effective population number. *Evolution* **17**: 279-288.
- , AND T. OHTA. 1969. The average number of generations until fixation of a mutant gene in a finite population. *Genetics* **61**: 763-771.
- LYDEARD, C., M. MULVEY, J. M. AHO, AND P. K. KENNEDY. 1989. Genetic variability among natural populations of the liver fluke, *Fascioloides magna*, in white-tailed deer, *Odocoileus virginianus*. *Canadian Journal of Zoology* **67**: 2021-2025.
- LYMBERY, A. J., AND R. C. A. THOMPSON. 1988. Electrophoretic analysis of genetic variation in *Echinococcus granulosus* from domestic hosts in Australia. *International Journal for Parasitology* **18**: 803-811.
- MARGOLIS, L., G. W. ESCH, J. C. HOLMES, A. M. KURIS, AND G. A. SCHAD. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). *Journal of Parasitology* **68**: 131-133.

- MARUYAMA, T., AND M. KIMURA. 1974. Geographical uniformity of selectively neutral polymorphisms. *Nature (London)* **249**: 30–32.
- , AND —. 1980. Genetic variability and effective population size when local extinction and recolonization of subpopulations are frequent. *Proceedings of the National Academy of Sciences USA* **77**: 6710–6714.
- MAY, R. M. 1973. Stability and complexity in model ecosystems. Princeton University Press, Princeton, New Jersey, 235 p.
- MAYR, E. 1954. Change of genetic environment and evolution. In *Evolution as a process*, J. Huxley, A. C. Hardy, and E. B. Ford (eds.). Allen and Unwin, London, U.K., p. 157–180.
- MCMANUS, D. P. 1985. Enzyme analysis of natural populations of *Schistocephalus solidus* and *Ligula intestinalis*. *Journal of Helminthology* **59**: 323–332.
- MULVEY, M., J. M. AHO, C. LYDEARD, P. L. LEBERG, AND M. H. SMITH. 1991. Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. *Evolution* **45**: 1628–1640.
- NADLER, S. A. 1990. Molecular approaches to studying helminth population genetics and phylogeny. *International Journal for Parasitology* **20**: 11–29.
- , M. S. HAFNER, J. C. HAFNER, AND D. J. HAFNER. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). *Evolution* **44**: 942–951.
- , R. L. LINQUIST, AND T. J. NEAR. 1995. Genetic structure of midwestern *Ascaris suum* populations: A comparison of isoenzyme and RAPD markers. *Journal of Parasitology* **80**: (in press).
- NAGYLAKI, T. 1975. Conditions for the existence of clines. *Genetics* **80**: 595–615.
- NASCETTI, G., R. CIANCHI, S. MATTIUCCHI, S. D'AMELIO, P. ORECCHIA, L. PAGGI, J. BRATTEY, B. BERLAND, J. W. SMITH, AND L. BULLINI. 1993. Three sibling species within *Contracaecum osculatum* (Nematoda, Ascaridida, Ascaridoidea) from the atlantic arctic-boREAL region: Reproductive isolation and host preferences. *International Journal for Parasitology* **23**: 105–120.
- NEI, M., AND A. CHAKRAVARTI. 1977. Drift variances of  $F_{ST}$  and  $G_{ST}$  statistics obtained from a finite number of isolated populations. *Theoretical Population Biology* **11**: 307–325.
- PAGGI, L., G. NASCETTI, R. CIANCHI, P. ORECCHIA, S. MATTIUCCHI, S. D'AMELIO, B. BERLAND, J. BRATTEY, J. W. SMITH, AND L. BULLINI. 1991. Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the north Atlantic and Norwegian and Barents seas. *International Journal for Parasitology* **21**: 195–212.
- PRICE, P. W. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, New Jersey, 237 p.
- SCHAD, G. A., AND R. M. ANDERSON. 1985. Predisposition to hookworm infection in humans. *Science* **228**: 1537–1539.
- SHIELDS, W. M. 1993. The natural and unnatural history of inbreeding and outbreeding. In *The natural history of inbreeding and outbreeding*, N. W. Thornhill (ed.). University of Chicago Press, Chicago, Illinois, p. 143–169.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* **39**: 53–65.
- . 1987. Gene flow and the geographic structure of natural populations. *Science* **236**: 787–792.
- . 1991. Inbreeding coefficients and coalescence times. *Genetical Research* **58**: 167–175.
- . 1993. Isolation by distance in equilibrium and nonequilibrium populations. *Evolution* **47**: 264–279.
- , AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**: 1349–1368.
- , AND W. P. MADDISON. 1989. A cladistic measure of gene flow from the phylogenies of alleles. *Genetics* **123**: 603–613.
- SMEAL, M. G., AND A. D. DONALD. 1981. Effects of inhibition of development on the transfer of *Ostertagia ostertagi* between geographical regions of Australia. *Parasitology* **82**: 389–399.
- TAKAHATA, N. 1983. Gene identity and genetic differentiation of populations in the finite island model. *Genetics* **104**: 497–512.
- VAN DEN BUSSCHE, R. A., M. J. HAMILTON, AND R. K. CHESSER. 1986. Problems of estimating gene diversity among populations. *Texas Journal of Science* **38**: 281–287.
- WARD, R. D., D. O. F. SKIBINSKI, AND M. WOODWARD. 1992. Protein heterozygosity, protein structure, and taxonomic differentiation. *Evolutionary Biology* **26**: 73–159.
- WEIR, B. S. 1990. *Genetic data analysis*. Sinauer Associates, Sunderland, Massachusetts, 377 p.
- , AND C. C. COCKERHAM. 1984. Estimating  $F$ -statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
- WILLIAMS, J. C., J. W. KNOX, B. A. BAUMANN, T. G. SNIDER, M. G. KIMBALL, AND T. J. HOERNER. 1983. Seasonal changes of gastrointestinal nematode populations in yearling beef cattle in Louisiana with emphasis on prevalence of inhibition in *Ostertagia ostertagi*. *International Journal of Parasitology* **13**: 133–143.
- WRIGHT, S. 1912. Notes on the anatomy of the trematode, *Microphallus opacus*. *Transactions of the American Microscopical Society* **31**: 167–175.
- . 1922. Coefficients of inbreeding and relationship. *American Naturalist* **56**: 330–338.
- . 1931. Evolution in Mendelian populations. *Genetics* **16**: 97–159.
- . 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Proceedings of the Sixth International Congress of Genetics I*: 356–366.
- . 1938. Size of population and breeding structure in relation to evolution. *Science* **87**: 430–431.
- . 1940. Breeding structure of populations in relation to speciation. *American Naturalist* **74**: 232–248.
- . 1951. The genetical structure of populations. *Annals of Eugenics* **15**: 323–354.
- . 1956. Modes of selection. *American Naturalist* **90**: 5–24.
- . 1965. The interpretation of population structure by  $F$ -statistics with special regard to systems of mating. *Evolution* **19**: 395–420.
- . 1978. *Evolution and the genetics of populations*, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, Illinois, 580 p.
- . 1982. The shifting balance theory and macroevolution. *Annual Review of Genetics* **16**: 1–19.



# History of microevolutionary thought in parasitology: The integration of molecular population genetics

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I never cease to marvel that the DNA and protein markers magically emerging from molecular-genetic analyses in the laboratory can reveal so many otherwise hidden facets about the world of nature.

—John C. Avise, *Molecular Markers, Natural History, and Evolution* (2004).

Molecular population genetics has had two major impacts in biology. It has opened the door to many questions in areas such as genetic inheritance modes and mapping, reproductive modes (sexual vs. asexual reproduction), mating systems (random vs. nonrandom), relatedness, population demography (growth/decline) and connectivity (gene flow), inference of selection, phylogeography, and delimitation of species. It has also mixed the disciplines of ecology, evolution, genetics, and molecular biology. As a result, we have a better understanding of microevolution (theory and empirical), and a means (albeit often indirectly) to study the population biology of any living organism. The latter is especially pertinent for parasites which, “because of their small size, location, biology, and behavior, direct observation of their population biology is almost impossible” (de Meeûs et al., 2007).

In the present chapter, I have been tasked with reflecting on the development of microevolutionary concepts in the field of parasitology over the past 100 years, since publication of the first volume of *The Journal of Parasitology* (*JP*). My goal is not to review or make broad generalizations about parasite microevolution, but rather to recap the history of thought and applications of molecular population genetics in parasitology. As chapter authors, we were also asked to pick an early paper from *JP* to highlight its relevance or impact in its respective field. I have selected Steven Nadler's (1995) review, “Microevolution and the genetic structure of parasite populations,” because I believe, personally, that it is one of the most under-recognized papers with regards to parasite microevolution. Indeed, I recall early in my graduate career reading his elegant analysis, but filing it away. When I started to write my dissertation, I felt that several of my ideas were novel. However, upon revisiting Nadler (1995), I realized otherwise and remember begrudgingly yelping, “Nadler!” (in much the same way that Jerry Seinfeld would exclaim “Newman!” in the comedy TV show *Seinfeld*). So, why feature an under-recognized paper and why choose a paper from 1995 as opposed to one early in *JP*'s history? To answer these questions, we need to step back in time.

I will first provide a brief history of population genetics in general and then expand the discussion to include parasites. I will then recount the history of some specific topics to show how molecular population genetics has resonated in different aspects of parasite population biology and evolution. To save space,

reviews are referenced when possible rather than original publications. I will attempt to cover various protozoans and metazoan parasites of animals, but admittedly my own experiences bias the review towards helminths. Stealing the words from a historical account of population genetics by James Crow (1987), a renowned population geneticist, “Space limitations dictate that this review be selective... The choice of subjects is arbitrary; they are topics that I think are interesting and historically important.”

# A very brief recap of population genetics history

Modern evolutionary theory has its origins with Darwin (1859), who focused on natural selection, but also hinted at the evolutionary mechanisms of inbreeding, gene flow, and drift. But, Darwin did not have correct explanations for two factors that are at the heart of what we now call genetics, namely, the mode of inheritance and mutation, the ultimate source of heritable variation. Although contemporaneous with Darwin's, Gregor Mendel's work on inheritance (segregation and independent assortment) in 1866 was not brought to light until its rediscovery in 1900 (Bowler, 2010). In 1908, two publications would formulate Mendelian segregation at the population level in what we recognize today as the Hardy–Weinberg Law (HWL); however, Weinberg's paper was neglected for 35 years (Crow, 1999). The status of HWL in modern evolutionary theory is well stated by Crow (1987): “Although the principle is trivially simple, it is nevertheless the foundation for theoretical population genetics.”

From the 1920s to the 1950s, population genetics was dominated by, and gained its quantitative base from, its founding “fathers,” Sewall Wright, Ronald Fisher, and J. B. S. Haldane (Crow, 1987). Their work, of which there was overlap among the three, was prominent in melding Mendel's inheritance principles with Darwin's theory of natural selection (Crow, 1987). By the 1940s, modern evolutionary thought had its start (Bowler, 2010). As a brief flash forward, Wright's work on inbreeding, population structure, and genetic drift has probably had the greatest impact on the field of molecular ecology. I believe this impact is obvious in the near universal use of hierarchical *F*-statistics and analogs, which have their origin with Wright. Crow (1987) noted two newcomers to the discipline in the 1950s. Motoo Kimura, who is known for the neutral theory of molecular evolution, and Gustave Malécot, who actually had several publications in the 1940s that were novel or extended Wright's work (Crow, 1987). Malécot's recognition, however, as with Mendel and Weinberg, came late (Epperson, 1999). Obviously, microevolutionary theory has advanced since the 1950s, but the basics were in place by then. Thus, I shift now to recount the history of some important molecular genetic markers/techniques as these provided the empirical means to test or implement population genetics theory.

In 1966, three papers published genetic diversity data based on gel electrophoresis of proteins (Lewontin, 1991). Much of the initial population genetic applications in the 1960s and 1970s focused on the Classical-Balance debate. Under the “classic” view, genetic variation would be low due to purifying selection, whereas the “balance” view held that variation would be high due to balancing selection, e.g., heterozygote superiority. This discussion morphed into the Neutralist-Selectionist debate, in which Neutralists proposed that most genetic variation was the product of mutation and genetic drift rather than natural selection; see Lewontin (1991) and Avise (2004) for details regarding these disputes). Here, I would emphasize that electrophoresis provided the first methodology for accessing co-dominant

genetic data “at many independent loci, chosen without bias with respect to magnitude of genomic variability” (Avise, 2004).

Biologists hit the ground running with electrophoretic genotyping and, in about 10 years, reviews were already meta-analyzing polymorphism and heterozygosity data from plant and animal populations (Avise, 2004). For example, Nevo (1978) examined broad scale patterns of enzyme-based genetic variation across 228 animal species, but, as a prelude to my discussion next, none was parasitic! Lewontin (1991) remarked that electrophoresis “marked the first stage in a new path of evolutionary genetics” and “provided for the first time the possibility of including virtually any organism in the study of evolutionary variation on the basis of a common denominator across species.” Was the latter statement true for parasites? As a sizable amount of tissue was needed to obtain multilocus genotypes (MLGs), tiny organisms that could not be cultured clonally were still not fully available for such research.

By the mid-1970s, direct DNA based approaches, such as restriction fragment-length polymorphisms, were being developed. These advances spawned the field of phylogeography, which in its origin (late 1970s) had more of a phylogenetic base, and hence, a historical approach to studying populations (Avise, 2000). In 1977, Sanger-based DNA sequencing was developed (Nelson et al., 2011), although electrophoresis remained a prominent means to obtain genotypic data through the 1980s.

The next major advance came about in 1985, with the polymerase chain reaction (PCR) (Bartlett and Stirling, 2003). From the perspective of a parasite population geneticist, PCR was truly significant because one could not only target DNA directly (as opposed to the phenotypic expression of a protein), but the technology did not require large quantities of tissue. With the advance of microsatellite markers in 1989, there was now a direct DNA marker that provided the equivalent co-dominant genotypic data of protein electrophoresis. To me, microsatellite scoring was probably the last significant genotyping method developed until the advent of next generation sequencing (NGS) 16 years later in 2005 (Nelson et al., 2011). I will return to NGS in my closing personal reflections.

In summary, I have two comments to close this section. Note how the work of important pioneers, e.g., Mendel, Weinberg, and Malécot, often went unrecognized, sometimes even neglected for long periods. Second, I hope it is clear why I do not highlight a *JP* paper published in 1914 to the early 1920s, i.e., the field of population genetics was just starting!

# The snail's pace flow of molecular population genetics into parasitology

Modern microevolutionary thought in parasitology can also be traced back to Darwin (1859), who makes a few statements about parasite traits that could reflect adaptations and co-adaptions to hosts. For example, in talking about a reduced head region in a parasitic barnacle, Darwin (1859) speculated on the potential selective advantage of the loss of morphological features: “Each individual *Proteolepas* would have a better chance of supporting itself, by less nutriment being wasted in developing a structure now become useless.” I chose this quote because parasitologists can surely attest to their study organisms' limited morphology, a primary factor that will later contribute to key applications of molecular population genetics. Microevolutionary thought (mostly related to natural selection) in parasitology was therefore present from Darwin's time to the advent of electrophoresis, e.g., for co-evolutionary matching alleles, selection model, see Mode (1958). However, I skip over this part of microevolutionary history in parasitology in order to focus on the integration of molecular population genetics.

The use of electrophoresis in parasite studies began in the late 1960s and early 1970s. Most applications assessed the marker itself and would ask questions such as were all Mendelian genotypes observed, was it a dimer, etc. (e.g., Zee et al., 1970). Or, researchers used banding profiles as more of a phenotypic trait, e.g., strain typing of protozoan parasites (Reeves and Bischoff, 1968), rather than estimate population genetic parameters. Some studies started to examine the distribution of allele frequencies among parasite populations (e.g., Carter and Voller, 1975), but little to no population genetic statistics or tests were used specifically. Thus, the 1970s were largely devoid of parasite population genetics (*sensu stricto*) even though such studies were commonly published on plants and free-living animals (Avise, 2004). The first papers (at least for helminths) that used molecular markers and population genetics theory or statistics to infer something about parasite biology were authored by Beverley-Burton et al. (1977), Beverley-Burton (1978), Vrijenhoek (1978), and Bullini et al. (1978).

However, before discussing these contributions, I feel the need to first underscore Peter Price's (1977, 1980) treatise on parasite evolutionary biology. He was the first to use parasite life history characteristics to make predictions about evolutionary mechanisms. He had to rely heavily upon an inductive process because no parasite population genetic data were available and, being an entomologist, most of his examples were of phytophagous insects. He largely viewed parasites as existing in ephemeral fragmented environments, i.e., the host (Price, 1980). In Price (1980), he stated [from here on, my analogies are in brackets] “For very small organisms [parasites] a wide dispersion of resources [individual hosts] within patches [host populations] and considerable distances between patches makes colonization of new hosts hazardous.” Because he saw

hermaphroditism and asexuality as common reproductive modes among parasites (Price, 1980), he believed these modes were adaptations to facilitate colonization by individual parasites. As a result, these traits would cause increased homozygosity via inbreeding and/or bottlenecking.

Although Price (1977) recognized life history variation among parasites, his final conclusion was one-sided when he stated, “The general patterns envisaged for parasitic species include small, relatively homozygous populations with little gene flow between populations, which results in many specialized races, rapid evolution and speciation without geographic isolation, and an abundance of sibling species.” Overall, I consider many of Price's arguments to be at the macroevolutionary scale, at which hypotheses are best tested within a phylogenetic framework. Nonetheless, the first part of the quote does provide what I view as the first non-natural selection related predictions, i.e., a focus on mating system, genetic drift, and gene flow, in parasites. Price (1977) also readily admitted that his generalizations “need critical evaluation” and outlined several core microevolutionary questions that required attention. For example,

In sexually reproducing parasites population structure requires much attention. What is the effective population size, the distance moved by the dispersal phase of parasites, the frequency of gene flow from one population to another, the behavior of individuals which influence mating patterns within and between populations?

He followed by saying, “The genetics of parasite species and races should receive much more attention.”

Not until the 1980s do we see a rise in the use of electrophoretic data in population genetic studies of parasites, although numbers of papers still pale in comparison to free-living organisms. Thus, compared to the population genetics literature on free-living plants and animals, there is about a 10-year lag before a synthesis of polymorphism and heterozygosity data in parasites. Nadler (1990) listed 23 helminth species in a review that really is the first to consider parasites in the Neutralist-Selectionist debate, which by this time had started to fade away in the primary literature as it really had no ultimate resolution (see Avise, 2004). A significant insight that stemmed from the Nadler review was that “Most endoparasitic helminth ‘populations’ or species surveyed have levels of genetic variation similar to those of free-living invertebrates.” Though Nadler (1990) does not discuss Price (1977, 1980) specifically, this review may be the first collective hint that Price's predictions may not apply across all parasites. In Nadler's (1990) final comments, he states the obvious: “Parasitologists have rarely used the full potential of biochemical and molecular methods to study the population genetics and phylogeny of helminths.” In the same year, a parallel review on molecular population genetics of protozoan parasites was published (Tibayrenc et al., 1990). All references were from the 1980s, thereby illustrating the 10-year lag in population genetics of protozoan parasites as well. The microevolutionary thought

of Tibayrenc et al. (1990) was unique from previous population genetic reviews on free-living organisms and of Nadler (1990) in that the attention was on mode of reproduction (clonal vs. sexual). Here, we see the statistical application of HWL and linkage disequilibrium (LD), the nonrandom association of alleles among loci, in order to make inferences on the assorted patterns of reproductive modes found among protozoan parasites.

The trickle of papers on parasite population genetics continued into the early 1990s and, in addition to electrophoresis, DNA based methods were starting to be used. Since Price (1980), however, no one had revisited the collective expectations of population genetic patterns in relation to parasite ecology and life history. Nadler (1995) stated,

Although the paucity of available studies makes it premature to develop general conclusions about the genetic structure of parasite populations, it is likely that new technologies [such as PCR] will soon promote groundbreaking research in this area. On the other hand, it is not premature to consider the types of questions that might be addressed in parasite population genetics.

He gave a broad overview (though a focus on metazoan parasites) that related theoretical and empirical population genetics to the extensive variation in parasite lifestyles. Continuing, Nadler wrote,

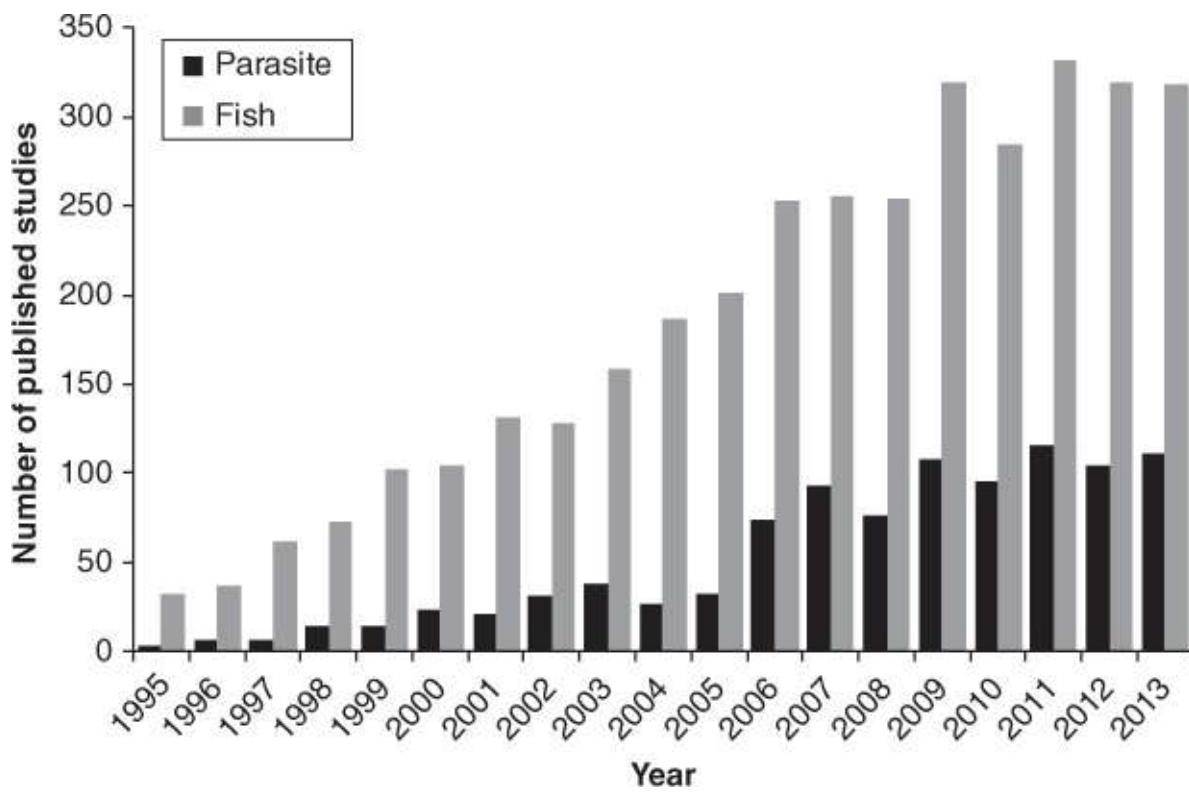
The genetic structure of parasite populations will be shaped strongly by ecological factors of individual species, including their demography, life cycle, mechanisms of dispersal, and host specificity. Characteristics of species such as the mating system and population-level attributes such as the effective size will affect certain aspects of demes, including the likelihood of random genetic drift.

In particular, he laid out how different parasite traits would act to increase or decrease genetic structuring (see Table I in Nadler, 1995). Thus, in comparison to Price (1977, 1980), Nadler's (1995) discourse provided a more holistic perspective in that parasite microevolutionary patterns are predicted to be as multifarious as the life histories of the parasites themselves. While several ideas in Nadler (1995) can be found in earlier or other, current publications, he did a great job of synthesizing the available data, tying them to population genetics theory, presenting novel ideas, and highlighting major gaps.

By establishing specific links between parasite life history and genetic drift, gene flow, and non-random mating, Nadler (1995) also provided a user-friendly framework for the application of molecular population genetics to parasite biology. In retrospect, it seems to me that he garnered less attention than what I would have assumed. For example, a parasite molecular ecology review I co-authored 10 years later (Criscione et al., 2005) has gotten more attention than Nadler (1995) (average citations per year are 16.1 and 4.8, respectively, as of 6 March 2014 in Web of Science). I think [jokingly] I heard Steve grumble “Criscione!” In Criscione and Blouin (2004), I too was guilty of not citing

[unintentionally] Nadler's (1995) ideas regarding the influence of life cycle patterns on gene flow. As noted previously, literature neglect seems common in evolutionary biology, so I will blame my lapse on that. On a sincere note, the reason I chose to highlight Nadler (1995) was to give it the credit due for providing a collective microevolutionary perspective of parasites that reflects the diversity of parasites themselves. It is a must read for anyone in the field and of broad interest to those outside.

With a well laid out framework, a list of questions to address, and a genetic toolkit that could be used on any organism, i.e., PCR and microsatellites, one would think that research on parasite population genetics would have skyrocketed. But, it did not. Again, there is an approximate 10-year lag in the use of microsatellites for parasite population genetics compared to free-living species. To illustrate (but see [Fig. 1](#) legend for major caveats), I used a Web of Science search on “microsatellite” and either “fish” or “parasite” ([Fig. 1](#)). Notice that in 1999 and 2000, there were  $\sim 103$  papers/year for “fish” and  $\sim 18$ /year for “parasite”. Not until 2009 and 2010 (10 years later) are there  $\sim 102$  papers/year for “parasite”. Sequence data, mainly mitochondrial (mtDNA) data in a phylogeographical framework, are also in extensive use at this time; a similar pattern can be found by replacing “phylogeography” for “microsatellite” in the previously mentioned search (data not shown).



**Figure 1** I conducted a Web of Science search on the terms “microsatellite” and either “fish” or “parasite” on March 6, 2014. The search was for each year separately. Extreme caution is advised in strictly interpreting results. All papers may not actually be population genetic studies or may not be using microsatellites in the respective organisms. Indeed, I know the three papers in 1995 under “parasite” are not studies on the population genetics of parasites two are not even on parasites. The figure is mainly of exploratory value and, while the exact numbers are incorrect, I suspect an approximate 10-year lag would be present even if proper scientific scrutiny were used. Keep in mind the search was only for one group of vertebrates as no one uses “free-living” as a keyword. Inclusion of other vertebrates would only increase the discrepancy. Thus, if anything, I suspect the figure gives a gross underestimate of parasites relative to free-living animals. I was not able to do a similar analysis for allozymes as Web of Science does not have abstracts or author provided keywords for most papers prior to 1995; thus, word searches will be less inclusive based on titles alone.

Despite these lags, there has been progress in the application of molecular population genetics to parasite biology from Nadler (1995) onward. It is just much less than one might expect given that parasitism is at least as common as a free-living lifestyle (Dobson et al., 2008). Various topics were being addressed and the next broad scale review to come along agglomerated these topics to emphasize how population genetics was applicable across a range of parasitology disciplines (Criscione et al. 2005). Specifically, we highlighted a hierarchical range of subjects (species identification, phylogeography, host specificity and speciation, population genetic structure, modes of reproduction and transmission patterns, and searching for loci under selection) that could be addressed in parasitology using a molecular ecology approach. By this time, available genetic data made it evident

that Price's (1977, 1980) predictions were not applicable across all parasites. Criscione et al. (2005) postulated why:

Many of Price's examples were phytophagous insects that can have many recurrent generations on a single host plant. In contrast, most animal macroparasites [metazoan parasites] release offspring into the external environment. Offspring are mixed and then recruited back into new definitive hosts. So the question of whether the component population (all the parasites of a given species in an entire host population; Bush et al., 1997) or the infrapopulation [conspecifics in/on a host] is best considered the relevant unit of evolution has been raised repeatedly (Lydeard et al., 1989; Nadler, 1995; Sire et al., 2001). In reality there is probably a continuum. If offspring are well mixed, then the transmission process only separates adult breeders into infrapopulations each generation but does not result in recurrent generations within individual infrapopulations. On the other end of the continuum, if offspring reinfect their natal host, e.g., lice, pinworms, or if offspring are transmitted as a clump from host to host over several generations, then the component population behaves more like a traditional subdivided population with infrapopulations as demes. Such species would be more likely to fit Price's predictions.

The use of population genetics to infer parasite transmission, a topic discussed next, is rooted in these concepts of the parasite deme.

Looking back on the history of the integration of molecular population genetics into parasitology, no one microevolutionary topic seemed to dominate the field at any single time. Moreover, each topic, like loci in a genome, has had its own historical path; some are parallel, but some diverge. Thus, I shift focus to the history of some of these topics that have resonated over the years in parasite population genetics.

# History of a few parasite population genetics topics

## How many species?

This question is one of the most fundamental, i.e., a necessary prerequisite before any downstream population genetics study, and maybe oldest questions in parasitology. Because reduced morphology in parasites (something Darwin noticed) can lead to ambiguity in species delimitation, here was an issue for which molecular population genetics could make significant contributions. Thus, it seems natural that the earliest papers (Beverley-Burton et al., 1977; Vrijenhoek, 1978) to use molecular population genetics addressed the potential for what we phrase today as “cryptic species” (morphologically similar, but genetically distinct). The conceptual basis behind this application is eloquently stated by Vrijenhoek (1978),

Populations diverge genetically as a result of adaptive and random processes. Often, individual gene loci diverge completely, such that distinct electromorphs are diagnostic of different species. Not all independent gene loci are expected to diverge to this extent, however. For example, genetic divergence need not produce concomitant morphological differentiation, resulting in cryptic or sibling species. When cryptic populations are sampled and analyzed as if they were a single randomly mating population (panmictic unit), deficiencies in the number of heterozygous genotypes commonly are observed as compared to the numbers expected by the Hardy-Weinberg equilibrium model [i.e., the Wahlund effect].

The principle of the Wahlund effect was used in several electrophoresis studies in the 1980s and 1990s (e.g., Renaud and Gabrion, 1988; Reversat et al., 1989; see nematode review by Anderson et al., 1998). A limitation of electrophoresis was that tissue requirements or low allozyme variation often precluded the ability to obtain variable MLGs. Thus, studies often relied on fixed alleles at a single locus between the cryptic groups, i.e., no heterozygotes were found. Sequence data became prominent in the late 1990s onward, especially mtDNA for “molecular prospecting” of cryptic species (Blouin 2002; Vilas et al., 2005). Such studies were largely based on single locus (mtDNA or rDNA) percent divergence or clade designation; hence, the term “prospecting” rather than “delimitation.” More recent approaches using MLGs have taken advantage of LD patterns in addition to HWL to identify cryptic species or populations (for examples with sequence data and microsatellites see Criscione and Blouin, 2004; Criscione et al., 2011, respectively). In the past few years, there has been a resurgence of attention given to the biological importance of recognizing cryptic species and the molecular methods that have been used to help identify and delimit (Perez-Ponce de Leon and Nadler, 2010; Nadler and Perez-Ponce de Leon, 2011). Criscione et al. (2005) stated, “The finding of cryptic parasite species has become very common as more phylogeographical and genetic structure studies are carried out on parasites.” I suspect this trend will continue.

## Hybridization

Curiosity about this topic in parasitology also predates molecular population genetics. Studies were based on laboratory crosses and interest seemed to be driven, in part, by the potential for hybrid sterility to lead to genetic assimilation or replacement of one species over another (e.g., LeRoux, 1954; Southgate et al., 1976; Le Jambre, 1979). By identifying hybrids of horse ascarids, Bullini et al. (1978) demonstrated how molecular markers could be used to assess parasite hybrids in nature (see also Vrijenhoek, 1978). In fact, molecular methods to identify hybrids paralleled cryptic species research due to conceptual similarities. Thus, early studies relied on fixed alleles between parental species, but looked for evidence of heterozygotes. As sequencing became available, nuclear-mtDNA discordance was used as a means to identify potential hybrids in nature. However, this discordance may result from incomplete lineage sorting or historical introgression rather than contemporary hybridization (reviewed in Detwiler and Criscione, 2010).

In the 2000s, the advent of genetic assignment tests, which use MLG data to assess HWL and LD, enabled the identification of contemporary (within ~3 generations) natural hybrids (for helminth examples see Criscione et al., 2007; Steinauer et al., 2008). As shown by the latter two studies, an important epidemiological consideration that stems from evidence of hybridization among two, host-associated parasite species/populations is that cross-transmission among host species must have happened. Hybridization studies on parasites themselves are still relatively few in number. Nonetheless, the current body of studies indicates it may be more common in nature than appreciated (Detwiler and Criscione, 2010). This situation is especially true when considering natural hybridization between clonal lineages of some protozoan parasites (e.g., Zingales et al., 2012), which I discuss next as a separate topic. Thus, more attention is warranted as,

...hybridization between species or diverged populations could result in the transfer of adaptive traits, promote divergence via reinforcement when hybrids are less fit than parentals, lead to homogenization across the genomes of the interbreeding populations, or promote rapid adaptive diversification via the formation of hybrid species. In relation to host-parasite interactions, such reticulate dynamics are of particular interest because host or parasite hybridization may impact host resistance/susceptibility or parasite infectivity, virulence, transmission, or host specificity.

(Detwiler and Criscione, 2010)

## Mode of reproduction in parasitic protozoa

If there is an area where parasite microevolution has been at the forefront of diploid population genetics (or at least on par), I would have to say it would have to be with respect to inference of asexual versus sexual as a primary means of

reproduction. Just as with helminths, electrophoretic studies on protozoans in the 1970s largely focused on taxonomic issues such as strain identification, but there was also interest in trying to determine if strains were associated with ecological/epidemiological variables (e.g., Miles et al., 1977, and references therein). In the 1980s, there began a focus on whether protozoan parasites had genetic exchange (meiosis and recombination) and this came to the forefront with the “clonal theory of parasitic protozoa” proposed by Tibayrenc et al. (1990). It sparked controversy via its inclusion of malarial parasites, which have an obligate sexual reproductive phase in its mosquito host (Dye, 1991). I will not delve into this debate, but simply state that the review stimulated many molecular population genetic analyses that explored parasitic protozoan reproductive modes in nature. By focusing on the effects clonal reproduction has on segregation and recombination, Tibayrenc et al. (1990) provided a framework to study the microevolution of protozoan parasites. Fascinating patterns that reflect complex reproductive histories involving some lineages with variable levels of genetic exchange, persistence of clonal lineages, and hybridization among these lineages have been observed among protozoan parasites (e.g., Miles et al., 2009; Zingales et al., 2012).

Important questions that remain today are: what drives the variable modes of reproduction and what is the ecological and evolutionary, and hence epidemiological, significance of these lineages (referred to as discrete typing units, DTUs)? For instance, Miles et al. (2009) stated,

Increasing evidence supports the idea that the 6 *T. cruzi* DTUs are historically and currently associated with distinct ecological niches, with concomitant implications for the epidemiology of Chagas disease. The niches are not fully understood, partly due to limited sampling and genotyping of *T. cruzi* isolates. As to be expected, interaction between niches occurs, changing as ecologies are disturbed, as evidenced by mixed DTU infections in vectors and mammals, including humans.

Interestingly, although a lot of empirical work on clonal reproduction in parasitic protozoans had been carried out since 1990 (see review on *Leishmania* and *Trypanosoma* by Tibayrenc and Ayala, 2013), theoretical work in terms of how population genetic statistics reflected clonal reproduction did not appear until the mid-2000s. This population genetics theory of clonal diploids, which had its origins in parasitology thanks to de Meeûs, Balloux, and colleagues (reviewed in de Meeûs et al., 2006), provided a framework to use measures of LD and inbreeding coefficients to assist in inferring modes of reproduction from population studies.

## **Comparative approach to studying population genetic structure**

“A comparative approach using both sexually and asexually reproducing parasites should be employed to examine the variation inherent in siblings and

populations” (Price, 1977). It was recognized early on that comparing parasites with different life histories could be useful for illuminating parasite traits that affect evolutionary mechanisms (see also Nadler, 1995), but 9 years passed before such a study was forthcoming. Bullini et al. (1986) used the comparative approach to determine whether parasite gene diversity is correlated with habitat heterogeneity and found “a significantly higher level of genetic variability exists in multiple-host ascaridoid species relative to single-host ones.” The authors argued that selection maintained polymorphism in parasites with complex life cycles, “one allozyme may work better in a certain step of the life cycle (e.g., when the host is a fish), and some other allozyme in another step (e.g., when the host is a marine mammal).” I view their conclusion as a product of the time because studies of this period often used natural selection as an explanation for observed differences in electrophoretic polymorphism (e.g., Nevo, 1978). Nadler (1990) criticized their conclusion on the basis that even direct life cycle parasites may encounter complex environments during within host migrations. I note here that the natural selection argument itself was flawed. In Bullini et al.’s (1986) scenario, selection would have to be for the heterozygote to maintain both alleles; otherwise, there would be directional selection for the allele with the greatest lifetime fitness, i.e., a reduction in diversity. I suspect the observed gene diversity patterns were, in part, driven by differences in effective population size ( $N_e$ ), a topic I address next. Today, it is recognized that “molecular measures of genetic diversity [neutral diversity] have only a very limited ability to predict quantitative genetic variability [adaptive diversity]” (Reed and Frankham, 2001).

It was 9 years later before another truly comparative paper appeared, but this time the focus was on a non-selective evolutionary mechanism. Blouin et al. (1995) published a seminal paper comparing patterns of genetic structure among trichstrongylid nematodes of domestic hosts and a wild host. Their approach enabled inference of host movement as a major factor affecting parasite gene flow. In the 2000s, comparative studies revealed how host or parasite characteristics, life cycle patterns, or host specificity could impact gene flow among parasite populations (reviewed in Criscione, 2008; Falk and Perkins, 2013). This body of work supports predictions by Nadler (1995). To date, comparative studies have focused on among-population dynamics, but within-population comparisons would be useful to highlight what factors affect mating systems or  $N_e$  (Criscione, 2008). As an aside, to address how organismal traits affect evolutionary mechanisms such as gene flow and  $N_e$ , studies must sample natural populations. As natural systems often have confounding factors, it may be difficult to find ideal comparative systems. In my opinion, one of the greatest strengths of using parasites as model systems in evolutionary studies is that their diverse life histories enable comparative studies.

## Inference of transmission and epidemiological monitoring

Nadler et al. (1990) noted that genetic differentiation of lice among individual

gophers is promoted by “transmission of relatively small number of lice from a female host to her offspring.” Inherent in this study, which addressed the scale of the parasite deme, was that patterns of genetic variation informed about transmission. The principal is nicely stated by Sire et al. (2001), “Genetic substructuring at the level of individual hosts from a transmission site would mainly result from the recruitment histories experienced by each of the individual host.” A human roundworm study by Anderson et al. (1995) brought population genetics as a means to infer transmission to the forefront of epidemiological studies, “Patterns of fine-scale population structure may provide information on transmission processes... Nonrandom distribution of parasite genotypes [based on neutral markers] could be generated if genetically related infective eggs are clumped in space” (Anderson et al., 1995). A study on malarial parasites 5 years later revealed a correlation between “high levels of self-fertilization [inferred from high LD and low MLG diversity] in populations with low levels of transmission [inferred from low prevalence]” (Anderson et al., 2000).

This latter observation had two large implications. Biologically, the result showed how clumped transmission could also interact with the mating system as co-transmission of related individuals could lead to biparental inbreeding or selfing (union of malaria gametocytes of a single parental oocyst is self-mating).

Epidemiologically, the population genetic patterns resulting from the high inbreeding when there is low transmission are now recognized as a means to monitor if malarial parasite populations decline in response to control (Volkman et al., 2012). Indeed, Nkhoma et al. (2013) provided empirical support for the latter. At this point I am reminded of a comment I received just 5 years ago on my rejected proposal that was aimed at using population genetics to monitor chemotherapy treatment of schistosomes: “Ultimately, it is not genetic diversity that will guide intervention and control programs, but egg counts in stool” (anonymous grant reviewer). I suspect that history will bypass this reviewer.

It is now recognized that molecular population genetics is a necessary tool in epidemiological studies from viruses to schistosomes (Pybus and Rambaut, 2009; Steinauer et al., 2010). For example, in a study on human roundworms Criscione et al. (2010) used evolutionary model-based, genetic assignment methods to identify transmission foci. Subsequent incorporation of these results into landscape genetics analyses revealed epidemiological insights such as temporally stable, focal transmission around households. Although the latter conclusion was not obtainable from worm counts alone, i.e., we cannot directly observe worm dispersal and acquisition, I do not view genetics data as a replacement for infection intensity data. Both provide different information and, thus, are complimentary (Criscione, 2013).

## Clonal transmission in trematodes

“Potential complicating effects of parasite genetic structure such as asexual amplification within intermediate hosts (digeneans)...have rarely been

investigated” (Nadler, 1995). Though largely specific to trematodes, I chose to discuss the history of this topic as an example to emphasize the role different life parasite life histories may play in impacting their microevolution. Trematode asexual reproduction is fundamentally different than that in many parasitic protozoa because adults have obligate sexual reproduction (except rare parthenogenetic forms). Thus, larval clonal lines produced in the first host do not persist over generations. Although the actual mode of reproduction in the larval propagation stage of mollusk hosts has been of historical interest in parasitology (e.g., Whitfield and Evans, 1983), I am not aware of any studies prior to the early 1990s that addressed fluke clonality with molecular population genetics.

A likely hurdle to be cleared before such work could be done was the need for MLGs, which was not possible with enzyme electrophoresis and small flukes. Indeed, the first study to examine clonal transmission in trematodes was conducted with *Fascioloides magna*, a large deer liver fluke, by Mulvey et al. (1991). These workers found that clonemates (individuals of the same clone) co-occurred within individual hosts more often than expected, a situation that in turn inflated genetic differentiation among hosts. They also concluded that aggregated clonemates resulted in a reduced observed heterozygosity within hosts, but later simulation modeling would show this conclusion to be wrong. PCR and microsatellites should have opened the door to additional work on fluke clonal transmission, but the next studies were not published until the 2000s (e.g., Prugnolle et al., 2002; Theron et al., 2004; Criscione and Blouin, 2006). Also during this time, Prugnolle et al. (2005) developed a theoretical framework for the population genetics of trematode clonal transmission. They found that a high variance in clonal reproduction did increase genetic differentiation among hosts, but actually created heterozygote excess within hosts (contrast to interpretation of Mulvey et al., 1991). The latter illustrates the need for proper theory to interpret population genetic patterns from organisms with life cycles that “depart from those used in theoretical population genetic models” (Prugnolle et al., 2005). In the same year, the observation of a lack of clonemate aggregation in second hosts led to an interesting hypothesis by Rauch et al. (2005), namely, that complex life cycles evolved to reduce inbreeding by decreasing the chance of clonemates ending up in the same final host (clonemate mating equals self-mating in hermaphrodites). Based on this hypothesis, Rauch et al. (2005) predicted that congeners with truncated life cycles would show greater clonemate aggregation in definitive hosts compared to species with a full life cycle. Gorton et al. (2012) provided a recent review on clonal transmission and proposed that clonal aggregation may be greater in trematodes with semi-terrestrial than fully aquatic life cycles; the latter providing an environment conducive to cercariae dispersal. The hypotheses of Rauch et al. (2005) and Gorton et al. (2012) are based on a limited number of studies, thus more data are needed to understand how clonal transmission may vary among trematodes with different life cycles. As noted by Rauch et al. (2005) and as I discussed previously, a comparative framework would be ideal to test these hypotheses.

## Effective population size

Effective population size directly quantifies the evolutionary mechanism of genetic drift. Populations with larger  $N_e$  will have greater gene diversities (assuming the same mutation rate). Although Nadler (1990) showed helminths had gene diversities similar to free-living invertebrates, Blouin et al. (1992) provided the first estimate of  $N_e$ . “Long-term  $N_e$  in these populations [of *Ostertagia ostertagi*] is estimated to be four to eight million individuals. This is a very large number given that long-term  $N_e$  is the harmonic mean of  $N_e$  in past years” (Blouin et al., 1992). Clearly, parasite populations could be larger than perceived by Price (1977, 1980). So, what have we learned about parasite  $N_e$  since the early 1990s? Even though  $N_e$  is one of the most important parameters in evolutionary biology, research on what impacts parasite  $N_e$  and actual estimates of parasite  $N_e$  are mostly recent. Long-term estimates of  $N_e$  were made for malarial parasites, but conflicting estimates stem from the use of different sets of loci that may have experienced different selective histories (Hartl, 2004). Prugnolle et al. (2005) modeled how increased selfing or variance in clonal reproductive reduced  $N_e$  in trematodes. Criscione and Blouin (2005) applied a subdivided breeders model, which highlighted how “several features of [metazoan] parasite life cycles probably function in concert to reduce  $N_e$  below that expected in a single free-living population of equivalent census size.” Note this statement is not to be misconstrued (though it already has) as meaning parasite populations will have small  $N_e$ . Criscione et al. (2005) showed a positive correlation between nucleotide diversity and mean intensities of some nematodes, but this observation provided a crude approximation of how parasite population parameters might impact  $N_e$ . Thus, the result is better viewed as a hypothesis to test than as an established relationship. Because little was (and is still) known about parasite  $N_e$ , we also suggested “comparisons of short-term genetic estimates of  $N_e$  among parasite populations that differ in key traits would help identify the ecological determinants of  $N_e$ ” (Criscione et al., 2005). The latter idea will be facilitated by recent developments of genetic, single-sample estimators of contemporary  $N_e$  (Wang, 2009; Waples and Do, 2010).

Criscione (2013) advocated that these single-sample estimators should also enable  $N_e$  estimation as a tool to monitor control programs. As these methods are beginning to be applied, it is important to note for history yet to pass that inference of  $N_e$  is dependent on what is sampled. For example, if parasite eggs from a definitive host are used, then a subcomponent of  $N_e$ , i.e., the effective number of breeders ( $N_b$ ) within that host, is estimated. Steinauer et al. (2013) estimated a range from the tens to low hundreds for the  $N_b$ s of *Schistosoma mansoni* in individual people and concluded that estimation of infrapopulation  $N_b$ s could be a useful means to “to depict relative worm burdens in patients.”

Moreover, they noted it would be incorrect to combine larval (miracidia) samples across individual human hosts to generate one genetic estimate (as in Gower et al., 2013). The reason this latter sampling is incorrect is because LD generated by combining sibling groups (which exist in schistosome egg/miracidial samples; Steinauer et al., 2013) across different hosts would be in excess of that caused by breeders in a host. In turn, this would create artifactual estimates based the LD-method (Waples and Do, 2010). More appropriately the  $N_b$ s would be used in the subdivided breeders model to estimate  $N_e$  (Criscione and Blouin, 2005). Criscione (2013) recently highlighted how life history could also influence interpretation of  $N_e$  genetic estimates. In particular, I discussed how long-lived eggs (a trait that creates a “seed-bank” effect as noted by Nadler, 1995) of *Ascaris lumbricoides* leads to overlapping generations in definitive hosts. With adult *A. lumbricoides*, I estimated subpopulation  $N_e$ s of about 100, which was in accord with the low intensity-low nucleotide diversity relationship given in Criscione et al. (2005).

## Hermaphroditic mating systems

Even though hermaphroditism is ubiquitous throughout the Neodermata (parasitic flatworms), which is estimated to have over 130,000 species (Strona and Fattorini, 2014), we know virtually nothing about their primary mating systems, i.e., whether they self-mate or outcross. Evolutionary significance of self-mating stems from the fact that it is the most extreme form of inbreeding, a situation that in turn magnifies the effect of drift, alters selection efficiency, and affects population levels of genetic diversity. In addition, inbreeding or outbreeding depression can be manifested in a single generation of mating. There was research on flatworm self-mating in the 1960s, about the same time as the advent of electrophoretic methods (see references in Nollen, 1971). However, progeny genotyping to estimate adult selfing-rates was not used until two decades later (Trouve et al., 1996). Again, I attribute the slow development to the tissue requirements of electrophoretic methods.

These early studies relied on radiolabeled sperm to assess self-insemination (reviewed in Nollen, 1983), but oddly there were no explicit links to inbreeding. Rather, focus was on whether cross-fertilization was required “for normal development of the life cycle” (Nollen, 1971). For instance, there was no mention of inbreeding depression by Nollen (1971), but he clearly compared several fitness traits between inbred and outbred lines. As another example, Fried and Harris (1971) found that flukes raised alone took longer to produce eggs than when worms occurred in pairs, but stated, “no explanation is available to explain the lag in development and the significant reduction in numbers of fully developed eggs in single-worm infections.” If only they had a time machine to read up on the evolutionary hypotheses of delayed selfing (Escobar et al., 2011).

I do not have space to reference a complete history but, as a backdrop, it is fundamental to know that most evolutionary theory and empirical work on

hermaphroditism has stemmed from plant research (see Goodwillie et al., 2005). Theory, progeny-array methods to estimate primary selfing-rates from field samples, and empirical work blossomed in the plant literature during the 1970s and 1980s based on electrophoretic analysis. In reference to the evolution of the primary mating system, Schemske and Lande (1985) reviewed selfing rates of 55 plant species and, in Goodwillie et al. (2005), this number expanded to 345, all based on progeny-array data. Work on animals lagged behind. Jarne (1995) listed 55 animal species and Jarne and Auld (2006) had 142 based on progeny-array and indirect population estimates (selfing rate calculated from  $F_{IS}$ ). How many of these species were parasites? There were 2 and 14 parasitic species, respectively. Even in relation to other evolutionary topics of hermaphroditic animal mating systems such as sex allocation, sex role, and inbreeding depression, parasites (mostly based on *Schistocephalus solidus*) make up only 2–5% of the studies in reviews; most of the others employ snails (Scharer, 2009; Anthes et al., 2006; Escobar et al., 2011).

In general, molecular population genetics work on flatworm mating systems has been sparse and largely inferred from indirect population estimates (Jarne and Auld, 2006; Gorton et al., 2012). Thus, no consensus mating patterns at the population level have emerged. Gorton et al. (2012) hypothesized that aquatic transmission may promote more outcrossing and, therefore, panmixia, than terrestrial life cycle patterns where clumped transmission may promote more biparental inbreeding. However, more work is needed to see what drives flatworm mating systems. Our knowledge regarding the primary mating system is even less extensive. In fact, there are only four flatworm species for which we have estimates of the primary mating system (Trouve et al., 1996; 1999; Luscher and Milinski, 2003; Schelkle et al., 2012; Rieger et al. 2013). All these studies show that mixed-mating (outcrossing and selfing) is possible, but they are laboratory studies and only work by Trouve and colleagues report individual worm selfing rates. To date, we do not have any direct estimates of the primary mating system for any hermaphroditic parasite in nature.

## Mode of inheritance and genetic mapping

Genetics started with Mendel and, with this last topic, I will have come full circle. Generating genetic crosses in parasites is not an easy task. Difficulties include life cycle maintenance, controlled crosses, access to purebred phenotypes, and large numbers of progeny. Thus, classic crosses examining the mode of inheritance of parasite phenotypes are historically rare. With the exception of work on lice coloration (Busvine, 1946), I have not found any examples that predate the 1960s. Via several cross-based studies in the 1970s, electrophoretic markers in combination with drug-resistant phenotypic markers enabled proof of recombination in malarial parasites (reviewed in Walliker, 1983). The next step was to use these data to generate genetic linkage maps and locate genes that underlie parasite phenotypes. Indeed, the first protozoan linkage map was for

*Plasmodium falciparum* (Su et al., 1999). This map has subsequently been used to map phenotypes associated with drug resistance, pathogenesis, and mosquito infectivity (reviewed in Volkman et al., 2012). Ten years later, the first linkage map for a helminth, *Schistosoma mansoni*, was generated (Criscione et al., 2009). This map has subsequently been used to correct genome assembly errors and map a drug-resistant phenotype (Protasio et al., 2012; Valentim et al., 2013). The population level extension of linkage mapping includes association-based studies and genome scans for regions of recent selection, i.e., population genomics studies. If there was another area where parasitology has led the field in population genetics (as with the clonal research), it would be with the population genomics studies on human malaria. In addition to revealing much about malaria biology, e.g., population history, local transmission, and inference of selection, population genomics studies of malaria have served as excellent examples for genome evolution in general. This literature is too extensive to review here, so, for an excellent review, I refer readers to Volkman et al. (2012).

In addition to mapping, crosses can test two assumptions that underlie all population genetics studies, i.e., Mendelian segregation and independent assortment. For example, de Meeûs et al. (2004) used tick crosses to show that size-based allelic dropout affected microsatellite scoring and Detwiler and Criscione (2011) used nature-provided tapeworm crosses to identify duplicated microsatellite loci, “by taking advantage of the fact that hosts represent closed mating systems for endoparasites, we were able to exploit natural crosses to test Mendelian inheritance.”

## A few personal thoughts

It was a learning experience for me to write this historical account. I came across papers I was not familiar with and re-affiliated myself with some classics. Unfortunately, there was not room to discuss them all. I also apologize to those who work on protozoan and non-helminth metazoans since I am certain that I have not adequately covered the history of molecular population genetics for these organisms. However, I suspect general patterns parallel those of the helminths.

So after recounting the history of molecular population genetics in parasitology, the succeeding question is: what next? At the end of Criscione et al. (2005), I stated, “Parasite molecular ecology is still in its infancy.” In my opinion, molecular population genetics of protozoan and metazoan parasites is still a young sub-discipline. Perhaps we are now at the toddler stage. Yes, we have gained a lot of knowledge from studies on malarial parasites and other medically important species. But, in terms of our general knowledge of parasite microevolution, we have only scratched the tip of parasite biodiversity and life history. Nadler (1995) noted, “Given the range of ecological diversity that is characteristic of parasites, a broad spectrum of genetic architectures is likely to be revealed as more empirical studies are undertaken.” For many of the topics I discussed previously, only a few species have been examined. Thus, the full gamut of parasite life history has yet to be explored. These gaps must be filled before there is enough data to determine if broad scale population genetic patterns or generalities are present across parasite life histories.

I also feel that more theoretical work needed is needed to tie traditional epidemiological models with population genetics. The purpose would be to determine which genetic statistics are most useful for inferring transmission and designing or monitoring control programs. Parasite life history may also dictate what, and how, parasite life stages are sampled. Simulations may also be needed to see how sampling might impact inference (e.g., Steinauer et al., 2013).

It is cliché to say these days that NGS will greatly change molecular population genetics, but I cannot deny this to be true; e.g., the Wellcome Trust Sanger Institute currently has draft genomes for 50 helminth species. Clearly, the topics I covered above will be enhanced by possession of genome-wide data. Nonetheless, to prevent artifactual results, utilization of NGS will require accurate genome assemblies. Moreover, several population genetic analyses assume independence among markers, thereby necessitating estimates of recombination rates across the assembly. NGS will also facilitate additional topics such as searching for signatures of adaptive evolution. We already see this with human malaria (Volkman et al., 2012). Insight into the evolution of the genome itself is now a hot topic. For example, in a review on how mating systems could impact genome evolution Glémén and Galtier (2012) state, “A major current molecular evolution challenge is to link comparative genomic patterns to species' biology and ecology.” With over four decades of research on the primary mating systems of

hermaphroditic plants in nature, there is a biological context with which NGS data can facilitate genome evolution studies in plants. In contrast, there are only four species of hermaphroditic parasites with primary mating system data, all of which are lab-based. My point is that the limitations of applying NGS data to parasites will not be the technology itself, but rather the biology of the parasites themselves. Granted, NGS data may be able to elucidate the biology, but whether NGS data are necessary or overkill versus other genotyping methods will depend on the nature of the question.

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I would like to end on a personal note by thanking those who have shaped my own academic history. I have been fortunate to learn from two of the pioneers in parasite molecular population genetics: Mike Blouin, my Ph.D. advisor, and Tim Anderson, my postdoc advisor. They are both still active in applying modern molecular methods to study parasite population biology. Of course, I would have never gotten into parasitology in the first place if it were not for my M.Sc. advisor, Bill Font. As a joke, I now blamably exclaim “Font!” In the true spirit of population genetics I am proud to say that I can trace my academic pedigree to a pioneer in North American Parasitology, Henry Baldwin Ward. Ward is my academic great-great grandfather on my M.S. side (William Font-Kenneth Corkum-Harry Bennett-Henry Ward). Interestingly, this relationship makes Sewell Wright (one of the fathers of population genetics) my great-grand uncle as he was a M.Sc. student of Ward, an interesting fact I first learned from reading Nadler (1995). Now, if only I could trace either of my other advisors' lineages back to Wright, then I could calculate my own academic inbreeding coefficient!

## Literature cited

- Anderson, T. J. C., M. S. Blouin, and R. N. Beech. 1998. Population biology of parasitic nematodes: Applications of genetic markers. *Advances in Parasitology* **41**: 219–283.
- \_\_\_\_\_, B. Haubold, J. T. Williams, J. G. Estrada-Franco, L. Richardson, R. Mollinedo, M. Bockarie, J. Mokili, S. Mharakurwa, N. French et al. 2000. Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Molecular Biology and Evolution* **17**: 1467–1482.
- \_\_\_\_\_, M. E. Romero-Abal, and J. Jaenike. 1995. Mitochondrial DNA and *Ascaris*: The composition of parasite populations from individual hosts, families and villages. *Parasitology* **110**: 221–229.
- Anthes, N., A. Putz, and N. K. Michiels. 2006. Sex role preferences, gender conflict and sperm trading in simultaneous hermaphrodites: A new framework. *Animal Behaviour* **72**: 1–12.
- Avise, J. C. 2000. *Phylogeography: The history and formation of species*. Harvard University Press, Cambridge, Massachusetts, 447 p.
- \_\_\_\_\_. 2004. *Molecular markers, natural history, and evolution*. 2nd edn. Sinauer Associates, Sunderland, Massachusetts, 684 p.
- Bartlett, J. M. S., and D. Stirling. 2003. A short history of the polymerase chain reaction. In *PCR protocols*, J. M. S. Bartlett, and D. Stirling (eds.). Humana Press, Totowa, New Jersey, p. 3–6.
- Beverley-Burton, M. 1978. Population genetics of *Anisakis simplex* (Nematoda: Ascaridoidea) in Atlantic salmon (*Salmo salar*) and their use as biological indicators of host stocks. *Environmental Biology of Fishes* **3**: 369–377.
- \_\_\_\_\_, O. L. Nyman, and J. H. C. Pippy. 1977. The morphology, and some observations on population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of North Atlantic. *Journal of the Fisheries Research Board of Canada* **34**: 105–112.
- Blouin, M. S. 2002. Molecular prospecting for cryptic species of nematodes: Mitochondrial DNA versus internal transcribed spacer. *International Journal of Parasitology* **32**: 527–531.
- \_\_\_\_\_, J. B. Dame, C. A. Tarrant, and C. H. Courtney. 1992. Unusual population genetics of a parasitic nematode: mtDNA variation within and among populations. *Evolution* **46**: 470–476.

\_\_\_\_\_, C. A. Yowell, C. H. Courtney, and J. B. Dame. 1995. Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* **141**: 1007–1014.

Bowler, P. J. 2010. *Evolution: History*. In Encyclopedia of life sciences, John Wiley & Sons, Ltd, Chichester, U.K., p. 1–6.

Bullini, L., G. Nascetti, S. Ciafre, F. Rumore, E. Biocca, S. G. Montalenti, and G. Rita. 1978. Ricerche cariologiche ed elettroforetiche su *Parascaris univalens* e *Parascaris equorum*. *Atti della Accademia Nazionale dei Lincei Rendiconti Classe di Scienze Fisiche Matematiche e Naturali* **65**: 151–156.

\_\_\_\_\_, \_\_\_\_\_, L. Paggi, P. Orecchia, S. Mattiucci, and B. Berland. 1986. Genetic variation of ascaridoid worms with different life cycles. *Evolution* **40**: 437–440.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**: 575–583.

Busvine, J. R. 1946. On the pigmentation of the body louse *Pediculus humanus* L. **21**: 98–103.

Carter, R., and A. Voller. 1975. The distribution of enzyme variation in populations of *Plasmodium falciparum* in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**: 371–376.

Criscione, C. D. 2008. Parasite co-structure: Broad and local scale approaches. *Parasite* **15**: 439–443.

\_\_\_\_\_. 2013. Genetic epidemiology of *Ascaris*: Cross-transmission between humans and pigs, focal transmission, and effective population size. In *Ascaris: The neglected Parasite*, C. V. Holland (ed.) Academic Press, London, U.K., p. 203–230.

\_\_\_\_\_, J. D. Anderson, D. Sudimack, W. Peng, B. Jha, S. Williams-Blangero, and T. J. C. Anderson. 2007. Disentangling hybridization and host colonization in parasitic roundworms of humans and pigs. *Proceedings of the Royal Society, B* **274**: 2669–2677.

\_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, J. Subedi, R. P. Upadhyay, B. Jha, K. D. Williams, S. Williams-Blangero, and T. J. C. Anderson. 2010. Landscape genetics reveals focal transmission of a human macroparasite. *PLOS Neglected Tropical Diseases* **4**: e665.

\_\_\_\_\_, and M. S. Blouin. 2004. Life cycles shape parasite evolution: Comparative population genetics of salmon trematodes. *Evolution* **58**: 198–202.

\_\_\_\_\_, and \_\_\_\_\_. 2005. Effective sizes of macroparasite populations:

A conceptual model. *Trends in Parasitology* **21**: 212–217.

\_\_\_\_\_, and \_\_\_\_\_. 2006. Minimal selfing, few clones, and no among-host genetic structure in a hermaphroditic parasite with asexual larval propagation. *Evolution* **60**: 553–562.

\_\_\_\_\_, R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Molecular Ecology* **14**: 2247–2257.

\_\_\_\_\_, C. L. L. Valentim, H. Hirai, P. T. LoVerde, and T. J. C. Anderson. 2009. Genomic linkage map of the human blood fluke *Schistosoma mansoni*. *Genome Biology* **10**: R71.

\_\_\_\_\_, R. Vilas, E. Paniagua, and M. S. Blouin. 2011. More than meets the eye: Detecting cryptic microgeographic population structure in a parasite with a complex life cycle. *Molecular Ecology* **20**: 2510–2524.

Crow, J. F. 1987. Population genetics history: A personal view. *Annual Review of Genetics* **21**: 1–22.

\_\_\_\_\_. 1999. Anecdotal, historical and critical commentaries on genetics: Hardy, Weinberg and language impediments. *Genetics* **152**: 821–825.

Darwin, C. 1859. *On the origin of species by means of natural selection*. Murray, London, U.K., 502 p.

de Meeûs, T., P. F. Humair, C. Grunau, C. Delaye, and F. Renaud. 2004. Non-Mendelian transmission of alleles at microsatellite loci: An example in *Ixodes ricinus*, the vector of Lyme disease. *International Journal for Parasitology* **34**: 943–950.

\_\_\_\_\_, L. Lehmann, and F. Balloux. 2006. Molecular epidemiology of clonal diploids: A quick overview and a short DIY (do it yourself) notice. *Infection Genetics and Evolution* **6**: 163–170.

\_\_\_\_\_, K. D. McCoy, F. Prugnolle, C. Chevillon, P. Durand, S. Hurtrez-Bousses, and F. Renaud. 2007. Population genetics and molecular epidemiology or how to “debusquer la bête”. *Infection Genetics and Evolution* **7**: 308–332.

Detwiler, J. T., and C. D. Criscione. 2010. An infectious topic in reticulate evolution: Introgression and hybridization in animal parasites. *Genes* **1**: 102–123.

\_\_\_\_\_, and \_\_\_\_\_. 2011. Testing Mendelian inheritance from field-collected parasites: Revealing duplicated loci enables correct inference of reproductive mode and mating system. *International Journal for Parasitology* **41**: 1185–1195.

Dobson, A., K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz. 2008.

Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences* **105**: 11482–11489.

Dye, C. 1991. Population genetics of nonclonal, nonrandomly mating malaria parasites. *Parasitology Today* **7**: 236–240.

Epperson, B. K. 1999. Anecdotal, historical and critical commentaries on genetics: Gustave Malecot, 1911–1998: Population Genetics Founding Father. *Genetics* **152**: 477–484.

Escobar, J. S., J. R. Auld, A. C. Correa, J. M. Alonso, Y. K. Bony, M. A. Coutellec, J. M. Koene, J. P. Pointier, P. Jarne, and P. David. 2011. Patterns of mating-system evolution in hermaphroditic animals: Correlations among selfing rate, inbreeding depression, and the timing of reproduction. *Evolution* **65**: 1233–1253.

Falk, B. G., and S. L. Perkins. 2013. Host specificity shapes population structure of pinworm parasites in Caribbean reptiles. *Molecular Ecology* **22**: 4576–4590.

Fried, B., and K. R. Harris. 1971. Reproduction in single-worm and double-worm infections of *Leucochloridiomorpha constantiae* (Mueller, 1935) (Trematoda) in chick. *Journal of Parasitology* **57**: 866.

Glémén, S., and N. Galtier. 2012. Genome evolution in outcrossing versus selfing versus asexual species. In *Evolutionary genomics: Statistical and computational methods*, Vol. 1, M. Anisimova, (ed.). Springer, *Methods in Molecular Biology* **855**: 311–335.

Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology Evolution and Systematics* **36**: 47–79.

Gorton, M. J., E. L. Kasl, J. T. Detwiler, and C. D. Criscione. 2012. Testing local-scale panmixia provides insights into the cryptic ecology, evolution, and epidemiology of metazoan animal parasites. *Parasitology* **139**: 981–997.

Gower, C. M., A. N. Gouvas, P. H. L. Lamberton, A. Deol, J. Shrivastava, P. N. Mutombo, J. V. Mbuh, A. J. Norton, B. L. Webster, J. R. Stothard, et al. 2013. Population genetic structure of *Schistosoma mansoni* and *Schistosoma haematobium* from across six sub-Saharan African countries: Implications for epidemiology, evolution and control. *Acta Tropica* **128**: 261–274.

Hartl, D. L. 2004. The origin of malaria: Mixed messages from genetic diversity. *Nature Reviews Microbiology* **2**: 15–22.

Jarne, P. 1995. Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals. *Genetical Research* **65**: 193–207.

\_\_\_\_\_, and J. R. Auld. 2006. Animals mix it up too: The distribution of self-

fertilization among hermaphroditic animals. *Evolution* **60**: 1816–1824.

Le Jambre, L. F. 1979. Hybridization studies of *Haemonchus contortus* (Rudolphi, 1803) and *Haemonchus placei* (Place, 1893) (Nematoda, Trichostrongylidae). *International Journal for Parasitology* **9**: 455–463.

LeRoux, P. L. 1954. Hybridization of *Schistosoma mansoni* and *S. rodhaini*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **48**: 3–4.

Lewontin, R. C. 1991. Twenty-five years ago in genetics: Electrophoresis in the development of evolutionary genetics: Milestone or millstone. *Genetics* **128**: 657–662.

Luscher, A., and M. Milinski. 2003. Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: An experimental test of predictions of mixed mating and Hermaphrodite's Dilemma theory. *Journal of Evolutionary Biology* **16**: 1030–1037.

Lydeard, C., M. Mulvey, J. M. Aho, and P. K. Kennedy. 1989. Genetic variability among natural populations of the liver fluke *Fascioloides magna* in white-tailed deer, *Odocoileus virginianus*. *Canadian Journal of Zoology* **67**: 2021–2025.

Miles, M. A., M. S. Llewellyn, M. D. Lewis, M. Yeo, R. Baleela, S. Fitzpatrick, M. W. Gaunt, and I. L. Mauricio. 2009. The molecular epidemiology and phylogeography of *Trypanosoma cruzi* and parallel research on *Leishmania*: Looking back and to the future. *Parasitology* **136**: 1509–1528.

\_\_\_\_\_, P. J. Toye, S. C. Oswald, and D. G. Godfrey. 1977. The identification by isoenzyme patterns of 2 distinct strain-groups of *Trypanosoma cruzi*, circulating independently in a rural area of Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **71**: 217–225.

Mode, C. J. 1958. A mathematical model for the co-evolution of obligate parasites and their hosts. *Evolution* **12**: 158–165.

Mulvey, M., J. M. Aho, C. Lydeard, P. L. Leberg, and M. H. Smith. 1991. Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. *Evolution* **45**: 1628–1640.

Nadler, S. A. 1990. Molecular approaches to studying helminth population genetics and phylogeny. *International Journal for Parasitology* **20**: 11–29.

\_\_\_\_\_. 1995. Microevolution and the genetic structure of parasite populations. *Journal of Parasitology* **81**: 395–403.

\_\_\_\_\_, M. S. Hafner, J. C. Hafner, and D. J. Hafner. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). *Evolution* **44**: 942–951.

\_\_\_\_\_, and G. Perez-Ponce de Leon. 2011. Integrating molecular and morphological approaches for characterizing parasite cryptic species: Implications for parasitology. *Parasitology* **138**: 1688–1709.

Nelson, F. K., M. Snyder, A. F. Gardner, C. L. Hendrickson, J. A. Shendure, G. J. Porreca, G. M. Church, F. M. Ausubel, J. Ju, J. Kieleczawa et al. 2011. Introduction and historical overview of DNA sequencing. *Current Protocols in Molecular Biology* **96**: 7.0.1–7.0.18.

Nevo, E. 1978. Genetic variation in natural populations: Patterns and theory. *Theoretical Population Biology* **13**: 121–177.

Nkhoma, S. C., S. Nair, S. Al-Saai, E. Ashley, R. McGready, A. P. Phy, F. Nosten, and T. J. C. Anderson. 2013. Population genetic correlates of declining transmission in a human pathogen. *Molecular Ecology* **22**: 273–285.

Nollen, P. M. 1971. Viability of a self-fertilizing strain of *Philophthalmus megalurus* (Trematoda: Digenea). *Journal of Parasitology* **57**: 1222–1226.

\_\_\_\_\_. 1983. Patterns of sexual reproduction among parasitic platyhelminths. *Parasitology* **86**: 99–120.

Perez-Ponce de Leon, G., and S. A. Nadler. 2010. What we don't recognize can hurt us: A plea for awareness about cryptic species. *Journal of Parasitology* **96**: 453–464.

Price, P. W. 1977. General concepts on the evolutionary biology of parasites. *Evolution* **31**: 405–420.

\_\_\_\_\_. 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton, New Jersey, 237 p.

Protasio, A. V., I. J. Tsai, A. Babbage, S. Nichol, M. Hunt, M. A. Aslett, N. De Silva, G. S. Velarde, T. J. C. Anderson, R. C. Clark et al. 2012. A systematically improved high quality genome and transcriptome of the human blood fluke *Schistosoma mansoni*. *PLOS Neglected Tropical Diseases* **6**: e1455.

Prugnolle, F., T. de Meeûs, P. Durand, C. Sire, and A. Theron. 2002. Sex-specific genetic structure in *Schistosoma mansoni* evolutionary and epidemiology implications. *Molecular Ecology* **11**: 1231–1238.

\_\_\_\_\_, H. Liu, T. de Meeûs, and F. Balloux. 2005. Population genetics of complex life-cycle parasites: An illustration with trematodes. *International Journal of Parasitology* **35**: 255–263.

Pybus, O. G., and A. Rambaut. 2009. Evolutionary analysis of the dynamics of viral infectious disease. *Nature Reviews Genetics* **10**: 540–550.

Rauch, G., M. Kalbe, and T. B. H. Reusch. 2005. How a complex life cycle can

- improve a parasite's sex life. *Journal of Evolutionary Biology* **18**: 1069–1075.
- Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* **55**: 1095–1103.
- Reeves, R. E., and J. M. Bischoff. 1968. Classification of *Entamoeba* species by means of electrophoretic properties of amebal enzymes. *Journal of Parasitology* **54**: 594–600.
- Renaud, F., and C. Gabrion. 1988. Speciation of cestoda: Evidence for two sibling species in the complex *Bothrimonius nylandicus* (Schneider 1902) (Cestoda: Cyathocephalidae). *Parasitology* **97**: 139–147.
- Reversat, J., F. Renaud, and C. Maillard. 1989. Biology of parasite populations: The differential specificity of the genus *Helicometra* Odhner, 1902 (Trematoda, Opecoelidae) in the Mediterranean Sea demonstrated by enzyme electrophoresis. *International Journal for Parasitology* **19**: 885–890.
- Rieger, J. K., D. Haase, T. B. H. Reusch, and M. Kalbe. 2013. Genetic compatibilities, outcrossing rates and fitness consequences across life stages of the trematode *Diplostomum pseudospathaceum*. *International Journal for Parasitology* **43**: 485–491.
- Scharer, L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution* **63**: 1377–1405.
- Schelkle, B., P. J. Faria, M. B. Johnson, C. van Oosterhout, and J. Cable. 2012. Mixed infections and hybridisation in monogenean parasites. *PLOS One* **7**: e39506.
- Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* **39**: 41–52.
- Sire, C., P. Durand, J.-P. Pointier, and A. Theron. 2001. Genetic diversity of *Schistosoma mansoni* within and among individual hosts (*Rattus rattus*): Infrapopulation differentiation at microspatial scale. *International Journal of Parasitology* **31**: 1609–1616.
- Southgate, V. R., H. B. Vanwijk, and C. A. Wright. 1976. Schistosomiasis at Loum, Cameroun; *Schistosoma haematobium*, *S. intercalatum* and their natural hybrid. *Zeitschrift Fur Parasitenkunde-Parasitology Research* **49**: 145–159.
- Steinauer, M. L., M. S. Blouin, and C. D. Criscione. 2010. Applying evolutionary genetics to schistosome epidemiology. *Infection Genetics and Evolution* **10**: 433–443.
- \_\_\_\_\_, M. R. Christie, M. S. Blouin, L. E. Agola, I. N. Mwangi, G. M. Maina, M. W. Mutuku, J. M. Kinuthia, G. M. Mkoji, and E. S. Loker. 2013. Non-invasive

sampling of schistosomes from humans requires correcting for family structure. *PloS Neglected Tropical Diseases* **7**: e2456.

\_\_\_\_\_, B. Hanelt, I. N. Mwangi, G. M. Maina, L. E. Agola, J. M. Kinuthia, M. W. Mutuku, B. N. Mungai, W. D. Wilson, G. M. Mkoji et al. 2008. Introgressive hybridization of human and rodent schistosome parasites in western Kenya. *Molecular Ecology* **17**: 5062–5074.

Strona, G., and S. Fattorini. 2014. Parasitic worms: How many really? *International Journal for Parasitology* **44**: 269–272.

Su, X., M. T. Ferdig, Y. Huang, C. Q. Huynh, A. Liu, J. You, J. C. Wootton, and T. E. Wellems. 1999. A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. *Science* **286**: 1351–1353.

Theron, A., C. Sire, A. Rognon, F. Prugnolle, and P. Durand. 2004. Molecular ecology of *Schistosoma mansoni* transmission inferred from the genetic composition of larval and adult infrapopulations within intermediate and definitive hosts. *Parasitology* **129**: 571–585.

Tibayrenc, M., and F. J. Ayala. 2013. How clonal are *Trypanosoma* and *Leishmania*? *Trends in Parasitology* **29**: 264–269.

\_\_\_\_\_, F. Kjellberg, and F. J. Ayala. 1990. A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, and *Trypanosoma* and their medical and taxonomical consequences. *Proceedings of the National Academy of Sciences USA* **87**: 2414–2418.

Trouve, S., F. Renaud, P. Durand, and J. Jourdane. 1996. Selfing and outcrossing in a parasitic hermaphrodite helminth (Trematoda, Echinostomatidae). *Heredity* **77**: 1–8.

\_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1999. Reproductive and mate choice strategies in the hermaphroditic flatworm *Echinostoma caproni*. *Journal of Heredity* **90**: 582–585.

Valentim, C. L. L., D. Cioli, F. D. Chevalier, X. H. Cao, A. B. Taylor, S. P. Holloway, L. Pica-Mattoccia, A. Guidi, A. Basso, I. J. Tsai, et al. 2013. Genetic and molecular basis of drug resistance and species-specific drug action in schistosome parasites. *Science* **342**: 1385–1389.

Vilas, R., C. D. Criscione, and M. S. Blouin. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology* **131**: 839–846.

Volkman, S. K., D. E. Neafsey, S. F. Schaffner, D. J. Park, and D. F. Wirth. 2012. Harnessing genomics and genome biology to understand malaria biology. *Nature*

*Reviews Genetics* **13**: 315–328.

Vrijenhoek, R. C. 1978. Genetic differentiation among larval nematodes infecting fishes. *Journal of Parasitology* **64**: 790–798.

Walliker, D. 1983. *The contribution of genetics to the study of parasitic protozoa*. Research Studies Press, Ltd., U.K., 228 p.

Wang, J. L. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology* **18**: 2148–2164.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary  $N_e$  using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* **3**: 244–262.

Whitfield, P. J., and N. A. Evans. 1983. Parthenogenesis and asexual multiplication among parasitic platyhelminths. *Parasitology* **86**: 121–160.

Zee, D. S., H. Isensee, and W. H. Zinkham. 1970. Polymorphism of malate dehydrogenase in *Ascaris suum*. *Biochemical Genetics* **4**: 253–257.

Zingales, B., M. A. Miles, D. A. Campbell, M. Tibayrenc, A. M. Macedo, M. M. G. Teixeira, A. G. Schijman, M. S. Llewellyn, E. Lages-Silva, C. R. Machado, et al. 2012. The revised *Trypanosoma cruzi* subspecific nomenclature: Rationale, epidemiological relevance and research applications. *Infection Genetics and Evolution* **12**: 240–253.



# **Chapter 8**

## **A Probable Case of Non-Specific Immunity Between Two Parasites of Ciscoes of the Trout Lake Region of Northern Wisconsin**

It has been noted by several investigators that the presence of large numbers of one species of parasites has a tendency to limit infection by other parasites. Ward (Trans. Amer. Fish. Soc., 1911, 207) states: "Few fishes shelter equal numbers of all kinds of parasites and no species is recorded as heavily infected with all four groups of intestinal worms." Wilson (Bull. Bur. Fish., 34: 333, 1914) in a study of the glochidia of fresh-water mussels, showed that fish heavily infected with copepods were either very lightly or not at all infected with glochidia. He found also from the examination of field specimens that those fish harboring a large number of glochidia had few or no copepods. Leigh-Sharpe (Parasit., 25, 1933) says: "Monogenetic trematodes were found in greater abundance on the gills of fishes than parasitic copepoda and seemed mutually exclusive." Nolf and Cort (Jour. Parasit., 20: 38, 1933) found, in the case of groups of snails infested with larval stages of a schistosome, evidence of a non-specific immunity to infection by metacercariae of a strigeid trematode, *Cotylurus flabelliformis*.

As part of the fish parasite investigation fostered by the Wisconsin Geological and Natural History Survey, a catch of 92 ciscoes from Silver Lake were examined in 1933. In this group no parasites were found other than adult cestodes of the species *Proteocephalus exigus* and Acanthocephala of the genus *Neoicanthorinchus*. In the series of 92 fish the average number of tapeworms harbored was 16.92 and the average number of acanthocephala was 8.3. Of these fish 84 harbored tapeworms, 73 harbored acanthocephala and every fish had one or both parasites.

The degree of infection of this group of fish is plotted in Figure 1. From this graph it will be seen that those fish having 15 or more acanthocephala had a very limited infection with tapeworms. Conversely those harboring more than 25 tapeworms had few or no acanthocephala. The fish with fewer parasites often have them quite evenly divided between the two types.

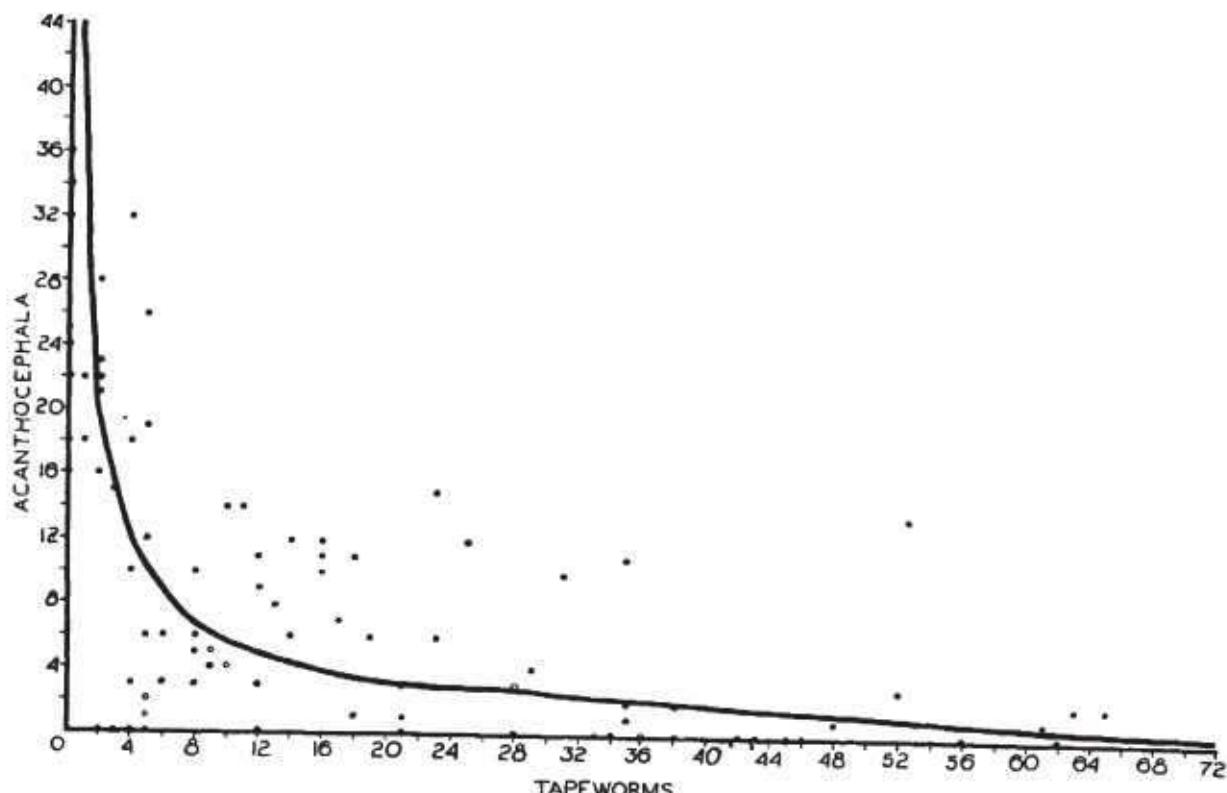


Fig. 1. Degree of infection of individual fish. The curve on this graph was located from a free hand line drawn on a logarithmic graph.

One dot represents 1 fish.

One circle represents 2 fish with similar infections.

In Table 1 the first group is made up of all fish harboring 28 or more tapeworms. Remembering the average tapeworm and acanthocephala infections it is evident that the tapeworm infection in this group is far higher than average

TABLE I.—*Averages of infections in arbitrary groups from Figure 1*

Limits of infection	Number of fish	Average tape-worm infection	Average acanthocephala infection
1. 28 tapeworms or more .....	23	42.09	1.9
2. 20 acanthocephala or more ....	12	1.33	27
3. 15–25 tapeworms .....	13	19.53	7.3
4. 10–20 acanthocephala .....	22	11.7	13.59
5. 1–15 tapeworms .....	45	6.62	9.62
6. 1–10 acanthocephala .....	45	19.87	4.21

while the acanthocephala infection is only a fraction of the average for these worms. In the second item in Table 1 the high acanthocephala infections are averaged using those cases with 20 or more of these worms. Here the infection with acanthocephala is far above the average with tapeworms very low.

That this phenomenon is not due to crowding of the parasites in the intestine is evident when we consider that the two species occupy different parts of the alimentary tract, the tapeworms preferring the gastric caeca as points of attachment while the acanthocephala attach themselves near the middle of the intestine. With this in mind it seems reasonable to think of this situation as a result of a non-specific immunity limiting either tapeworms or acanthocephala when one of these parasites is present in large numbers.—SAMUEL X. CROSS, *Limnological Laboratory, Wisconsin Geological and Natural History Survey*. (Note No. 53.)



# The worm's eye view of community ecology

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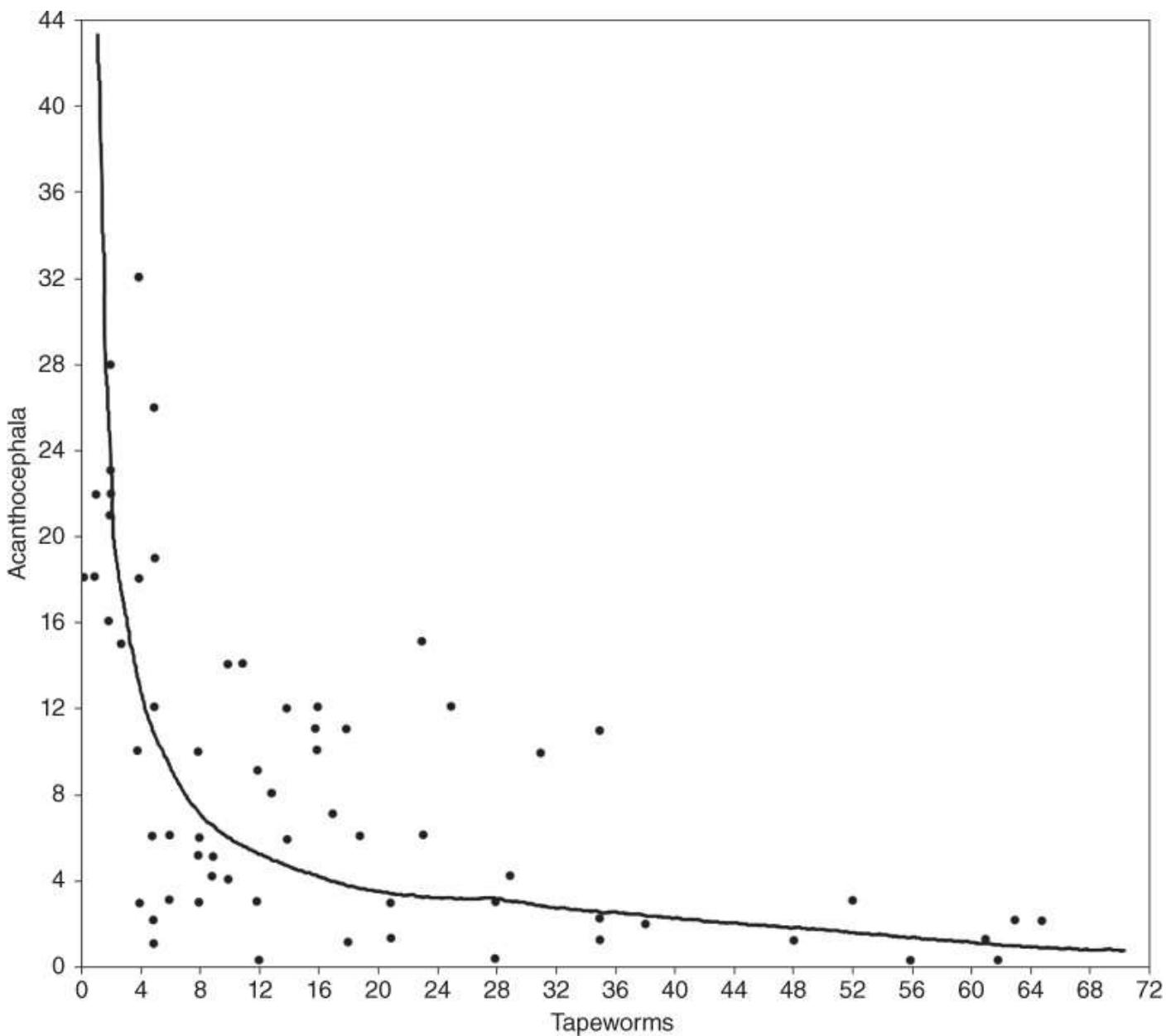
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The study of parasites in the context of community level organization, either as parasites embedded within host communities, or as parasite communities themselves, is now quite prevalent in parasitology and ecology today. However, this was not always the case. In terms of publications, there was almost no consideration of parasite interactions at the community level for most of the first half of the last century. Papers in *The Journal of Parasitology* by Clark Read (1951) and John Holmes (1961) were the defining contributions to the beginning of the field, and the ideas elaborated by these two parasitologists still inspire current debates on parasite community structure today. There are several probable explanations for why investigation of parasite communities was not popular during the early part of the century. Most likely, it was related to funding sources, and the strong biomedical rubric that has guided parasitological studies for most of the last century. The intensity of focus on treatment and control of parasites of medical and veterinary importance often resulted in indifference to the natural and evolutionary histories of the parasites. Fortunately, this situation has changed, and there has been an exponential growth of ideas based on ecological and evolutionary theory, especially since the beginning of this century. We are now entering into areas of inquiry that the early investigators probably never dreamed of. For example, it was only in 1997 that “Food webs: A plea for parasites” by Marcogliese and Cone (1997) made eloquent arguments for why parasites should be included in studies of food webs and ecosystems. This paper was an explicit call to action for community-minded parasitologists, and it ignited a huge transformation in the way ecologists and evolutionary biologists began to look at parasites in nature. Studies on the roles of parasites in food webs, an area that was almost totally ignored by ecologists for eight decades, are now fairly common (Sukhdeo, 2012).

The first significant article in *The Journal of Parasitology* to report the existence of interactions between parasite species was a Research Note by Cross (1934). He studied natural co-infections of two phylogenetically distant species, an acanthocephalan and a tapeworm, sharing the same fish host. The tendency of large numbers of one species of parasites to limit infection by other parasite species was reported by other parasitologists (Ward, 1912; Wilson, 1916), but Cross was the first to unambiguously demonstrate the existence of negative interactions between two parasite species under natural conditions. He necropsied

92 cisco fish from Silver Lake in Wisconsin that were infected with only two parasite species, a cestode *Proteocephalus exigus* and an acanthocephalan identified to genus *Neoacanthorinchus*. His interesting observation was that although co-infections occurred, fish with more than 15 individual acanthocephalans rarely had any tapeworms, whereas fish with more than 25 tapeworms rarely had any acanthocephalans (fish with fewer parasites of each species had co-infections with similar intensities of each parasite). Cross' graph of the relationship between the two parasite species ([Fig. 1](#)) is one of the most elegant demonstrations of negative parasite interactions that we have seen, and it is a major reason why we think this paper is the appropriate seed paper for a chapter on parasite community ecology.



unclear. He ruled out interspecific competition as the cause for these negative interactions because each species occupied different parts of the small intestine. At this time, mainstream ecologists were still struggling with defining interspecific competition in a meaningful way. The mathematical extension of Pearl and Read's 1920 logistic equation to describe the influence of one species on another had only been recently developed (Lotka, 1925; Volterra, 1926). These descriptive models appeared to fit the types of dynamics observed in experimental studies with free living species, including the famous study by Gause (1932) showing that yeast grown in a mixed population achieved lower densities than when grown in monoculture. However, it is unlikely that these ideas had yet trickled down to parasitology. An earlier article in the journal reported that snails infected with *Schistosoma* sp. were resistant to infection with the metacercariae of the *Cotylurus flabelliformis*, because of a non-specific immune response (Nolf and Cort, 1933), and Cross tentatively invoked a similar idea of indirect non-specific immune responses to explain his observations. Nevertheless, his questions on whether the negative interspecific effect was the result of direct competition, or if it was indirectly mediated through the host immune response, would presage much of the future work on parasite co-infections. This was somewhat unfortunate for the biological study of direct interactions between parasite species, because over the next several decades, the field would be taken over by the hundreds of studies on the indirect immunological regulation of concurrent infections between all possible combinations of protozoan and helminth parasites (Cox, 2001).

An earlier iteration of this chapter used a seed paper that exhaustively detailed a 3-year study of cattle infected with several co-occurring nematode species that persisted in a single pasture (Field 27) into which no other cattle were allowed after the first colonizing group (Stoll, 1936). The study explored whether the cattle parasites could persist without re-infection, whether cattle parasites could infect sheep, and if fecal flotation was a valid diagnostic tool. Stoll's conclusions that cattle parasites could persist "naturally" without re-infections and that sheep could be infected with cattle parasites may sound trivial today, but it had significant meaning for farmers and agriculturalists in the 1930s. In addition, fecal flotation is now a standard diagnostic tool. Nevertheless, that was as far as his interest in interspecific parasite interactions went and although he tracked multiple infections in his animals, he did only the barest of analyses. It was a sign of the times because ecological frameworks for interspecific competition were only just being developed, although not for parasites. If available today, the enormous amount of information he collected on the seasonality, prevalence and intensity of infections among the parasite communities, could be the basis for several theses.

## Crowding and competition

The Cross paper laid the foundations for the classic 1961 paper on tapeworm/acanthocephalan interactions by John Holmes. This Holmes paper is a classic in the real sense of the word, because it is still cited by almost every author now working on interspecific parasite interactions. However, before we get to Holmes, we must first discuss a paper published in 1951 by C. P. Read on the “crowding effect” in *H. diminuta*. This was a transformative paper published in our journal, and one of the first studies to create a meaningful linkage between the fields of parasitology and ecology. Read reported that in crowded infections, individual tapeworms were smaller than individuals from uncrowded infections, and it was this demonstration of competition in parasites that opened the door for ecological ideas in parasitology. Competition and predation are the two major forces thought to regulate free living populations, and these forces are still the subjects of intense scrutiny by ecologists today. Although it is easy to invoke competition in biological systems, competition is extremely difficult to demonstrate.

The crowding effect in tapeworms was an interesting model because, unlike most free-living systems, it is not confounded by the effects of predation. The idea that parasites could compete for resources was already established prior to Read's work, and several investigators had reported that in cestode infections, the size of the worms was inversely proportional to the number of worms in a given infection. This was seen with *Hymenolepis nana* infections of rats and mice (Woodland, 1924; Shorb, 1933; Hunninen, 1935) and also with infections by *H. diminuta* in rats (Chandler, 1939; Hager, 1941). However, these ideas really only coalesced with Read's carefully controlled studies of worm length and weight in tapeworms of the same age (38–44 days post infection) infecting male rats of similar size (190–208 g). This paper provided indisputable evidence that multiple infections of a single species produced worms were individually smaller than the tapeworms from single infections. In the absence of predators, density-dependent competition for scarce resources could be the only explanation, and in subsequent papers, Read was able to demonstrate that the resources being competed for were carbohydrates that occurred in limited concentrations in the gut (Read, 1959). The enormous advantage was that this study provided a laboratory model that was easy to work with and totally reliable. In fact, the demonstration of the crowding effect in tapeworms is so reliable it is often used as a standard laboratory exercise in many undergraduate parasitology courses.

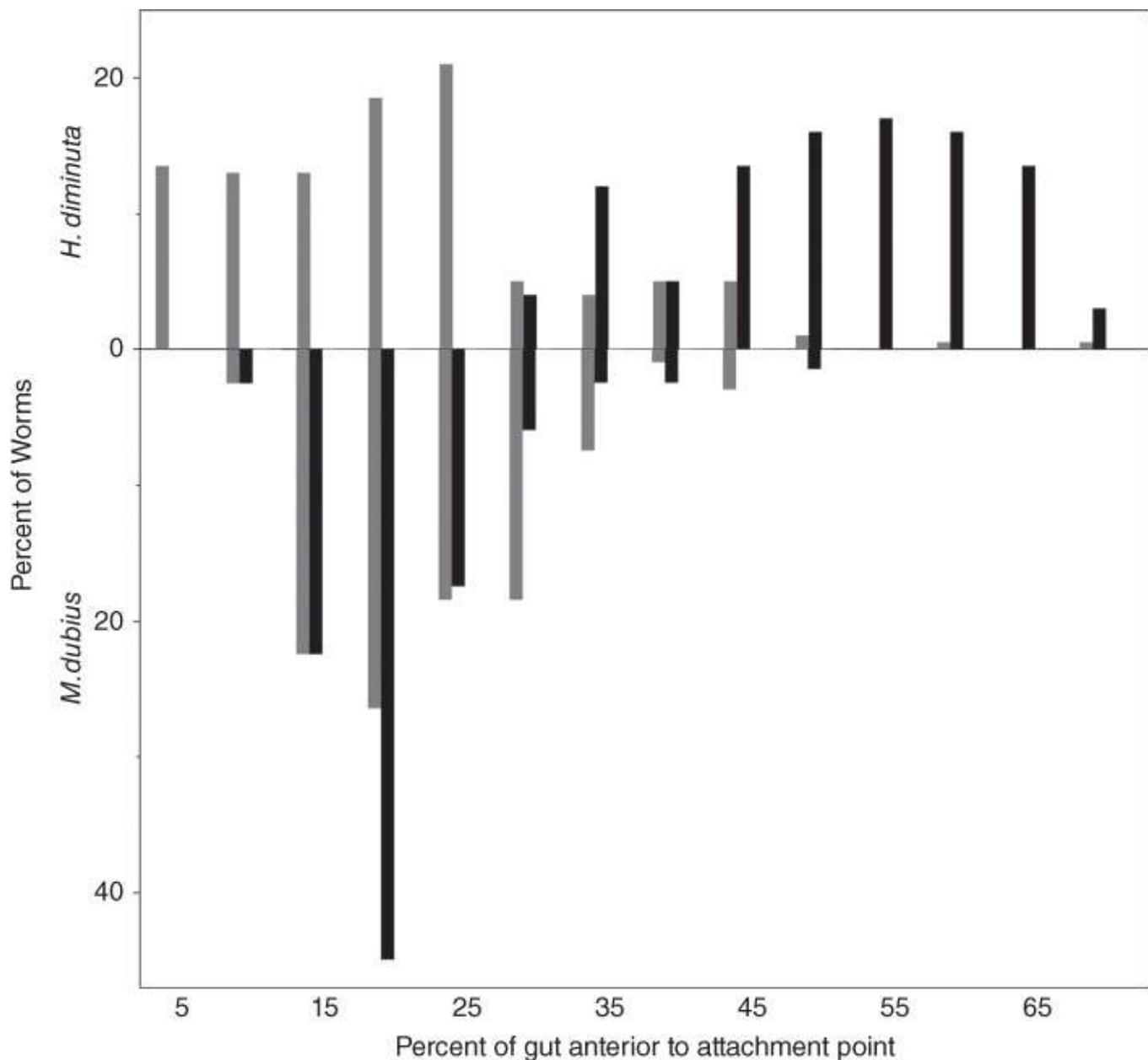
There was a huge bolus of papers following Read's paper (many in *The Journal of Parasitology*) that demonstrated the crowding phenomenon in almost all classes of helminths, although the best results came from tapeworm studies. Competition for limited resources is called exploitative competition, and while this mechanism is not the exclusive one in intraspecific interactions, it is the most common proximate mechanism for crowding effects in parasites. In the case of *H.*

*diminuta*, competition is for limited carbohydrate resources (Read, 1959; Read and Phifer, 1959), but in other parasites it may be for space for attachment sites or for mates (Bansemir and Sukhdeo, 2001; Sukhdeo and Sukhdeo, 2004). Although reductions in size or body weight are the most common response to crowding, this may not always be the case, and instead, as seen in crowded pinworms of insects, there may be significant reductions in per capita egg production (Zervos, 1988a; b). Intraspecific competition may also be mediated through chemical means, or interference competition, and there are several examples of parasites which secrete toxins to specifically target their conspecifics (Poulin, 2007). The situation is complicated further in many situations by the indirect effects of immune responses precipitated by the parasite's infection (Cox, 2001).

Crowding is by definition, competition, pure and simple. So, it is perhaps not surprising that as we have dug deeper into the subject, the ripple effect of crowding has proven to be quite wide-ranging. The effects may be positive: for example, crowding may benefit some host-manipulating metacercariae where the appropriate changes in host behavior are dependent on parasite-intensity (Weinersmith et al., 2014); or effects may be negative, as on conspecifics (Read, 1951). Defining and measuring crowding has been a problem, and the great variety of parasite models used in crowding studies have raised the call for an objective definition of crowding in parasites, especially since responses may be species-specific and density-dependent regulation may depend on threshold infrapopulation sizes of each species (Poulin, 2007). Indeed, there are now several indices and statistical tools to define crowding in parasites, but these have yet to be validated (Neuhäuser and Poulin, 2004; Reiczigel et al., 2005). Nevertheless, despite the imprecision of our current metrics, an exciting realization has been that crowding-induced inequalities in body size among adult helminths can result in inequalities in reproductive output via their impacts on growth and mating probabilities (Poulin, 2007). For example, for some larval helminths in intermediate hosts, crowded conditions early in life can have a significant negative effect on the fitness of adult parasites (Fredensborg and Poulin, 2005; Heins et al., 2010), thus having considerable consequences for the parasite's population dynamics. These long term biological effects of parasite crowding are in an area that is ripe for research, and it is a testament to Read's work that his paper is cited in almost all of these studies.

## Interspecific interactions among parasites

From Read's paper, we fast forward 10 years to a paper by John Holmes who published his Ph.D. thesis on interspecific interactions as a series of papers starting in 1961. Interspecific interactions in parasites had been demonstrated in a series of experiments on concurrent infections between *H. diminuta* and the closely related *Hymenolepis citelli*, that demonstrated loss of weight for each species, and this result was clear evidence of interspecific effects (Read and Phifer, 1959). However, Holmes' (1961) demonstration of negative interspecific effects between two phylogenetically distant parasites, tapeworms and acanthocephalans, became the definitive work. Again, his advantage was a laboratory model that allowed careful experimental control of co-infections, and quantitative measures of each worm's position provided indisputable evidence that in the presence of the acanthocephalan parasite, the tapeworm relocated in the intestine (Holmes, 1961, 1962a, 1962b); and his graphical representation of this response is elegant ([Fig. 2](#)).



**Figure 2** Effects of concurrent infection on the intraintestinal distribution of *Hymenolepis diminuta* and *Moniliformis dubius*. Light bars are single infections; dark bars are concurrent infections. Redrawn from [Figure 1](#) of Holmes (1961) Journal of Parasitology 47: 209–216. Reproduced with permission of Allen Press Publishing Services.

Following Holmes, much of parasite community ecology has continued to explore infracommunity processes, i.e. all parasite infrapopulations in a single host (Bush et al., 1997), because experimental manipulations of entire infrapopulations are possible, and because it is the only level at which different parasite populations meet and interact. Thus, the interactions between species within infracommunities can determine how parasite species can coexist within a single host, and one of Holmes' major contributions was a public and prolonged debate over whether infracommunities of parasites formed isolationist (non-interactive) versus interactive communities. These two possibilities formed the basis of a long-running, good-natured debate between Holmes and Peter Price (see Janovy, 2002), which stimulated much discussion, and provided a huge incentive for

continued experimentation by young scientists. The debate is now mostly moot, because the cumulative evidence suggests that interactions among parasite species can range along a continuum from isolationist to interactive, and that much depends on the evolutionary and ecological history of each species (Goater et al., 1987; Cabaret and Hoste, 1998; Dove, 1999; Poulin, 2007). However, at the time, the debate often became polarized. The interactions between parasite species were thought to be key to understanding of infracommunity structure in nature, but it was not at all clear that lab results translated appropriately into natural infections. Holmes himself had reported that the results from the rat studies could not be repeated in hamsters (Holmes, 1962a, 1962b), indicating a role for other factors in the responses.

In the years following Holmes' seminal paper, there were hundreds of publications on concurrent infections, mostly products of rigorous experimental protocols in the lab, and that more often than not, were primarily concerned with the immune responses involved. Thus, the process of dissecting the biological relevance of interspecific interactions was slow and tortuous, and over the decades, there have been many articles and scores of reviews on the subject. A general theory of parasite infracommunity structure has remained elusive, but the profusion of literature on the subject makes it challenging to deal comprehensively with the topic of interspecific parasite competition in this short chapter, so we will focus only on a few highlights of the debate. For more details, readers are referred to the following: Holmes and Price (1986); Price (1987); Esch et al. (1990a, 1990b); Sousa (1994); Poulin (1997); Janovy (2002); Poulin (2007).

The isolationist view was that, in nature, parasites most likely did not encounter each other frequently enough for interactions to evolve. Parasites that are spatially separated, e.g., ticks and tapeworms in the same host, were unlikely to interact. Even if they were close to each other in the host, parasite site specificity is usually very narrow, and interspecific interactions would be unlikely to occur even among parasites in the same organ (Price, 1980). The almost complete spatial separation of eight co-occurring related pinworm species crowded into the rectum of turtles (Schad, 1963) was often used to support this idea. In cases like these, it was thought that in order to maximize reproductive success, selection may have favored a narrowing of the niche. For instance, the reproductive success of helminths may vary as a function of each worm's position in the gut (Sukhdeo, 1991). This specialization could produce isolationist parasite communities if location of the niche of one species is independent of the presence of other species.

In fact hosts may have many vacant niches (Rohde, 1993a), obviating the need for competitive interactions. Furthermore, parasite infrapopulations are aggregated in their hosts (Crofton, 1971; Shaw and Dobson, 1995), and large parasite infrapopulations tend to occur in only a relatively few hosts. Theoretically, if the aggregated distributions of different parasite species are independent of one another, competition is unlikely to occur because there are likely to be very few opportunities for two or more species of parasites to encounter each other (Poulin,

2007). Indeed, there have been strong arguments against any significant roles for interspecific interactions in determining infracommunity structure in natural assemblages (Esch et al., 1990b; Fernandez and Esch, 1991a, 1991b).

Comprehensive long-term studies by Esch and colleagues suggested that the mortality rates of most parasites in hosts under natural conditions were independent of infrapopulation density (Esch and Fernandez, 1994). Instead, parasite density and prevalence tended to be related to seasonal changes in temperature and other physical factors in the environment, or of natural senescence and mortality within the host population, and not the result of interspecific interactions. For example, in freshwater snails studied by Esch and Fernandez, a new cohort may replace the existing one every year, and as snails die, so do their parasite communities. Replacement of their infracommunities was an annual event that occurred independent of any interspecific competitive effects (Esch and Fernandez, 1994).

However, it was equally clear since the work of Cross (1934), that negative interspecific parasite interactions do occur in nature, but it was not evident whether it was as common and pervasive as Holmes suggested. Co-occurrences of pairs of species that are more or less frequent than expected by chance, can provide strong evidence that species interactions exist and act on community structure. However, deducing the presence of interspecific interactions between helminth species from patterns in community structure is a convoluted process (Esch et al., 1990). In laboratory experiments, changes in numbers of parasite individuals or in their function roles related to niche utilization or reproductive output are easily demonstrated by comparing with controls. Field studies can only provide indirect, circumstantial inferences based on patterns in the distribution of species richness or species composition in infracommunities from naturally infected hosts. Controls are usually the random patterns predicted by appropriate null models (Poulin, 2007), and in many cases, the alternative explanations for presumed patterns may be equally as plausible as the effect of interactions among helminth species.

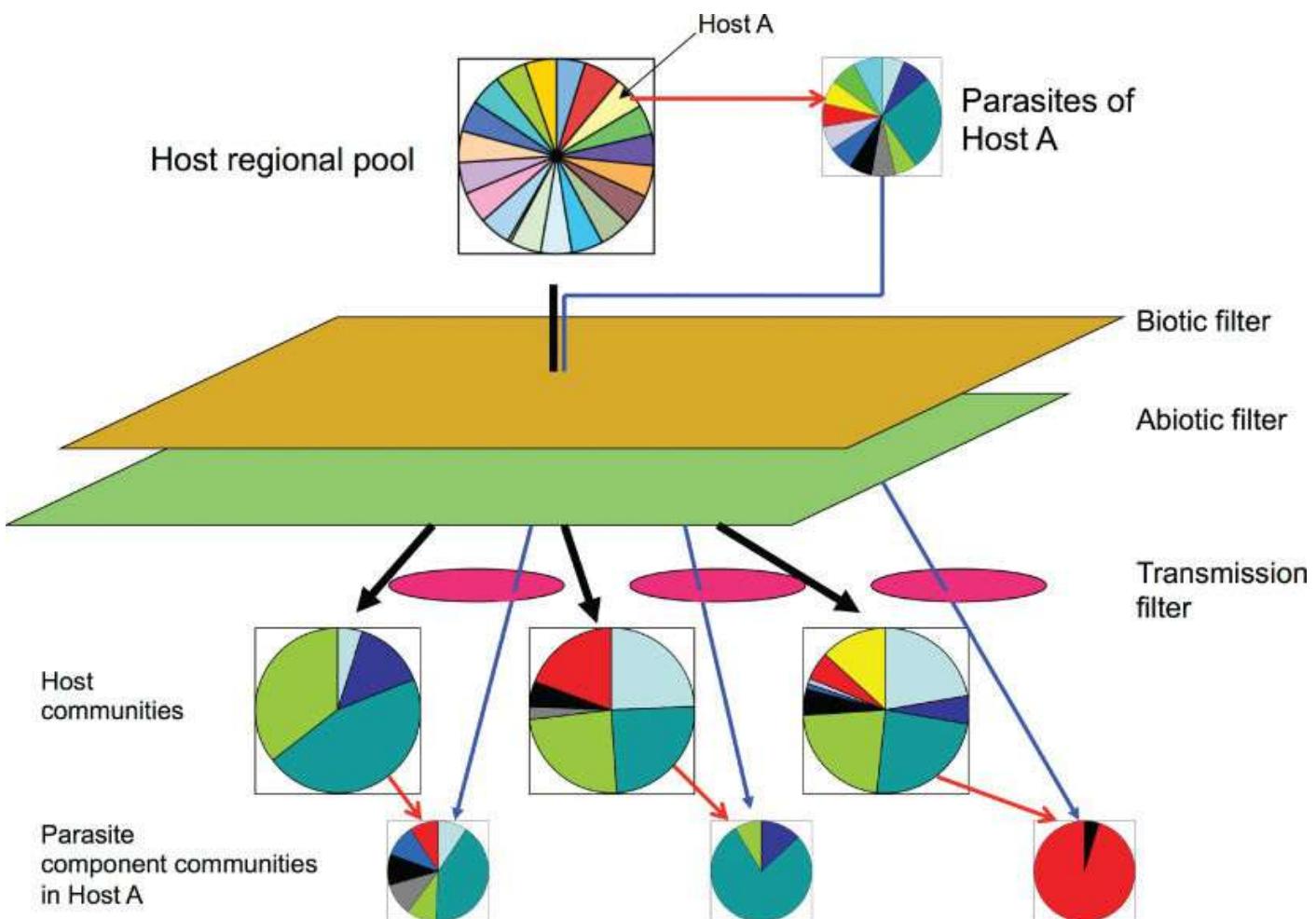
When parasite species interact negatively, the outcome can take various forms, including spatial displacement across potential sites, or reduction in numbers and biomass of one or more participants. Most negative interactions are the result of exploitative or interference competition, but there can be other mechanisms, including predation. For example, in some host snails from natural populations, mixed species infections with larval trematode species are less frequent than expected by chance. While several mechanisms might generate such negative associations, laboratory studies demonstrated the effects of strong predatory interactions between larval stages of species that infect the same host snail (Sousa, 1992, 1993). More recent studies have demonstrated that some species of these interactive trematodes may have evolved a caste system, producing warrior castes (small forms with aggressive behavior and large oral suckers) that will specifically attack and destroy competitors (Hechinger et al., 2011b).

However, not all interactions between parasite species are negative, and there are several situations where positive interactions might occur (Dobson and Pacala, 1992; Bucknell et al., 1996; Cabaret and Hoste, 1998; Sanmartín et al., 2000; Janovy, 2002; Luque et al., 2004). An exciting example is seen in the hitchhiking strategy, where a non-manipulating trematode preferentially infects intermediate hosts that are already infected with a manipulating parasite (Thomas et al., 1998). Parasite species sharing an intermediate host population with a manipulator species would benefit by associating with the manipulator, as they would obtain a cost-free ride to a shared definitive host (Thomas et al., 1998; Lafferty, 1999). In many cases like these, positive interactions have to be inferred from changes in species composition and abundance. Thus we must be cautious with these inferences because there are several factors that can generate spurious covariances and affect the sign of associations, e.g., a major source of bias is the number of hosts sampled (Lotz and Font, 1994; Dove, 1999).

Regardless of whether there are negative, positive, or no interactions between parasite species, at the heart of the matter is the potential evolutionary costs of such interactions. Interactive strategies would require the sensory apparatus to recognize competitors or cooperators, and behavioral or other mechanisms (toxins, weapons) to deal with the competitor or cooperator. These mechanisms would be very costly investments for only rare encounters with the enemy or friend. Interspecific interactions are a product of coexistence strategies, and these are determined by the forces that assemble parasite communities. Thus, if parasite species co-occur at the same site in the same host at the same time, and this situation occurs frequently over evolutionary periods, these parasites might evolve interactive strategies. For example, in systems where snails concurrently serve as hosts to several trematode species, antagonistic competition ensues (Sousa, 1993). However, in systems where different species only co-occur infrequently or where the parasite species are subject to extensive spatial and temporal heterogeneity, interspecific interactions are unimportant (Curtis and Hubbard, 1993; Curtis, 1997; Esch et al., 1997).

Much of this will be dealt in a later section of this chapter, and the reader is referred to our hierarchical model of parasite community assembly ([Fig. 3](#)). Our model argues that several biotic and abiotic factors acting on the host and on the success of parasite transmission stages, determine infracommunity structure in nature. The list of potential forces that can have significant impacts on community structuring processes is quite long, and at a minimum these factors include host longevity, host size, host diet, pool of available parasites, shared intermediate hosts, temporal and spatial heterogeneities, various abiotic conditions, generalist versus specialist natures of parasites, and host phylogenies. These influences are further complicated by various stochastic events related to local occurrences such as the probability of infection, host populations size, and colonization strategies by regional parasite pools (Esch et al., 1988; Kennedy, 1990; Valtonen et al., 2001; Janovy, 2002; Poulin, 2003; Bagge et al., 2004; Bauer and Whipps, 2013).

Realistically, to be a significant factor in structuring the parasite infrapopulations, interactions between parasite species would have to override these ecological forces that distribute parasite species into distinct infracommunities. This situation can only occur under conditions that repeatedly favor co-infections by competitors in the same host over evolutionary time, but parasite infracommunities within hosts are rarely replicated in time and space! Our view is that the rules of assembly for parasite infracommunities is a complicated process that can only be solved by multi-pronged approaches that integrate several levels of biological organization, and this topic will be the subject of most of the remainder of this chapter.



**Figure 3** Parasite community assembly is influenced by processes operating at a range of spatial and temporal scales. Parasite species are found within a regional species pool that is constrained by evolutionary processes. A subset of the species from the regional pool will colonize a particular site depending on dispersal and exposure probability. This, in essence, suggests that the observed parasite community within a host is the result of infective stages passing through abiotic and biotic filters. Modified from HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. *Ann. Rev. Ecol. Evol. Syst.*, 43: 227–248.

## A new paradigm

Finally, every author acknowledges the key role Holmes played in creating the field of parasite community ecology, often with laudatory prose regarding his experimental methods and analytical savvy, but few have recognized one of the most significant transformations brought about by the Holmes paper—a change in how parasitologists perceive and think about parasites! Small as it may seem, this change was a major paradigm shift without which there would be no study of parasite community ecology. In “Structure of Scientific Revolutions,” Kuhn (1962) argued for an episodic model where the pace of normal science is interrupted by periods of revolutionary science. We think that Holmes' (1961) work qualifies for this distinction because after him, parasites became the subject of investigations rather than the object. To answer questions Holmes raised, one had to imagine how the parasites saw their world, and how each individual responded to environmental cues and potential competitors or cooperators (Sukhdeo, 1990).

There was a time when the idea that parasites “behaved” was unimaginable, but after 1961, it became acceptable to wonder how parasites might navigate through their hosts, select mates, or even respond to competitors (Sukhdeo, 1990). Holmes himself explored these ideas by focusing on site specificity as a major factor in structuring parasite communities (Holmes, 1973) and Janovy (2002) argues this is one of the reasons Holmes' 1961 paper has such an enduring quality. However, the shift in tapeworm niche that Holmes reported from co-infections with acanthocephalans turned out to be less than remarkable when it was found out that the tapeworm species in these studies (*H. diminuta*) had a daily migration up and down the gut (Read and Kilejian, 1969). With the benefit of hindsight, the evidence suggests that the ultimate causation for site or niche specificity is multivariate, and might include competition interactions, physiological or morphological specialization, or mate selection (Holmes, 1973; Sukhdeo, 1990; Rohde, 1991; Sukhdeo and Sukhdeo, 2004).

More importantly, we now recognize that hosts are predictable homeostatic islands that provide a “third environment” that is distinct from free-living aquatic and terrestrial environments (Sukhdeo, 1990). The host environment is so predictable that cestodes, trematodes and nematodes rely on genetically fixed behaviors and strategies to navigate and find their specific sites (Sukhdeo, 1990, 1997, 2000). Navigation through their hosts to find their sites requires complex nervous systems, which are sometimes equivalent or better than their free-living counterparts, with impressive arrays of sensory apparatus (Sukhdeo and Mettrick, 1987; Sukhdeo, 1992). As an example, the brain of *Fasciola hepatica* contains the first evidence of giant neurons and specialized glial cells called trophospongium (adaptations for rapid conduction) that do not appear again until much higher taxa evolved (Sukhdeo et al., 1988a, 1988b; Sukhdeo, 1992). It is interesting that free-living stages of parasites also seem to follow genetically fixed and programmed host-finding behaviors that optimize their transmission across both

time and space (Sukhdeo, 1990, 1997; Combes, 2001). Nevertheless, it has become increasingly clear that to understand parasite strategies, from the proximate mechanisms of behavior to higher levels of organization in food web structure and ecosystem function, we have to consider the world from the parasite's point of view. It is an evolutionary perspective that drives parasite community ecology today, and this is one of Holmes' most important legacies.

# Parasite infracommunities

We believe that the second major advancement in parasite community ecology was the integration of parasites into mainstream ecological concepts of host communities, mostly occurring over the past 20 years. This period in parasite community ecology is best captured by the most cited paper in *The Journal of Parasitology* history: Bush et al., (1997) “Parasitology Meets Ecology on its Own Terms: Margolis et al. Revisited.” Though not a research article, in its exhaustive detailing of terms to describe patterns in parasite communities for an ecological audience, this paper grappled with a larger issue—how to integrate parasite ecology into mainstream ecological theory, and what form and direction should that integration should take.

Early observational studies to explain patterns in the richness and abundance of observed parasite communities used standard abiotic versus biotic dichotomies: for example, the abundance and diversity of definitive and intermediate hosts (Hoff, 1941; Smith, 2001), along with environmental constraints on transmission (abiotic conditions, e.g., Lafferty, 1997). Unfortunately, many of these studies addressed only a single factor within this mix of factors (e.g., temperature or intermediate host availability) resulting in equivocal results that confounded the underlying processes (Anderson and Sukhdeo, 2013b). Those studies that did focus on the interplay between biotic and abiotic factors on the structure of parasite communities tended to be mathematical, and these have become progressively more complex over time (e.g., Dobson, 1990; Hochberg and Holt, 1990; Greenman and Hudson, 1997, 1999, 2000). A consequence of the theory-driven studies was that general insights into the ecology and evolution of the observed parasite community became obscured. For one, the models were so analytically challenging that the results were often not biologically realistic. In addition, because of specificity of the model systems, there were usually no correlates in biological systems (see review in Keesing et al., 2006). Despite these limitations, the parameters and component processes that are described have enabled parasitologists to connect with community ecologists (e.g., Bush et al., 1997) by generating testable conceptual frameworks that describe parameters thought to be important in parasite establishment and persistence.

Two central concepts have come to define the main paradigm of parasite community ecology, and these tap into two universal evolutionary themes; namely, the basic reproductive rate of an individual,  $R_o$ , and the threshold host population size necessary to sustain a viable population,  $N_T$  (Anderson, 1982). In their seminal work, Anderson (1982) and Anderson and May (1979, 1982; May and Anderson, 1979) tied the intrinsic rate of population growth,  $R_o$ , to host dynamics using a simple model combining the density of susceptible and infected hosts with transmission rate. In doing so, they revealed how the persistence of an individual parasite population was dependent on the density of hosts. Consequently, a natural extension of the model was the identification of a

deterministic criterion for the long term persistence of a parasite species within a host population (Anderson and May, 1991). With this model, it has been possible to generalize criteria for sustained parasite transmission to one biological process: the instantaneous growth rate of parasite infection as a function of host density and proportion of susceptible individuals (Holt and Pickering, 1985; Begon et al., 1992; Holt et al., 2003).

Although this model was based at the population level, the general framework has been and continues to be extended to communities of parasites to identify the general conditions under which host community diversity would alter parasite dynamics (Holt et al., 2003; Dobson, 2004; Rudolf and Antonovics, 2005). These studies have revealed two key features tied to mechanisms of transmission that appears to alter parasite establishment and dynamics: (1) density-, or (2) frequency-dependent transmission whereby the parasite population is a function of the absolute density of the host population, or the proportion of infected hosts within the population (Keesing et al. 2006). Density-dependent models of transmission are typically used to describe parasites that are spread through environmental propagules or random contact (e.g., Gao and Hethcote, 1992). Frequency-dependent models are normally used to describe the transmission of sexually transmitted diseases and epidemiology in human systems (see Getz and Pickering, 1983; Thrall et al., 1993). Vector-borne diseases conform broadly to the structure of frequency-dependent models of transmission and theoretical work has demonstrated that contact between vector and host is a function of search rate and infected host density (Antonovics et al., 1995; Rudolf and Antonovics, 2005). It has been argued that if parasite transmission is density-dependent, a more diverse community will result in lower parasite transmission only if the increased diversity reduces the density of the focal host species (Dobson, 2004; Rudolf and Antonovics, 2005). Conversely, in parasites that follow the frequency-dependent mode of transmission, an increase in host community diversity will always result in reduced transmission and establishment success (Dobson, 2004; Rudolf and Antonovics, 2005).

A second consequence of increasing host diversity for metazoan parasites is the potential for transmission success to change depending upon whether the parasite species uses a single- or multi-host life cycle. The assumption that transmission is higher for single-host parasites than for species that rely on multiple hosts is common in almost all models of disease transmission. Begon et al. (1999) and Woolhouse et al. (2001) argue that this assumption is appropriate and a requirement for hosts to coexist in mathematical models. There are only a few examples where multi-host pathogen transmission is higher than single host transmission; importantly, these examples are restricted to a virus (Rhodes et al., 1998) and a bacterium (Caley and Hone, 2004). Evidence suggests that all other metazoan parasites and vector-borne diseases fit the assumption that transmission is higher for single-host parasites (see reviews in Kuris and Lafferty, 2000; Keesing et al., 2006).

Using a simple graphical isocline framework, Holt et al. (2003) further explored the consequences of single- and multi-host pathogen transmission and described a series of critical thresholds for parasites to establish in host communities. In a single-host parasite, the density of the host provides a single threshold to establishment, whereas parasites that use multiple hosts have various combined host densities that enable establishment. Under the assumption that single-host transmission is higher than multi-host transmission, an increase in diversity increases the probability of parasite establishment when compared to lower diversity systems. Furthermore, Holt et al. (2003) demonstrated that increasing the diversity of the system resulted in higher critical host population thresholds; i.e., as the density of a non-target host increases there must be a concomitant increase in the target host density for the parasite to establish—the non-target host dilutes the pool of target hosts (for empirical examples see Norman et al., 1999; Ostfeld and Keesing, 2000; Schmidt and Ostfeld, 2001). In general, additional host species within a system are likely to inhibit the establishment of diverse parasite species because of higher critical thresholds in host density; this may be offset by the higher probability of encountering a target host in more diverse systems, a phenomenon similar to the sampling effect (Loreau et al., 2001).

In spite of the considerable discussion and development of these theoretical frameworks, they have mostly been studied in single-host and single-parasite systems, with the assumption that the observed dynamics scale can be extended to multi-host, multi-parasite communities (Pedersen and Fenton, 2007). This practice stems principally from the difficulty in estimating core parameters such as  $R_0$  for all parasite species, and the critical host thresholds for all hosts in the system, a necessity given that multiple host-parasite systems are the norm (Anderson and May, 1991). Consequently, over the last 10 years, the literature has detailed a litany of factors, some biotic and others abiotic, that may have an impact upon parasite communities, with no unifying patterns identified (reviewed in Poulin and Morand, 2000). Biotic factors have been demonstrated to drive the dynamics of parasite communities in sea birds (e.g., Bush and Holmes, 1986), and in salmonid and rocky reef fishes (Holmes, 1990; Kennedy and Bush, 1994). In contrast, there are examples where abiotic factors such as “harsh” environmental conditions (Galaktionov, 1996; Marcogliese and Cone, 1996; Biserkov and Kostadinova, 1998; Marcogliese and Pietrock, 2011) and anthropogenic perturbations (Marcogliese, 2001; 2005; Marcogliese and Pietrock, 2011) determine the dynamics of a parasite community. In all cases, successful description of parasite community dynamics within the host environment has relied on local processes with relatively small spatial scales (e.g., Anderson and Sukhdeo, 2010). We assert that by tackling parasite community dynamics on a local scale, parasitologists have gained considerable insight into host factors such as host age and density that may have an impact on parasite community dynamics, but these studies do not have the universal appeal of the early population biology studies of Anderson and May (1991).

In contrast to the parasitological focus on fine scale processes that has defined parasite community ecology, mainstream community ecology has explicitly considered community dynamics within a framework consisting of regional and local processes (e.g., Ricklefs and Schlüter, 1993; Brown, 1999; Lawton, 1999; Gaston et al., 2000). Although the dominant forces structuring communities varies by system, by including regional processes alongside local processes, ecologists have developed a solid theoretical framework and considerable empirical evidence explaining patterns and processes in many free-living communities. Further, the approach for integrating regional and local processes is frequently in the form of food webs (reviews in Pascual and Dunne, 2005). We contend that it is the “disconnect” between local and regional processes that has stymied parasitologists, and the methods and relative success of community ecology has driven a generation of parasite ecologists to adopt and ask questions in a similar manner, even if they may not be suitable for parasite ecology!

The methods in community ecology were adopted because they were intuitive: regional and local approaches are complementary and were likely to provide important insight into parasite community assembly and diversity patterns. Indeed, the potential benefit of studying parasite community dynamics using multiple scales stems from advances made in the field of complex system analysis (Bar-Yam, 1997; Pascual and Dunne, 2005). This approach suggests that units (molecules, cells, cells with organelles, multicellular organisms, herds, or other organismal groupings) and the relationship between these individual units, may effectively describe system level behaviors. This approach seems particularly useful in parasite study. Recent work has demonstrated the interaction between global environmental change and local parasite dynamics (Harvell et al., 1999) and there is evidence that local transmission dynamics may scale up to affect global disease dynamics (Hahn et al., 2000; Daszak and Cunningham, 2002). It seems plausible to suggest that regional processes coupled with detailed population and community studies, and the relationship between the two scales, will provide more insight into parasite community dynamics than either approach alone.

# Parasites and food webs

Though not immediately obvious, this “complex system” approach has been used in mainstream ecology since the work of Charles Elton in the 1920s (Elton, 1927). This approach describes trophic interactions between consumers and resources which, in effect, unifies local and regional dynamics. Local interactions between species—trophic links—are in part determined by regional dynamics for two reasons: resource competition and energy. The topology of webs and the interactions within them influence the dynamics and persistence of populations through resource availability and mortality caused by predation (De Ruiter et al., 2005; Pascual and Dunne, 2005; Neutel et al., 2007). Moreover, trophic interactions represent transfer rates of energy and matter, a fundamental concept in ecosystem and community processes. Food webs, therefore, provide a way to analyze the relationship between populations, communities, and ecosystems and core ecological concepts such as stability, diversity, and community assembly (Sukhdeo and Hernandez, 2005; Lafferty et al., 2008; Anderson and Sukhdeo, 2013a).

The use of food web analysis to reveal underlying concepts in parasite ecology has exploded following Marcogliese and Cone's call to arms in the late 1990s. Nevertheless, there are still only a small number of food webs in the literature that contain parasites (e.g., Lafferty et al., 2006b; Anderson and Sukhdeo, 2011; Preston et al., 2013). The lack of taxonomic resolution and inclusion of parasites in food web descriptions and theory is a major criticism leveled at food web ecology (Huxham et al., 1995; Marcogliese and Cone, 1997). Frequently, published webs include relatively few of the species present in the system; further, they rarely describe all the potential interactions. The most common consumer strategy, parasitism, is generally left out of food web analyses (Lafferty and Kuris, 2002). Given the difficulty quantifying parasite-host interactions using standard ecological techniques this situation is not surprising, yet parasitism appears to be a fundamental feature of all natural systems. Price (1980) estimates that parasitism is a strategy used by over 50% of all species at some point in their life history. Esch and Fernandez (1993) make the claim that the number of non-parasitic species that are parasitized approaches 100%. Similarly Rohde (1993b) suggests that all marine species are infected with parasites. Despite the obvious ubiquitous nature of parasitism, there remain few food webs in the literature that contain metazoan parasites (Huxham et al., 1995; Lafferty et al., 2006b; Hernandez and Sukhdeo, 2008; Mouritsen et al., 2011; Thielges et al., 2011; Preston et al., 2012), and those that are included represent a small fraction of possible species and potential trophic links likely to be present. In some cases, there is acknowledgement of the omission of parasites from community webs, but the diverse sizes and feeding strategies used by parasites make it very difficult to retrofit them into the 50 years of food web theory.

It is possible to construct food webs anew including parasites (e.g., Lafferty et al.,

2006b) or add parasite information to existing food webs using parasite-host records (e.g., Anderson and Sukhdeo, 2011). Despite the promise of this approach, it is a non-trivial pursuit that should be embarked upon with caution. Systematic inclusion and consideration of all parasites for all free-living species in food webs would be ideal but it is clearly intractable to include all species in a system. Further, not all food web datasets are appropriate for expansion to include parasites because of the high degree of taxonomic aggregation, i.e., functional groups, whereas others are dominated by species with few historical parasite-host records. Consequently, including parasites in food webs and understanding how they impact upon food web dynamics and topology can result in exhaustive multi-year empirical studies (Hernandez and Sukhdeo, 2008; Kuris et al., 2008). Further, integrating parasites has generally taken the approach of developing a parasite subweb (*sensu* Lafferty et al., 2006a) which does not take into account the delightfully elegant complexities of trophically transmitted parasites (Sukhdeo, 2012). Including parasites then seems to run counter to the initial goal of food web analyses: the description of complex multi-scale processes in simple webs that provide insight into patterns and processes. Consequently, a more fruitful question may then be what can host food webs and community ecology tell us about parasites?

For several decades a dominant paradigm in community ecology was that complex communities are more stable than simple ones (MacArthur, 1955; Hutchinson, 1959; Elton, 2000). MacArthur (1955) postulated that a large number of “paths” through each species is necessary to ameliorate the effects of dominant or overpopulated species. He concluded that “stability increases as the number of links increases,” tying together the concept of community stability with two core food web properties, trophic linkage and number of species. May (1972, 1973) challenged this general paradigm using dynamic models of abstract communities, finding that communities tended towards unstable behavior as system complexity increased. He made the observation that stability in food webs is conditional on the interaction between species diversity ( $S$ ), connectance between species ( $C$ ), and interaction strength ( $i$ ) and that systems would be stable if  $i(SC)^{1/2} < 1$ . Several papers since May (1972, 1973) have pointed out the limitations in his analyses of abstract communities (e.g., Lawlor, 1980; Cohen and Newman, 1985; Polis, 1991), stemming largely from evidence suggesting that species interactions in biological systems are not random. Regardless, May's work provided a framework to empirically address two universal parameters: the interplay between diversity and connectance and the ratio of species to links within the food web (linkage density).

The core metric in much of this discussion, connectance  $C$ , has not been ignored by parasite ecologists. Fundamentally,  $C$  measures the proportion of potential links among species that are realized, and it is predicted to decrease hyperbolically as species richness increases to maintain system stability (Warren, 1989; Dunne, 2006). Original analyses of community dynamics using  $C$  provoked a flurry of

criticism because they included only a fraction of species present in natural systems (Paine, 1988; Polis, 1991; Hall and Raffaelli, 1993). However, subsequent analyses of food webs with higher taxonomic resolution detailed how an increase in species, including parasite links, resulted in a decrease in connectance fitting the community ecology paradigm (Huxham et al., 1995; Memmott et al., 2000; Thompson et al., 2005). And it was then that parasitologists became a focal point in the debate. Lafferty and colleagues (Lafferty et al., 2006a) re-analyzed these data along with other parasite-host webs and documented that a startling number of food-web links are parasite derived. Further, by omitting illogical parasite-parasite and predator-parasite links from analysis and they found an increase in connectance. These data do not fit the expected inverse relationship between connectance and species diversity and created a conundrum for parasite ecologists: a food web with parasites is not unstable! *Post-hoc* explanations such as suggesting that increased web cohesiveness offset the increase species richness and concomitant increase in connectance were proposed (Lafferty et al. 2006a, 2006b), but were relatively underwhelming because the metrics used in these arguments were derived from topological matrices rather than biological observation.

A more plausible explanation is that the true biology of parasites with complex life cycles solves the apparent paradox of a diverse, highly connected communities retaining stability. A major impact of including parasites in food web analyses is the extension of trophic chains (Williams and Martinez, 2004), which should decrease system stability. However, complex life cycle parasites, though extending the length of trophic chains, introduce relatively weak interactions into “long loops” that may offset the effect of increasing connectance (Neutel et al., 2002). Many parasitic helminths with complex life cycles have strong impacts on some species in their life cycle (e.g., Lafferty and Morris, 1996) but have weak or non-detectable impacts on others. Further, when parasitic helminths infect intermediate hosts in their life cycle, they parasitize a small fraction of the total population of that host and a smaller fraction of that goes on to infect the next host in the life cycle. A consequence of this situation is that the interaction link between a parasite and host is a relatively weak one (Dobson et al., 2006). Additionally, direct life cycle parasites may be a strong stabilizing force because their dynamics are typically frequency dependent and the most common host species may suffer the greatest pathology (Dobson, 2004; Lafferty et al., 2008). Though the net effect of including parasites in food webs is an increase in species diversity and an increase in connectance that would seemingly result in lower system stability, any effects may be offset by relatively weak links with a significant number of free-living species on multiple trophic levels (Neutel et al. 2002; Dobson et al. 2006). This narrative reveals a critical component of our thesis: incorporating an evolutionary perspective of parasitism reveals a potentially fundamental, though untested, property in community ecology—parasites may be the glue that binds food webs together!

Considerable effort has gone into explaining food web regularities beyond diversity and the distribution and density of feeding links between species (see reviews in (Belgrano, 2005; Pascual and Dunne, 2005). Beginning in the early 2000s, a series of studies in community ecology began to apply analytical methods derived from graph theory. Specifically these researchers began to search for regular patterns of species interactions within ecological communities (Dunne 2006). Montoya and Sole (2002) used three empirical food webs and found that web topology was very similar to those of small world, scale-free networks like the World Wide Web. Contradicting these results was a meta-analysis conducted on seven food webs by Camacho et al. (2002) who found that the degree of clustering in empirical food webs was no higher than would be expected from random expectations, and significantly lower than clustering in small world networks. In an attempt to bridge this conflict, Dunne et al. (2002) expanded analysis to 16 food webs, including those used by Montoya and Sole (2002) and Camacho et al. (2002), and concluded that most food webs displayed low clustering coefficients and link distributions that deviated from those of scale-free networks.

Although these studies may appear to be esoteric mathematical explorations, the implication derived from them is that food webs deviate from physical networks and are not randomly connected “graphs” or regular lattices in which every species has the same number and pattern of links. Instead, the majority of food webs seem to have a unique topology, one that has relatively low diversity, high connectance, a degree distribution that is exponential in form and a short path length between species. That is, within a food web there are a few highly connected species that give the appearance of compartments in webs. Given the partial reliance of parasites on trophic interactions for the successful completion of their life cycles, we would expect these free-living regularities to be reflected in subsequent patterns of parasite diversity observed in extant systems (Marcogliese, 2003).

The search for tightly interacting compartments in food webs, however, has presented conflicting accounts of their presence (e.g., Paine, 1966) or absence (Pimm and Lawton, 1980). We think it likely that this conflict is likely caused by analytical difficulties: high connectance, a trait of most food webs, may obscure the presence of compartments. Recent methodological advances have provided a variety of effective algorithms that identify compartments by searching for nestedness, modularity, or “groups.” Particularly relevant for parasites is the presence of clusters of species that may be core components of the food web network. Intuitively, host species that are central within ecological networks experience fewer fluctuations in abundance relative to those that fall in the periphery of a network providing a reliable resource for parasites. Second, stable ecological interactions between predators and prey may ensure successful completion of the parasite life cycle, and this dynamic will be represented by clusters of tightly interacting species that form the mathematical foundation for food web nestedness and modularity.

These characteristics will be particularly important for helminth parasites with complex life cycles involving two or more hosts where transmission occurs via predation. The reliance on this form of transmission, over evolutionary time, is likely to have favored parasitism of host species that are central to the structure of food webs, and fall within interactions that are relatively “strong” (Anderson and Sukhdeo, 2011, 2013a). Using this logic, parasite ecologists have been able to identifying patterns in the topology of ecological networks and link these regularities in the networks to parasite community dynamics (Chen et al., 2008; Anderson and Sukhdeo, 2011). The argument has been that a reliable and stable source of energy is required for the persistence over evolutionary time (Anderson, 2009; Anderson and Sukhdeo, 2010, 2013a).

These data have allowed the parasite ecology community to rephrase the discussion towards asking what the parasite needs to survive and reproduce, rather than what the parasite is doing to the host. This approach is similar to the metabolic theory of ecology (Brown et al., 2004), which proposes that energy metabolism is the central unifying theme in ecology. Remarkably, Hechinger and colleagues (Hechinger et al., 2011a) applied this framework to parasite and free-living species and were able to demonstrate that biomass production within trophic levels was invariant of body size across all species and functional groups! The critical implication is that the flow of energy to parasites operates under the same thermodynamic rules that govern energy flows to every organism in the food web (Sukhdeo and Hernandez, 2005; Sukhdeo, 2010). Indeed, thinking of parasites as energy-limited may explain disparate results such as highly diverse salt marsh systems in California (Hechinger and Lafferty, 2005) and Chilean intertidal zones (Hechinger et al., 2008) supporting a high diversity of parasites, whereas a comparable salt marsh system in New Jersey documented no such association (Anderson and Sukhdeo, 2013b). Parasites have high host fidelity, and there should be a correlation between the success of establishment in a specific host population and the local stability of that community. Logically, a community that is locally stable (Ives and Carpenter, 2007; Allesina and Tang, 2012) represents a predictable resource for complex life cycle parasites to establish in or upon. Thompson et al. (2013) were able to track energetic resources through a food web and find patterns that suggested a relationship between energy flow and parasite community dynamics and diversity. Anderson (2009) and Anderson and Sukhdeo (2013a) used a qualitative measure of community stability as a proxy of energy flow and were able to document a positive correlation between system stability and parasite community assembly. And perhaps more convincingly, Sukhdeo (2012) and Rossiter (2012) presented data documenting biomass fluctuation over time in a New Jersey river, and were able to show that parasites preferred hosts that were the most stable in their seasonal biomass values, clearly supporting the idea that reliable and stable energetic resources are an important component for parasitism. This observation clearly represents a success: mainstream ecological theory has provided insight into parasite community ecology—but is this an exception?

## Final comments

Despite the increasing attention parasites have received over the past decade, and the successes we have documented earlier, our understanding of parasite community assembly mechanisms remains rudimentary. Generally the establishment of parasites in host systems has not been explored beyond epidemiological settings and the invasion of novel environments (e.g., Kennedy, 1990; Kennedy and Fitch, 1990). Intuitively, the absence of necessary host resources will be reflected in the absence of parasite species (Hudson et al., 2006). And similarly, it is plausible to suggest that presence of a diverse parasite community reflects the presence of a diverse host community; not only definitive species but also all species involved in the functioning of the free-living community (Anderson and Sukhdeo, 2010). We suggest that a necessary part of host-parasite community ecology is the study of all links, and the distribution of these links, in the network of host-host and host-parasite interactions and how patterns of energy flow underlay all of these dynamics! A simple task, no doubt, but given the wealth of food web theory (see reviews in De Ruiter et al., 2005; Pascual and Dunne 2006) describing community regularities, and the tight evolutionary link between host and parasite, there are likely predictable structures within the host food web that facilitate establishment and persistence of parasites: a framework that considers each of these steps, from a parasite's perspective is hopefully in our future ([Fig. 3](#)).

This chapter is a preliminary step towards synthesizing an almost overwhelming number of studies that have been conducted on parasite community ecology over the past 100 years. As we reflect on the development of the field, we realize that we are only at the start of the journey towards understanding of how parasites establish and persist within ecological communities. There have been elegant experimental demonstrations of parasite interactions or lack thereof, observational descriptions of how free-living species diversity and dynamics and the transmission environment determines parasite community dynamics or does not, and how modern ecological theory—primarily in the field of food web ecology—is a poor fit for what we know of the biology of parasites unless filtered through an evolutionary perspective. After Holmes' seminal experimental, our approach was to shoehorn ideas from the community ecologists, and to try to force these ideas (especially regarding competition) on to parasites. We continued to do this at the community level, and now parasites are mired as a “pseudonode” within food webs, despite the demonstration of their critical importance in modifying energy flow and species interactions. Our future history will be to disentangle parasites from the theoretical mess, and to strike out on our own. It is an evolutionary perspective which drives parasite community ecology today, and this is one of most important legacies of the early papers in *The Journal of Parasitology*: if you understand how your parasite perceives and responds to its world, you can truly make a contribution to our understanding of parasite community ecology.

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## Literature cited

- Allesina, S., and S. Tang. 2012. Stability criteria for complex ecosystems. *Nature* **483**: 205–208.
- Anderson, R. M. 1982. *Population dynamics of infectious diseases: theory and applications*. Chapman and Hall, London, U.K., 368 p.
- \_\_\_\_\_, and R. May. 1982. Coevolution of hosts and parasites. *Parasitology* **85**: 411–426.
- \_\_\_\_\_, and \_\_\_\_\_. 1979. Population biology of infectious diseases: Part I. *Nature* **280**: 361–367.
- \_\_\_\_\_, and \_\_\_\_\_. 1991. *Infectious diseases of humans*. Oxford University Press, Oxford, U.K., 768 p.
- Anderson, T. K. 2009. *Food web networks and parasite diversity*. Ph.D. Thesis, Rutgers University, New Brunswick, New Jersey, 185 p.
- \_\_\_\_\_, and M. V. K. Sukhdeo. 2010. Abiotic versus biotic hierarchies in the assembly of parasite populations. *Parasitology* **137**: 743–754.
- \_\_\_\_\_, and \_\_\_\_\_. 2011. Host centrality in food web networks determines parasite diversity. *PLoS One* **6**: e26798.
- \_\_\_\_\_, and \_\_\_\_\_. 2013a. Qualitative community stability determines parasite establishment and richness in estuarine marshes. *Peer J* **1**: e92.
- \_\_\_\_\_, and \_\_\_\_\_. 2013b. The relationship between community species richness and the richness of the parasite community in *Fundulus heteroclitus*. *Journal of Parasitology* **99**: 391–396.
- Antonovics, J., Y. Iwasa, and M. P. Hassell. 1995. A generalized model of parasitoid, venereal, and vector-based transmission processes. *American Naturalist* **145**: 661–675.
- Bagge, A. M., R. Poulin, and E. T. Valtonen. 2004. Fish population size, and not density, as the determining factor of parasite infection: a case study. *Parasitology* **128**: 305–313.
- Bansemir, A. D., and M. V. K. Sukhdeo. 2001. Intestinal distribution of worms and host ingesta in *Nippostrongylus brasiliensis*. *Journal of Parasitology* **87**: 1470–1472.
- Bar-Yam, Y. 1997. *Dynamics of complex systems*. Westview Press, Boston, 864 p.
- Bauer, E. F., and C. M. Whipps. 2013. Parasites of two native fishes in adjacent Adirondack Lakes. *Journal of Parasitology* **99**: 603–609.

Begon, M., R. G. Bowers, N. Kadianakis, and D. E. Hodgkinson. 1992. Disease and community structure: The importance of host self-regulation in a host-host-pathogen model. *American Naturalist* **139**: 1131–1150.

Begon, M., S. M. Hazel, D. Baxby, K. Bown, R. Cavanagh, J. Chantrey, T. Jones, and M. Bennett. 1999. Transmission dynamics of a zoonotic pathogen within and between wildlife host species. *Proceedings of the Royal Society of London: Series B* **266**: 1939–1945.

Belgrano, A. 2005. *Aquatic food webs: An ecosystem approach*. Oxford University Press, Oxford, 272 p.

Biserkov, V., and A. Kostadinova. 1998. Intestinal helminth communities in the green lizard, *Lacerta viridis*, from Bulgaria. *Journal of Helminthology* **72**: 267–271.

Brown, J. H. 1999. Macroecology: Progress and prospect. *Oikos* **87**: 3–14.

\_\_\_\_\_, J. Gillooly, A. Allen, V. Savage, and G. West. 2004. Toward a metabolic theory of ecology. *Ecology* **85**: 1771–1789.

Bucknell, D., H. Hoste, R. B. Gasser, and I. Beveridge. 1996. The structure of the community of strongyloid nematodes of domestic equids. *Journal of Helminthology* **70**: 185–192.

Bush, A. O., and J.C. Holmes. 1986. Intestinal helminths of lesser scaup ducks: An interactive community. *Canadian Journal of Zoology* **64**: 142–152.

\_\_\_\_\_, K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**: 575–583.

Cabaret, J., and H. Hoste. 1998. Comparative analysis of two methods used to show interspecific associations in naturally acquired parasite nematode communities from the abomasum of ewes. *Veterinary Parasitology* **76**: 275–285.

Caley, P., and J. Hone. 2004. Disease transmission between and within species, and the implications for disease control. *Journal of Applied Ecology* **41**: 94–104.

Camacho, J., R. Guimerà, and L. A. N. Amaral. 2002. Robust patterns in food web structure. *Physical Review Letters* **88**: 228102.

Chandler, A. C. 1939. The effects of number and age of worms on development of primary and secondary infections with *Hymenolepis diminuta* in rats, and an investigation into the true nature of “premunition” in tapeworm infections. *American Journal of Epidemiology* **29**: 105–114.

Chen, H.-W., W. -C. Liu, A.J. Davis, F. Jordan, M. -J. Hwang, and K. -T. Shao. 2008. Network position of hosts in food webs and their parasite diversity. *Oikos*

**117:** 1847–1855.

Cohen, J. E., and C. M. Newman. 1985. When will a large complex system be stable? *Journal of Theoretical Biology* **113**: 153–156.

Combes, C. 2001. *Parasitism: The ecology and evolution of intimate interactions*. University of Chicago Press, Chicago, I.L., 552 p.

Cox, F. E. 2001. Concomitant infections, parasites and immune responses. *Parasitology* **122**: S23–38.

Crofton, H. D. 1971. A model of host–parasite relationships. *Parasitology* **63**: 343–364.

Cross, S. X. 1934. A probable case of non-specific immunity between two parasites of ciscoes of the Trout Lake region of northern Wisconsin. *Journal of Parasitology* **20**: 244–245.

Curtis, L. A. 1997. *Ilyanassa obsoleta* gastropoda as a host for trematodes in Delaware estuaries. *Journal of Parasitology* **83**: 793–803.

\_\_\_\_\_, and K. M. Hubbard. 1993. Species relationships in a marine gastropod-trematode ecological system. *The Biological Bulletin* **184**: 25–35.

Daszak, P., and A. A. Cunningham. 2002. Emerging infectious diseases: A key role for conservation medicine. In *Conservation medicine: Ecological health in practice*, A. A. Aguirre, R. S. Ostfeld, G. M. Taylor, C. House, and M. C. Pearl (eds.). Oxford University Press, Oxford, U.K., p. 40–61.

De Ruiter, P. C., V. Wolters, J. C. Moore, and K. O. Winemiller. 2005. Food web ecology: Playing Jenga and beyond. *Science* **309**: 68–71.

Dobson, A. P. 1990. Models for multi-species parasite-host communities. In *Parasite communities: Patterns and processes*, G. W. Esch, A. O. Bush, and J. M. Aho (eds.). Springer, The Netherlands, p. 261–288.

\_\_\_\_\_. 2004. Population dynamics of pathogens with multiple host species. *American Naturalist* **164**: S64–78.

\_\_\_\_\_, and S.W. Pacala. 1992. The parasites of *Anolis* lizards the northern Lesser Antilles. II. *The structure of the parasite community*. *Oecologia* **92**: 118–125.

Dobson, A. P., K. D. Lafferty, and A. M. Kuris. 2006. Parasites and food webs. In *Ecological networks: Linking structure to dynamics in food webs*, M. Pascual, and J. A. Dunne (eds.). Oxford University Press, Oxford, U.K., p. 119–135.

Dove, A. D. 1999. A new index of interactivity in parasite communities. *International Journal for Parasitology* **29**: 915–920.

Dunne, J. A. 2006. The network structure of food webs. In *Ecological networks: Linking structure to dynamics in food webs*, M. Pascual, and J. A. Dunne (eds.). Oxford University Press, Oxford, U.K., p. 27–86.

\_\_\_\_\_, R. J. Williams, and N. D. Martinez. 2002. Food-web structure and network theory: The role of connectance and size. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 12917–12922.

Elton, C. S. 1927. *Animal ecology*. University of Chicago Press, Chicago, I.L., 296 p.

\_\_\_\_\_. 2000. *The ecology of invasions by animals and plants*. University of Chicago Press, Chicago, I.L., 196 p.

Esch, G. W., and J. C. Fernandez. 1993. *A functional biology of parasitism: Ecological and evolutionary implications*. Chapman and Hall, London, U.K., 337 p.

\_\_\_\_\_, and \_\_\_\_\_. 1994. Snail-trematode interactions and parasite community dynamics in aquatic systems: A review. *The American Midland Naturalist* **131**: 209–237.

\_\_\_\_\_, Bush, A. O. and Aho, J. M. 1990a. *Parasite communities: patterns and processes*. Chapman and Hall, London, U.K., 335 p.

\_\_\_\_\_, C. R. Kennedy, A. O. Bush, and J. M. Aho. 1988. Patterns in helminth communities in freshwater fish in Great Britain: Alternative strategies for colonization. *Parasitology* **96**: 519–532.

\_\_\_\_\_, A. W. Shostak, D. J. Marcogliese, and T. M. Goater. 1990b. Patterns and processes in helminth parasite communities: an overview. In *Parasite communities: patterns and processes*, G. W. Esch, A. O. Bush, and J. M. Aho (eds.). Springer, The Netherlands, p. 1–19.

\_\_\_\_\_, E. J. Wetzel, D. A. Zelmer, and A. M. Schotthoefer. 1997. Long-term changes in parasite population and community structure: A case history. *The American Midland Naturalist* **137**: 369–387.

Fernandez, J. C., and G.W. Esch. 1991a. Guild structure of larval trematodes in the snail *Helisoma anceps*: Patterns and processes at the individual host level. *Journal of Parasitology* **77**: 528–539.

\_\_\_\_\_, and \_\_\_\_\_. 1991b. The component community structure of larval trematodes in the pulmonate snail *Helisoma anceps*. *Journal of Parasitology* **77**: 540–550.

Fredensborg, B. L., and R. Poulin. 2005. Larval helminths in intermediate hosts: Does competition early in life determine the fitness of adult parasites? *International Journal for Parasitology* **35**: 1061–1070.

Galaktionov, K. V. 1996. Life cycles and distribution of seabird helminths in arctic and sub-arctic regions. *Bulletin of the Scandinavian Society of Parasitology* **6**: 31–49.

Gao, L. Q., and Hethcote, H. W. 1992. Disease transmission models with density-dependent demographics. *Journal of Mathematical Biology* **30**: 717–731.

Gaston, K. J., T. M. Blackburn, J. J. Greenwood, R. D. Gregory, R. M. Quinn, and J. H. Lawton. 2000. Abundance–occupancy relationships. *Journal of Applied Ecology* **37**: 39–59.

Gause, G. F. 1932. Experimental studies on the struggle for existence I. *Mixed population of two species of yeast*. *Journal of Experimental Biology* **9**: 389–402.

Getz, W. M., and J. Pickering, J. 1983. Epidemic models: Thresholds and population regulation. *American Naturalist* **121**: 892–898.

Goater, T. M., G. W. Esch, and A. O. Bush. 1987. Helminth parasites of sympatric salamanders: Ecological concepts at infracommunity, component and compound community levels. *The American Midland Naturalist* **118**: 289–300.

Greenman, J. V., and P. J. Hudson. 1997. Infected coexistence instability with and without density-dependent regulation. *Journal of Theoretical Biology* **185**: 345–356.

\_\_\_\_\_, and \_\_\_\_\_. 1999. Host exclusion and coexistence in apparent and direct competition: An application of bifurcation theory. *Theoretical Population Biology* **56**: 48–64.

\_\_\_\_\_, and \_\_\_\_\_. 2000. Parasite-mediated and direct competition in a two-host shared macroparasite system. *Theoretical Population Biology* **57**: 13–34.

Hager, A. 1941. Effects of dietary modifications on the tapeworm *Hymenolepis diminuta*. *Iowa State Journal of Research* **16**: 60.

Hahn, B. H., G. M. Shaw, K. M. De, and P. M. Sharp. 2000. AIDS as a zoonosis: Scientific and public health implications. *Science* **287**: 607–614.

Hall, S. J., and D. G. Raffaelli. 1993. Food webs: Theory and reality. *Advances in Ecological Research* **24**: 187–239.

Harvell, C., K. Kim, J. Burkholder, R. Colwell, P. Epstein, D. Grimes, E. Hofmann, E. Lipp, A. Osterhaus, R. Overstreet, et al. 1999. Emerging marine diseases—Climate links and anthropogenic factors. *Science* **285**: 1505–1510.

Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: Bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society of London: Series B* **272**: 1059–1066.

\_\_\_\_\_, \_\_\_\_\_, and A. M. Kuris. 2008. Trematodes indicate animal biodiversity in the Chilean intertidal and Lake Tanganyika. *Journal of Parasitology* **94**: 966–968.

\_\_\_\_\_, \_\_\_\_\_, A. P. Dobson, J. H. Brown, and A. M. Kuris. 2011a. A common scaling rule for abundance, energetics, and production of parasitic and free-living species. *Science* **333**: 445–448.

\_\_\_\_\_, A. C. Wood, and A. M. Kuris. 2011b. Social organization in a flatworm: Trematode parasites form soldier and reproductive castes. *Proceedings of the Royal Society of London: Series B* **278**: 656–665.

Heins, D. C., J. A. Baker, M. A. Toups, and E. L. Birden. 2010. Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode *Schistocephalus solidus*. *Biological Journal of the Linnean Society* **100**: 835–846.

Hernandez, A. D. and Sukhdeo, M. V. K. 2008. Parasites alter the topology of a stream food web across seasons. *Oecologia* **156**: 613–624.

Hochberg, M., and R. Holt. 1990. The coexistence of competing parasites. I. The role of cross-species infection. *American Naturalist* **136**: 517–541.

Hoff, C. C. 1941. A case of correlation between infection of snail hosts with *Cryptocotyle lingua* and the habits of gulls. *Journal of Parasitology* **27**: 539–539.

Holmes, J. C. 1961. Effects of concurrent infections on *Hymenolepis diminuta* Cestoda and *Moniliformis dubius* Acanthocephala. I. General effects and comparison with crowding. *Journal of Parasitology* **47**: 209.

\_\_\_\_\_. 1962a. Effects of concurrent infections on *Hymenolepis diminuta* Cestoda and *Moniliformis dubius* Acanthocephala. III. Effects in hamsters. *Journal of Parasitology* **48**: 97–100.

\_\_\_\_\_. 1962b. Effects of concurrent infections on *Hymenolepis diminuta* Cestoda and *Moniliformis dubius* Acanthocephala. II. Effects on growth. *Journal of Parasitology* **48**: 87–96.

\_\_\_\_\_. 1973. Site selection by parasitic helminths: Interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology* **51**: 333–347.

\_\_\_\_\_. 1990. Helminth communities in marine fishes. In *Parasite communities: Patterns and processes*, G. W. Esch, A. O. Bush, and J. M. Aho (eds.). Springer, The Netherlands, p. 101–130.

\_\_\_\_\_, and P.W. Price. 1986. Communities of parasites. In *Community ecology: Pattern and process*, J. Kikkawa, and D. J. Anderson (eds.). Blackwell Publishing, Oxford, U.K., p. 187–213.

- Holt, R. D., and J. Pickering. 1985. Infectious disease and species coexistence: A model of Lotka-Volterra form. *American Naturalist* **126**: 196–211.
- \_\_\_\_\_, A. P. Dobson, M. Begon, R. Bowers, and E. Schauber. 2003. Parasite establishment in host communities. *Ecology Letters* **6**: 837–842.
- Hudson, P. J., A. P. Dobson, and K. D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends In Ecology & Evolution* **21**: 381–385.
- Hunnenin, A. V. 1935. Studies on the life history and host-parasite relations of *Hymenolepis fraterna* (*H. nana*, var. *fraterna*, Stiles) in white mice. *American Journal of Epidemiology* **22**: 414–443.
- Hutchinson, G. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? *American Naturalist* **93**: 145–159.
- Huxham, M., D. Raffaelli, and A. Pike. 1995. Parasites and food web patterns. *Journal of Animal Ecology* **64**: 168–176.
- Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. *Science* **317**: 58–62.
- Janovy, Jr, J. 2002. Concurrent infections and the community ecology of helminth parasites. *Journal of Parasitology* **88**: 440–445.
- Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. *Ecology Letters* **9**: 485–498.
- Kennedy, C. R. 1990. Helminth communities in freshwater fish: Structured communities or stochastic assemblages? In *Parasite communities: Patterns and processes*, G. W. Esch, A. O. Bush, and J. M. Aho (eds.). Springer, The Netherlands, p. 131–156.
- \_\_\_\_\_, and A. O. Bush. 1994. The relationship between pattern and scale in parasite communities: A stranger in a strange land. *Parasitology* **109**: 187–196.
- \_\_\_\_\_, and D. J. Fitch. 1990. Colonization, larval survival and epidemiology of the nematode *Anguillicola crassus*, parasitic in the eel, *Anguilla anguilla*, in Britain. *Journal of Fish Biology* **36**: 117–131.
- Kuhn, T. S. 1962. *The structure of scientific revolutions*. University of Chicago Press, Chicago, I.L., 172 p.
- Kuris, A. M., and K. D. Lafferty. 2000. Parasite-host modeling meets reality: Adaptive peaks and their ecological attributes. In *Evolutionary biology of host-parasite relationships: Theory meets reality*, R. Poulin, S. Morand, A. Skorping (eds.). Elsevier Science, New York, N.Y., p. 9–26.
- \_\_\_\_\_, R. F. Hechinger, J. C. Shaw, K. L. Whitney, L. Aguirre-Macedo, C. A.

Boch, A. P. Dobson, E. J. Dunham, B. L. Fredensborg, T. C. Huspeni, et al. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* **454**: 515–518.

Lafferty, K. and Morris, A. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* **77**: 1390–1397.

\_\_\_\_\_. 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today* **13**: 251–255.

\_\_\_\_\_. 1999. The evolution of trophic transmission. *Parasitology Today* **15**: 111–115.

\_\_\_\_\_, and A. M. Kuris. 2002. Trophic strategies, animal diversity and body size. *Trends in Ecology & Evolution* **17**: 507–513.

\_\_\_\_\_, S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. J. Johnson, A. M. Kuris, D. J. Marcogliese, et al. 2008. Parasites in food webs: The ultimate missing links. *Ecology Letters* **11**: 533–546.

\_\_\_\_\_, A. P. Dobson, and A. M. Kuris. 2006a. Parasites dominate food web links. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 11211–11216.

\_\_\_\_\_, R. F. Hechinger, and J. C. Shaw. 2006b. Food webs and parasites in a salt marsh ecosystem. In *Disease ecology: Community structure and pathogen dynamics*, S. K. Collinge, and C. Ray (eds.). Oxford University Press, Oxford, U.K., p. 119–134.

Lawlor, L. 1980. Structure and stability in natural and randomly constructed competitive communities. *American Naturalist* **116**: 394–408.

Lawton, J. 1999. Are there general laws in ecology? *Oikos* **84**: 177–192.

Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. Grime, A. Hector, D. Hooper, M. Huston, D. Raffaelli, B. Schmid, et al. 2001. Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* **294**: 804–808.

Lotka, A. J. 1925. *Elements of physical biology*. Plenum Press, New York, N.Y., 492 p.

Lotz, J. M., and W. F. Font. 1994. Excess positive associations in communities of intestinal helminths of bats: A refined null hypothesis and a test of the facilitation hypothesis. *Journal of Parasitology* **80**: 398–413.

Luque, J., D. Mouillot, and R. Poulin. 2004. Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. *Parasitology* **128**: 671–682.

- MacArthur, R. 1955. Fluctuations of animal populations and a measure of community stability. *Ecology* **36**: 533–536.
- Marcogliese, D. J., and D. K. Cone, 1997. Food webs: A plea for parasites. *Trends In Ecology & Evolution* **12**: 320–325.
- \_\_\_\_\_. 2003. Food webs and biodiversity: Are parasites the missing link? *Journal of Parasitology* **89**: S106–113.
- \_\_\_\_\_. 2001. Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology* **79**: 1331–1352.
- \_\_\_\_\_. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology* **35**: 705–716.
- \_\_\_\_\_, and D. K. Cone. 1996. On the distribution and abundance of eel parasites in Nova Scotia: Influence of pH. *Journal of Parasitology* **82**: 389–399.
- \_\_\_\_\_, and M. Pietrock. 2011. Combined effects of parasites and contaminants on animal health: Parasites do matter. *Trends in Parasitology* **27**: 123–130.
- May, R. M. 1972. Will a large complex system be stable? *Nature* **238**: 413–414.
- \_\_\_\_\_. 1973. *Stability and complexity in model ecosystems*. Princeton University Press, Princeton, N.J., 304 p.
- \_\_\_\_\_, and R. M. Anderson. 1979. Population biology of infectious diseases: Part II. *Nature* **280**: 455–461.
- Memmott, J., N. Martinez, and J. Cohen. 2000. Predators, parasitoids and pathogens: Species richness, trophic generality and body sizes in a natural food web. *Journal of Animal Ecology* **69**: 1–15.
- Montoya, J. M., and R. V. Solé. 2002. Small world patterns in food webs. *Journal of Theoretical Biology* **214**: 405–412.
- Mouritsen, K. N., R. Poulin, J. P. McLaughlin, and D. W. Thieltges. 2011. Food web including metazoan parasites for an intertidal ecosystem in New Zealand: Ecological Archives E092–173. *Ecology* **92**: 2006–2006.
- Neuhäuser, M., and R. Poulin. 2004. Comparing parasite numbers between samples of hosts. *Journal of Parasitology* **90**: 689–691.
- Neutel, A.-M., J. A. P. Heesterbeek, and P. C. De Ruiter. 2002. Stability in real food webs: Weak links in long loops. *Science* **296**: 1120–1123.
- \_\_\_\_\_, J. A. P. Heesterbeek, J. van de Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, and P. C. De Ruiter. 2007. Reconciling complexity with stability in naturally assembling food webs. *Nature* **449**: 599–602.

- Nolf, L. O., and W. W. Cort. 1933. On immunity reactions of snails to the penetration of the cercariae of the strigeid trematode, *Cotylurus flabelliformis* Faust. *Journal of Parasitology* **20**: 38–48.
- Norman, R., R. G. Bowers, M. Begon, and P. J. Hudson. 1999. Persistence of tick-borne virus in the presence of multiple host species: Tick reservoirs and parasite mediated competition. *Journal of Theoretical Biology* **200**: 111–118.
- Ostfeld, R., and F. Keesing. 2000. Biodiversity and disease risk: The case of lyme disease. *Conservation Biology* **14**: 722–728.
- Paine, R. 1988. Road maps of interactions or grist for theoretical development? *Ecology* **69**: 1648–1654.
- \_\_\_\_\_. 1966. Food web complexity and species diversity. *American Naturalist* **100**: 65–75.
- Pascual, M., and J. A. Dunne. 2005. *Ecological networks: Linking structure to dynamics in food webs*. Oxford University Press, Oxford, U.K., 416 p.
- Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution* **22**: 133–139.
- Pimm, S., and J. Lawton. 1980. Are food webs divided into compartments? *Journal of Animal Ecology* **49**: 879–898.
- Polis, G. 1991. Complex trophic interactions in deserts: An empirical critique of food-web theory. *American Naturalist* **138**: 123–155.
- Poulin, R. 2003. Information about transmission opportunities triggers a life-history switch in a parasite. *Evolution* **57**: 2899–2903.
- \_\_\_\_\_. 2007. Are there general laws in parasite ecology? *Parasitology* **134**: 763–776.
- \_\_\_\_\_. 1997. Species richness of parasite assemblages: Evolution and patterns. *Annual Review of Ecology and Systematics* **28**: 341–358.
- \_\_\_\_\_, and S. Morand. 2000. The diversity of parasites. *Quarterly Review of Biology* **75**: 277–293.
- Preston, D. L., A. Z. Jacobs, S. A. Orlofske, and P. T. J. Johnson. 2013. Complex life cycles in a pond food web: Effects of life stage structure and parasites on network properties, trophic positions and the fit of a probabilistic niche model. *Oecologia* **174**: 953–968.
- \_\_\_\_\_, S. A. Orlofske, J. P. McLaughlin, and P. T. J. Johnson. 2012. Food web including infectious agents for a California freshwater pond: Ecological Archives E093–153. *Ecology* **93**: 1760–1760.

Price, P. W. 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton, N.J., 256 p.

\_\_\_\_\_. 1987. Evolution in parasite communities. *International Journal for Parasitology* **17**: 209–214.

Read, C. P. 1951. The “crowding effect” in tapeworm infections. *Journal of Parasitology* **37**: 174–178.

\_\_\_\_\_. 1959. The role of carbohydrates in the biology of cestodes. VIII. Some conclusions and hypotheses. *Experimental Parasitology* **8**: 365–382.

\_\_\_\_\_, and A. Z. Kilejian. 1969. Circadian migratory behavior of a cestode symbiont in the rat host. *Journal of Parasitology* **55**: 574–578.

\_\_\_\_\_, and K. Phifer. 1959. The role of carbohydrates in the biology of cestodes VII. Interactions between individual tapeworms of the same and different species. *Experimental Parasitology* **8**: 46–50.

Reiczigel, J., Z. Lang, L. Rózsa, and B. Tóthmérész. 2005. Properties of crowding indices and statistical tools to analyze parasite crowding data. *Journal of Parasitology* **91**: 245–252.

Rhodes, C. J., R. Atkinson, R. M. Anderson, and D. W. Macdonald. 1998. Rabies in Zimbabwe: Reservoir dogs and the implications for disease control. *Philosophical transactions of the Royal Society of London Series B* **353**: 999–1010.

Ricklefs, R. E. and Schlüter, D. 1993. *Species diversity in ecological communities: historical and geographical perspectives*. University of Chicago Press, Chicago, I.L., 414 p.

Rohde, K. 1993a. Niche restriction in parasites: Proximate and ultimate causes. *Parasitology* **109**: S69–84.

\_\_\_\_\_. 1991. Intra-and interspecific interactions in low density populations in resource-rich habitats. *Oikos* **60**: 91–104.

\_\_\_\_\_. 1993b. *Ecology of marine parasites: An introduction to marine parasitology*. CAB International, Wallingford, U.K., 298 p.

Rossiter, W. D. 2012. *Impacts of space, abundance and food web structure on parasite life cycles*. Ph.D. Thesis, Rutgers University, New Brunswick, New Jersey, 190 p.

Rudolf, V. H., and J. Antonovics. 2005. Species coexistence and pathogens with frequency-dependent transmission. *American Naturalist* **166**: 112–118.

Sanmartín, M. L., M. F. Alvarez, D. Peris, R. Iglesias, and J. Leiro. 2000. Parasite community study of the undulate ray *Raja undulata* in the Ría of Muros Galicia,

- northwest Spain. *Aquaculture* **184**: 189–201.
- Schad, G. A. 1963. Niche diversification in a parasitic species flock. *Nature* **198**: 404–406.
- Schmidt, K., and R. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. *Ecology* **82**: 609–619.
- Shaw, D. J., and A. P. Dobson. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: A quantitative review. *Parasitology* **111**: S111–S133.
- Shorb, D. A. 1933. Host-parasite relations of *Hymenolepis fraterna* in the rat and the mouse. *American Journal of Epidemiology* **18**: 74–113.
- Smith, N. 2001. Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia* **127**: 115–122.
- Sousa, W. P. 1992. Interspecific interactions among larval trematode parasites of freshwater and marine snails. *Integrative and Comparative Biology* **32**: 583–592.
- Sousa, W. P. 1993. Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. *Ecological Monographs* **63**: 104–128.
- Sousa, W. P. 1994. Patterns and processes in communities of helminth parasites. *Trends in Ecology & Evolution* **9**: 52–57.
- Stoll, N. R. 1936. Observations on cattle nematode infections, with a demonstration of their secondary transmission to grazing sheep. *Journal of Parasitology* **22**: 386–407.
- Sukhdeo, M. V. K. 1992. The behavior of parasitic flatworms *in vivo*: What is the role of the brain? *Journal of Parasitology* **78**: 231–242.
- \_\_\_\_\_. 1990. Habitat selection by helminths: A hypothesis. *Parasitology Today* **6**: 234–237.
- \_\_\_\_\_. 1991. The relationship between intestinal location and fecundity in adult *Trichinella spiralis*. *International Journal for Parasitology* **21**: 855–858.
- \_\_\_\_\_. 1997. Earth's third environment: The worm's eye view. *Bioscience* **47**: 141–149.
- \_\_\_\_\_. 2000. Inside the vertebrate host: Ecological strategies by parasites living in the third environment. In *Evolutionary biology of host–parasite relationships: Theory meets reality*, R. Poulin, S. Morand, and A. Skorping (eds.). Elsevier Science, New York, p. 43–62.
- \_\_\_\_\_, and Mettrick, D. F. 1987. Parasite behaviour: Understanding

platyhelminth responses. *Advances in Parasitology* **26**: 73–144.

\_\_\_\_\_, and Hernandez, A. D. 2005. Food web patterns and the parasite's perspective. In *Parasitism and ecosystems*, F. Thomas, F. Renaud, and J.-F. Guegan (eds.). Oxford University Press, Oxford, U.K., p. 54–67.

\_\_\_\_\_, and Sukhdeo, S. C. 2004. Trematode behaviours and the perceptual worlds of parasites. *Canadian Journal of Zoology* **82**: 292–315.

\_\_\_\_\_. 2010. Food webs for parasitologists: A review. *Journal of Parasitology* **96**: 273–284.

\_\_\_\_\_. 2012. Where are the parasites in food webs? *Parasites and Vectors* **5**: 239.

Sukhdeo, S. C., M. V. K. Sukhdeo, and D. F. Mettrick. 1988. Histochemical localization of acetylcholinesterase in the cerebral ganglia of *Fasciola hepatica*, a parasitic flatworm. *Journal of Parasitology* **74**: 1023–1032.

\_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1988b. Neurocytology of the cerebral ganglion of *Fasciola hepatica* (Platyhelminthes). *Journal of Comparative Neurology* **278**: 337–343.

Thielges, D. W., K. Reise, K. N. Mouritsen, J. P. McLaughlin, and R. Poulin. 2011. Food web including metazoan parasites for a tidal basin in Germany and Denmark: Ecological Archives E092–E172. *Ecology* **92**: 2005–2005.

Thomas, F., F. Renaud, T. de Meeūs, and R. Poulin. 1998. Manipulation of host behaviour by parasites: Ecosystem engineering in the intertidal zone? *Proceedings of the Royal Society of London: Series B* **265**: 1091–1096.

Thompson, R. M., R. Poulin, K. N. Mouritsen, and D. W. Thielges. 2013. Resource tracking in marine parasites: Going with the flow? *Oikos* **122**: 1187–1194.

\_\_\_\_\_, K. Mouritsen, and R. Poulin. 2005. Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *Journal of Animal Ecology* **74**: 77–85.

Thrall, P. H., J. Antonovics, and D. W. Hall. 1993. Host and pathogen coexistence in sexually transmitted and vector-borne diseases characterized by frequency-dependent disease transmission. *American Naturalist* **142**: 543–552.

Valtonen, E. T., K. Pulkkinen, R. Poulin, and M. Julkunen. 2001. The structure of parasite component communities in brackish water fishes of the northeastern Baltic Sea. *Parasitology* **122**: 471–481.

Volterra, V. 1926. Fluctuations in the abundance of a species considered mathematically. *Nature* **118**: 558–560.

Ward, H. B. 1912. The distribution and frequency of animal parasites and parasitic diseases in North American fresh-water fish. *Transactions of the American Fisheries Society* **41**: 207–244.

Warren, P. H. 1989. Spatial and temporal variation in the structure of a freshwater food web. *Oikos* **55**: 299–311.

Weinersmith, K. L., C. B. Warinner, V. Tan, D. J. Harris, A. B. Mora, A. M. Kuris, K. D. Lafferty, and R. F. Hechinger. 2014. A lack of crowding? Body size does not decrease with density for two behavior-manipulating parasites. *Integrative and Comparative Biology* **54**: 184–192.

Williams, R. J., and N. D. Martinez. 2004. Limits to trophic levels and omnivory in complex food webs: Theory and data. *American Naturalist* **163**: 458–468.

Wilson, C. B. 1916. Copepod parasites of fresh-water fishes and their economic relations to mussel glochidia. *Bulletin of the Bureau of Fisheries* **824**: 331–374.

Woodland, W. N. F. 1924. On a new Bothriocephalus and a new Genus of Proteocephalidae from Indian fresh-water fishes. *Parasitology* **16**: 441–451.

Woolhouse, M., L. Taylor, and D. Haydon. 2001. Population biology of multihost pathogens. *Science* **292**: 1109–1112.

Zervos, S. 1988a. Evidence for population self-regulation, reproductive competition and arrhenotoky in a thelastomatid nematode of cockroaches. *Parasitology* **96**: 369–379.

Zervos, S. 1988b. Population dynamics of a thelastomatid nematode of cockroaches. *Parasitology* **96**: 353–368.



# **Chapter 9**

## **The Life History of *Gongylonema Scutatum***

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In April, 1911, the writers undertook some investigations in regard to insects as intermediate hosts of parasites. The investigations of the senior author were carried on at the Experiment Station of the Bureau of Animal Industry at Bethesda, Md., and in the laboratories of the Bureau at Washington, and later at Colorado Springs, Colo. Those of the junior author were carried on at Colorado Springs, Colo. Particular attention was paid to the dung beetles, as it seemed evident that these insects in working through fresh feces as is their habit, would have the first opportunity to ingest eggs of worms parasitic in the digestive tract of cattle, sheep, and other live stock. Such beetles as species of *Aphodius*, furthermore, appeared small enough to be readily ingested by grazing animals, and the beetles' habit of flight from one manure deposit to another offered chances for such ingestion, as the beetles' flight commonly terminates on grass and herbage with which they might readily be swallowed by cattle or sheep.

The dissections by the senior author of *Aphodius femoralis*, *A. granarius*, *A. fimetarius* and *Onthophagus hecate* resulted in the finding of encysted larval nematodes in the body cavity. These cysts were about 0.5 mm. in diameter and as many as 8 were found in one *Aphodius* and 15 in one *Onthophagus hecate*. One larva which was measured was 2 mm. in length and 50 $\mu$  in thickness. Viewed from in front the head shows a narrow mouth aperture elongated dorso-ventrally and surrounded by a chitinous border, which is oblong quadrangular in outline with rounded corners, and measures about 12 $\mu$  dorso-ventrally and about 8 $\mu$  from side to side. A short distance posterior of the edge of the chitinous border are 2 sub-dorsal and 2 sub-ventral papillae. The chitinous border of the mouth is raised above the surrounding surface of the head and resembles a projecting flange when the head is viewed from the side. The slender pharynx is 40 $\mu$  long. The esophagus measures about 1.5 mm. in length, and is differentiated into a slender anterior portion about 225 $\mu$  long, and a more granular posterior portion of somewhat larger diameter. The anus is about 100 $\mu$  from the tip of the tail. The latter is blunt and is supplied with two or three very small short conical processes. The excretory

pore is about  $200\mu$  from the anterior end of the body. About  $140\mu$  from the anterior end of the body the esophagus is surrounded by a nerve ring.

In June a number of larvae of *Aphodius* spp. were examined, and in these were discovered some young nematodes which agreed perfectly with the unhatched embryos of *Gongylonema scutatum*. The embryo of *Gongylonema scutatum* is very distinctly annulated in the head region on the side opposite the mouth. The mouth is not terminal, but is a triangular aperture on one side of the head, with a curved hook-like process projecting from it. The tip of the tail is bluntly pointed.

The finding of these newly hatched larvae in the same host beetles as the encysted larvae was fairly good evidence that they were of the same species and, in view of the failure to find them in adult beetles, seemed to indicate that the eggs of the worm were principally ingested by the beetle while it was in the larval stage.

In July both of the writers were in Colorado and the examination of *Aphodius coloradensis*, *A. vittatus*, and *A. granarius* showed the presence of an encysted larval nematode exactly similar to that found at Bethesda. These beetles were collected from sheep manure at points 17 miles east and 75 miles northeast of Colorado Springs.

The observations in regard to the finding of *Gongylonema* larvae in larval and adult dung beetles were briefly alluded to in the Twenty-eighth Annual Report of the Bureau of Animal Industry for the year 1911, as follows:

"Important facts have been determined bearing upon the life history of the gullet worm of sheep and cattle."

In 1912 the encysted larval forms of *Gongylonema* were again observed in the dissection of insects in connection with other investigations in Colorado, but no further work was carried on that year.

In 1911 an experimental feeding was made by the senior author. Four or five larval *Gongylonema* were fed to a white mouse on May 13 and 14 more were fed to this mouse on May 17. On June 14, 32 and 28 days, respectively, after these feedings, the mouse was killed and examined. No worms were found. This result tended to show that the parasite is not transmissible to mice and that the larvae were not those of *Spiroptera obtusa* whose larvae, occurring in the meal worm, show very striking similarities to those which the present writers assumed belonged to *Gongylonema*.

In 1913 an experimental feeding was made by the junior author at Colorado Springs. During the two months from May 10 to July 9 inclusive, a sheep was fed a total of over 250 specimens of *Alphodius coloradensis*, *A. congregatus*, *A. fimetarius*, *A. granarius*, *A. inquinatus*, and *A. vittatus*. A dissection of a large proportion of these beetles showed a very small number of them to contain encysted *Gongylonema* larvae.

Three of the larvae obtained on dissection were also fed to this sheep. This sheep was killed November 18 and was found to have 7 *Gongylonema scutatum* in the esophagus, together with the characteristic lesions showing where a few others had been at one time. A companion sheep kept under identical conditions for a year and a half, but not fed any specimens of *Aphodius* did not develop any infection with *Gongylonema*. Thirty-five lambs raised under experiment conditions very much the same as those of the two sheep mentioned above were killed during the course of the three years 1911 to 1913 inclusive, and these were all free from *Gongylonema*. This furnishes an abundance of checks in support of the experimental finding that species of *Aphodius* act as intermediate hosts of *Gongylonema scutatum*.

During the summer of 1914 some additional work was done investigation of this life history. Cattle weasands heavily infested with *Gongylonema scutatum* were sent in from Indianapolis, Ind., by Dr. G. W. Butler, the egg-bearing worms cut into small fragments, mixed with small quantities of bread or other food and fed to specimens of *Aphodius* and to croton bugs, *Ectobia germanica*. The day after the insects were exposed to infection in this way, empty shells of *Gongylonema* eggs were found in the intestine, and the following day numbers of free embryos were found. Other eggs were found unhatched in the intestine and in the feces of the insects, but these were obviously eggs which had not yet developed to the infective stage. These findings demonstrated that the eggs would hatch when ingested by the adult as well as by the larval *Aphodius*.

The young larvae found two days after exposure to infection were about  $250\mu$  long and were apparently increasing in size. In the course of a week the larvae found were very much thicker. At the end of two weeks the larvae were about three times as thick as the original embryos and were apparently on the verge of an ecdysis. At this time they show a complete alimentary tract. The esophagus is about three-eighths to four-ninths of the entire body length, and is surrounded by a nerve ring a short distance in front of its middle. The rectum is a well-marked structure of rather large diameter, and posteriorly is closed by a plug of tissue which projects from the ventral surface of the body. This plug marks the location of the anus and is one-sixth of the body length from the posterior end. About this time there is an ecdysis and the cephalic annulation is lost.

At the end of three weeks the head is more pointed but the flange-like margin of the lips is not yet developed. The rectum is no longer prominent, but the button marking the position of the anus persists. The larvae are now much longer.

At the end of about a month the larvae are encysted in the final stage. The head has the structure described in the first part of the paper and the anal button is lost. In favorable specimens cervical papillae may be seen about half way between the nerve ring and the anterior end of the body.

An experimental feeding to croton bugs of eggs of *Gongylonema* from the gullet of a hog, gave substantially the same results. The larvae were encysted in the final stage at the end of a month.

A rabbit was fed with three *Gongylonema* larvae on one occasion and with two on another. Two months after the first feeding and one month after the second, the rabbit was killed and the mouth, pharynx, and stomach were examined. No worms were found. A guinea-pig was fed with three *Gongylonema* larvae on one occasion and three more on another. Five weeks after the first feeding and three weeks and two days after the second feeding it died. No worms were found.

August 18 a sheep was fed eleven *Gongylonema* larvae from a croton bug and a hog was fed a croton bug containing possibly fifty larval *Gongylonema*, the larvae having been developed by feeding eggs of *Gongylonema* collected from cattle. On August 25 the same sheep and hog were fed more croton bug material heavily infested with similar larvae. The hog was killed October 17, but showed no infection. The failure to infect the hog with the nematode from sheep and cattle is suggestive of a specific infectivity and strengthens the idea that the hog nematode is a distinct species. The sheep was killed November 23 and the gullet found heavily infested with *Gongylonema*, the females of which were mature and full of eggs.

While the work noted above was in progress, a very interesting paper appeared, dealing with the life history of another species of *Gongylonema*. Fibiger (1913) published a note in which he stated that he had found in rats a gastric carcinoma etiologically related to a species of *Spiroptera*. A year later, Fibiger and Ditlevsen (1914) published their complete study of the worm itself and the lesions attributed to it. The worm in question, called by them *Spiroptera (Gongylonema) neoplastica*, should be called *Gongylonema neoplasticum*. *Gongylonema* is a well established genus and there is no reason to question the propriety of including this species in *Gongylonema*, notwithstanding its lack of one characteristic of this genus, namely, the presence of cervical papillae. It is even not impossible that cervical papillae may be present, as these structures are frequently very difficult to distinguish in some species of nematodes and may be overlooked in numerous specimens, finally being discovered when a specimen happens to be turned into just the right position.

Fibiger and Ditlevsen have made an excellent study of the life history of this worm. It was found in the first instance in rats, but it appears to be communicable to rodents generally as it was transmitted to the following: *Mus decumanus*, *Mus rattus*, *Mus musculus*, *Lepus cuniculus*, *Cavia cobaya*. The parasite occurred in the squamous-celled epithelium of the anterior portion of the digestive tract, including the mouth, tongue, esophagus and fundus of the stomach. In these regions the worm gave rise to a proliferation of the epithelial elements, originating as a circumscribed or diffuse hypertrophy associated with a slight inflammation, going on to the formation of papilloma, and terminating in distinct carcinoma with occasional metastases.

The eggs produced by the female worm are passed in the feces of the infested rodent and were first found to be ingested by *Periplaneta americana*, but were also found infective for *Periplaneta orientalis*, *Ectobia germanica* and *Tenebrio molitor*. Twenty days after the ingestion of the eggs by the insects, the fully developed larvae are found coiled in the muscles of the prothorax and limbs. It will be noted that this site is different from that of *Gongylonema scutatum* larvae. The location of the embryonic and larval forms after the first day following the ingestion of the eggs and up to the time they are found in the musculature of the prothorax and limbs was not determined.

It is evident from the above that the life history of the two species, *Gongylonema scutatum* and *Gongylonema neoplasticum* is much the same in that the larval stage is spent in insects, at least one of which, *Ectobia germanica*, is common to both, and that the adult worm is found in the epithelium of the gullet in the primary host in both cases. The worms differ in that the larval stage of the rodent nematode is found in the musculature of the insect host, while the larval stage of the ruminant nematode is found encysted in the body cavity. They also differ in that the rodent nematode commonly occurs in the tongue, mouth and cardiac portion of the stomach as well as in the esophagus. Finally, the rodent nematode has the unusual power of producing neoplastic changes in its primary host, while there is yet no evidence that the ruminant nematode is more than a rather innocuous parasite.

The life history of *Gongylonema scutatum* and *G. neoplasticum* is strikingly similar to that of *Spiroptera obtusa*, which occurs in its adult stage in the intestine of rats, mice and similar rodents.

Leuckart (1867: 113-115) and Marchi (1871) found that the larval development of this parasite occurs in the meal worm (larva of *Tenebrio molitor*). The eggs, which resemble those of *Gongylonema* and contain similar embryos, when swallowed by meal worms hatch out and release the embryos. These embryos pass through the wall of the

alimentary tract, and develop in the midst of the fat surrounding it, becoming enclosed in connective tissue cysts. The larval development is complete in about six weeks after ingestion of the eggs. The fully developed larva measures from two-thirds of a millimeter to nearly a millimeter in length. The head, as described, is supplied with two triangular papillae curved on their inner surfaces, and surrounding the mouth except laterally. The tip of the tail is supplied with several small conical papillae. The excretory pore is about  $100\mu$  and the base of the esophagus about  $300\mu$  from the anterior end of the body.

Judging from Marchi's description and figures one of the most striking differences between the full-grown larvae of *Spiroptera obtusa* and *Gongylonema* is that the esophagus of the former is only about one-third the length of the body, whereas the esophagus of the latter is fully two-thirds the body length.

As a postscript it may be noted that since this paper was read at a meeting of the Helminthological Society of Washington, Dec. 17, 1914, an additional intermediate host of *G. scutatum* has been found, namely, *Onthophagus pennsylvanicus*. Beetles of this species collected from sheep pastures near Vienna, Va., during the summer of 1915 were found to be commonly infested with the encysted larvae.

#### SUMMARY

The eggs of *Gongylonema scutatum* present in the feces of sheep and cattle infested with the adult parasite, hatch out when swallowed by insects of various species.

The larvae thus released from the eggs, pass into the body cavity and reach the final larval stage in about a month. In this stage the larva is coiled into a spiral and is enclosed in a capsule about half a millimeter in diameter. The length of the fully developed larva is about 2 mm. and the esophagus equals about two-thirds the body length. The mouth, elongated dorso-ventrally, is surrounded by a flange-like chitinous border.

Sheep fed upon insects containing these larvae became infested with *Gongylonema*. A hog fed upon croton bugs artificially infested by feeding with eggs of *Gongylonema* from cattle failed to become infested. A mouse, rabbit and guinea-pig fed with *Gongylonema* larvae from beetles found in sheep manure, or from croton bugs artificially infested by feeding *Gongylonema* eggs from cattle, also failed to become infested. Failure to produce infestation in these various animals indicates that the *Gongylonema* of sheep and cattle (*G. scutatum*) is not transmissible to hogs, mice, rabbits or guinea-pigs.

*Gongylonema* larvae have been found in various species of dung beetles collected from sheep manure, namely, *Aphodius femoralis*, *A. granarius*, *A. fimentarius*, *A. coloradensis*, *A. vittatus*, *Onthophagus*

*hecate*, and *O. pennsylvanicus*. They have been developed in various species of *Aphodius* and in croton bugs (*Ectobia germanica*) by feeding the eggs of *Gongylonema scutatum* from cattle. The feeding of eggs of *Gongylonema* from the gullet of a hog (presumably *G. pulchrum*) to croton bugs also resulted in the development to encysted larvae.

Under natural conditions the usual intermediate hosts of *Gongylonema scutatum* are probably dung beetles of various species.

The life history of *G. scutatum* is similar to that of *G. neoplasticum* of rats, mice and other rodents, the intermediate stage of the latter having been found by Fibiger and Ditlevsen to develop in roaches (*Periplaneta americana*, *P. orientalis*, and *Ectobia germanica*) and in a beetle (*Tenebrio molitor*). It is also similar to that of another rat and mouse parasite, *Spiroptera obtusa*, whose intermediate host was found by Leuckart and Marchi to be the larva of a beetle (*Tenebrio molitor*).

#### REFERENCES

- Fibiger, J. 1913. Ueber eine durch Nematoden (*Spiroptera* sp. n.) hervorgerufene papillomatöse und carcinomatöse Geschwulstbildung im Magen der Ratte. Berl. klin. Wchnschr., 50: 289-298, figs. 1-12.
- Fibiger, J., and Ditlevsen, H. 1914. Contributions to the biology and morphology of *Spiroptera (Gongylonema) neoplastica* n. sp. 28 pp., 4 pls. København.
- Leuckart, R. 1867. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten, v. 2, 1. Lief., 256 pp., 158 figs. Leipzig & Heidelberg.
- Marchi, P. 1871. Monografia sulla storia genetica e sulla anatomia della *Spiroptera obtusa* Rud. Mem. r. Accad. sci. Torino, cl. sci. fis. e mat., 2. s., v. 25, 30 pp., 2 pls.
- Ransom, B. H., and Hall, M. C. 1915. The life history of *Gongylonema scutatum*. [Abstract.] J. Parasitol., 1: 154.
- Stiles C. W. 1892. On the anatomy of *Myzomimus scutatus* (Mueller, 1869) Stiles, 1892. Festsch. z. R. Leuckart, pp. 126-133, pl. 17.



# The iron wheel of parasite life cycles: Then and now!

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When a scientific achievement is stripped of its genealogical record and historical background it becomes an impersonal thing, without face, voice or spirit.

—Edward G. Reinhard, (1957)

The year was 1915, and the first paper on a complete life cycle of a parasite titled “The Life History of *Gongylonema scutatum*” (Ransom and Hall, 1915), was published in *The Journal of Parasitology*. This work sets the stage in *JP* for the many other original and fascinating papers on parasite life cycles to follow. Each of these papers describes original ideas and illustrates the many “A-ha” moments that are all wrapped up into fascinating stories. One can argue that our current understanding of parasite community structure, life cycle evolution, and the spread of diseases through populations have been shaped by these historical studies. However, over the years, the study of life cycles has been looked upon by many as being too risky of an undertaking for graduate research problems. The difficulty in studying parasite life cycles can be summarized in a statement made by Wendell Krull in a letter to Miriam Rothschild where he stated, “Experiences with life cycles are unique in one way: I do not think the average biologist or even some parasitologists have any idea of the amount of confining work that is necessary in completing one” (Ewing, 2001). Because of these challenges, the scientific community as a whole has mostly avoided this area of parasitology. As a result, the common perception of parasite life cycles is that of rigid iron wheels, with little or no room for plasticity. However, over the last decade we, and our collaborators, have attempted to disassemble this common misperception of the iron wheel of parasite life cycles. This endeavor has been an evolving experience supported by many fascinating lessons that are all available for free at most university libraries in the 100 volumes of *The Journal of Parasitology*.

In this centennial overview of research on parasite life cycles, we share some of the lessons that parasite life cycles can teach us about how parasites live out their lives in their world, which is in contrast to how we, as humans, think parasites should live out their lives in our world. This distinction is extremely important and logical but surprisingly difficult to decipher (Janovy, 2014). In our review, we selected a few historical life cycle studies on helminths (nematodes and trematodes) by parasitologists who published some of their work in the *Journal*. We then discuss the history of those parasitologists and their critical insights that made them so successful at solving these life cycle puzzles. Finally, we discuss how recent helminth life cycle studies have added to our understanding of how we perceive parasite life cycles to work.

# **General insights about helminth life cycles and how to solve them**

A curious feature of helminth life cycle investigations, which strikes anyone who has seriously examined the literature, is that few people pursue this line of research for more than a year or two. Additionally, once a life cycle is solved, few ever question its validity (Bolek et al., 2009a; Bolek et al., 2010). One reason for this oddity is that most parasitologists, and scientists in general, assume that the chief element of success in solving these life cycle puzzles is dumb luck. In fact, some eminent parasitologists have compared life cycle investigations to an activity bordering on sorcery or alchemy (Esch, 2007). Yet all helminth life cycles are puzzles with a solution; you start with an egg (zygote) and end up with a worm. The problem is well defined, conventionally delineated, and seems quite simple in retrospect. However, most helminth life cycles have not been solved, and, in fact, some life cycle studies have not stood up to the test of time. So, what makes certain individuals very successful at solving these life cycle puzzles and does luck have anything to do with it?

If anything, a century of parasitology teaches us that there are key elements that emerge from the 100 years of scientific publications on life cycles in *JP*. In general, solving helminth life cycles is time-consuming and full of uncertainty. However, if one would compile a list of key elements or develop a tool kit used by the successful parasitologists to reveal the secret lives of helminths and their life cycle puzzles, it would at least include the following: First, make critical field observations about the hosts, the potential hosts, and their parasites. Second, understand the taxonomy of your hosts, the parasites, and be familiar with the literature. Finally, in the laboratory, have the appropriate controls (time-*t*, positive, and negative), and always figure out a way to keep your, usually uncooperative, experimental animals alive!

## Ransom and Hall and the tool kit for elucidating the life cycle of *Gongylonema scutatum*

Brayton Howard Ransom was born in Missouri Valley, Iowa on 24 March 1879 and was educated in the public school system of Bancroft, Nebraska. He received his B.Sc. degree from the University of Nebraska in 1899 and his M.A. and Ph.D. from the University of Nebraska in 1900 and 1908, respectively (Hall, 1925).

In June of 1903, Ransom was appointed as a Scientific Assistant in charge of the Zoological Division of the Hygiene Laboratory of the Public Health and Marine Hospital Service, now the U.S. Public Health Service at the Bureau of Animal Industry (BAI). In this capacity he helped carry out various scientific investigations in parasitology. In 1906, he was promoted to the position of Principal Zoologist and Chief of the Zoological Division, which he held until his unexpected death on September 17, 1925 at the age of 46 after an illness of 3 weeks. Incredibly, he produced over 160 publications on various aspects of parasitism during his short life (Hall, 1925).

While serving as Chief of the Zoological Division, Ransom made many important scientific contributions to the field of parasitology. These included monographic systematic works on various veterinary helminth parasites, the discovery and the documentation of the distribution of many of the most economically important parasites in the USA at the time, contributions to the understanding of the basis of parasite-induced pathology, and the development of protocols for controlling stomach worms in sheep. As a result of these experiences, Ransom developed a broad knowledge of host-parasite relationships and a clear understanding of the natural history of the many helminths he investigated. This tool kit was essential for his continuous success at solving some of the most complex helminth life cycle puzzles. Throughout all of his work, Ransom was constantly engaged in an attempt to elucidate the complete life cycles of the various nematodes and tapeworms that he investigated. His argument was a simple one, in order to control the transmission of the parasites, the detailed knowledge of their life cycles must be known. Incredibly, during his short life he was involved in solving and/or filling in the gaps for not only the life cycle of *Gongylonema scutatum* (now *Gongylonema pulchrum*), but also the life cycles for *Haemonchus contortus*, *Ascaris lumbricoides*, *Syngamus trachea*, *Habronema muscae*, *Strongyloides ovocinctus*, and *Taenia ovis* (Hall, 1925; Cort, 1926; Mohler, 1926).

Maurice Crowther Hall was born on July 15, 1881 in Golden, Colorado. He obtained his B.A. at Colorado College in 1905, and his M.Sc. degree on gregarines from the University of Nebraska where he studied under Henry Baldwin Ward. He received his Ph.D. and D.V.M. degrees from George Washington University in 1915 and 1916, respectively. He was also well versed in studies on the relationships of helminths, and his doctorate thesis was on the morphology and classification of nematode parasites of rodents. During 1906 and 1907, Hall worked as a high school science instructor at Canon City, Colorado. Like Ransom before him, he

joined the Zoological Division of the BAI with the title of Junior Zoologist in 1907. He later served as Chief of the Zoology Division from 1925 to 1936 following the death of his boss and mentor, Brayton Ransom (Schwartz, 1938; Schwartz and Harwood, 1938; Otto and Foster, 1981).

In April 1936, Hall left the BAI to assume the position as Head of the Zoological Laboratory at the National Institutes of Health, where he remained until his death in 1938. He was renowned by his colleagues as a witty man and a master of verse who also published articles on the philosophical and social aspects of parasitism, government civil service, animal experimentation, and poetry in various venues including *Scientific Monthly* (Schwartz, 1938; Esch, 2004). Like Ransom, Hall was an active worker in many professional societies, and was a founder and Charter member of the Helminthological Society of Washington and the American Society of Parasitologists and served as president of ASP in 1932 (Esch et al., 2014).

During his relatively short career, Hall made numerous discoveries in the field of parasitology. Although he died on 1 May 1938 at the early age of 56, following an operation for a gastric ulcer, he published 577 papers, notes, and reports, as well as three books (Otto and Foster, 1981). Unlike his co-author Ransom, Hall did not continue his work on solving parasite life cycles. Instead, he concentrated on the descriptions and control of tapeworms and nematodes. His major research efforts were on the improvements of anthelmintic medications and the discovery of new and better anthelmintics and insecticides (Schwartz and Harwood, 1938). One of his most significant discoveries was that the chemical compound carbon tetrachloride was incredibly effective as an anthelminthic in eradicating hookworms. His discovery played a vital role in the worldwide destruction of hookworm. Hall's other most important publication on helminth life cycles was a paper on arthropods as intermediate hosts of helminths, published in 1929 by the Smithsonian Institution (Otto and Foster, 1981).

In 1915, Ransom and Hall convincingly showed that various species of small 2–8 mm dung beetles (*Aphodius* spp.) and the German cockroach served as intermediate hosts for the sheep gullet worm, *G. scutatum*. Although in retrospect, their life cycle paper seems like a typical life cycle study, at the time, it was the first of any species of *Gongylonema* elucidated (Anderson, 2000). The question remains: how did Ransom and Hall decode the life cycle without previous examples to reference, and did dumb luck have anything to do with their discovery? A detailed look at this paper will shed some light on how they accomplished this masterful feat and that luck had little, if anything, to do with it. In fact, Ransom and Hall used their critical skills in field observations, taxonomy, natural history, experimental infections, and their comprehensive knowledge of the literature to provide the first evidence of the life history of any *Gongylonema* species.

First, Ransom and Hall (1915) made the argument that they had identified

potential intermediate hosts which overlap in their habitat with the eggs of *G. scutatum* by stating,

Particular attention was paid to the dung beetles, as it seemed evident that these insects in working through fresh feces as is their habits, would have the first opportunity to ingest eggs of worms parasitic in the digestive system of cattle, sheep and other live stock.

Second, they provided a convincing argument on how cattle and sheep, the definitive hosts for *G. scutatum*, can come in contact with an infected intermediate host by stating,

Such beetles as species of *Aphodius*, furthermore, appeared small enough to be readily ingested by grazing animals, and the beetles' habit of flight from one manure to another offered chances for such ingestion, as the beetles' flight commonly terminates on grass and herbage with which they might readily be swallowed by cattle or sheep.

Next, using their skills in taxonomy and morphology they provide critical information relating the morphology of the embryos of *G. scutatum* recovered from the manure of ruminants with the morphology of young larval nematodes recovered from field collected dung beetles by stating,

In June a number of larvae of *Aphodius* spp. were examined, and in these were discovered some young nematodes which agreed perfectly with the unhatched embryos of *Gongylonema scutatum*.

Finally, they provided field observations on the distribution of different life stages of what they assumed was *G. scutatum* and how beetle intermediate hosts became infected with *G. scutatum* in nature by indicating that,

Examinations of adult and larval *Aphodius* spp. indicated that newly hatched nematode larvae were only found in larval beetles whereas encysted nematodes were found in both larval and adult beetles strongly suggesting that the eggs of these nematodes were principally ingested by the beetle while it was in the larval stage.

These basic observations set the stage for their experimental infections in solving the life cycle puzzle of *G. scutatum*.

To resolve the puzzle, and for their first set of experimental infections, Ransom and Hall exposed an uninfected sheep and a laboratory reared white mouse by feeding their experimental animals' encysted larval nematodes recovered from naturally infected beetles and/or entire beetles assumed to be infected with these nematodes. As a negative control, companion sheep and 35 lambs were kept under identical conditions to the exposed sheep for a period of 3 years, but not fed any nematode larvae or beetles. Their results indicated that only the sheep exposed to nematode larvae and beetles became infected with *G. scutatum*. Once again, in this rather simple but elegant experiment, you can see the life cycle tool kit at

work. Although the taxonomy of *Gongylonema* was not well understood at that time, no reports of any species of *Gongylonema* were known from house mice, leading one to ponder why they chose to expose mice? Their explanation for exposing mice was clever and demonstrates their detailed knowledge of the literature and careful attention to details when they stated,

This result tended to show that the parasite is not transmissible to mice and that the larvae were not those of *Mastophorus muris* (= *Spiroptera obtuse*) whose larvae, occurring in the meal worm, show very striking similarities to those which the present writers assumed belong to *Gongylonema*.

With that experiment they were able to exclude the possibility of other species of spirurid nematodes of rodents, which might have been present in their naturally infected beetle hosts.

For their second set of experimental infections, Ransom and Hall concentrated on the arthropod intermediate host part of the puzzle. At the time of their study, little to no information was available on the life histories or culturing techniques for dung beetles, as the only comprehensive work on *Aphodius* species predominantly dealt with European taxa (Schmidt, 1910). To overcome the inability to culture their beetles in the laboratory, they used specimens of *Aphodius* spp. collected in the field and German cockroaches as positive controls that, based on their field-work, were never infected with any spirurid nematodes. They fed both groups of arthropods embyonated eggs of *G. scutatum* mixed with small quantities of bread and other food. Importantly, 24 hours after exposure, they examined the frass of exposed beetles and roaches for hatched nematode eggs, followed by detailed dissections and recovery of young nematode larvae from the intestines of both groups of hosts 48 hours post exposure. Subsequently, and over a period of a month, they documented the growth, development, and encystment of nematode larvae in both beetles and roaches. This ability of using positive controls allowed Ransom and Hall to control for the possibility that their exposed, but field-collected, beetles were already infected. This technique of using positive controls followed by detailed observations on the hatching and development of helminths within their intermediate hosts became the standard part of the tool kit for future life cycle studies.

Finally, to complete the puzzle, they exposed an uninfected sheep with encysted nematode larvae recovered from laboratory-infected roaches. The sheep was killed 3 months after exposure and the gullet was heavily infected with adult *G. scutatum*, of which the females were full of eggs. With this last experiment, they were able to complete the puzzle under controlled laboratory conditions. Since their work, partial life cycles of seven other *Gongylonema* species have been elucidated (Anderson, 2000). However, their study is the only one that combines critical observations on the natural history of the definitive and intermediate hosts with experimental infections of both the intermediate and definitive hosts under controlled laboratory conditions. As a result, it is the only complete

*Gongylonema* species life cycle published and has stood the test of time (Ransom and Hall, 1915, 1917; Anderson, 2000).

# **Wendell Krull, miniature zoos, and elucidation of trematode life cycles**

Arguably, one of the most successful parasitologists of the early part of the twentieth century at solving trematode life cycle puzzles was Wendell H. Krull (Ewing, 2001). Krull was born on December 8, 1897 in Tripoli, Iowa and attended Iowa public schools. After high school, he completed a couple of years as an undergraduate student at Cornell College in Iowa before entering the United States Navy. He served in the Navy from October 1917 until being honorably discharged in 1920, after which he completed his B.A. degree in 1921 at Upper Iowa University in Fayette. He then received his M.Sc. from the University of Iowa in 1924. During his Master's degree, Krull was a teaching assistant in invertebrate zoology where he apparently developed an interest in parasitology. However, after completion of his M.Sc., he took a position as Head of the Department of Zoology at Northwestern College in Naperville, Illinois for a year, followed by a teaching position at Kansas Wesleyan in Salina, Kansas. After spending a year teaching at Kansas Wesleyan, Krull moved to the University of Michigan in Ann Arbor to pursue his Ph.D. under the direction of George R. LaRue, where he conducted his first life cycle studies on frog lung flukes at the Michigan Biological Station at Douglas Lake in Pellston, Michigan.

Upon completing his Ph.D. in 1929, Krull accepted a position as an assistant zoologist in the Zoology Division of the BAI in Washington D.C. and came under the influence of many renowned parasitologists including Maurice C. Hall who was his boss and the Chief of the Zoology Division. At that time, Krull and many other BAI parasitologists moved to a research station opened in nearby Beltsville, where Krull continued his work on various trematode life cycles until 1938. Over the next 9 years (1929–1937), he was incredibly productive by publishing 62 papers on trematode life cycles and/or techniques for rearing their intermediate hosts! In 1938, Krull moved west to the BAI unit in Logan, Utah where he was appointed as Chief of the Zoology Division and remained there until 1942 (Ewing, 2001).

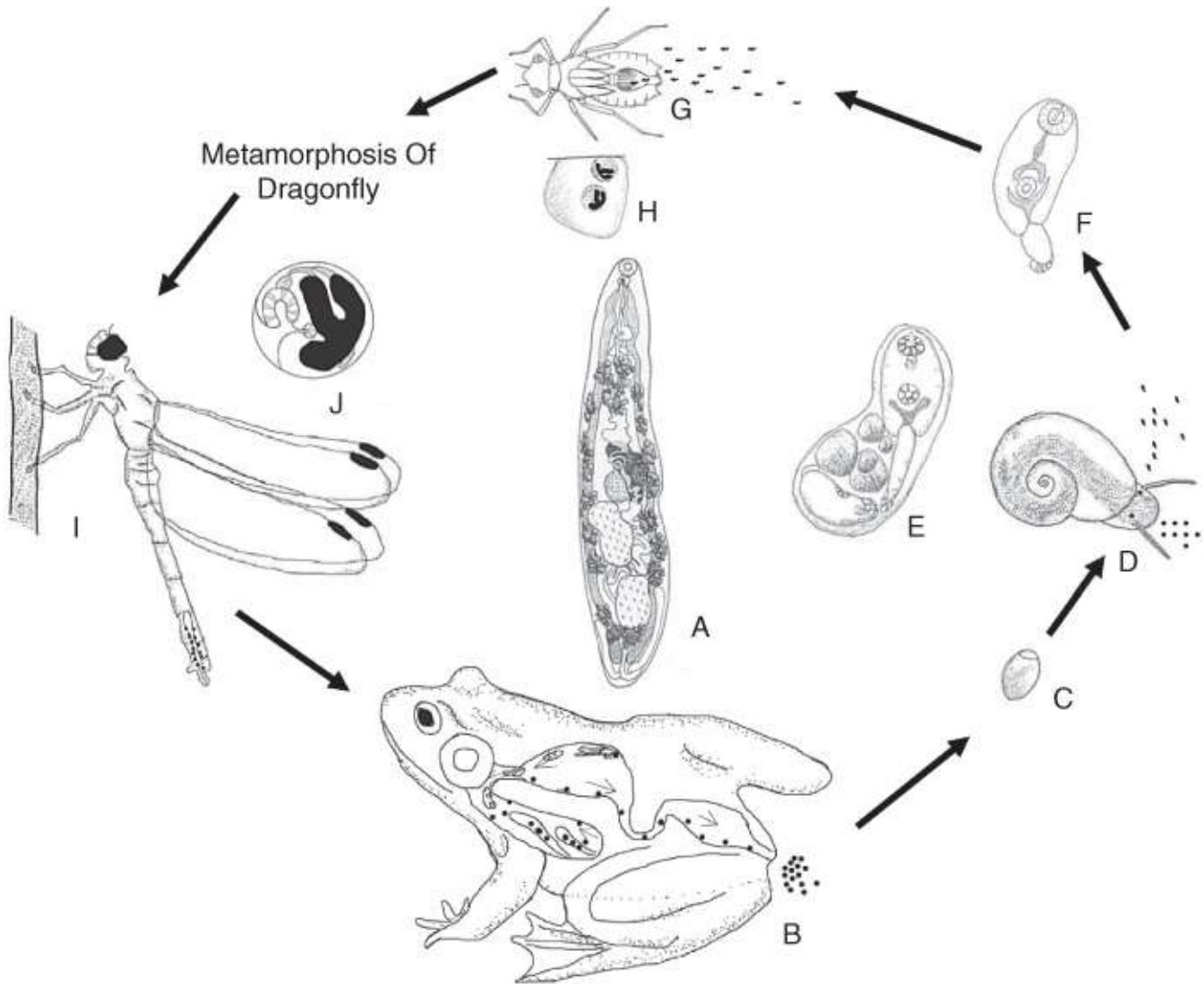
In 1942, Krull changed his career path once again and began studies toward a D.V.M. degree at Auburn University where he was also a part time instructor for the veterinary parasitology course. However, he did not care for the heat and humidity of the south, and within a year he moved to Colorado State University (CSU) in Fort Collins and completed his D.V.M. in 1945. After completing this degree, he joined the Department of Zoology at CSU as a faculty member.

Incredibly, within a year, Krull advanced from being a part-time instructor to Professor and Department Head at CSU. In addition, during this time he served as the Parasitologists at CSU's Agricultural Experimental Station where he remained for 3 years. He then moved to Oklahoma A&M (now Oklahoma State University [OSU]) where he became Professor and Head of the fledgling Department of Veterinary Parasitology. Because of his previous experience at CSU, and to justify

his salary, he was also jointly appointed as the Head of the Department of Zoology in the School of Arts and Sciences at OSU. During this time, he predominantly concentrated on his teaching. However, even then, he was able to solve one of the most difficult and economically important trematode life cycles, that of the sheep lancet fluke *Dicrocoelium dendriticum* which, at the time, was erroneously described as a two-host life cycle (Cameron, 1931). He remained as the Head of Veterinary Parasitology at OSU until 1964 when academic politics forced him into retirement at the age of 67. He quickly accepted a teaching position at the Veterinary School at Kansas State University and eventually retired at the age of 70.

By the end of his career, Krull had published 80 papers, and only once was he a junior author on a publication! Almost all of his research dealt with trematode life cycles or techniques for rearing various invertebrate groups used to complete those life cycles. So, what made him so successful at unraveling these life cycle puzzles? First, he was a master at maintaining miniature zoos by culturing snails and other invertebrates for his experimental infections. Second, he was a patient man who made critical observations and allowed the life cycles to do the work for him. Of his numerous life-cycle publications, two on frog lung flukes and frog tongue worms standout in particular and demonstrate his masterful skills at using the life cycle tool kit—combining detailed field observations, followed by controlled laboratory infections. Importantly, those publications have inspired numerous graduate students to continue other life cycle investigations and to fill hundreds of pages with “A-ha” life cycle moments in *JP*.

The first life cycle papers published in *JP* by Krull was his dissertation work on frog lung flukes. The first frog lung fluke was described in 1737, but it was not until 1930, almost 200 years later that the life cycles of two species were first elucidated (Krull, 1930). In his work, Krull was able to show that the eggs of *Haematoloechus medioplexus* (= *Pneumonoeces medioplexus*) and *H. parvplexus* (= *P. parvplexus*) hatched into miracidia in the intestine of two laboratory reared freshwater planorbid snails *Gyralus armigera* and *Gyralus parvus*. Over approximately 30 days, the miracidia developed within the hepatopancreas of their snail first intermediate hosts through two generations of sporocysts and produced free-swimming cercariae. The second intermediate host in the life cycle was a dragonfly larva. Again, Krull was able to show that the cercariae of these two species were drawn in into the branchial basket of his laboratory reared dragonfly larva second intermediate host, where they encysted as metacercariae. The final host was a frog, which ingested either an infected larva or metamorphosed dragonfly. The worms then migrated to the lungs of the frog where they matured. The entire life cycle (from egg to adult worm) takes just over 2 months, and amazingly, he was able to solve this puzzle within a single year ([Fig. 1](#)).

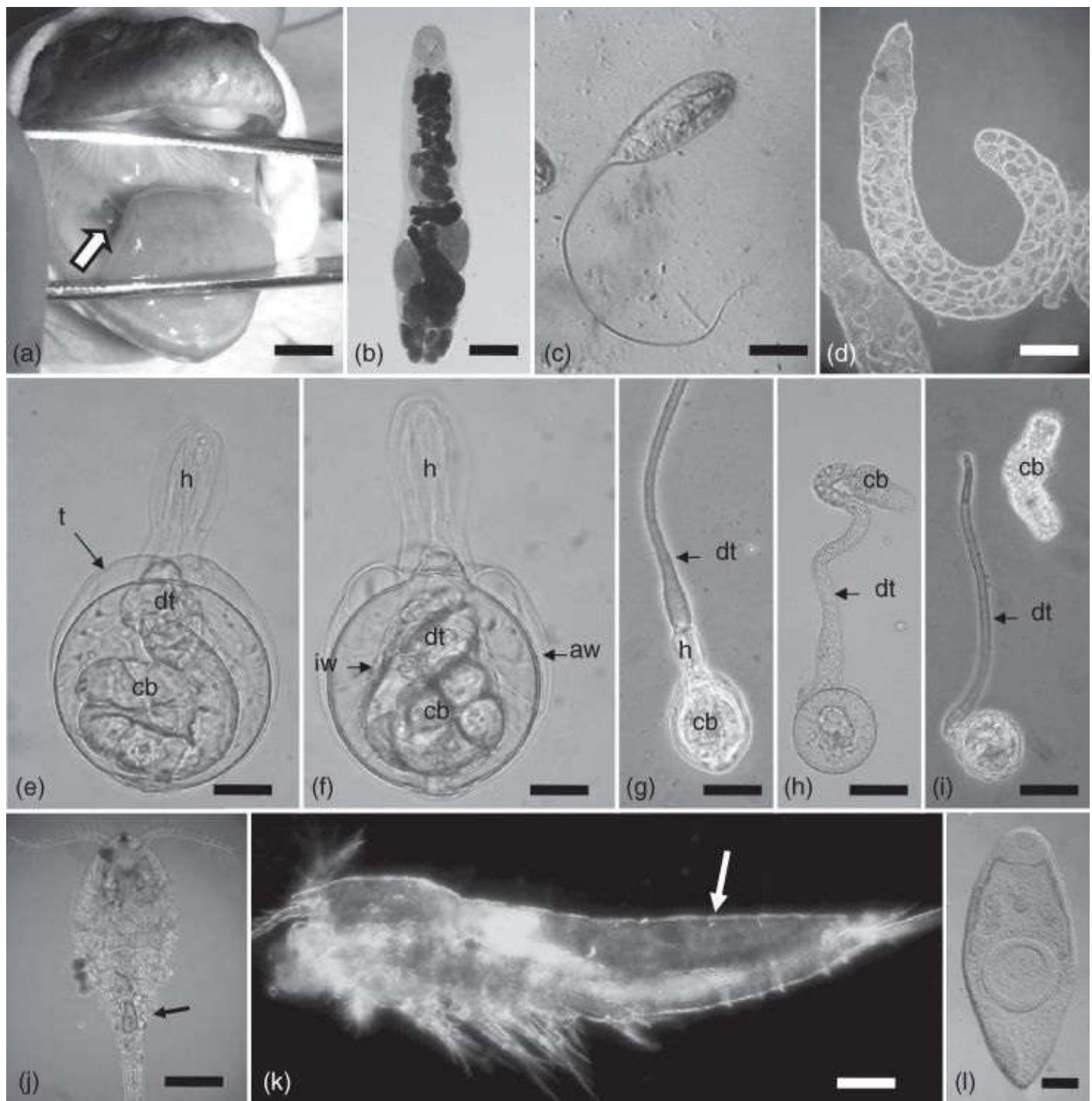


**Figure 1** Life cycle of *Haematoloechus parvplexus* (=*Pneumonocercus parvplexus*). (A) 32-day-old adult *H. parvplexus* from the lungs of the bullfrog, *Rana catesbeiana*. (B) Bullfrog, showing the escape of the metacercariae from the dragonfly in the stomach and their migration to the lungs; adult worms depositing eggs and the route of the eggs to the external environment. (C) Egg. (D) *Gyraulus parvus* eating eggs; releasing cercariae. (E) Sporocyst from the digestive gland of *Gyraulus parvus* with cercariae in various stages of development. (F) Cercaria showing body, tail, stylet, oral sucker, pharynx and cecae, ventral sucker, and excretory bladder. (G) Larva of the eastern pondhawk dragonfly, *Erythemis simplicicollis*, showing the swimming cercariae being taken into the branchial basket respiratory organ of the larva. (H) Lamella from the branchial basket of eastern pondhawk dragonfly larva containing two encysted metacercariae. (I) Teneral eastern pondhawk dragonfly with encysted metacercariae within the vestige of the branchial basket of the larva. (J) Encysted metacercaria. Drawings not to scale. All drawings are original but modified after Krull (1930).

Krull was able to make this work look so easy because of his innate ability at culture and infect his snail hosts. Over the last 15 years, one of us (Bolek) has worked on various trematode life cycles and, of these, the frog lung flukes are the most difficult to maintain in the laboratory. The most challenging step is infecting

the snail host. *Gyraulus parvus*, the first intermediate host for frog lung flukes, is a small fragile snail, which is difficult to culture and can be easily over-infected. However, Krull developed methods for both culturing and infecting these snails (Krull, 1931a, 1931b). To accomplish this, he demonstrated incredible patience and skill. For example, in the process of infecting his snails, Krull handled each snail individually. With an eye dropper, he placed a snail into a dish containing fluke eggs and immediately brought the snail into focus under a microscope to watch it consume the eggs. In this way, he found that each snail moved its radula 80 to 120 times a minute and, in the process, it consumed an average of five eggs at a time. He then determined that the snail had to consume between 10 and 200 eggs to insure that snails became infected without becoming overly infected and that they survived long enough to shed cercariae. At first, these detailed observations may seem excessive. However, one of us (Bolek) can attest that these steps are crucial and involve an extraordinary amount of patience to complete one of these life cycles in the laboratory. As a first year Ph.D. student at the University of Nebraska-Lincoln, and totally clueless about life cycle investigations, Bolek attempted to infect *G. parvus* with *H. parviplexus*. Krull's work provided all the details needed to accomplish this, therefore, how difficult could it be? After spending 6 months perfecting techniques for culturing snails, Bolek had enough snails to begin his infections; however, 2 months of infecting and killing hundreds of snails produced only 12 snails that survived past the 30-day prepatent period. Additionally, the 12 surviving snails were under-infected and shed a total of 14 measly cercariae before the snails all died. It took another 3 months of trial and error before he could consistently infect *G. parvus* with patent infections that produced enough cercariae to complete the life cycle!

The second, and arguably one of the most bizarre and complex trematode life cycles elucidated by Wendell Krull was that of the hemiurid, *Halipegus occidualis* (Krull, 1935a). The worm was described by Stafford in 1905; most species in the family infect the stomachs of marine fish. However, unlike its marine relatives, *H. occidualis* resides under the tongue of green frogs and, surprisingly, has four hosts in its life cycle! At the time, Krull was studying the life cycles of two species of frog lung flukes and, as part of his work, he examined various species of dragonflies and damselflies for metacercariae (Krull, 1932–1934). We can only imagine that, on one of those occasions, he must have discovered a metacercaria in a dragonfly, which he assumed was *H. occidualis*. Knowing that frogs commonly eat dragonflies, he collected tadpoles of green frogs and reared them in the laboratory through metamorphosis. Next, he exposed his metamorphosed green frogs with field-collected metacercariae from dragonflies and recovered adult worms from under the tongue of frogs 22 days post exposure (DPE) ([Fig. 2](#)).



**Figure 2** Original photomicrographs of the developmental stages of *Halipegus occidualis*. (a) Adult worm (arrow) under the tongue of a green frog, *Rana clamitans*. Scale bar = 1.0 cm. (b) Adult worm full of eggs. Scale bar = 1.5 mm. (c) Egg. Note the long abopercular filament and well developed miracidia. Scale bars = 20  $\mu$ m. (d) Redia. Note the developing cercariae. Scale bar = 100  $\mu$ m. (e–f) Cystophorous cercaria. Note the coiled cercaria body (cb), coiled delivery tube (dt), handle (h), encysted tail (t), and inner (iw) and outer (ow) membranes. Scale bars = 20  $\mu$ m. (g–i) Expulsion of the delivery tube (dt) through the handle (h) followed by the cercaria body (cb) traveling through the delivery tube until its expulsion. Scale bars = 50  $\mu$ m. (j–k) Metacercariae (arrows) within the hemocoel of *Cyclops* sp. and *Phyllognathopus* sp. copepods. Scale bars = 200  $\mu$ m in (j) and 10  $\mu$ m in (k). (l) Metacercaria removed from the intestine of a dragonfly larva. Scale bar = 85  $\mu$ m.

To complete the description of the life cycle of this species, Krull began by obtaining eggs of *H. occiduialis* from an adult worm he removed from under the tongue of an infected green frog. Unlike many other trematodes, the eggs of *H. occiduialis* have long abopercular filaments, and Krull made the critical observation that the eggs contained fully formed miracidia that did not hatch ([Fig. 2](#)). This observation led him to place six *Helisoma anceps* (= *Helisoma antrosa*) snails (raised from eggs in the laboratory) in a covered stender dish containing filtered pond water, *H. occiduialis* eggs, and calcium carbonate. Twelve hours later, the snails had completely ingested the eggs, which he confirmed by observing hatched eggs in their feces. Over the next 3 months, he maintained these snails in a large aquarium, and occasionally they were examined for shedding cercariae. Of the six snails he exposed, five died before they began shedding cercariae. One may think that because all but one of his exposed snails died before they began shedding cercariae his experiment was a failure. However, Krull made detailed observations, even on his dead snails, which ultimately made his experiment one of success. His first snail died 7 days post-exposure, followed by the others on 13, 17, 29, and 49 DPE. By examining each snail after it died, he was able to determine that all of the snails were infected with intramolluscan stages, and he was able to fully document the development of the intramolluscan stages of this trematode, including a sporocyst and redia generation. His sixth and only remaining snail finally began shedding cercariae 94 DPE and continued to shed cercariae for an additional 28 days before it died.

Krull next turned his attention to the cercaria stage, the morphology of which is exceptionally strange. The cercariae of *H. occiduialis*, as in all hemiurids, are of the cystophorous type. They are non-motile and reside on the pond bottom once released from their snail host. The cercaria body, along with a delivery tube, is tightly folded up and maintained under great pressure within the double walled tail, which encloses the two as a cyst. On one side of the cystophorous tail is an appendage, which Krull referred to as a handle ([Fig. 2](#)). He provided detailed observation of how the cercariae behaved after leaving their snail hosts where he stated,

After leaving the snail, the cercaria sinks to the bottom of the container where it rests in such a position that the axis of the handle is at an obtuse angle, and rarely at a right angle with the substratum.

Next, he was able to demonstrate by using a teasing needle and by touching the cercaria's handle that the delivery tube emerged first through the handle, followed by the cercaria body traveling through the delivery tube until its expulsion ([Fig. 2](#)). He was then able to demonstrate that the cercariae of *H. occiduialis* remained viable for up to 2 weeks, unlike motile cercariae, which only live for 24–48 hours (Krull, 1935a).

To tackle the next part of the life cycle, Krull used his laboratory-reared cercariae to expose two species of laboratory-reared copepods (*Cyclops vernalis* and *C.*

*serrulatus*). Based on his dragonfly work, it was obvious that Krull was well aware that larval odonates fed on microcrustaceans, but why did he choose copepods as the next potential host in the life cycle? Again, the answer was simple; he was familiar with the literature. In 1932, Lyell J. Thomas published an abstract, as part of the American Society of Parasitologists annual meeting program that reported *Cyclops* sp. could be infected by exposing them to cystophorous cercariae of another frog hemiurid, *Haliipegus eccentricus*. Krull was aware of this work because he cited it in his 1935 publication. So, even before he started his experimental work on that part of the life cycle, Krull knew what to expect, and now he just had to demonstrate it.

He began his experimental infections by placing cercariae in containers of water with copepods and made detailed observations on how these microcrustaceans became infected. He first demonstrated that copepods commonly consume the cercariae when he stated,

Both mature and young cyclops appear to be very fond of the cercariae, and the larger cyclops eat one cercaria after another, the cercaria body being discharged into the mouth of the cyclops by a manipulation of the handle, after which the cystoid portion is eaten.

Next, he provided detailed information on the infection process with the following information:

In many cases the smaller cyclops eat the cercariae as above described, but occasionally when the cystoid cercaria is discharged into the mouth of the cyclops the later makes a terrific spurt for a moment and lies motionless with the appendages widely separated, as if dead on the bottom of the container for as long as a minute in some cases. While in this condition a part of the delivery tube with the attached cyst of the cercariae usually projects from the mouth. The cyclops suddenly regains its equilibrium and appears normal except that it now contains an active larval fluke which is almost always in the body cavity and occasionally in the intestine.

Even more impressively, Krull was able to document the infection process by observing copepods using a compound microscope and provided detailed information on the location of the worms within their hosts through serial sections. Although cyclopoid copepods are relatively easy to culture, trying to manipulate or capture one with a pipette is one of the most frustrating tasks one can imagine. To overcome this difficulty, Krull used the life cycle process to do the work for him. After some practice, he was able to isolate individual copepods for microscopic and histological examinations by collecting individuals after they were stunned by the discharged cercariae. With that simple technique, he was able to show that the metacercariae are unencysted and typically develop in the hemocoel of their microcrustacean host ([Fig. 2](#)).

Surprisingly, Krull never attempted to expose dragonflies with infected copepods, and he provides no explanation for this omission, nor is there any information

provided on this subject in Sidney Ewing's biographical sketch of Wendell Krull (Krull, 1935a; Ewing, 2001). However, it is one part of the life cycle that has been the most difficult to complete. Since Krull's study, all attempts at infecting odonates with the metacercariae of *H. occidualis* have been unsuccessful (Rankin, 1944; Macy et al., 1960; Goater et al., 1990). Clearly something was missing, and it was not until one of Jerry Esch's graduate students, Derek Zelmer, got involved and the mystery was solved. As part of his dissertation, Zelmer examined the development of *H. occidualis* metacercariae in their microcrustacean host and compared their development to metacercariae recovered from field-collected odonates (Zelmer and Esch, 1998a, 1998b, 1998c). He reasoned that infective metacercariae from microcrustacean hosts should have a similar morphology to infective metacercariae from odonate hosts. He discovered that the metacercariae of *H. occidualis* needed a considerable amount of development time (4 weeks post exposure) in the microcrustacean host before they were morphologically similar to infective metacercariae from odonate hosts. To test his assumption, Zelmer exposed a green frog with microcrustaceans containing 4-week-old metacercariae and examined the frog's buccal cavity every 48 hours over several weeks. Zelmer experienced one of the life cycle "A-ha" moments 22 days post-exposure when he discovered adult *H. occidualis* under the tongue of his exposed green frog!

Although Zelmer never exposed larval dragonflies with infected microcrustaceans, his study provided the first plausible explanation of why so many previous trials had failed (Zelmer and Esch, 1998a). It was another 12 years before Zelmer's hypothesis was supported when Bolek et al. (2010) finally infected larval damselflies with mature metacercariae of *Halipegus eccentricus* from microcrustacean hosts. Thus, an entire *Halipegus* life cycle within all four hosts was finally completed in the laboratory.

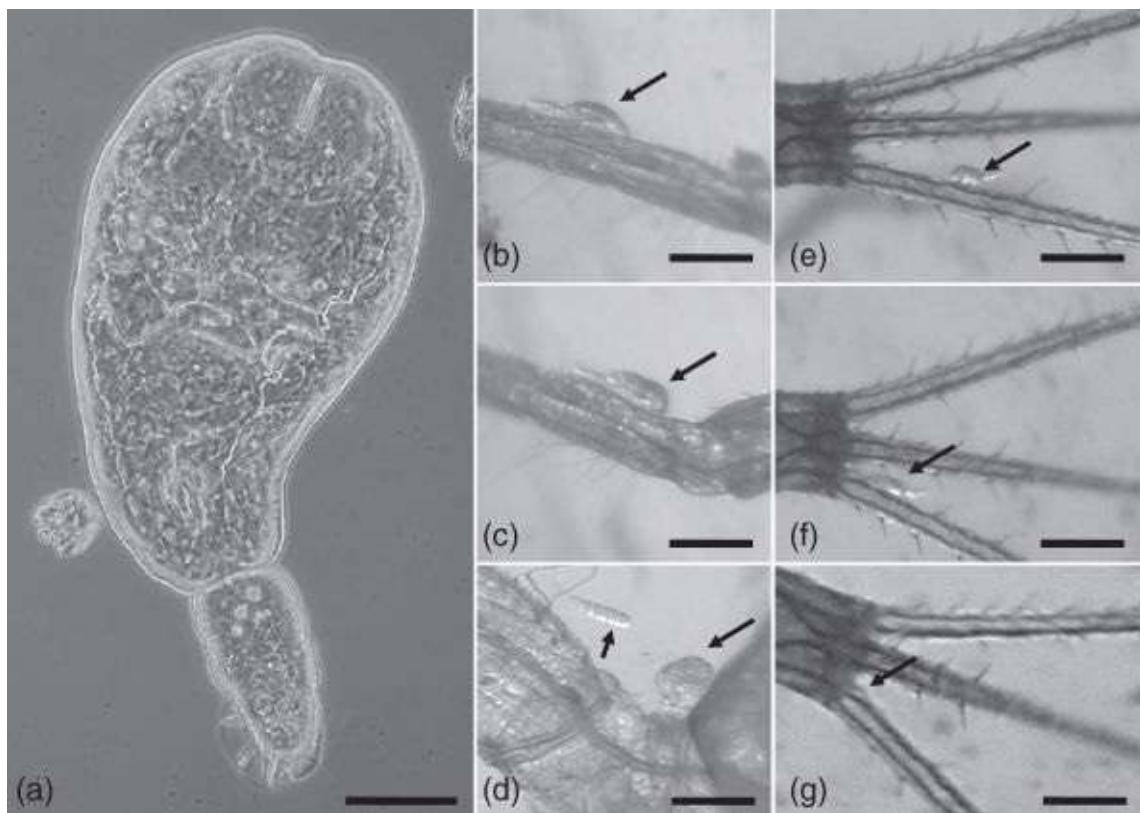
# Life cycles then and now: Using a comparative approach

Over the last 100 years, many other fascinating parasite life cycles have been resolved by the individuals that pursued such endeavors. However, through time, the details of those life cycles have been overlooked and forgotten, and the typical “iron wheel” diagrams have emerged. However, these illustrations only provide a snapshot of how these parasites actually live out their lives in nature (see [Fig. 1](#)) and, therefore, it is even more concerning that very few scientists ever question the validity of those iron wheels. Fortunately, over the last four decades, a few individuals have continued to question the validity of some of those life cycles (Goater, et al., 1990; Zelmer and Esch, 1998a, 1998b, 1998c; Snyder and Janovy, 1994, 1996; Bolek and Janovy, 2007a, 2007b, 2008; Bolek et al., 2009a–2010; Langford and Janovy, 2009, 2013). As a result of those investigations, a new way of looking at life cycles has emerged. Instead of attempting to just solve a life cycle, the attention was placed on the life cycle itself and how that life cycle influenced the ecology and evolutionary history of the parasites that employ it. To accomplish this work, these parasitologists made use of the standard tool kit equipped by their predecessors while also incorporating a comparative approach to life cycle studies.

Upon examining the numerous publications from the last 30 years in *JP*, a number of intriguing comparative life cycle studies appear on various helminth groups. However, from a comparative perspective, a number of complimentary studies on (1) frog lung flukes and (2) frog bladder flukes stand out because they provide a realistic snapshot of how flukes live out their lives on the western plains of Nebraska. Furthermore, those studies demonstrate how unrealistic the representative iron wheel life cycle diagrams are in deciphering the real transmission strategies of parasites.

The first example on frog lunge flukes was conducted by John Janovy's Ph.D. students, Scott Snyder, and one of us, Matthew Bolek. Snyder's dissertation examined frog lung fluke host specificity at the arthropod second intermediate host level, whereas Bolek's dissertation examined the role of arthropod second intermediate hosts in the transmission of frog lung flukes to their anuran hosts. Their combined studies indicate that distinct patterns of arthropod host specificity have evolved in North American frog lung flukes, ranging from generalists to specialists (Snyder and Janovy, 1994, 1996; Bolek and Janovy, 2007a, 2007b). *Haematoloechus medioplexus* and *H. parviplexus* are dragonfly specialists. The cercariae of these species are passive host invaders. For a dragonfly larva to be infected, the cercariae must be drawn into the dragonfly's branchial basket where they encyst as metacercariae (Snyder and Janovy, 1996). In contrast, *Haematoloechus complexus* and *H. coloradensis* are generalists at the second intermediate host level and can infect any aquatic arthropod. The cercariae of these species are active host invaders. Upon contact with an arthropod host, the cercariae use their suckers to attach to any region of an arthropod host. Next, they

actively search for an inter-segmental membrane on the exoskeleton of their host, where they penetrate using their stylet ([Fig. 3](#)). As a result, the metacercariae of these two species are found as a thin hyaline cyst within any part of the arthropod's body (Snyder and Janovy, 1994, 1996; Bolek and Janovy, 2007a). Finally, *H. longiplexus* is intermediate in its arthropod host specificity and can infect both dragonflies and damselflies, but no other arthropods. Dragonflies become infected when they draw cercariae of *H. longiplexus* into their branchial basket. However, when the cercariae of *H. longiplexus* encounter a larval damselfly, they attach to the caudal gills with their suckers and actively move to the base of the damselfly's caudal gills and enter through the anus ([Fig. 3](#)). Even more fascinating, Snyder (1996) was able to show that cercariae of frog lung fluke species that only infect dragonflies developed into metacercariae when injected into damselflies. This simple yet elegant experiment provided convincing evidence that species-specific cercarial behavior patterns are the major factors responsible for second intermediate host specificity in frog lung flukes.



**Figure 3** Original photomicrographs of cercariae of *Haematoloechus* spp. in the process of attaching and penetrating a damselfly host. (a) Free swimming cercaria of *Haematoloechus coloradensis*. Note the stylet. Scale bar = 40 µm. (b-d) Attachment, crawling behavior and penetration of a cercaria of *Haematoloechus coloradensis* on the leg of a larval damselfly. Note the loss of the tail (small arrow) from the cercarial body (large arrow) in the process of penetration in (d). Scale bars = 200 µm. (e-g) Attachment, crawling behavior and penetration of a cercaria of *Haematoloechus longiplexus* on the anal gill of a larval damselfly. Scale bars = 300 µm.

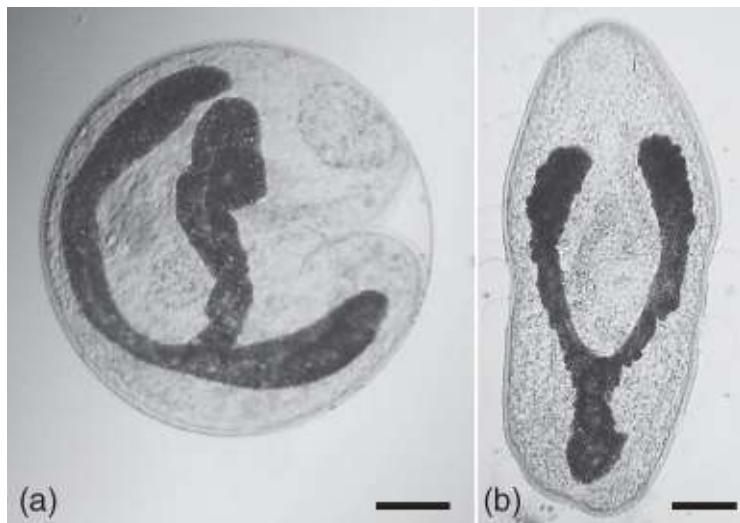
As with arthropod second intermediate host specificity, the Nebraska frog lung

flukes exhibit distinct anuran definitive host specificity. Semi-terrestrial leopard frogs are commonly infected with *H. complexus* and *H. coloradensis*, but aquatic bullfrogs are never infected with these two species. Additionally, of the three species of frog lung flukes (*H. medioplexus*, *H. parvplexus*, and *H. longiplexus*) that strictly use odonates as second intermediate hosts, only *H. medioplexus* commonly infects semi-terrestrial leopard frogs; whereas *H. parvplexus* and *H. longiplexus* infect aquatic bullfrogs (Brooks, 1976; Snyder, 1996; Bolek and Janovy, 2007a, 2007b). More surprisingly, semi-terrestrial leopard frogs can be easily infected with *H. longiplexus* in the laboratory although they are almost never infected in nature (Brooks, 1976; Snyder, 1996; Bolek and Janovy, 2007a, 2007b). Clearly, anuran frog lung fluke specificity is more complex than a typical iron wheel life cycle diagram would suggest.

Upon examining the literature on the diet habits of frogs, some tantalizing clues emerge that may help explain this odd pattern of host specificity. Studies on the diet of semi-terrestrial leopard frogs and aquatic bullfrogs indicate that, although they both commonly feed on odonates, leopard frogs predominantly ingest terrestrial stages and bullfrogs predominantly feed on aquatic stages (Korschgen and Baskett, 1963; Dronen, 1977). These observations suggest that second intermediate odonate hosts may act as filters or sieves (Euzet and Combes, 1980). Only certain lung fluke species are able to pass through all filters and end up in the appropriate terrestrial environment and infect leopard frogs. Therefore, Bolek and Janovy (2007b) hypothesized that differences in second intermediate host specificity and the life histories of the second intermediate hosts play an important role in parasite movement, distribution, and the observed host specificity in definitive frog hosts.

To test their hypotheses, Bolek and Janovy (2007b) exposed larval dragonflies to cercariae of *H. complexus*, *H. coloradensis*, *H. longiplexus*, and *H. parvplexus*. After exposure, odonates were maintained in the laboratory through metamorphoses and examined for metacercariae. As the dragonflies began to metamorphose, three of the four groups contained encysted metacercariae of *H. complexus*, *H. coloradensis*, and *H. parvplexus*. However, *H. longiplexus* was nowhere to be found. Could the infections with this species not have worked? This was unlikely because repeated trials produced identical results. To solve this mystery, Bolek decided to follow Krull's rules and make critical observations on his odonate hosts. Because odonates metamorphose at night, Bolek spent a number of weeks watching his dragonflies throughout the night. On one of those nights, a male dragonfly exposed to cercariae of *H. longiplexus* began to emerge from his exuvia. As the dragonfly metamorphosed, the remnants of the branchial basket were clearly visible in the exuvia, and when the remnants of the branchial basket were examined with a compound microscope another "A-ha" moment occurred. The metacercariae of *H. longiplexus* were left behind in the exuviae when odonates metamorphosed! But, why did the other dragonflies not lose their infections of the three other frog lung fluke species during metamorphosis? The

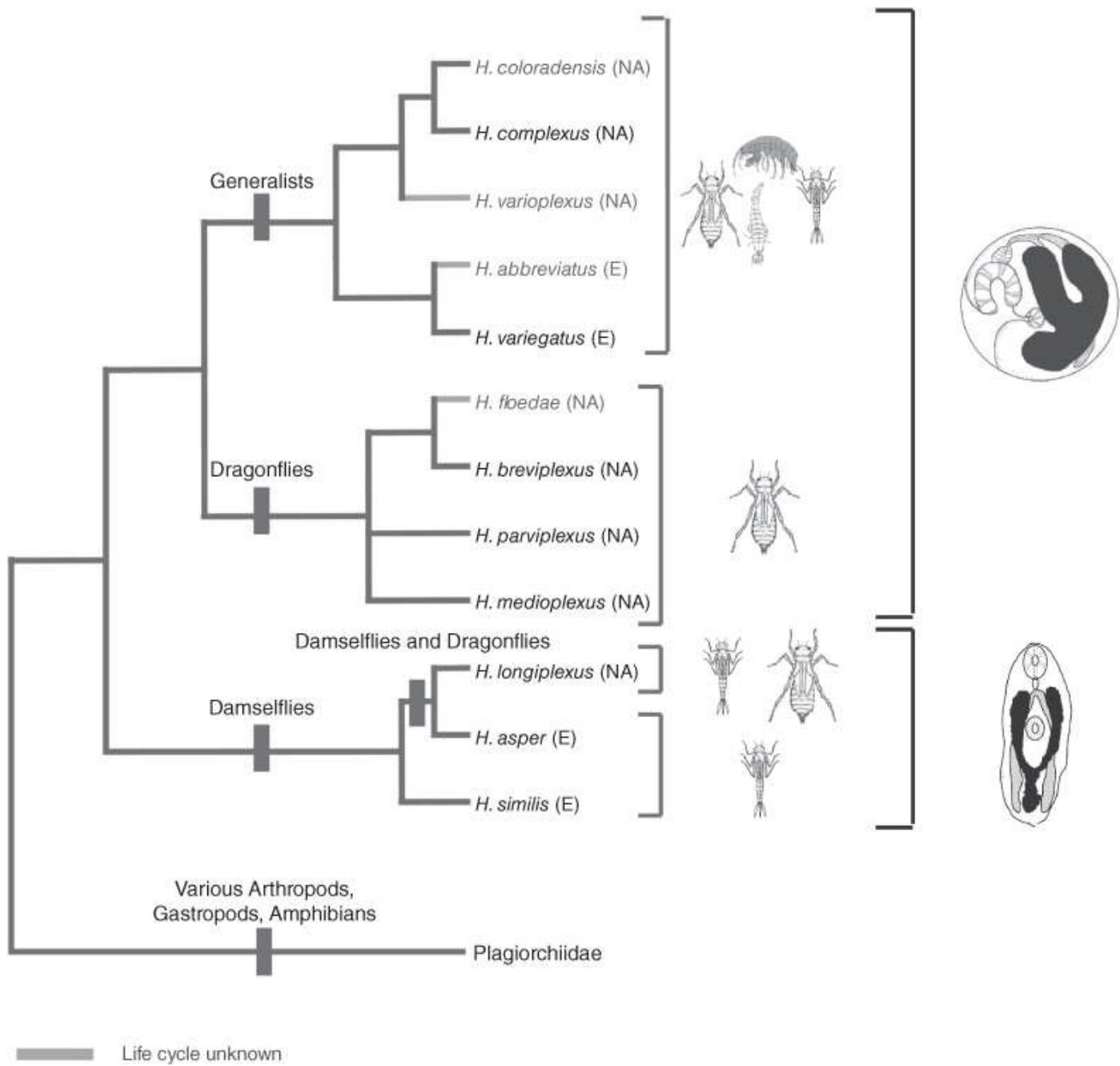
answer to this question was provided by Wendell Krull in 1932. Unlike all other North American frog lung flukes that have encysted metacercariae, Krull (1932) showed that the metacercariae of *H. longiplexus* are unencysted ([Fig. 4](#)) and, as a result, are lost during dragonfly metamorphoses.



**Figure 4** Original photomicrographs of metacercariae of *Haematoloechus* spp. removed from larval dragonflies. (a) Encysted metacercaria of *Haematoloechus coloradensis*. (b) Unencysted metacercaria of *Haematoloechus longiplexus*. Scale bars = 20 µm.

To complete their study, Bolek and Janovy exposed groups of laboratory reared leopard frogs and bullfrogs to metacercariae of the four species of lung flukes. Their results indicated that bullfrogs were resistant to infections of *H. complexus* and *H. coloradensis* and leopard frogs were resistant to infections of *H. parviplexus*. However, both bullfrogs and leopard frogs became infected with *H. longiplexus*. Those experimental results clearly suggested that semi-terrestrial leopard frogs are never infected with *H. longiplexus* because they most likely never encounter their metacercariae in the adult dragonflies they feed on.

The frog lung fluke story is especially intriguing when considered from an evolutionary perspective. Molecular phylogenetic studies by Snyder and Tkach (2001) and Bolek (2006) on 12 species of European and North American frog lung flukes indicate that two of the three clades are comprised of both European and North American species, suggesting that the lineages of *Haematoloechus* spp. arose before the breakup of Laurasia and then radiated after Eurasia and North America separated. Interestingly, within each of the evolutionary lineages, members share similar patterns of arthropod host specificity and metacercaria morphology, and they are distinct from patterns found in the other lineages ([Fig. 5](#)). This suggests that second intermediate host specificity and metacercaria morphology are traits that have been conserved through evolutionary time.



**Figure 5** Arthropod host specificity, metacercaria type, and geographical distribution among species of *Haematoloechus* indicated on a phylogenetic tree derived from internal transcribed spacer rDNA data by Snyder and Tkach (2001) and Bolek (2006). Generalist parasites have the ability to form metacercariae in odonate and non-odonate arthropods. Species in gray indicate that the life cycle is unknown. NA = North America; E = Europe.

The second example we discuss below demonstrates that life cycle variations can also exist within a single species of trematode. The conceptual strength of the following study rests with the examination of alternative routes of infections by a single species of frog bladder fluke, *Gorgoderina attenuata*, to different anuran species that vary in their life histories and phylogenetic relationships (Bolek et al., 2009a).

In North America, 12 species of frog bladder flukes (Gorgoderidae) have been described from anurans (see Bolek et al., 2009b), and most of these appear to be

relatively host specific by infecting a single or a few closely related frog species. Studies on the life cycles and epizootiology of some of those species indicate that they use sphaeriid clams as the first intermediate host, but can vary in the type of second intermediate host used in their life cycles. A number of frog bladder fluke species, from which life cycles are known, use tadpoles as second intermediate hosts whereas other species use odonates or molluscs as second intermediate hosts. Adult amphibians become infected by ingesting arthropods, snails, tadpoles, or other frogs infected with encysted metacercariae. Once metacercariae are ingested by a frog definitive host, worms reside in the kidney where they develop; they eventually migrate to the frogs' urinary bladder and begin egg production (Krull, 1935b; Rankin, 1939; Goodchild, 1943, 1948, 1950; Ubelaker and Olsen, 1972). Additionally, field studies on amphibian gorgoderids indicate that, within individual anuran species, newly metamorphosed and juvenile anurans are less commonly infected with bladder flukes than larger adult frogs because of their relatively small gape size. The limited gape size ultimately affects the size of potential intermediate hosts that can be ingested by these frogs (Bolek and Janovy, 2007a).

Contrary to these studies, *G. attenuata* has been reported from 24 species of amphibian and reptilian hosts (Bolek et al., 2009b). Even more surprising is that newly metamorphosed (1–4 cm snout vent length) anuran species, such as leopard frogs and toads, that never feed on tadpoles or other anurans are commonly infected with this species and prevalence can be as high as 80%. Clearly the life cycle of this species must be distinct from the typical frog bladder fluke life cycle to account for these observed differences. However, work by Rankin (1939) indicates that the life cycle of *G. attenuata* is a typical frog bladder fluke life cycle: adult anurans become infected after ingesting a tadpole or a metamorphosed frog infected with metacercariae. As a result of these contradictory observations, Bolek et al. (2009a) questioned the validity of Rankin's (1939) "iron wheel" and re-evaluated the life cycle.

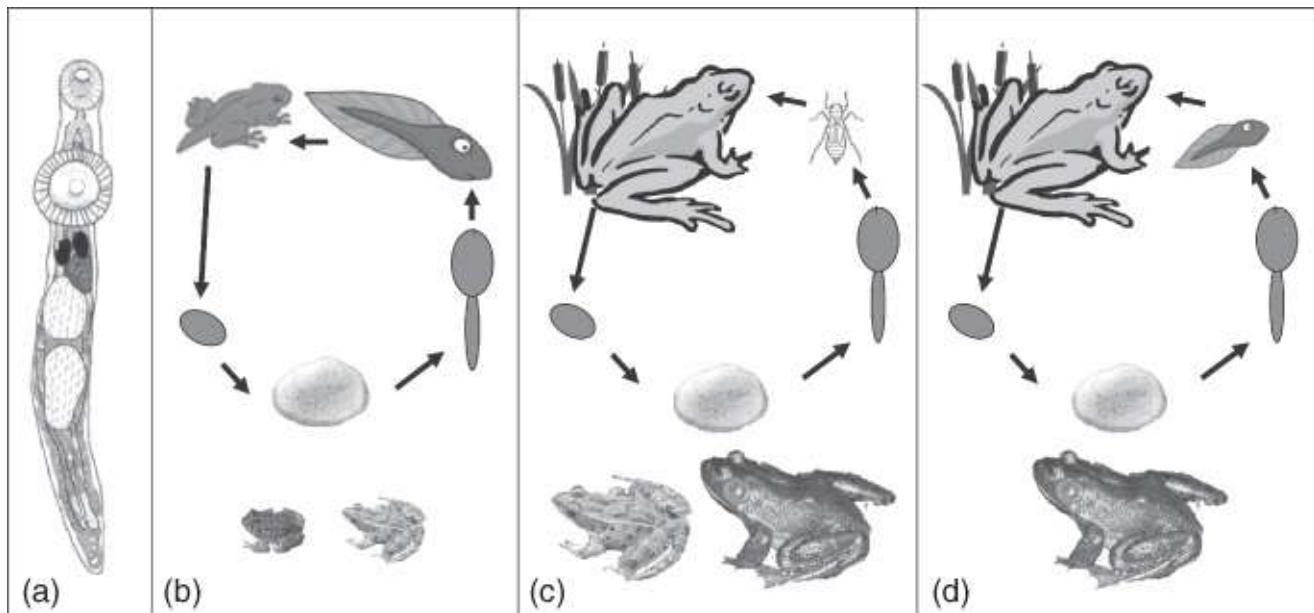
They began their study by examining field-collected tadpoles, frog/toadlets, and adults of leopard frogs, Woodhouse's toads, and bullfrogs for metacercariae and adult stages of *G. attenuata* from a single location in western Nebraska. To their surprise, no gorgoderid metacercariae were found! Instead, anurans were infected with unencysted non-gravid worms in the kidneys and adult worms in the urinary bladder. More importantly, their field data indicated that there was a significant difference in the prevalence, mean abundance, and degree of maturation of *G. attenuata* between tadpoles and metamorphs of leopard frogs and Woodhouse's toads, as well as tadpoles, metamorphs, and adults of bullfrogs. All infected tadpoles contained non-gravid worms in their kidneys. In contrast, only a single metamorphosed leopard frog and none of the metamorphosed toads had worms in the kidneys, but all contained adult worms in their bladders. They reasoned that lack of worms in the kidneys of metamorphosed leopard frogs and toads indicated that both of these anuran species acquire infections with *G. attenuata* primarily

during the tadpole stage and that worms migrated from the kidneys to the urinary bladder as soon as tadpoles metamorphose into froglets or toadlets. Finally, because tadpoles of bullfrogs were less commonly infected with gorgoderids in the kidneys, but adult bullfrogs contained hundreds of non-gravid worms in their kidneys, they hypothesized that adult bullfrogs were actively recruiting *G. attenuata* after metamorphosis.

To confirm their field observations, they brought the life cycle into the laboratory. They began by collecting naturally infected fingernail clams with what they assumed were the cercariae of *G. attenuata*. To confirm the identification of the cercariae, they sequence the cercariae and compared those sequences to sequences of juvenile and adult worms recovered from the kidneys and urinary bladders of field collected tadpoles and metamorphosed anurans, respectively. Their sequence data confirmed that all stages belonged to *G. attenuata*. They then exposed tadpoles of leopard frogs with the cercariae of this species, and examined them for the locations of worms at different stages through metamorphoses. They discovered that tadpoles became infected by accidentally ingesting cercariae, and that worms entered tadpole kidneys, eventually migrating to the urinary bladder as tadpoles metamorphosed into froglets. Their combined field and laboratory data indicated that in leopard frogs and toads, *G. attenuata* has a truncated, two-host life cycle that includes fingernail clams and anurans. However, the question still remained as to how adult bullfrogs and adult leopard frogs became infected with this species?

To resolve the second part of the puzzle, and knowing that adult leopard frogs commonly feed on odonates, whereas adult bullfrogs commonly feed on odonates and tadpoles, Bolek et al. (2009a) expanded their study. They began by conducting additional field surveys of arthropods for the presence of gorgoderid metacercariae and adult leopard frogs and adult bullfrogs for the presence worms in their kidneys. They discovered that gorgoderid metacercariae were present in larval odonates and non-gravid worms were present in the kidneys of adult leopard frogs and bullfrogs. To confirm their field observations, they conducted two critical laboratory experiments. For their first experiment, they exposed uninfected damselfly larvae with the cercariae of *G. attenuata* and recovered encysted metacercariae from these hosts. Next, they exposed laboratory-reared frogs with metacercariae recovered from damselflies and found non-gravid *G. attenuata* worms in the kidneys of their exposed frogs. With that experiment, they were able to show a second transmission strategy where adult leopard frogs and adult bullfrogs can become infected by *G. attenuata* when they ingest metacercariae from damselfly second intermediate hosts. For their second experiment, they fed laboratory-reared bullfrogs with leopard frog tadpoles infected with non-gravid *G. attenuata* worms in their kidneys. Surprisingly, the results of those infections revealed a third transmission strategy; *G. attenuata* can also infect adult bullfrogs when bullfrogs feed on other infected anurans possessing worms in their kidneys! More importantly, this comparative approach

allowed Bolek et al. (2009a) to demonstrate that in nature different “iron wheels” of the life cycle were operating simultaneously, allowing for infections of different life stages and different species of anurans (see Fig. 6). As a consequence, the bladder fluke example clearly demonstrates that life cycles are more plastic than previously thought and simple, human-constructed, iron wheel parasite life cycles should be viewed with caution and questioned when data suggests otherwise.



**Figure 6** Variations on the life cycle of *Gorgoderina attenuata* and the anuran hosts which are infected. (a) Line drawing of an adult *G. attenuata* from the urinary bladder of a northern leopard frog, *Rana pipiens*. (b) Truncated two host life cycles where the cercaria is ingested by a tadpole. This transmission strategy is used to infect newly metamorphosed leopard frogs and Woodhouse's toads. (c) Three host life cycle where the cercaria is ingested by an odonate second intermediate host. This transmission strategy is used to infect adult leopard frogs and adult bullfrogs. (d) Three host life cycle using a tadpole as a transport host. This transmission strategy is used to infect adult bullfrogs. Adapted from Bolek, Snyder & Janovy, Jr (2009a). Reproduced with permission of *Journal of Parasitology*, Allen Press Publishing Services.

Clearly, over the last century of publications in *JP*, our concepts of helminth life cycles have changed dramatically. They began with Ransom and Hall developing the standard tool kit for elucidating life cycles, followed by Wendell Krull's mastery of culturing uncooperative invertebrates for completing those life cycles, to our current understanding of the variation and plasticity that exists within life cycles. However, what all those studies encompass is an intellectual elegance that provides a childlike wonder about how parasites really live out their lives in nature.

## Conclusions and lessons

We hope that our mini-review on the fascinating history of helminth life cycle work published in *JP* over the last century provides the background and perspective on how this type of work is conducted. More importantly, this review provides a list of tools that should be stocked in one's tool kit in order to solve a life cycle and demonstrates that elucidating a life cycle is not based on just dumb luck! To solve one, it takes an incredible amount of patience, observational skills, animal love and care, and general understanding of the natural world. One goal of this review was to demonstrate that the typical “iron wheel life cycle” diagrams that inevitably emerge only represent a snapshot of how parasites truly live out their complex lives, and, hopefully, future parasitologists will view these life cycles with intrigue rather than as absolute truth.

Additionally, we hope our review inspires the next generation of parasitologists to continue in this endeavor by elucidating new life cycles and always question the completeness of known life cycles. Clearly, there are many other fascinating life cycle studies published in *The Journal of Parasitology* that we were unable to include in our review because of page limitations. However, we recommend that parasitology students that are just beginning their work examine some of the life cycle papers published in *JP* in great detail and question the validity of the iron wheels presented and, to those students, we recommend beginning their search with three review papers on the transmission strategies of nematodes, cestodes, and trematodes by Anderson (1988), Mackiewicz (1988), and Shoop (1988), respectively.

Finally, we leave you with a statement made by Wendell Krull in a letter to Miriam Rothschild almost 60 years ago that provides a starting point for any life cycle investigation (Ewing, 2001):

Life cycle investigation is somewhat simpler now than it was twenty years ago because enough cycles have been completed in many of the groups to indicate what a related unknown cycle may be like; on the other hand one should not pin his hopes too closely on what is known, for cycles of closely related species may at times be quite different, and you certainly don't want to exclude any possibilities that are in the realm of procedure.

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## Literature cited

- Anderson, R. C. 1988. Nematode transmission patterns. *Journal of Parasitology* **74**: 30–45.
- . 2000. *Nematode parasites of vertebrates: their development and transmission*, 2nd ed. CABI International, Oxford, U.K., 650 p.
- Bolek, M. G. 2006. The role of arthropod second intermediate hosts as avenues for and constraints on the transmission of frog lung flukes (Digenea: Haematoloechidae). Ph.D. Thesis. University of Nebraska–Lincoln, Lincoln, Nebraska, 198 p.
- , and J. Janovy, Jr 2007a. Small frogs get their worms first: The role of non-odonate arthropods in the recruitment of *Haematoloechus coloradensis* and *Haematoloechus complexus* in newly metamorphosed northern leopard frogs, *Rana pipiens*, and Woodhouse's toads, *Bufo woodhousii*. *Journal of Parasitology* **93**: 300–312.
- , and —. 2007b. Evolutionary avenues for and constraints on the transmission of frog lung flukes (*Haematoloechus* spp.) in dragonfly second intermediate hosts. *Journal of Parasitology* **93**: 593–607.
- , and —. 2008. Alternative life cycle strategies of *Megalodiscus temperatus* in tadpoles and metamorphosed anurans. *Parasite Journal De La Societe Francaise De Parasitologie* **15**: 396–401.
- , S. D. Snyder, and J. Janovy, Jr. 2009a. Alternative life-cycle strategies and colonization of young anurans by *Gorgoderina attenuata* in Nebraska. *Journal of Parasitology* **95**: 604–615.
- , —, and —. 2009b. Redescription of the frog bladder fluke, *Gorgoderina attenuata* from the northern leopard frog, *Rana pipiens*. *Journal of Parasitology* **95**: 665–668.
- , H. R. Tracy, and J. Janovy, Jr 2010. The role of damselflies (Odonata: Zygoptera) as paratenic hosts in the transmission of *Halipegus eccentricus* (Digenea: Hemiuridae) to anurans. *Journal of Parasitology* **96**: 724–735.
- Brooks, D. R. 1976. Parasites of amphibians of the Great Plains: Part 2. *Platyhelminthes of amphibians in Nebraska*. *Bulletin of the University of Nebraska State Museum* **10**: 65–92.
- Cameron T. W. M. 1931. Experimental infection of sheep with *Dicrocoelium dendriticum*. *Journal of Helminthology* **9**: 41–44.
- Cort, W. W. 1926. Dr. Ransom's contributions to parasitology. *Journal of Parasitology* **8**: 1–4.

- Dronen, N. O., Jr. 1977. Studies on the population structure of two species of *Haematoloechus* Looss, 1899 (Digenea: Plagiorchiidae) in raniid frogs in New Mexico. *Proceedings of the Helminthological Society of Washington* **44**: 68–72.
- Edward, G. R. 1957. Landmarks of parasitology I. The discovery of the life cycle of the liver fluke. *Experimental Parasitology* **6**: 208–232.
- Esch, W. G. 2004. *Parasites, people, and places: Essays on field parasitology*. Cambridge University Press, Cambridge, U.K., 235 pp.
- . 2007. *Parasites and infectious diseases: Discovery by serendipity, and otherwise*. Cambridge University Press, Cambridge, U.K., 355 p.
- , S. Desser, and B. Nickol. 2014. A history of The Journal of Parasitology. *Journal of Parasitology* **100**: 1–10.
- Euzet, L., and C. Combes. 1980. Les problèmes de l'espèce chez les animaux parasites. *Bulletin de la Société Zoologique Francaise* **40**: 239–285.
- Ewing, S. A. 2001. *Wendell Krull: Trematodes and naturalists*. Stillwater, Oklahoma: College of Veterinary Medicine, Oklahoma State University, 115 pp.
- Goater, T. M., C. L. Brown, and G. W. Esch. 1990. On the life history and functional morphology of *Halipegus occidualis* (Trematoda: Hemiuridae), with emphasis on the cystophorous cercaria stage. *International Journal for Parasitology* **20**: 923–934.
- Goodchild, C. G. 1943. The life-history of *Phyllodistomum solidum* Rankin, 1937, with observations on the morphology, development and taxonomy of the Gorgoderinae (Trematoda). *Biological Bulletin* **84**: 59–96.
- . 1948. Additional observations on the life history of *Gorgodera amplicava* Looss, 1899 (Trematoda: Gorgoderidae). *Journal of Parasitology* **34**: 407–427.
- . 1950. Establishment and pathology of gorgoderid infections in anuran kidneys. *Journal of Parasitology* **36**: 439–446.
- Hall, M. C. 1925. Brayton Howard Ransom. *Science* **62**: 319–320.
- Janovy, J., Jr 2014. Why American higher education needs parasitologists. *Journal of Parasitology* **100**: 700–707.
- Korschgen, L. J., and T. S. Baskett. 1963. Foods of impoundment and stream-dwelling bullfrogs in Missouri. *Herpetologica* **19**: 89–99.
- Krull, W. H. 1930. The life history of two North American frog lung flukes. *Journal of Parasitology* **16**: 207–212.
- . 1931a. Life history studies on two frog lung flukes, *Pneumonectes medioplexus* and *Pneumonectes parvplexus*. *Transactions of the American*

*Microscopial Society* **50**: 215–277.

—. 1931b. Importance of laboratory-raised snail in helminthology with life history notes on *Gyraulus parvus*. *Occasional Papers of the Museum of Zoology, University of Michigan* **226**: 1–10.

—. 1932. Studies on the life history of *Pneumobites longiplexus* (Stafford). *Zoologischer Anzeiger* **99**: 231–239.

—. 1933. Studies on the life history of a frog lung fluke, *Haematoloechus complexus* (Seely, 1906) Krull, n. comb. *Zeitschrift für Parasitenkunde* **6**: 193–206.

—. 1934. Some additional notes on the life history of a frog lung fluke, *Haematoloechus complexus* (Seely, 1906) Krull. *Transactions of the American Microscopical Society* **53**: 196–199.

—. 1935a. Studies on the life history of *Halipegus occidualis* Stafford, 1905. *American Midland Naturalist* **16**: 129–143.

—. 1935b. Studies on the life history of a frog bladder fluke, *Gorgodera amplicava* Looss, 1899. *Papers of the Michigan Academy of Sciences Arts and Letters* **20**: 697–710.

Langford, G. J., and J. Janovy, Jr 2009. Comparative life cycles and life histories of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae): lungworms of frogs and snakes. *Journal of Parasitology* **95**: 1145–1155.

—, and —. 2013. Host specificity of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae): Combining field data and experimental infections with a molecular phylogeny. *Journal of Parasitology* **99**: 277–286.

Mackiewicz, J. S. 1988. Cestode transmission patterns. *Journal of Parasitology* **74**: 60–71.

Macy, R. W., W. A. Cook, and W. R. Demott. 1960. Studies on the life cycle of *Halipegus occidualis* Stafford, 1905 (Trematoda: Hemiuridae). *Northwest Science* **34**: 1–17.

Mohler, J. R. 1926. Dr. Ransom and the Bureau of Animal Industry. *Journal of Parasitology* **8**: 5–9.

Otto, F. G., and A. O. Foster. 1981. Centenary biographical note Maurice Crowther Hall, 1881–1938. *International Journal for Parasitology* **11**: 341–342.

Ransom, H., and M. C. Hall. 1915. The life history of *Gongylonema scutatum*. *Journal of Parasitology* **2**: 80–86.

—, and —. 1917. Note on the life-history of *Gongylonema scutatum*.

*Journal of Parasitology* **3**: 177–181.

Rankin, J. S. 1939. The life cycle of the frog bladder fluke, *Gorgoderina attenuata* Stafford, 1902 (Trematoda: Gorgoderidae). *American Midland Naturalist* **21**: 476–488.

—. 1944. A review of the trematode genus *Halipegus* Loss, 1899, with an account of the life history of *H. amherstensis* n. s. *Transactions of the American Microscopical Society* **63**: 149–164.

Schmidt, A. 1910. Coleopterorum Catalogus, pars 20. *Scarabaeidae, Aphodiinae*. W. Junk and S. Schenkling, Berlin, Germany, 111 p.

Schwartz, B. 1938. Maurice Crowther Hall. *Science* **87**: 451–453.

—, and P. D. Harwood. 1938. Maurice Crowther Hall as a parasitologist. *Journal of Parasitology* **24**: 283–290.

Shoop, W. L. 1988. Trematode transmission patterns. *Journal of Parasitology* **74**: 46–59.

Snyder, S. D. 1996. Host specificity among species of *Haematoloechus* (Digenea: Haematoloechidae). Ph.D. Thesis. University of Nebraska–Lincoln, Lincoln, Nebraska, 112 p.

—, and J. Janovy, Jr 1994. Second intermediate host-specificity of *Haematoloechus complexus* and *Haematoloechus medioplexus* (Digenea: Haematoloechidae). *Journal of Parasitology* **80**: 1052–1055.

—, and —. 1996. Behavioral basis of second intermediate host specificity among four species of *Haematoloechus* (Digenea: Haematoloechidae). *Journal of Parasitology* **82**: 94–99.

—, and V. V. Tkach. 2001. Phylogenetic and biogeographical relationships among some Holarctic frog lung flukes (Digenea: Haematoloechidae). *Journal of Parasitology* **87**: 1433–1440.

Stafford, J. 1905. Trematodes from Canadian vertebrates. *Zoologischer Anzeiger* **28**: 681–694.

Ubelaker, J. E., and O. W. Olsen. 1972. Life cycle of *Phyllodistomum bufonis* (Digenea: Gorgoderidae) from the boreal toad, *Bufo boreas*. *Proceedings of the Helminthological Society of Washington* **39**: 94–100.

Zelmer, D. A., and G. W. Esch. 1998a. Bridging the gap: The odonate naiad as a paratenic host for *Halipegus occidualis* (Trematoda: Hemiuridae). *Journal of Parasitology* **84**: 94–96.

—, and —. 1998b. Interactions between *Halipegus occidualis* and its

ostracod second intermediate host: evidence for castration. *Journal of Parasitology* **84**: 778–782.

—, and —. 1998c. The infection mechanism of the cystophorous cercariae of *Halipegus occidualis* (Digenea: Hemiuridae). *Invertebrate Biology* **117**: 281–287.



# **Chapter 10**

## **A Survey of Meat Samples from Swine, Cattle, and Sheep for the Presence of Encysted *Toxoplasma***

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A number of workers have contributed data to or have expressed the hypothesis that meat may serve as a source of human *Toxoplasma* infection. Miller and Feldman (1953) and Feldman and Miller (1956) reported the occurrence of dye test antibodies in goats, cattle, swine, and sheep in various localities in the United States. Farrell et al (1952) and Momberg-Jorgensen (1956) reported toxoplasmosis in pigs in the United States and in Norway, respectively; and Varela et al (1956) in a study of ocular toxoplasmosis in Mexico, mentioned that they had found *Toxoplasma* in 87 of 817 pigs' eyes examined. Weinman and Chandler (1956) found *Toxoplasma* antibodies in a high percentage of swine fed on uncooked garbage in Connecticut, and a much lower prevalence among pigs fed on cooked garbage or grain. Sanger et al (1953) isolated *Toxoplasma* from cattle in Ohio. Olafson and Monlux (1942) described toxoplasmic encephalomyelitis in a sheep in New York; Wickham and Carne (1950) reported on a similar disease in a sheep in Australia; and Hartley (1957) has recorded perinatal mortality, due to *Toxoplasma*, in sheep in New Zealand. Roever-Bonnet (1957) performed a serological survey of 22 sheep, 29 cows, and 26 pigs at an Amsterdam slaughter-house and found 14 sheep, 6 cows, and 13 pigs with dye test titers of 1:20 or higher; only 11 animals, of the total of 77 studied, gave completely negative reactions. He also isolated *Toxoplasma* from the brains of 4 sheep, out of 30 animals whose brain and heart were inoculated into mice.

Suum (1956) mentioned the possibility that meat, milk, or eggs might serve as the sources of human toxoplasmosis. Van Thiel and Van der Waaij (1956) suggested that pseudocysts in meat or other animal tissues may be responsible for oral infection of man and animals. Weinman and Chandler (1956) presented an impressive case for incriminating undercooked pork as one of the important sources of human infection. Kayhoe et al (1957) mentioned that one of two cases of lymphadenopathic toxoplasmosis they reported occurred in a woman who liked to eat raw meat.

It was pointed out by Jacobs (1957) that the main gap in the evidence thus far was the lack of information on the occurrence of cysts of *Toxoplasma* in the edible flesh of meat animals. On the basis of studies on the resistance of cysts of *Toxoplasma* to digestive juices (Jacobs and Melton, 1957a, Jacobs et al, to be published) and on the distribution of the parasites in the muscle of rats with chronic infections (Jacobs and Melton, 1957b), a survey procedure was developed for demonstrating *Toxoplasma* in meat samples (Jacobs and Melton, 1957c) and preliminary results were presented on pork and beef. It is the purpose of this paper to describe the procedures of our survey work in more detail, and to present data on sheep in addition to more complete data on swine and beef. Some corollary information on the

virulence of the *Toxoplasma* strains isolated, and some incidental findings on *Sarcocystis* will also be given.

#### MATERIALS AND METHODS

On the basis of our study of infections in the muscle of rats, it appeared that the diaphragm could be expected to be infected as frequently as other striated muscles. This made it convenient to choose the diaphragm as the meat sample to be investigated. The advantages were economic and administrative; there was no charge for the samples, and there were no memoranda required to justify the purchase of edible meats. The diaphragms of swine and cattle were provided free of charge by the Schluderberg-Klein Co. of Baltimore. We are grateful to Dr. Somers, Regional Office, U. S. D. A. in Baltimore, for making the arrangements with the S.-K. Company. We are also very thankful to the S.-K. Company for their kindness in transporting the specimens to Washington. The sheep specimens were obtained, also gratis, from the George R. Reid Co. of Baltimore.

The specimens were received fresh on the day of slaughtering or on the next day. They were either processed immediately or were packed in plastic bags and kept in ice for up to 3 days. Fat and fascia were stripped from the muscle, which was then ground in an ordinary household-type meat grinder. The trimmed hog diaphragm specimens ranged in weight from 48 to 137 g, with an average of 91 g. The beef specimens weighed from 55 to 205 g, with an average of 130 g; and the sheep specimens weighed from 12 to 113 g, with an average of 40 g. The ground meat was placed in a 2-liter flask and covered with a volume of peptic digestive solution about 10 $\times$  its weight. The flask was placed on a magnetic stirring device in the hot room at 37° C, and digestion was allowed to proceed for 2 hours. After digestion, the suspension was filtered through several layers of coarse cheese cloth and centrifuged in 250-ml bottles for 15 minutes at 2000 rpm in the International No. 2 Centrifuge. The digestive fluid was poured off, the sediments were pooled into a bottle, resuspended in saline as a wash, and recentrifuged under the same conditions. Finally, the sediment was suspended in 10 to 20 ml of saline, depending on its volume, and inoculated intraperitoneally into 10 mice. Each mouse received 1 ml of the inoculum. Any residual suspension was kept in the refrigerator overnight and the same 10 mice received additional injections on the following day.

The mice were observed for 3 to 4 weeks, during which time any that died were examined by means of fresh smears of peritoneal exudate, if present, and Giemsa-stained impression smears of brain, liver, spleen, and lung. At the end of 1 month, about one-half of the survivors were bled to death or bled from the orbital sinus, and the serum specimens were tested at dilutions of 1:16 and 1:64 in the dye test for toxoplasmosis. In some but not all cases, if positive titers were obtained among these serum specimens, the remaining mice were killed and their tissues were subinoculated into fresh mice.

Estimations of the virulence of various strains of *Toxoplasma* isolated by these procedures were made by counting parasites from peritoneal exudates in a Neubauer-Levy counting chamber, and preparing appropriate dilutions in 10 percent serum-saline solution to give the desired numbers of parasites per ml. Standard amounts of these suspensions were inoculated intraperitoneally into mice, and the number of deaths and the survival times were noted.

#### RESULTS

Table I presents the results of examinations of 50 swine, 60 cattle, and 86 sheep. In some instances, serological tests were positive on the serum specimens of some of the mice, but *Toxoplasma* was not demonstrated in the remaining animals which were killed and subinoculated into fresh animals. In those cases in which *Toxoplasma* was demonstrated in the originally inoculated mice, not all of the mice were found positive. The same was true with second-passage mice. Apparently, the infections in the muscle were relatively light in most animals; and even though an attempt was made to prepare homogeneous suspensions of the digest, some of the inocula given to the mice did not contain parasites. Since our aim, however, was mainly to demonstrate *Toxoplasma* in these samples, once such a demonstration was accomplished no attempt was made to check closely all the mice inoculated with the same specimen. The low dye test titers found in some pools of blood from the mice may have been due to the dilution of a positive sample with a negative one.

TABLE I. Results of studies on mice inoculated with digests of muscle from swine, cattle, and sheep.

Animal	No. of specimens	Groups* of mice found positive by dye test	by demonstration of parasites	Total
Swine	50	4**	8	12
Cattle	60	1†	0	1
Sheep	86	8	4††	8

\* Ten mice (= 1 group) were inoculated from the digest of each specimen.

† One pool of serum from 2 mice gave a titer of 1:16. Two other pools, from 2 mice each, were negative serologically. No isolation was attempted from the remaining 4 mice.

\*\* Isolation was not attempted.

†† Of the 8 groups of mice positive serologically, 6 were subinoculated into fresh mice, and in 4 of these groups toxoplasmas were demonstrated. The titers of the 2 groups negative on isolation were 1:16 (pools of serum from 2 mice each); the titers of the 4 groups positive on isolation were 1:1024 or higher.

Since numerous uninfected mice have been uniformly negative serologically, we believe these titers, although low, are indicative of infection with low numbers of organisms of an avirulent strain of *Toxoplasma*.

On the basis of the total number of samples found positive, either parasitologically or serologically, or by both methods, 24 percent of the swine and 9.3 percent of the sheep harbored encysted *Toxoplasma*. One positive dye test resulted from a pool of serum from 2 mice inoculated from 1 cattle specimen; thus the prevalence rate in these cattle specimens was 1.7 percent.

None of the strains of *Toxoplasma* isolated from sheep was virulent enough to kill mice of the original passage. On the other hand, 7 of the porcine strains killed all or some mice in the groups originally inoculated with them. Table II gives the results of tests of the virulence of some of the porcine strains, as compared to the well known RH strain. These data are more definite than percentages of kill of the originally inoculated mice, which could have varied with the intensity of infection in the samples. One of the strains tabulated, S-6, was highly virulent, producing death of mice as rapidly as the RH strain. The others were less pathogenic. Since in most cases the tests of virulence were made with parasites from chick embryos, which were infected as soon as possible after the parasites were demonstrated, these differences in virulence are considered natural, and not due to continuous passage through mice. The latter sometimes results in enhanced virulence of originally slow-growing strains, while little change occurs on passage in embryonated eggs (Jacobs and Melton, 1954).

TABLE II. Comparison of porcine strains with the RH strain as to their virulence for mice.

Strain	No. of mice dead/no. inoculated with designated number of parasites, and survival days	10	100	1000
S 2	—		10/12 (13.1)	11/12 (12.7)
S 4	17/20 (14.6)*		17/20 (13.5)	20/20 (11.9)
S 5	19/20 (12.3)		18/19 (10.9)	19/19 (10.8)
S 6	18/20 ( 8.7)		19/20 ( 7.8)	19/20 ( 7.2)
S 7	8/10 (13.1)		16/18 (11.7)	18/20 (10.3)
R H	10/10 ( 8.6)		10/10 ( 7.7)	10/10 ( 7.0)

\* Figures in parentheses are the average survival days of the mice that succumbed to the infection.

One of the strains listed in Table II, S-2, was originally very slow-growing. It required 20 passages in cortisonized mice before an adequate number of parasites was available for use in a virulence test; it was not established in chick embryos until it had undergone 24 passages in mice. The data shown in table II are there-

fore not indicative of the original virulence of this strain which was very low. The strains isolated from sheep were similarly avirulent, and were not obtained in large enough numbers for virulence tests even after several passages in mice.

Microscopic examination of the washed sediment, after digestion and centrifuging, was carried out on the majority of the specimens. This was done with a 40X objective and 10X ocular. In no instance was *Toxoplasma* seen by this technique. However, a remarkable number of *Sarcocystis* infections were found. Of 39 hog specimens thus examined, 17 or 43.6 percent had *Sarcocystis*. All of the cattle and sheep digests were examined microscopically, and 98.3 percent and 97.7 percent, respectively, showed *Sarcocystis*.

In addition to the diaphragm of sheep, portions of the esophagus of some of these animals were examined macroscopically and with the use of the dissecting microscope in an attempt to find the Miescher's sacs. These were never seen, despite the finding of the individual spores in the digest. It is considered possible that the animals were too young, when slaughtered, for the *Sarcocystis* to have developed the large sacs described for *S. tenella* of sheep. This experience also suggests, however, that studies of the biology and transmission of *Sarcocystis* can be carried on more profitably by a digestion technique to reveal the parasites, than by attempts to see the Miescher's sacs in the muscle. The spores seen in the concentrated digest were motile and apparently unharmed by the treatment.

#### DISCUSSION

It is believed that the above results represent the first definitive demonstration of *Toxoplasma* cysts in the flesh of meat animals. They lend considerable support to the hypothesis that man can acquire the infection by consuming undercooked meat. While our sample of beef yielded only one positive serological result, it is believed that *Toxoplasma* may possibly be demonstrated to occur more frequently in other samples. It is to be remembered that Feldman and Miller (1956) found, in the same geographic locale, two herds of cattle with significant amounts of *Toxoplasma* antibody and two with none. It is quite possible that our small sample was derived principally from negative herds, and that a larger sample from more herds will reveal a higher prevalence rate for the parasite in this host.

Our studies of the effects of digestion on proliferative and encysted forms of *Toxoplasma* (Jacobs and Melton, 1957a, and Jacobs et al, to be published) indicate that the parasites demonstrated by the present technique were encysted organisms. The ability of these forms to survive a 2-hour digestion in artificial gastric juice furnishes additional support for the meat-to-man hypothesis of transmission. However, the importance of this avenue in the epidemiology of human toxoplasmosis requires evaluation in the light of certain serological data on human beings in various areas. For example, vegetarians in the United States have been shown to have *Toxoplasma* antibodies in relatively high titer (Jacobs, 1957). Also, there is as high a prevalence rate of serologic positives among Hindus, who are strict vegetarians, as among Moslems, who eat mutton (Rawal, 1959); indeed this rate is as high as that in the English midlands (Beverley et al, 1954). It seems obvious that, since herbivores acquire toxoplasmosis, there must be some route of transmission other than by the ingestion of infected flesh, and it is possible that *Toxoplasma* can also be acquired by humans by such a route.

It now becomes clear that more detailed studies are necessary to evaluate, in various geographic areas, the role of meat animals in the epidemiology of toxoplasmosis. The hypotheses of Weinman and Chandler (1956) of a rat-to-swine cycle, and a swine-to-swine cycle through the medium of uncooked garbage, require further study. Although these authors found the highest prevalences of *Toxoplasma* antibodies among swine fed uncooked garbage, epidemics of toxoplasmosis have occurred in localized herds of grain-fed swine as well. It may be possible to elucidate the life cycle of *Toxoplasma gondii* by studying local epidemics among herds of domestic animals.

The demonstrations of *Toxoplasma* in sheep and cattle were mainly on the basis of serologic tests of mice inoculated with the digests. The strains were so low in virulence that they did not kill the mice. On the other hand, some of the strains from swine were highly virulent and killed a high proportion of the mice inoculated with them. The variations found among strains of *Toxoplasma* isolated from swine were very striking. At the present time, all we know about differences in virulence, beyond the descriptions of pathogenicity for mice and other laboratory animals, is that these are correlated with invasiveness and rate of multiplication in tissue culture (Kaufman et al, 1958). It would be most interesting and important to understand what factors, if any, in the physiology of a host account for the differences in strains seen in nature. It would be most important, also, to investigate the possibility that the severity of the disease toxoplasmosis is correlated more with one source of infection than with another.

Beyond these points, our corollary data on the high prevalence of *Sarcocystis* in these meat animals are of considerable interest in regard to the serology of toxoplasmosis. No serologic survey of *Toxoplasma* antibodies in swine, cattle, or sheep has revealed any such high prevalence rate as we found for *Sarcocystis* by microscopic examination of the digests. Unfortunately, it was not possible for us to obtain blood specimens corresponding to the diaphragm specimens; so we cannot make a direct comparison here. However, there were obviously many *Sarcocystis* spores in the inoculum given to the mice, and these must have provided some antigenic stimulus. Despite this, the number of positive dye test reactions in the mice bears no relation at all to the number of samples found positive for *Sarcocystis*. Since *Sarcocystis* has been reported (Scott, 1943) to be very prevalent in meat animals, the cross-reactions between *Sarcocystis* and *Toxoplasma* related by Mühlfordt (1951) and by Awad (1954) seem highly unlikely.

#### SUMMARY

By means of a peptic digestion technique for concentrating parasites from relatively large samples, a survey of *Toxoplasma* infection in diaphragm muscle from 50 swine, 60 beef cattle, and 86 sheep was conducted. After digestion and washing, the sediment from the digests was inoculated into mice. *Toxoplasma* was isolated from 8 samples from swine and 4 from sheep. In addition, 4 pork, 1 beef, and 4 mutton specimens produced dye test antibodies in the mice inoculated with them. Thus, 24 percent of swine, 1.7 percent of cattle, and 9.3 percent of sheep were demonstrated to have *Toxoplasma* infection. The parasite strains from swine varied from low to high virulence; those from sheep were all of low virulence. *Sarcocystis* was found in 43.6 percent of swine, 98.3 percent of cattle, and 97.7 percent of sheep.

The relation of these findings to the epidemiology of human toxoplasmosis and to the specificity of serological tests for this infection is discussed.

#### REFERENCES

- AWAD, F. I. 1954 The diagnosis of toxoplasmosis. Lack of specificity of Sabin-Feldman dye test. *Lancet* **267**: 1055-6.
- BEVERLEY, J. K. A., BEATTIE, C. P. AND ROSEMAN, C. 1954 Human *Toxoplasma* infection. *J. Hyg.* **52**: 37-46.
- FARRELL, R. L., DOCTON, F. L., CHAMBERLAIN, D. M. AND COLE, C. R. 1952 Toxoplasmosis I. *Toxoplasma* isolated from swine. *Am. J. Vet. Res.* **13**: 181-185.
- FELDMAN, H. A. AND MILLER, L. T. 1956 Serological study of toxoplasmosis prevalence. *Am. J. Hyg.* **64**: 320-335.
- HARTLEY, W. J. 1957 Toxoplasmosis as a cause of ovine perinatal mortality. *N. Z. Vet. J. (Dec.)*: 119-124.
- JACOBS, L. 1957 The interrelation of toxoplasmosis in swine, cattle, dogs, and man. *Pub. Health Rept.* **72**: 872-882.
- AND MELTON, M. L. 1954 Modifications in virulence of a strain of *Toxoplasma gondii* by passage in various hosts. *Am. J. Trop. Med. Hyg.* **3**: 447-457.
- AND — 1957a Studies on the resistance of pseudocysts of *Toxoplasma gondii*. *J. Parasit.* **43** (supp.) : 38.
- AND — 1957b The distribution of *Toxoplasma gondii* in the muscles of rats with chronic infections. *J. Parasit.* **43** (supp.) : 41-42.
- AND — 1957c A procedure for testing meat samples for *Toxoplasma*, with preliminary results of a survey of pork and beef samples. *J. Parasit.* **43** (supp.) : 38-39.
- , REMINGTON, J. S. AND MELTON, M. L. The resistance of the encysted form of *Toxoplasma gondii*. *J. Parasitol.* **46**: 11-21.
- KAUFMAN, H. E., REMINGTON, J. S. AND JACOBS, L. 1958 Toxoplasmosis. The nature of virulence. *Am. J. Ophth.* **46**: 255-260.
- KAYHOE, D. E., JACOBS, L., BEYE, H. K. AND McCULLOUGH, N. B. 1957 Acquired toxoplasmosis. Observations on two parasitologically proved cases treated with pyrimethamine and triple sulfonamides. *New Eng. J. Med.* **257**: 1247-1254.
- MILLER, L. T. AND FELDMAN, H. A. 1953 Incidence of antibodies for *Toxoplasma* among various animal species. *J. Inf. Dis.* **92**: 118-120.
- MOMBERG-JORGENSEN, H. C. 1956 Toxoplasmosis in pigs. *Nord. Vet.-Med.* **8**: 227-238.
- MÜHLFFORDT, H. 1951 Das Verhalten Sarcosporidien-infizierter Tiere im Serofarbstest auf Toxoplasmose nach Sabin-Feldman. *Ztschr. Tropenmed. Parasit.* **3**: 205-215.
- OLAFSON, P. AND MONLUX, W. S. 1942 *Toxoplasma* infection in animals. *Cornell Vet.* **32**: 176-190.
- RAWAL, B. D. 1959 Toxoplasmosis. A dye test survey on sera from vegetarians and meat eaters in Bombay. *Trans. Roy. Soc. Trop. Med. Hyg.* **53**: 61-63.
- ROEVER-BONNET, H. DE 1957 Toxoplasmosis in slaughter-cattle, particularly in sheep. *Doc. Med. Geograph. Trop.* **9**: 336-338.
- SANGER, V. L., CHAMBERLAIN, D. M., CHAMBERLAIN, K. W., COLE, C. R. AND FARRELL, R. L. 1953 Toxoplasmosis V. Isolation of *Toxoplasma* from cattle. *J. Am. Vet. Med. Assoc.* **123**: 87-91.
- SCOTT, J. W. 1943 Economic importance of Sarcosporidia, with especial reference to *Sarcocystis tenella*. *Univ. Wyo. Agr. Exp. Sta. Bull.* No. 262.
- SIIM, J. C. 1956 Toxoplasmosis acquisita lymphonodosa: Clinical and pathological aspects. *Ann. N. Y. Acad. Sci.* **64**: 185-206.
- VAN THIEL, P. H. AND VAN DER WAALJ, D. 1956 The significance of pseudocysts in the oral infection of man and animals with *Toxoplasma gondii*. *Doc. Med. Geograph. Trop.* **8**: 392-396.
- WARELA, G., ROCH, E. AND TORROELLA, J. 1956 Estudio de toxoplasmosis ocular. *Rev. Inst. Salub. y Enferm. Trop.* **16**: 17-19.
- WEINMAN, D. AND CHANDLER, A. H. 1956 Toxoplasmosis in man and swine. An investigation of the possible relationship. *J. Am. Med. As.* **161**: 229-232.
- WICKHAM, N. AND CARNE, H. R. 1950 Toxoplasmosis in domestic animals in Australia. *Austral. Vet. J.* **26**: 1-3.



# **Transmission of *Toxoplasma gondii*—From land to sea, a personal perspective**

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*Toxoplasma gondii* is one of the most widespread parasitic microbes. It infects virtually all warm-blooded species throughout the world, including Antarctica. From its discovery in 1908 until 1970, its transmission had been a mystery. I have had the good fortune of doing research on this subject for more than 50 years, and being instrumental in unraveling the *T. gondii* life cycle in 1970. Therefore, I feel privileged to be a part of this centennial celebration of *The Journal of Parasitology*. Before the discovery of the oocyst stage, who would have thought that we were surrounded by a universe of *Toxoplasma* infections?

A paper by Jacobs, Remington, and Melton (1960b) titled, “A survey of meat samples from swine, cattle, and sheep for the presence of encysted *Toxoplasma*,” is considered a landmark discovery in the study of *T. gondii*. It took another decade to resolve the life cycle of this “euphonious and enigmatic parasite” as termed by the eminent parasitologist, Paul Weinstein (see Jacobs, 1979). Because I lived through the euphoria surrounding the discovery of its life cycle, I would like to share this story here, especially the lessons I learned, for the benefit of young scientists. The present paper concerns people, places, and difficulties encountered in solving problems, and is not about the priority of discoveries by different groups.

# The scientific scene in 1960 and significance of the quoted paper

Through the use of a novel serologic test, the “dye test” by Sabin and Feldman (1948), it had become clear that *T. gondii* infection was common in humans and other animals (Feldman and Miller, 1956), but its mode of transmission was still largely unknown. Transmission through ingestion of infected meat was suspected by Weinman and Chandler (1956), who found a higher prevalence in pigs fed uncooked garbage than in pigs fed cooked garbage. At the time of the publication by Jacobs, Remington, and Melton (1960b), we were in the golden age of science and the National Institutes of Health (NIH), Bethesda, Maryland was the source of many biomedical discoveries. *The Journal of Parasitology* was the most prestigious journal in parasitology. There was no internet and very few overseas libraries had foreign journals. *Biological Abstracts*, *Tropical Diseases Bulletin*, *Veterinary Bulletin*, and *Current Contents* were the main means of disseminating scientific information. At the time, I was a graduate student in veterinary parasitology in India. Believe it or not, I spent a day's allowance of my fellowship on postage to request the paper by Jacobs, Remington, and Melton (1960b). Within 4 weeks of this request, Dr. Jacobs sent me his paper—I still have it.

Now, a few words regarding the authors, before discussing the significance of their findings: Dr. Leon Jacobs was a senior scientist in the Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, Maryland. He was trained in classical parasitology at the George Washington University. Leon was 22 years old when he joined NIH in 1937 (NIH was established in 1930) and served there for 41 years. He was known for precision in experimentation and scientific writing. Leon had also served as the editor of *The Journal of Parasitology* and President of the American Society of Parasitologists, and had been awarded the Henry Baldwin Ward Medal. An example of his precise writing is illustrated in the materials and methods section of the paper describing the first isolation of viable *T. gondii* from the eye of a blind person (Jacobs et al., 1954). Beginning in 1966, Dr. Jacobs assumed administrative positions, first as Director of NIAID, then as Director of the Division of Biological Standards, and later as Associate Director of NIH, Deputy Assistant Secretary for Science in the Department of Health, Education, and Welfare, and Director of the Fogarty International Center. In these positions, he helped many young researchers, including me. According to Jack Remington, “honesty and integrity were his middle names.” Leon was a researcher at heart and regretted leaving the bench research when the discovery of *T. gondii* oocyst was so close (pers. obs., J. P. Dubey). He died in 1995 at the age of 80.

The second author of the paper, Jack S. Remington, had just joined the Jacobs laboratory, purely by chance. This 26-year-old had just completed his M.D. degree and wanted to become a neurosurgeon. But that all changed when he received a phone call regarding his selection as one of the 12 promising medical graduates

recruited from across the country for training in NIH laboratories in a unique Research Associate Fellowship Program. The program was unique in the sense that the fellows attended graduate seminars for half of each day and spent the other half in research in a laboratory of their choosing. The invited lectures were given by the most prominent scientists invited from throughout the U.S.A. While waiting for the interview outside the office of the Chief of the NIAID, he came across a paper by Jacobs and Lunde (1957) on an indirect hemagglutination test for toxoplasmosis in the May 1957, issue of *Science* magazine. This article interested him, and he therefore decided to work with Leon Jacobs. While at NIH from 1957–1959, Remington published several papers on congenital transmission of *T. gondii*. After a 3-year stay at Harvard University Medical School, Jack then joined the Palo Alto Medical Foundation and the Stanford University Medical School, where he trained numerous M.D.s in infectious diseases over the next 57 years. In the process, he became the world's foremost authority on human toxoplasmosis. An example of precision and careful editing of scientific papers, that surpassed even his mentor, is given in his own handwriting by J. G. Montoya (2002), one of his disciples.

Marjorie L. Melton, the third author of the paper, was a dedicated assistant to Leon Jacobs. She kept accurate records, and managed laboratory operations on a daily basis. She continued to carry the torch after Jacobs became an administrator, and Dr. Harley Sheffield became her supervisor. She retired from NIH in 1977, at the age of 59 years after 34 years of service. She will long be remembered for helping so many scientists, including Jack Remington, who visited NIH.

Jacobs, Remington, and Melton (1960a) discovered that the *T. gondii* tissue cyst wall is immediately dissolved by proteolytic enzymes (pepsin, trypsin) but that the released parasites (bradyzoites) survive for 2–3 hours in the presence of these enzymes. With this knowledge, they developed a method to isolate *T. gondii* from meat samples (Jacobs et al., 1960b—the quoted paper). The problem was the sparse distribution of cysts in tissues of asymptomatic animals (now estimated 1 cyst/50–100 g of meat: Dubey, 2010). They ground muscle tissue (diaphragm) in a household meat grinder, and incubated it in a pepsin-HCl mixture for 2 hours at 37°C. The digest was centrifuged 2–3 times and the digest was inoculated intraperitoneally into mice. The inoculated mice were examined for *T. gondii* infection, both for seroconversion and for viable parasites. Using this procedure, they isolated viable *T. gondii* from 8 out of 50 samples of pork and from 4 out of 86 samples of sheep meat, but none from 60 samples of beef. Subsequently, I modified their pepsin digestion procedure making it possible to test large numbers of samples in shorter time (Dubey, 1998).

Results of this investigation showed that *T. gondii* encysts commonly in meat, and that infected, uncooked meat could be a source of infection for humans. Two of the strains (M 7741, ME 49) of *T. gondii* isolated from diaphragm of sheep by Jacobs, Remington, and Melton (1960b) continue to be widely used for research.

Subsequently, we were to use the M 7741 strain to complete the *T. gondii* life cycle (Dubey et al., 1970a, 1970b; Frenkel et al., 1970; Dubey and Frenkel, 1976; Freyre et al., 1989; Dubey, 2002). The ME 49 strain (specific clones of which are called P strain and PLK strain) is now used worldwide as the standard type II genetic strain, and its genome has been characterized (Dubey, 2010). Credit goes to Marjorie Melton for the maintenance and distribution of these strains long after Leon Jacobs had retired from the lab.

# **Discovery of the organism now known as *Toxoplasma gondii***

## **Early work on *T. gondii***

Nicolle and Manceaux (1908) had originally found a protozoan in tissues of a rodent, the gundi (*Ctenodactylus gundi*), which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis, Tunisia. They initially believed the parasite to be a species of *Leishmania*, but soon realized that they had discovered a new organism and named it *Toxoplasma gondii* based on the morphology (mod. L. *toxo* = arc or bow, *plasma* = life) and the host (Nicolle and Manceaux, 1909). Splendore (1908) discovered the same parasite in rabbits in Brazil, also erroneously identifying it as *Leishmania*, but he did not name it. The rabbits were kept in his laboratory for research and they died of acute disease without prior clinical signs.

It is a remarkable coincidence that this disease was first recognized in laboratory animals and was first thought to be *Leishmania* by both groups of investigators. Nicolle and Splendore were both medical doctors. Charles Jules Henri Nicolle was a well-known French scientist and eventually received in 1928 the Nobel Prize for his work on typhus. Manceaux, also French, was an assistant to Nicolle. Alfonso Splendore had emigrated from Italy to Brazil, where he made numerous contributions in microbiology; he was to become the Chief Scientist at the Bacteriological Laboratory at Portuguese Hospital, São Paulo, Brazil. It is noteworthy that, although they were on different continents, they communicated with each other about their discoveries.

Transmission of *T. gondii* was at first a mystery. After the parasite's discovery, Chatton and Blanc (1917) found that gundis, native to the foothills and mountains of southern Tunisia, were not infected naturally, but acquired infection in captivity. They suspected that *T. gondii* was transmitted by arthropods because it was found in the blood of the host and transmission of *Plasmodium* and *Leishmania* species by arthropods was on the minds of researchers at the time. Chatton and Blanc (1917) in Tunis and Woke et al. (1953) and others in the U.S.A. investigated possible transmission by several species of arthropods with completely unsuccessful results (Frenkel, 1970, 1973).

## **Congenital transmission**

It is ironic that the congenital mode of transmission was recognized first, because it is not the most common mode of transmission of *T. gondii*. In 1939, three pathologists working in New York City conclusively identified *T. gondii* in an infant girl who was delivered full-term by Caesarean section on May 23, 1938, at Babies Hospital, New York (Wolf et al., 1939a, 1939b). The girl developed convulsive seizures at 3 days of age and lesions were noted through an ophthalmoscope in the maculae of both eyes. She died when she was only 1 month

old and an autopsy was performed. The brain, spinal cord, and the right eye were removed for examination. Free and intracellular *T. gondii* were found in lesions of encephalomyelitis and retinitis of the girl. Portions of cerebral cortex and spinal cord were homogenized in saline and inoculated intracerebrally into rabbits and mice, producing encephalitis. *Toxoplasma gondii* was demonstrated in the neural lesions and *T. gondii* from these animals was successfully passaged into other mice.

Congenital transmission was later found to occur in many species of animals, particularly sheep (Hartley and Marshall, 1957) and rodents. Congenital infections can recur through successive pregnancies in some strains of mice (Beverley, 1959), with infected mice producing congenitally infected offspring for at least 10 generations. Beverley discontinued his experiments because of high mortality in some lines of congenitally infected mice, and because the progeny from the last generation of infected mice were seronegative, even with undiluted serum (Pers. Commun. J. P. Dubey) and presumed not to be infected with *T. gondii*. Jacobs (1964) repeated these experiments and found that congenitally infected mice may lack antibodies due to immune tolerance. Dubey et al. (1995) isolated viable *T. gondii* from naturally-infected mice that were found to have no antibodies to *T. gondii*. These findings were to become of epidemiological significance because serological surveys in mice may underestimate the prevalence of *T. gondii*.

Unlike in mice, congenital transmission in humans, sheep, goats, rats, and many other species has been found to occur, for the most part, during primary infection of the mother during pregnancy and not during chronic infection. In contrast, transmission can occur during the chronic stage of infection in some species of rodents, Pallas and sand cats, and some other small mammals (Dubey, 2010).

## **Carnivory**

As noted earlier, Weinman and Chandler (1956) suggested that transmission might occur through the ingestion of undercooked meat. This hypothesis for transmission via the ingestion of infected meat was tested by Desmonts et al. (1965) in an experiment with children in a Paris sanatorium. They compared the acquisition rates of *T. gondii* infection in children before and after admission to the sanatorium. Of the 1,125 children admitted to the Children's Tuberculosis Department of the Hospital of Brévannes, 641 (56.9%) had no antibodies to *T. gondii* at the time of admission, whereas 204 of 641 children became seropositive during their hospital stay. The mean duration of hospitalization of these children was 9 months (from 3 months to more than 1 year). Children were serologically tested at admission and then every 4 months until the end of hospitalization (although some children had just one test).

All the children were given beef meat juice (one teaspoon/day) and raw horsemeat (20 to 60 g, two or three times/week); these children represented the control group. The monthly rate of acquisition of *T. gondii* infection was 4.8%, which was

five times higher than in the general population (deduced from data in children at the time of admission). For the experimental group (66 children), barely cooked lamb chops were added twice a week to the usual diet of beef meat juice and raw horsemeat. The monthly rate of acquisition of *T. gondii* infection was 9.2%, which was nearly twice that of the other hospitalized children.

Desmonts et al. (1965) estimated that the time required to infect 50% of the hospital population to be 5 years for a general population, a little more than 1 year for the hospitalized children (usual diet), but a little more than 6 months for the experimental group (those fed lamb chops). Because the prevalence of *T. gondii* is much higher in sheep than in horses or cattle (Dubey and Beattie, 1988; Dubey, 2010), the Desmonts et al. (1965) study illustrated the importance of carnivory in the transmission of *T. gondii*. Although beef is rarely infected with *T. gondii*, viable *T. gondii* has been isolated from horsemeat destined for human consumption and severe toxoplasmosis has been reported recently in humans in France after eating imported horsemeat (Al-Khalidi and Dubey, 1979; Pomares et al., 2011; Sobanski et al., 2013). The Desmonts et al. (1965) experiment was justified ethically because of the prevailing belief at that time that feeding of uncooked meat was beneficial in recovery from tuberculosis (pers. comm., G. Desmonts).

## **Lesson one: Important discoveries can be made using epidemiological data**

Several small outbreaks of clinical toxoplasmosis have been epidemiologically linked to the ingestion of undercooked meat (Dubey, 2010). Of these reports, two meat-associated episodes are described here. Five medical students developed clinical toxoplasmosis during the second week after eating hamburgers at the Cornell University Medical School snack bar (Kean et al., 1969). These five students lived and ate separately, except for one evening when they were in a hurry to attend a talk by the famous heart surgeon Dr. Christian Barnard, and the hamburgers were made in a hurry. It seems plausible that the beef used to prepare hamburgers may have been contaminated with infected pork. Not only is ingesting infected meat important in transmission, but contaminated meat grinders, the handling of raw meat, and knives can be sources of infection. A report from France illustrated this mode of transmission when a woman developed fatal toxoplasmosis, apparently after being savagely stabbed with a butcher's knife (Ferre et al., 1986).

## **Fecal-oral, sexual cycle, and discovery of the oocyst**

Although carnivory can explain some of the transmission of *T. gondii*, it does not explain the widespread infection in vegetarians and herbivores. A study in Bombay (now Mumbai), India found the prevalence of *T. gondii* in strict vegetarians to be similar to that in non-vegetarians (Rawal, 1959). The real breakthrough in the discovery of the life cycle of the parasite came when

Hutchison (1965) first discovered that *T. gondii* infection was associated with cat feces.

Dr. William McPhee Hutchison was a parasitologist in the Department of Biology, Strathclyde University, Glasgow, Scotland (Ferguson, 2009). He had a Ph.D. in parasitology, but had never done research on protozoans before. In a preliminary experiment, Hutchison (1965) fed the Beverley strain of *T. gondii* tissue cysts to a cat experimentally infected 2 months previously with *Toxocara cati*. He collected feces containing nematode ova, starting 2 weeks after feeding *T. gondii*. The feces, after floatation in 33% zinc sulfate solution and storage in tap water in an open beaker for 12 months to embryonate *T. cati* eggs, induced toxoplasmosis in mice. This discovery was a breakthrough because, until then, both known forms of *T. gondii*, i.e., tachyzoites and bradyzoites, were killed by water.

Microscopic examination of the feces revealed only *T. cati* eggs and *Isospora* oocysts. In Hutchison's (1965) published report, *T. gondii* infectivity was not attributed to either oocysts or *T. cati* eggs. He suspected transmission of *T. gondii* via the eggs of *T. cati*, similar to the transmission of the fragile flagellate *Histomonas meleagridis* by way of *Heterakis gallinae* eggs. He initially wanted to test the nematode hypothesis using *Toxocara canis* and *T. gondii* transmission in a dog, but decided on the cat and *T. cati* model because there was no place to house dogs (see Dubey, 2008). Transmission of *T. gondii* by *T. canis* eggs made more sense because of the known zoonotic potential of *T. canis*; *T. cati* was not, at that time, known to infect humans, but *T. canis* was. It is probable that the discovery of the life cycle of *T. gondii* would have been delayed if Hutchison had worked with dogs instead of cats.

Three other British scientists, J. K. A. Beverley, C. P. Beattie, and P. C. C. Garnham played important advisory roles in this discovery. Drs. Beverley and Beattie were experts on toxoplasmosis at the University of Sheffield, and Hutchison visited them in September 1964 with a draft of his proposed paper on *T. gondii*. Hutchison was convinced regarding the association of *T. cati* and *T. gondii*. On the advice of Beverley and Beattie, Hutchison added caution in the concluding paragraph of his paper: "At present it is impossible to say whether the infection induced by this material was transmitted by helminth ova or by some other means." (Hutchison, 1965) Hutchison had a great regard for Beverley and Beattie and brought with him a tape recorder to record their conversation.

Hutchison later contacted Dr. Garnham (who was the most famous and influential parasitologist in Britain at that time, and who was also an examiner for my Ph.D. thesis). Dr. Garnham helped Hutchison obtain funding for the following experiment. I should note that I am describing his experiment here for the benefit of future graduate students, to underscore that even a brilliantly planned experiment requires repetition to adequately test a hypothesis.

Hutchison (1967) used two cats (#5, #8) in the second experiment whose serological status for *T. gondii* had not been determined. Cat #5 had previously

been experimentally infected with *T. cati* and was shedding nematode ova in the feces. Cat #8 had not been infected with *T. cati*. Hutchison fed *T. gondii*-infected mice to each cat for 5 days and floated feces in zinc sulfate to collect ova. After incubating the ova in water to embryonate them, he orally inoculated fecal floats to groups of six mice. *Toxoplasma gondii* infection was achieved from the feces of cat #5 from day 13 through day 30, when the experiment was terminated; fecal floats from cat #8 were all negative. He then reversed the role of cat #5 and cat #8. He gave an anthelmintic to cat #5, to remove worms, and experimentally infected cat #8 with *T. cati*—and repeated *T. gondii* feeding and fecal infectivity testing. As he had expected, no fecal infectivity was detected in the feces of cat #5, but cat #8 was shedding *T. gondii* in feces from days 17 through 30 when the experiment was terminated. Thus, *T. gondii* was transmitted only in association with *T. cati* infection. On this basis, Hutchison (1967) hypothesized that *T. gondii* was transmitted through nematode ova.

Hutchison had a preconceived hypothesis of nematode transmission of *T. gondii*. In hindsight, we now know that the chance of success of Hutchison's second experiment was remote (perhaps 1 in 1,000, or worse) because cats usually pass oocysts only upon primary exposure; once they become immune, they usually do not pass oocysts upon re-exposure to the parasite (Dubey, 2010).

I have been involved with investigating transmission of *T. gondii* for more than 50 years. I became especially interested in the summer of 1961, but only by an accident at a Veterinary Institute in India (Dubey, 1996a). I wanted to work on toxoplasmosis in sheep for my Master's degree thesis, but was compelled to work on coccidians of animals, including the feral Indian cat (*Felis chaus*) under the supervision of legendary Professor B. P. Pande (Dubey and Pande, 1963). Little did I know then that I would be working on this parasite for the rest of my life. Fortunately, I was awarded a Commonwealth Scholarship for pursuing a Ph.D. and arrived in Sheffield in September 1964 to work with Dr. J. K. A. Beverley and Professor C. P. Beattie. Although Beverley was a physician, he had done pioneering research on toxoplasmosis abortion in sheep. He did postgraduate studies on toxoplasmosis under Professor Beattie who was a walking encyclopedia on toxoplasmosis. Professor Beattie wrote critiques and comments on articles on toxoplasmosis abstracted in *Tropical Diseases Bulletin*. Dr. John Kenneth Addison Beverley died in 1976 at the age of 66 from prolonged illness. He was a great thinker and planned his experiments very carefully. Beverley wrote several of his papers (with assistance from Professor Beattie) while hospitalized for terminal illness. Some of his unpublished research on ovine toxoplasmosis was posthumously included (with proper credit) in our book, *Toxoplasmosis of Animals and Man* (Dubey and Beattie, 1988).

## **Lesson two: I learned early in my career was not to delay publishing research**

As noted previously, I had planned to work on the transmission of *T. gondii* in

sheep, but all that changed when Beverley told me of Hutchison's initial finding. Thus, I learned of his *Toxocara-Toxoplasma* discovery 9 months before its publication in May 1965. I began research with this problem right away. Bill Hutchison (see photo in Dubey, 1977a) and I became friends and he knew that I was also working on *T. gondii*. Hutchison had not yet conducted his second experiment when I visited his laboratory at Strathclyde University in 1965; the "magic beaker" containing the *Toxoplasma* and *Toxocara* was sitting open on his laboratory bench (he added water to it from time to time).

Hutchison shared the results of his second experiment with cats #5, and #8 before it was submitted for publication. I was so impressed with the design of his second experiment that I was anxious to confirm rather than analyze the results critically. Moreover, I did not know how to interpret my isolation of *T. gondii* from feces of a *T. cati*-free cat (Dubey, 1966), but went ahead and published it (Dubey, 1968). I had invested an enormous effort looking for *T. gondii* inside *T. cati* eggs, and was seeking an association between *T. cati* and *T. gondii* in naturally infected cats (Dubey, 1966). However, I had to return to India in 1967 after completing my Ph.D. as part of the scholarship agreement. In the meantime, Professor J. K. Frenkel from the University of Kansas had obtained an NIH grant to work on Hutchison's hypothesis, and fortunately he accepted me as a research associate in his laboratory. I joined him in the summer of 1968.

Hutchison's (1965) report stimulated other investigators to examine fecal transmission of *T. gondii* through *T. cati* eggs (Jacobs and Melton, 1966; Jacobs, 1967; Hutchison et al., 1968; Frenkel et al., 1969; Sheffield and Melton, 1969). Jacobs at the NIH (Jacobs and Melton, 1966), and I at Sheffield University (Dubey, 1966) were the first to retest Hutchison's hypothesis of nematode transmission of *T. gondii* and to isolate *T. gondii* from *T. cati*-free cats, but again the focus was on *T. cati* and not *T. gondii* itself (Jacobs and Melton, 1966; Jacobs, 1967).

In 1968, Harley Sheffield assumed the leadership of the lab at NIH that Jacobs vacated when he took an administrative position. Harley was hired by Leon to study ultrastructure of protozoan parasites. Harley had a head start in this muddled *Toxocara-Toxoplasma* field because of Marjorie Melton, who had worked with Jacobs, and who possessed an immense knowledge of the biology of the parasite. In the meantime, Hutchison and his associate, Dunachie, collaborated with a research group in Denmark, headed by Dr. J. C. Siim and an ophthalmologist, Dr. Work (see Ferguson, 2009). Soon after, the nematode egg theory of transmission was discarded after *T. gondii* infectivity was dissociated from *T. cati* eggs (Frenkel et al., 1969) and *T. gondii* infectivity was found in feces of worm-free cats fed *T. gondii* tissue cysts (Frenkel et al., 1969; Sheffield and Melton, 1969).

Finally, in 1970, knowledge of the *T. gondii* life cycle was completed by discovery of the sexual phase of the parasite in the small intestine of the cat (Frenkel et al.,

1970). *Toxoplasma gondii* oocysts, the product of schizogony and gametogony, were found in cat feces and characterized morphologically and biologically (Dubey et al., 1970a, 1970b). Like other coccidians, *T. gondii* oocysts were demonstrated to be environmentally resistant (Dubey et al., 1970a, 1970b). Unlike other scientists working on *T. gondii* transmission, I had been trained in the life cycles and morphology of coccidian parasites, and I used this knowledge to fully characterize the coccidian part of the life cycle of *T. gondii*. Harley Sheffield used his electron microscopy experience to provide the first descriptions of the *T. gondii* sporozoite, and schizogony in the cat intestine (Sheffield, 1970; Sheffield and Melton, 1970). Sheffield also discovered that *T. gondii* sporozoites lack the crystalloid body present in other coccidian sporozoites; he died in 2008 at the age of 76.

### **Lesson three: Knowledge never goes to waste**

I am still perplexed about the success of the second experiment of Hutchison with cats #5, and #8. Knowing the life cycle of *T. gondii* now, the chances of success of Hutchison's experiment were remote because cats usually shed oocysts for approximately 1 week, and peak oocyst shedding occurs 5–8 days after ingesting tissue cysts (Dubey and Frenkel, 1972). Additionally, cats that have shed oocysts become immune to subsequently shedding more oocysts, especially when challenged with a homologous strain of *T. gondii*, as later shown (Dubey, 1995, 2010). For these reasons, only 1% of cats shed *T. gondii* oocysts at any given time.

Hutchison died in 1998 at the age of 74, but I have full faith that he recorded his results accurately. Eggs of *T. cati* are very sticky and pitted, and oocysts are very small; in retrospect, some of the discrepant findings (including my own) may be attributable to those physical characteristics; perhaps we discarded most of the 10 µm diameter oocysts in the filtrate (I used a 63 µm pore size sieve and Hutchison used 36 µm sieve to filter fecal floats to collect *T. cati* ova) down the drain and worked with those few oocysts remaining stuck to ova. Additionally, *T. gondii* oocysts are coated with lipids (cat food and feces are rich in lipids) and they even float in water. For this reason, we now autoclave or incinerate all water-sugar mixtures used to float cat feces, and do not dispose of any cat feces by pouring them down the drain. The reproductive potential of *T. gondii* in the cat intestine is very high, and I have collected as many as a million oocysts from a cat fed a single bradyzoite (Dubey, 2001). The Beverley strain that Hutchison and I used at Sheffield forms hundreds of cysts in mice, and we fed several infected mice to each cat.

*Toxocara cati* ova and *T. gondii* oocysts have different densities and it is therefore difficult to find them in same plane of focus. One successful attempt to do so is shown in [Fig. 1](#).



**Figure 1** Sugar fecal float of cat feces showing unsporulated oocysts of *T. gondii* (arrows), *Cystoisospora (Isospora) felis* (f), and *C. rivolta* (r). *Toxoplasma gondii* oocysts are the smallest of all feline coccidia, averaging  $10 \times 12 \mu\text{m}$ . *Cystoisospora felis* oocysts are pear-shaped and average  $40 \times 30 \mu\text{m}$ . *Cystoisospora rivolta* oocysts are ovoid and measure about  $25 \times 20 \mu\text{m}$ . All three coccidian oocysts are compared to the size of a *Toxocara cati* (c) egg, the common feline round worm (from Dubey, 2010).

Separating *T. gondii* from *T. cati* now may appear to like child's play, but it took a great scientific effort by several highly acclaimed laboratories to achieve it.

## Lesson four: Big discoveries can be made in a small laboratory

Hutchison deserves all the credit for discovering the fecal transmission of *T. gondii* and his work proves that big discoveries can be made in a small laboratory. Hutchison's research also shows that rigorous experimentation is needed to truly test a hypothesis.

### Discovery of *T. gondii* oocysts

Several groups of workers, independently and at about the same time, found *T. gondii* oocysts in cat feces (Hutchison et al., 1969, 1970, 1971; Frenkel et al., 1970; Dubey et al., 1970a, 1970b; Overdulve, 1970; Sheffield and Melton, 1970; Weiland and Kühn, 1970; Witte and Piekarski, 1970). The discovery of *T. gondii* oocysts in cat feces and its implications have been reviewed by Frenkel (1970, 1973), Garnham (1971), Jacobs (1973), and Ferguson (2009). There were ill feelings, precipitated by antagonistic personalities, and groups seeking priority, among some of the researchers involved in the discovery of the oocyst. I would like to cite the wisdom expressed by Leon Jacobs (1973) in his review paper; he stated that in addition to the timing of publication, "the reports must be judged on their completeness."

The papers by Sheffield and Melton (1970), and Frenkel et al. (1970) were submitted to *Science* independently in October 1969, but were published together in February 1970. These papers were supposed to be published in an earlier issue of the journal, but were delayed because of the publication of events related to

landing of man on the moon (although *T. gondii* is half-moon in shape, it was no match for the real thing). According to Garnham (1971), Hutchison phoned Professor Garnham in late December 1969, to tell him of the oocyst discovery and within a week a letter was printed in the last 1969 (December 27) issue of the *British Medical Journal*, followed by a full article 3 weeks later (Hutchison et al., 1969; 1970). Gordon Wallace presented his studies on the natural epidemiology of *T. gondii* in the Pacific islands (discussed later) and our discovery of the life cycle of *T. gondii* at the November 1969, Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) in Washington, D.C. Thus, many people came to know of the discovery of the oocyst before its official publication.

Several scientists including Leon Jacobs, William Trager, and Harley Sheffield contributed to discussion of this paper presented at the ASTMH meeting. Although I could not attend the meeting, Dr. R. L. Hoff (he was a postdoctoral fellow in Frenkel's laboratory at the same time as I was) recorded the presentation and discussion and provided me a typed script, which I still have. Thus, oocyst talk was "in the air" in the fall of 1969. I was just a post-doctoral fellow in Frenkel's laboratory at that time and had many sleepless nights due to all the excitement. Gordon Wallace was a very good friend and collaborator of Frenkel's and he was aware of our work on the discovery of the oocyst. Although discovery of the oocyst stage in the life cycle of a coccidian parasite would be expected, proving *T. gondii* to be a coccidian parasite was a big challenge at that time for several reasons. First, fecal infectivity (from oocysts) was linked with *T. gondii* infection (Hutchison, 1965, 1967). Second, the oocyst had been called a new cyst (Siim et al., 1969; Work and Hutchison, 1969a, 1969b). *Toxoplasma gondii* oocysts were morphologically identical to oocysts of the previously described coccidian parasite (*Isospora bigemina*) of cats and dogs (Dubey et al., 1970a).

In view of these facts, the evidence provided by us to link *T. gondii* oocysts to fecal infectivity is noteworthy (Dubey et al., 1970a; Frenkel et al., 1970). We subjected the newly discovered fecal stage (oocyst) to the following mutually independent tests to accumulate critical evidence for or against its similarity to *T. gondii*: (1) the use of newborn kittens and littermate controls used to avoid pre-existing coccidial infections as far as possible; (2) comparison of the development of oocysts and of infectivity in relation to heat, cold, oxygenation, and chemicals; (3) comparison by filtration of the size of the infection's entity with oocyst size; (4) comparison of the density characteristics of oocysts and of infectivity; (5) comparison of the electrophoretic characteristics of oocysts and infectivity; (6) antigenic comparison of oocysts with the standard RH strain of *Toxoplasma* by means of the fluorescent antibody test; (7) identification of the endogenous cycle preceding the development of oocysts and linking it, antigenically, to *Toxoplasma* infectivity of oocysts before and after excystation; and (8) comparison of the appearance of oocysts and *T. gondii* infectivity in feces of cats after feeding of cysts, tachyzoites, and oocysts (Dubey et al., 1970a). The full description of the oocyst was published in the *Journal of Experimental Medicine* (Dubey et al.,

1970a). This lengthy paper had gone through 50 drafts (there was no track change facility available at that time), but was published as submitted; Dr. René Dubos (famous bacteriologist and Pulitzer Prize Winner for General Non-Fiction), Editor of *The Journal of Experimental Medicine*, sent a hand written congratulatory note that I have cherished all my life.

Overdulve (1970) wanted to synonymize *T. gondii* with *Isospora bigemina*. In 1973, I obtained a faculty position in the Department of Veterinary Pathobiology at The Ohio State University (OSU). Germ-free cats and dogs were developed in the 1960s at OSU. Thus, I had access to laboratory raised cats and dogs and I used them to sort out the identity of *I. bigemina* and other feline and canine coccidians. I continued this research when I moved to my present position at USDA in 1982. It turned out that *I. bigemina* is *nomen nudum*, consisting of numerous species of *Toxoplasma*, *Sarcocystis*, *Hammondia*, and *Neospora* (for discussion of this issue, see Dubey et al., 2002; Dubey, 2009).

At the USDA, the focus of my research shifted to epidemiology, diagnosis, and control of toxoplasmosis and related protozoan diseases. I learned the value of collaborative research and timely responses to the needs of livestock producer groups, farmers, zoo veterinarians, and pregnant women worried about birth defects. I was awoken one night (there is a 4-hour time difference between Maryland and Alaska) by a telephone call from a concerned zoo veterinarian from Anchorage, Alaska, who suspected toxoplasmosis as cause of sudden death of two prized exhibited polar bears. It turned out that the bears had died of massive hepatitis due to *Sarcocystis canis* infection (Garner et al., 1997), a parasite that we had discovered a few years earlier as cause of fatal hepatitis in dogs (Dubey and Speer, 1991). An example of our collaborative research, involving 28 scientists from eight countries can be found in Dubey et al. (2002)

## **Lesson five: Scientific research is a joint effort, and mutual trust and fairness are the key elements for any successful collaboration**

I learned the value of consulting original papers, rather than relying on abstracts. I learned from Leon Jacobs' papers that all literature (good or bad) should be reviewed, and I have tried to uphold this ideal in preparing my books on toxoplasmosis in animals (Dubey and Beattie, 1988; Dubey, 2010). For 20 years, I had relied on Professor Beattie to translate many articles to English; he had a Master's degree in classics before he went to medical school and knew many languages. Professor Colin Panton Beattie died in 1987 at the age of 84, before publication of the book I co-wrote in his honor (Dubey and Beattie, 1988). When I visited Mrs. Beattie (herself a bacteriologist) in Sheffield in 2004, she told me that Colin was very happy about the book and that he had always wanted to write a book on toxoplasmosis, himself; he discarded many drafts because they were not perfect.

## **Lesson six: Even the mightiest can be afraid to publish**

For me, scientific papers are about telling the facts and imperfection is nothing to fear; others can help correct grammar as many have done for me.

Related to the subject of publication, Beattie had told me a story me about his colleague, Hans Adolf Krebs, Professor of Physiology at the University of Sheffield. Professor Krebs received Nobel Prize, in 1953, for discovering what is now known as the *Krebs Cycle*. His 1937 paper describing the citric cycle was rejected by *Nature* and subsequently published in a Dutch journal *Enzymologia*. This incident prepared me to accept then process rejection of my papers by a journal; sometimes such rejection was based on one reviewer's opinion.

In retrospect, the discovery and characterization of the *T. gondii* oocyst in cat feces was also delayed because until 1970 coccidian oocysts had been sporulated in 2.5% potassium dichromate. Chromation of the oocyst's wall interfered with excystation of the sporozoites when oocysts were fed to mice; thus the mouse infectivity titer of the oocysts was lower than expected from the number of oocysts administered (Dubey et al., 1970a). These findings led to the use of 2% sulfuric acid as the best medium for sporulation and storage of *T. gondii* oocysts. Unlike dichromate, which was difficult to wash off the oocysts, sulfuric acid could be easily neutralized and the oocysts could be injected directly into mice without washing (Dubey et al., 1972). Unlike other coccidians, *T. gondii* oocysts were found to excyst efficiently when inoculated parenterally into mice. This observation alleviated the need for oral inoculation for bioassay of oocysts.

Ben Rachid (1970) fed *T. gondii* oocysts to gundis, which died 6–7 days later from toxoplasmosis. This knowledge about the life cycle of *T. gondii* probably explains how gundis became infected in the laboratory of Nicolle. At least one cat was present in the Pasteur laboratory in Tunis (Frenkel, 1970; Dubey, 1977a). In the old times, cats were kept in animal houses to control rodents and we can surmise a cat-rodent-bird cycle.

All three of us in Frenkel's laboratory (Frenkel, Miller, and me) and scientists in other laboratories who worked with cat feces became infected with *T. gondii* during 1968 and 1969 before we realized the highly infectious nature of oocysts (Miller et al., 1972). I had no antibodies to *T. gondii* when tested on November 5, 1968. At the end of April 1969, over a weekend I developed sore throat, fatigue, and was found to be seropositive on 30 April 1969; my antibody titer rose 16-fold in next 3 weeks but attempts to isolate *T. gondii* from blood and feces were negative. I had bilateral enlargement of cervical lymph nodes for 6 months; the internist who examined me reached out for my neck to palpate lymph nodes every time I saw him down the hallway.

# **Discovery of the full life cycle**

## **Developmental stages in the intestine of cats**

As stated earlier, it became clear in 1970, that there was profuse multiplication of *T. gondii* in the intestine of cats after eating tissue cysts (Sheffield, 1970). The challenge for me was to sort out different asexual stages in enterocytes because the entire asexual and sexual cycle could be completed in 60 hours (Dubey and Frenkel, 1972). We found five morphologically distinct types of *T. gondii* could develop in enterocytes before gametogony. These stages are designated types A–E (instead of generations because there are several generations within each *T. gondii* type). These asexual stages in the feline intestine are structurally distinct from tachyzoites that also develop in the lamina propria. The enteroepithelial stages (Types A–E, gamonts) are formed in the intestinal epithelium and the development of types B–E schizonts in enterocytes has been confirmed ultrastructurally (Speer and Dubey, 2005). Occasionally, type B and C schizonts develop within enterocytes that are displaced beneath the epithelium into the lamina propria.

I would like to emphasize that the asexual types A–C are time-dependent stages, few in number, and difficult to find; they are not artifacts, abnormal stages or tachyzoites as some have suggested (Dubey, 2010). To find these early stages, I examined hundreds of sections from the small intestines of cats fed tissue cysts. To find the earliest stage, type A, I had sectioned at 5 µm the entire duodenum of a kitten fed tissue cysts and microscopically examined all sections at 1,000 × magnification. When this very long paper with 128 figures describing in detail the entero-epithelial cycle of *T. gondii* was submitted to *The Journal of Protozoology* for publication, one very famous reviewer remarked that he did not believe the existence of numerous generations but had no evidence to reject the paper. The main objection was that until our life cycle paper, the life cycles of all known coccidian parasites were thought to have fixed generations of schizonts with morphologically distinct merozoites. Professor N. D. Levine, editor of *The Journal of Protozoology*, spent a lot of effort in editing our paper. He also revealed to me that the reviewer for my *T. gondii* cycle was none other than Leon Jacobs; Jacobs' wisdom and fairness were reflected in the comments made to improve my paper. It turned out that other coccidians of cats and dogs follow a similar, asexual multiplication cycle as in *T. gondii*. Dr. Levine was the expert in coccidiosis at that time and although I never worked under him he edited many of my papers on coccidiosis and taught me how to describe coccidian stages starting in 1962.

## **Lesson seven: Not finding does not mean non-existence and one should have faith in one's findings**

### **Other aspects of transmission and life cycle**

Although *T. gondii* has a wide host range it has retained the definitive host

specificity restricted to felids. Dr. J. K. Frenkel deserves the credit for initiating the testing of many species of animals, including wild felids, for oocyst shedding under difficult housing conditions (Miller et al., 1972). It was an experience of a lifetime for me, handling bobcats and ocelots in cages. I was a post-doctoral fellow in Frenkel's laboratory and the memory of one incident is still vivid in my mind decades after it happened. My neighbor, who had a pet ocelot, loaned me the animal for testing for oocyst shedding. Three of us, Jack Frenkel, Nancy Miller (she was a biological technician), and I were sweating while trying to obtain a blood sample (without giving anesthesia) from this 15-kg ocelot before feeding it tissue cysts. The ocelot shed oocysts and we returned the animal to the owner. Of the many species of animals experimentally infected with *T. gondii*, only felids shed *T. gondii* oocysts.

Frenkel also suggested that we use newborn kittens to study the life cycle of *T. gondii*. I used many hundreds of kittens to work out the complete life cycle of *T. gondii*. We had a unique program of persuading cat owners in Kansas City to donate kittens for research. After a few months, word got around and owners recognized my Indian accent on the phone, and thus spared me a lengthy introduction. They loaned their cats with a promise that we would vaccinate the dams against feline pathogens, raise kittens, and return the dam to the owners. This procedure allowed me to infect part of the litter with *T. gondii* tissue stages and keep one or two littermates as controls. It turned out that we were fortunate as not to expose the dam to *T. gondii* infection. The litter was euthanized as soon as oocysts were shed, and before oocysts were sporulated; I later found that *T. gondii* oocysts have a very low infectivity for cats (Dubey, 1996b), and that *T. gondii* is not transmitted congenitally by a dam infected prior to pregnancy (Dubey, 1977b).

Additionally, the *T. gondii* cycle was the same in kittens whether or not they were born to exposed queens (Dubey and Frenkel, 1972). Prior to experimentation, I did not find *T. gondii* oocysts in the feces of any cat (Dubey, 1973). That was in the 1970s. With current regulations of animal welfare, such experimentation would be unthinkable. Frenkel was also correct in his opinion that "specific pathogen free" is exaggerated. It only means freedom from pathogens for which one can accurately test. It should not be confused with germ-free. Retrospectively, we know that *Cystoisospora (Isospora) felis*, the coccidian with the largest oocyst ([Fig. 1](#)), is universal in distribution, even in commercial cat colonies in the U.S.A. that are otherwise free of pathogens, even feline viruses.

Dr. Jacob Karl Frenkel died in 2013 at the age of 92. In 2008, both of us were awarded the Splendore and Nicolle Medal by the *Toxoplasma* Centennial Celebration Committee (and presented in Brazil by Splendore's grandson). Dr. Frenkel was a great researcher. He kept reminding me that scientists become famous for solving difficult problems and for providing conclusive evidence to support a hypothesis.

Seroepidemiological studies on isolated islands in the Pacific (Wallace, 1969), Australia (Munday, 1972), and the U.S.A. (Dubey et al., 1997) have shown an absence of *T. gondii* on islands without cats, confirming the important role of the cat in the natural transmission of *T. gondii*. Wallace's study on Pacific Islands was remarkable and full of challenges. These islands are really remote and their main contact with the outside world was via U.S. Coast Guard cutter ships. Wallace was allowed only 2 days to work on each island. Wallace was also one of the first to find *T. gondii* oocysts in the feces of naturally infected cats (Wallace, 1971a). Additional studies revealed that earthworms (Dubey et al., 1970b), flies and cockroaches (Wallace, 1971b–1972) could act as transport hosts and further disseminate oocysts in the environment. Like other coccidian oocysts, *T. gondii* oocysts were found to be highly resistant to environmental influences including commonly used disinfectants (Dubey et al., 1970b; Yilmaz and Hopkins, 1972). I am still searching for a chemical that will kill *T. gondii* oocysts without harming humans.

Vaccinating cats with a live mutant strain of *T. gondii* on eight pig farms in the U.S.A. reduced the transmission of *T. gondii* infection in mice and pigs (Frenkel et al., 1991; Mateus-Pinilla et al., 1999), again supporting the role of cats in natural transmission of *T. gondii*. Although *T. gondii* can be transmitted in several ways, it has adapted to be transmitted most efficiently by carnivory in the cat and by the fecal-oral (oocysts) route in other hosts. Pigs and mice (and presumably humans) can be infected by ingesting just one oocyst (Dubey et al., 1996), whereas a dose of 100 oocysts might not infect cats (Dubey 1996b). Additionally, the ingestion of only a few oocysts by certain hosts, e.g., mice and New World monkeys, can be lethal whereas oocysts are not pathogenic orally to cats, irrespective of the dose or the strain of *T. gondii*. Cats can shed millions of oocysts after ingesting only one bradyzoite, whereas ingestion of 100 bradyzoites may not infect mice when administered orally (Dubey, 2001). This information has proved very useful in conducting epidemiological studies and for the detection of low numbers of *T. gondii* in large samples of meat by feeding to cats (Dubey et al., 2005). Thus, it has been possible to detect a few bradyzoites in a 500 g piece of meat by feeding it to a cat and then examining cat feces for oocyst shedding (Dubey et al., 1995a).

## Oocyst-associated outbreaks

Oocysts shed into the environment have caused several outbreaks of toxoplasmosis in humans (Teutsch et al., 1979; Benenson et al., 1982; Bowie et al., 1997; de Moura et al., 2006; Baldursson and Karanis, 2011). The episode (known as the “stable outbreak”) described by Teutsch et al. (1979) added another dimension to the mode of transmission. In October 1977, an outbreak of acute toxoplasmosis occurred in patrons of a riding stable in Atlanta, Georgia, U.S.A. Thirty-five out of 37 patrons of the stable had clinical toxoplasmosis characterized by headache, fever, lymphadenopathy and abortion in one of three pregnant patrons. That woman was in her first trimester at the time of the outbreak. She

aborted in December, 1977, and viable *T. gondii* was isolated from the fetus' amniotic fluid (Teutsch et al., 1980; Dubey et al., 1981). An epidemiological investigation suggested that the patrons acquired *T. gondii* from oocysts aerosolized during the riding activity, although attempts to isolate oocysts from 29 samples of soil, sand and sawdust from different parts of the stable were unsuccessful (Dubey et al., 1981). Attempts were made to isolate *T. gondii* from animals trapped in and around stable. Viable *T. gondii* were isolated from tissues of two of four kittens, all of three adult cats, and all four mice trapped in the stable in November, 1977 (sample 1) but not from any of 12 mice, three rats or four cotton rats trapped in the stable in December, 1977 (sample 2). All four mice from whose tissues viable *T. gondii* was isolated had no detectable antibodies to *T. gondii* at a 1:2 serum dilution (prozone was excluded) using the dye test. Bulldozing of the arena between collection of samples 1 and 2 might have contributed to differences in results obtained with these two samplings.

In a Canadian waterborne outbreak of toxoplasmosis in humans (Bowie et al., 1997), the outbreak was epidemiologically linked to contamination of a municipal water reservoir with oocysts. *Toxoplasma gondii* oocysts were found in the feces of naturally-infected cougars (Aramini et al., 1998) trapped near the reservoir, but oocysts were not isolated from the water reservoir (Isaac-Renton et al., 1998).

A Brazilian outbreak was linked to the supply from a water reservoir that was not sealed, and at least one *T. gondii*-infected cat was around the reservoir (Dubey et al., 2004; de Moura et al., 2006). *Toxoplasma gondii* was isolated for the first time from drinking water stored in small water tanks on school roofs; these tanks were served by the reservoir epidemiologically linked to the Brazilian outbreak (de Moura et al., 2006). The mode of isolation is of interest. The water stored in tank was dirty and clogged the filters intended to remove large particles. Eluting oocysts from the clogged filters was a major hurdle. We fed these filthy filters to *T. gondii*-free pigs; the filters passed through the pig gut undigested but pigs developed antibodies to *T. gondii*, and cats fed pig meat shed *T. gondii* oocysts. Thus, the knowledge that pigs were highly susceptible to oocyst infection (Dubey et al., 1996) proved useful in isolating viable *T. gondii* from environmental samples. Subsequently, genetic typing of the *T. gondii* isolate from water linked it to infected humans using multilocus DNA sequencing and serotyping (Vaudaux et al., 2010).

After the discovery of the life cycle of *T. gondii* in the cat, it became clear why Australasian marsupials and New World monkeys are highly susceptible to clinical toxoplasmosis. The former evolved apparently in the absence of the cat (there were few or no cats in Australia and New Zealand before settlement by Europeans) and the latter live in tree tops, and thus not likely to be exposed to oocysts. In contrast, marsupials in America and Old World monkeys are resistant to clinical toxoplasmosis (Dubey and Beattie, 1988).

One of the unresolved questions is how people become infected with *T. gondii* in

nature, especially in geographically remote or culturally unique areas of the world. A limited survey by Feldman and Miller (1956) indicated that Eskimos in Alaska were not exposed to *T. gondii*. Subsequently, Peterson et al. (1974) reported that not only were *T. gondii* antibodies widely prevalent (28% of 1,572) in Alaskan natives, but also that domesticated cats were common, and the close association of cats, humans, and food animals favored transmission of *T. gondii*. Recently, Prestrud et al. (2007) reported that 43% of 594 arctic foxes (*Alopex lagopus*), 7% of 149 barnacle geese (*Branta leucopsis*), and 6% of 17 walruses (*Odobenus rosmarus*) from the Arctic of Svalbard, Norway had antibodies to *T. gondii* (but that 604 herbivores from the same region were seronegative). According to these authors, there are no wild felids in Svalbard, and domestic cats are prohibited, thus transmission via oocysts is unlikely.

Kristin Prestrud was brave enough to put a tent in the frozen Arctic of Svalbard, trap and serologically test 11 live foxes, seek permission to euthanize one of 11 seropositive foxes, and bring with her unfrozen tissues of the infected fox to my laboratory at USDA; her attempts to isolate *T. gondii* were successful (Prestrud et al., 2008). Elucidation of this apparently entirely carnivorous transmission of *T. gondii* is awaited. Comparison of sera from humans in such localities with sera from strict vegetarians in certain sects of people in India who are strict vegetarians (some Jains wear a mask on their face so as not to inadvertently swallow even insects, let alone eating meat) might provide standards for the differential serologic testing with the bradyzoite versus sporozoite ELISA developed by Hill et al. (2011).

One of the most intriguing recent findings related to oocyst biology concerns widespread *T. gondii* infections in marine mammals (Cole et al., 2000). Who would have thought that non-captive marine mammals would be dying of toxoplasmosis acquired from freshwater run-off from land contaminated with oocysts (Cole et al., 2000; Conrad et al., 2005; Thomas et al., 2007; Shapiro et al., 2010; VanWormer et al., 2013). Sea otters eat approximately 25% of their body weight in invertebrate prey each day. Cold blooded animals, including fish, are not regarded as intermediate hosts for *T. gondii*, as shown by Dubey and Beattie (1988) and Dubey (2010). With respect to oocysts, it is not known if the sporozoite excysts after ingestion of oocysts by ectothermic animals. Molluscs, anchovies, sardines, and other aquatic animals, however, can act as transport hosts for *T. gondii* oocysts (Lindsay et al., 2004; Miller et al., 2008; Massie et al., 2010). *Toxoplasma gondii* oocysts can sporulate and remain infectious in seawater for 2 years at 4°C and 1.5 years at room temperature (Lindsay and Dubey, 2009). In addition, sea otters might ingest oocysts directly from marine water. How much marine water is cycled through the gut of sea otters in a day is unknown; however, it is likely to be a large quantity. Among the other unexplained modes of transmission, is *T. gondii* prevalence in insectivorous and frugivorous bats (Cabral et al., 2013; Yuan et al., 2013).

Although there is only one species of *Toxoplasma*, many *T. gondii* genotypes are

now known and there are geographic differences among isolates that might have bearing on transmission and clinical disease (Lehmann et al., 2006; Su et al., 2012). Little is known of the transmission potential or characteristics of *T. gondii* in the wild and of sylvatic cycles. Historically, studies have been focused on the house mouse-domestic cat cycle. As an example, a strain from an Alaskan black bear produced few or no *T. gondii* oocysts in domestic cats (Dubey et al., 2010). More than 30% of 32 million white-tailed deer in the U.S.A. have antibodies to *T. gondii*, indicating widespread contamination of the environment with oocysts (Dubey et al., 2013).

Even though humans have solved some mysteries concerning the transmission of *T. gondii*, we must not forget that it is a very “clever” parasite and prevention of oocyst shedding by the “alley cat” is not going to be easy. Historically, it is thought that the parasite moved indoors when Egyptians brought the cat into their homes to control rats. I only hope that enthusiasts do not take the cat to other planets. Remember, the cat has more than nine lives and has been the last living creature standing alive in a famine.

### **Lessons to be learned from the colonization of the world by *T. gondii*: To be successful, adapt to the environment, and live and let live**

On a personal note, in 2010, I was elected to be a member of the United States National Academy of Sciences. I have been often asked about my success in research. I feel blessed to be at the right place at the right time, and while that may have been out of my control, I did make full use of the opportunity presented to me. I learned not to be afraid of being scientifically challenged or of publishing. I am truly grateful for the opportunities to conduct research, and the encouragement of my teachers. I emigrated to the U.S.A. in 1968 with eight dollars (the maximum exchange allowed by India at that time); Dr. Frenkel arranged for a loan from a church in Kansas City to pay for my airfare, and for that I am grateful.

### **Lesson eight: I learned that hard work alone is not enough for success**

In 1964, Professor Beattie was invited to give the Lister (the famous bacteriologist) Fellowship lecture by the Royal college of Physicians of Edinburgh. He chose to summarize what was known of toxoplasmosis at that time (Beattie, 1964). Soon after I arrived in Sheffield in 1964, Professor Beattie presented me copy of this 64 page publication with a hand written note “to J. P. Dubey in the hope that he will remove some of the ignorance shown in this.” He must have had a premonition.

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## Literature cited

- Al-Khalidi, N. W., and J. P. Dubey. 1979. Prevalence of *Toxoplasma gondii* infection in horses. *Journal of Parasitology* **65**: 331–334.
- Aramini, J. J., C. Stephen, and J. P. Dubey. 1998. *Toxoplasma gondii* in Vancouver Island cougars (*Felis concolor vancouverensis*): Serology and oocyst shedding. *Journal of Parasitology* **84**: 438–440.
- Baldursson, S., and P. Karanis. 2011. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks—an update 2004–2010. *Water Research* **45**: 6603–6614.
- Beattie, C. P. 1964. Toxoplasmosis. Royal College of Physicians of Edinburgh. Publication No. 28. Edinburgh, U.K. Published by T. and A. Constable Ltd., Printers to the University of Edinburgh. p. 1–64.
- Benenson, M. W., E. T. Takafuji, S. M. Lemon, R. L. Greenup, and A. J. Sulzer. 1982. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *New England Journal of Medicine* **307**: 666–669.
- Ben Rachid, M. S. 1970. Contribution à l'étude de la toxoplasmose du gondi. II. Comportement du *Ctenodactylus gundi* vis-à-vis de *Isospora bigemina*. *Archives de l'Institut Pasteur de Tunis* **47**: 33–35.
- Beverley, J. K. A. 1959. Congenital transmission of toxoplasmosis through successive generations of mice. *Nature* **183**: 1348–1349.
- Bowie, W. R., A. S. King, D. H. Werker, J. L. Isaac-Renton, A. Bell, S. B. Eng, and S. A. Marion. 1997. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* **350**: 173–177.
- Cabral, A. D., A. R. Gama, M. M. Sodré, E. S. M. M. Savani, M. A. Galvão-Dias, L. R. Jordão, M. M. Maeda, L. E. O. Yai, S. M. Gennari, and H. F. J. Pena. 2013. First isolation and genotyping of *Toxoplasma gondii* from bats (Mammalia: Chiroptera). *Veterinary Parasitology* **193**: 100–104.
- Chatton, E., and G. Blanc. 1917. Notes et reflexions sur le toxoplasme and le toxoplasmose du gondi (*Toxoplasma gundii* Ch. Nicolle et Manceaux 1909). *Archives de l'Institut Pasteur de Tunis* **10**: 1–41.
- Cole, R.A., D. S. Lindsay, D. K. Howe, C. L. Roderick , J. P. Dubey, N. J. Thomas, and L.A Baeten. 2000. Biological and molecular characterization of *Toxoplasma gondii* strains obtained from Southern Sea Otters (*Enhydra lutris nereis*). *Journal of Parasitology* **86**: 526–530.
- Conrad, P. A., M. A. Miller, C. Kreuder, E. R. James, J. Mazet, H. Dabritz, D. A. Jessup, F. Gulland, and M. E. Grigg. 2005. Transmission of *Toxoplasma*: Clues

from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *International Journal for Parasitology* **35**: 1155–1168.

de Moura, L., L. M. G. Bahia-Oliveira, M. Y. Wada, J. L. Jones, S. H. Tuboi, E. H. Carmo, W. M. Ramalho, N. J. Camargo, R. Trevisan, R. M. T. Graça, et al. 2006. Waterborne toxoplasmosis, Brazil, from field to gene. *Emerging Infectious Diseases* **12**: 326–329.

Desmonts, G., J. Couvreur, F. Alison, J. Baudelot, J. Gerbeaux, and M. Lelong. 1965. Étude épidémiologique sur la toxoplasmose: De l'influence de la cuisson des viandes de boucherie sur la fréquence de l'infection humaine. *Revue Fransçaise d'Études Cliniques et Biologiques* **10**: 952–958.

Dubey, J. P. 1966. Toxoplasmosis and its transmission in cats with special reference to associated *Toxocara cati* infestations. Ph.D. Thesis. University of Sheffield, Sheffield, U.K., 169 p.

\_\_\_\_\_. 1968. Isolation of *Toxoplasma gondii* from the feces of a helminth free cat. *Journal of Protozoology* **15**: 773–775.

\_\_\_\_\_. 1973. Feline toxoplasmosis and coccidiosis: A survey of domiciled and stray cats. *Journal of the American Veterinary Medical Association* **162**: 873–877.

\_\_\_\_\_. 1977a. *Toxoplasma*, *Hammondia*, *Besnoitia*, *Sarcocystis*, and other tissue cyst-forming coccidia of man and animals. In *Parasitic Protozoa*, J.P. Kreir, (ed.) Academic Press, New York, N.Y., **3**: 101–237.

\_\_\_\_\_. 1977b. Attempted transmission of feline coccidia from chronically infected queens to their kittens. *Journal of the American Veterinary Medicine Association* **170**: 541–543.

\_\_\_\_\_. 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. *Journal of Parasitology* **81**: 410–415.

\_\_\_\_\_. 1996a. WAAP and Pfizer Award for excellence in veterinary parasitology research pursuing life cycles and transmission of cyst-forming coccidia of animals and humans. *Veterinary Parasitology* **64**: 13–20.

\_\_\_\_\_. 1996b. Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. *Journal of Parasitology* **82**: 957–961.

\_\_\_\_\_, 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Veterinary Parasitology*. **74**: 75–77.

\_\_\_\_\_. 2001. Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. *Journal of Parasitology* **87**: 215–219.

- \_\_\_\_\_. 2002. Tachyzoite-induced life cycle of *Toxoplasma gondii* in cats. *Journal of Parasitology* **88**: 713–717.
- \_\_\_\_\_. 2008. The history of *Toxoplasma gondii*—the first 100 years. *Journal of Eukaryotic Microbiology* **55**: 467–475.
- \_\_\_\_\_. 2009. The evolution of the knowledge of cat and dog coccidia. *Parasitology* **136**: 1469–1475.
- \_\_\_\_\_. 2010. *Toxoplasmosis of animals and humans*, 2nd ed. CRC Press, Boca Raton, Florida, 313 p.
- \_\_\_\_\_, and C.P. Beattie. 1988. *Toxoplasmosis of animals and man*, CRC Press, Boca Raton, Florida, 220 p.
- \_\_\_\_\_, and J. K. Frenkel. 1972. Cyst-induced toxoplasmosis in cats. *Journal of Protozoology* **19**: 155–177.
- \_\_\_\_\_, and \_\_\_\_\_. 1976. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *Journal of Protozoology* **23**: 537–546.
- \_\_\_\_\_, and B. P. Pande. 1963. Observations on the coccidian oocysts from Indian jungle cat (*Felis chaus*). *Indian Journal of Microbiology* **3**: 103–108.
- \_\_\_\_\_, and C. A. Speer. 1991. *Sarcocystis canis* n. sp. (Apicomplexa: Sarcocystidae), the etiologic agent of generalized coccidiosis of dogs. *Journal of Parasitology* **77**: 522–527.
- \_\_\_\_\_, B. C. Barr, J. R. Barta, I. Bjerkås, C. Björkman, B. L. Blagburn, D. D. Bowman, D. Buxton, J. T. Ellis, B. Gottstein, et al. 2002. Redescription of *Neospora caninum* and its differentiation from related coccidia. *International Journal for Parasitology* **32**: 929–946.
- \_\_\_\_\_, D. E. Hill, J. L. Jones, A. W. Hightower, E. Kirkland, J. M. Roberts, P. L. Marcket, T. Lehmann, M. C. B. Vianna, K. Miska, et al. 2005. Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: Risk assessment to consumers. *Journal of Parasitology* **91**: 1082–1093.
- \_\_\_\_\_, J. K. Lunney, S. K. Shen, O. C. H. Kwok, D. A. Ashford, and P. Thulliez. 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. *Journal of Parasitology* **82**: 438–443.
- \_\_\_\_\_, N. L. Miller, and J. K. Frenkel. 1970a. The *Toxoplasma gondii* oocyst from cat feces. *Journal of Experimental Medicine* **132**: 636–662.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1970b. Characterization of the new fecal form of *Toxoplasma gondii*. *Journal of Parasitology* **56**: 447–456.

\_\_\_\_\_, I. T. Navarro, C. Sreekumar, E. Dahl, R. L. Freire, H. H. Kawabata, M. C. B. Vianna, O. C. H. Kwok, S. K. Shen, P. Thulliez, et al. 2004. *Toxoplasma gondii* infections in cats from Paraná, Brazil: Seroprevalence, tissue distribution, and biologic and genetic characterization of isolates. *Journal of Parasitology* **90**: 721–726.

\_\_\_\_\_, A. R. Randall, S. Choudhary, L. R. Ferreira, S. K. Verma, S. Oliveira, O. C. H. Kwok, and C. Su. 2013. Occurrence, isolation, and genetic characterization of *Toxoplasma gondii* from white tailed deer (*Odocoileus virginianus*) in New Jersey. *Journal of Parasitology* **99**: 763–769.

\_\_\_\_\_, C. Rajendran, L. R. Ferreira, O. C. H. Kwok, D. Sinnott, D. Majumdar, and C. Su. 2010. A new atypical highly mouse virulent *Toxoplasma gondii* genotype isolated from a wild black bear in Alaska. *Journal of Parasitology* **96**: 713–716.

\_\_\_\_\_, E. A. Rollor, K. Smith, O. C. H. Kwok, and P. Thulliez. 1997. Low seroprevalence of *Toxoplasma gondii* in feral pigs from a remote island lacking cats. *Journal of Parasitology* **83**: 839–841.

\_\_\_\_\_, S. P. Sharma, D. D. Juranek, A. J. Sulzer, and S. M. Teutsch. 1981. Characterization of *Toxoplasma gondii* isolates from an outbreak of toxoplasmosis in Atlanta, Georgia. *American Journal of Veterinary Research* **42**: 1007–1010.

\_\_\_\_\_, G. V. Swan, and J. K. Frenkel. 1972. A simplified method for isolation of *Toxoplasma gondii* from the feces of cats. *Journal of Parasitology* **58**: 1005–1006.

\_\_\_\_\_, P. Thulliez, and E. C. Powell. 1995a. *Toxoplasma gondii* in Iowa sows: Comparison of antibody titers to isolation of *T. gondii* by bioassays in mice and cats. *Journal of Parasitology* **81**: 48–53.

\_\_\_\_\_, R. M. Weigel, A. M. Siegel, P. Thulliez, U. D. Kitron, M. A. Mitchell, A. Mannelli, N. E. Mateus-Pinilla, S. K. Shen, O. C. H. Kwok , et al. 1995b. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. *Journal of Parasitology* **81**: 723–729.

Feldman, H. A., and L. T. Miller. 1956. Serological study of toxoplasmosis prevalence. *American Journal of Hygiene* **64**: 320–335.

Ferguson, D. J. P. 2009. Identification of faecal transmission of *Toxoplasma gondii*: Small science, large characters. *International Journal for Parasitology* **39**: 871–875.

Ferre, F., J. C. Desport, P. Peze, P. L. Ferrat, H. Bertrand, P. Grandcha., and P. Weinbrec. 1986. Toxoplasmose après plaie par arme blanche. *Presse Médicale* **15**: 929–930.

- Frenkel, J. K. 1970. Pursuing *Toxoplasma*. *Journal of Infectious Diseases* **122**: 553–559.
- \_\_\_\_\_. 1973. *Toxoplasma* in and around us. *BioScience* **23**: 343–352.
- \_\_\_\_\_, J. P. Dubey, and N. L. Miller. 1969. *Toxoplasma gondii*: Fecal forms separated from eggs of the nematode *Toxocara cati*. *Science* **164**: 432–433.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1970. *Toxoplasma gondii* in cats: Fecal stages identified as coccidian oocysts. *Science* **167**: 893–896.
- \_\_\_\_\_, E. R. Pfefferkorn, D. D. Smith, and J. L. Fishback. 1991. Prospective vaccine prepared from a new mutant of *Toxoplasma gondii* for use in cats. *American Journal of Veterinary Research* **52**: 759–763.
- Freyre, A., J. P. Dubey, D. D. Smith, and J. K. Frenkel. 1989. Oocyst-induced *Toxoplasma gondii* infections in cats. *Journal of Parasitology* **75**: 750–755.
- Garnham, P. C. C. 1971. The parasitic life. In *Progress of Parasitology*. Athlone Press. London, U.K. p. 116–124.
- Garner H. M., B. C. Barr, A. E. Packham, A. E. Marsh, K. A. Burek Huntington, R. K. Wilson, and J. P. Dubey. 1997. Fatal hepatic sarcocystosis in two polar bears (*Ursus maritimus*). *83*: 523–526.
- Hartley, W. J., and S. C. Marshall. 1957. Toxoplasmosis as a cause of ovine perinatal mortality. *New Zealand Veterinary Journal* **5**: 119–124.
- Hill, D., C. Coss, J. P. Dubey, K. Wroblewski, M. Sautter, T. Hosten, C. Muñoz-Zanzi, E. Mui, S. Withers, K. Boyer, G. Hermes, et al. 2011. Identification of a sporozoite-specific antigen from *Toxoplasma gondii*. *Journal of Parasitology* **97**: 328–337.
- Hutchison, W. M. 1965. Experimental transmission of *Toxoplasma gondii*. *Nature* **206**: 961–962.
- \_\_\_\_\_. 1967. The nematode transmission of *Toxoplasma gondii*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**: 80–89.
- \_\_\_\_\_, and K. Work. 1969. Observations on the faecal transmission of *Toxoplasma gondii*. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **77**: 275–282.
- \_\_\_\_\_, J. F. Dunachie, and K. Work. 1968. Brief report. The faecal transmission of *Toxoplasma gondii*. *Acta Pathologica et Microbiologica Scandinavica* **74**: 462–464.
- \_\_\_\_\_, \_\_\_\_\_, J. C. Siim, and K. Work. 1969. Life cycle of *Toxoplasma gondii*. *British Medical Journal* **4**: 806–806.

- \_\_\_\_\_, \_\_\_, \_\_\_, and \_\_\_\_\_. 1970. Coccidian-like nature of *Toxoplasma gondii*. *British Medical Journal* **1**: 142–144.
- Isaac-Renton, J., W. R. Bowie, A. King, G. S. Irwin, C. S. Ong, C. P. Fung, M. O. Shokeir, and J. P. Dubey. 1998. Detection of *Toxoplasma gondii* oocysts in drinking water. *Applied and Environmental Microbiology* **64**: 2278–2280.
- Jacobs, L. 1964. The occurrence of *Toxoplasma* infection in the absence of demonstrable antibodies. **1**: 176–177. Proceedings of the First International Congress of Parasitology. A. Corrdetti (ed.). Rome, Italy.
- \_\_\_\_\_. 1967. *Toxoplasma and toxoplasmosis*. In *Advances in parasitology*, B. Dawes (ed.) Academic Press, New York, N.Y. **5**: 1–45.
- \_\_\_\_\_. 1973. New knowledge of *Toxoplasma* and toxoplasmosis. In *Advances in parasitology*, B. Dawes (ed.) Academic Press, New York, N.Y. **11**: 631–669.
- \_\_\_\_\_. 1979. Presidential address. *Journal of Parasitology* **65**: 193–202.
- \_\_\_\_\_, and M. N. Lunde. 1957. Hemagglutination test for toxoplasmosis. *Science* **125**: 1035–1035.
- \_\_\_\_\_, J. R. Fair, and J. H. Bickerton. 1954. Adult ocular toxoplasmosis. Report of a parasitologically proved case. *American Medical association, Archives of Ophthalmology* **52**: 63–71.
- \_\_\_\_\_, and M. L. Melton. 1966. Toxoplasmosis in chickens. *Journal of Parasitology* **52**: 1158–1162.
- \_\_\_\_\_, J. S. Remington, and M. L. Melton. 1960a. The resistance of the encysted form of *Toxoplasma gondii*. *Journal of Parasitology* **46**: 11–21.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 1960b. A survey of meat samples from swine, cattle, and sheep for the presence of encysted *Toxoplasma*. *Journal of Parasitology* **46**: 23–28.
- Kean, B. H., A. C. Kimball, and W. N. Christenson. 1969. An epidemic of acute toxoplasmosis. *Journal of the American Medical Association* **208**: 1002–1004.
- Lehmann, T., P. L. Marcet, D. H. Graham, E. R. Dahl, and J. P. Dubey. 2006. Globalization and the population structure of *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 11423–11428.
- Lindsay, D. S., and J. P. Dubey. 2009. Long-term survival of sporulated *Toxoplasma gondii* oocysts in seawater. *Journal of Parasitology* **95**: 1019–1020.
- \_\_\_\_\_, M. V. Collins, S. M. Mitchell, C. N. Wetch, A. C. Rosypal, G. J.

Flick, A. M. Zajac, A. Lindquist, and J. P. Dubey. 2004. Survival of *Toxoplasma gondii* oocysts in eastern oysters (*Crassostrea virginica*). *Journal of Parasitology* **90**: 1054–1057.

Massie, G. N., M. W. Ware, E. N. Villegas, and M. W. Black. 2010. Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. *Veterinary Parasitology* **169**: 296–303.

Mateus-Pinilla, N. E., J. P. Dubey, L. Choromanski, and R. M. Weigel. 1999. A field trial of the effectiveness of a feline *Toxoplasma gondii* vaccine in reducing *T. gondii* exposure for swine. *Journal of Parasitology* **85**: 855–860.

Miller, M. A., W. A. Miller, P. A. Conrad, E. R. James, A. C. Melli, C. M. Leutenegger, H. A. Dabritz, A. E. Packham, D. Paradies, M. Harris et al. 2008. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *International Journal for Parasitology* **38**: 1319–1328.

Miller, N. L., J. K. Frenkel, and J. P. Dubey. 1972. Oral infections with *Toxoplasma* cysts and oocysts in felines, other mammals, and in birds. *Journal of Parasitology* **58**: 928–937.

Montoya, J. G. 2002. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *Journal of Infectious Diseases* **185**: S73–S82.

Munday, B. L. 1972. Serological evidence of *Toxoplasma* infection in isolated groups of sheep. *Research in Veterinary Science* **13**: 100–102.

Nicolle, C., and L. Manceaux. 1908. Sur une infection à corps de Leishman (ou organismes voisins) du gondi. *Comptes Rendus des Séances de l'Academie des Sciences* **147**: 763–766.

\_\_\_\_\_, and \_\_\_\_\_. 1909. Sur un protozoaire nouveau du gondi. *Comptes Rendus des Séances de l'Academie des Sciences* **148**: 369–372.

Overdulve, J. P. 1970. The identity of *Toxoplasma* Nicolle & Manceaux, 1909 with *Isospora* Schneider, 1881 (I). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, Series C* **73**: 129–151.

Peterson, D. R., M. K. Cooney, and R. P. Beasley. 1974. Prevalence of antibody to *Toxoplasma* among Alaskan natives: Relation to exposure to the Felidae. *Journal of Infectious Diseases* **130**: 557–563.

Pomares, C., D. Ajzenberg, L. Bornard, G. Bernardin, L. Hasseine, M. L. Dardé, and P. Marty. 2011. Toxoplasmosis and horse meat, France. *Emerging Infectious Diseases* **17**: 1327–1328.

Prestrud, K. W., K. Åsbakk, E. Fuglei, T. Mørk, A. Stien, E. Ropstad, M. Tryland,

- G. W. Gabrielsen, C. Lydersen, K. M. Kovacs, et al. 2007. Serosurvey for *Toxoplasma gondii* in arctic foxes and possible sources of infection in the high Arctic of Svalbard. *Veterinary Parasitology* **150**: 6–12.
- \_\_\_\_\_, J. P. Dubey, K. Åsbakk, E. Fuglei, and C. Su. 2008. First isolate of *Toxoplasma gondii* from arctic fox (*Vulpes lagopus*) from Svalbard. *Veterinary Parasitology* **151**: 110–114.
- Rawal, B. D. 1959. Toxoplasmosis. *A dye-test on sera from vegetarians and meat eaters in Bombay. Transactions of the Royal Society of Tropical Medicine and Hygiene* **53**: 61–63.
- Sabin, A. B., and H. A. Feldman. 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*). *Science* **108**: 660–663.
- Shapiro, K., P. A. Conrad, J. A. K. Mazet, W. W. Wallender, W. A. Miller, and J. L. Largier. 2010. Effect of estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from land to sea. *Applied and Environmental Microbiology* **76**: 6821–6828.
- Sheffield, H. G. 1970. Schizogony in *Toxoplasma gondii*: An electron microscope study. *Proceedings of the Helminthological Society of Washington* **37**: 237–242.
- \_\_\_\_\_, and M. L. Melton. 1969. *Toxoplasma gondii*: Transmission through feces in absence of *Toxocara cati* eggs. *Science* **164**: 431–432.
- \_\_\_\_\_, and \_\_\_\_\_. 1970. *Toxoplasma gondii*: The oocyst, sporozoite, and infection of cultured cells. *Science* **167**: 892–893.
- Siim, J. C., W. M. Hutchison, and K. Work. 1969. Transmission of *Toxoplasma gondii*. *Further studies in the morphology of the cystic form in cat faeces. Acta Pathologica et Microbiologica Scandinavica* **77**: 756–757.
- Sobanski, V., D. Ajzenberg, L. Delhaes, N. Bautin, and N. Just. 2013. Severe toxoplasmosis in immunocompetent hosts: Be aware of atypical strains. *American Journal of Respiratory and Critical Care Medicine* **187**: 1143–1145.
- Speer, C. A., and J. P. Dubey. 2005. Ultrastructural differentiation of *Toxoplasma gondii* schizonts (types B to E) and gamonts in the intestines of cats fed bradyzoites. *International Journal for Parasitology* **35**: 193–206.
- Splendore, A. 1908. Un nuovo protozoo parassita de conigli incontrato nelle lesioni anatomiche d'une malattia che ricorda in molti punti il Kala-azar dell'uomo. *Nota preliminare. Revista de Sociedade Scientifica de São Paulo* **3**: 109–112.
- Su, C., A. Khan, P. Zhou, D. Majumdar, D. Ajzenberg, M. L. Dardé, X. Q. Zhu, J. W. Ajioka, B. M. Rosenthal, J. P. Dubey, and L. D. Sibley. 2012. Globally diverse

*Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 5844–5849.

Teutsch, S. M., D. D. Juranek, A. Sulzer, J. P. Dubey, and R. K. Sikes. 1979. Epidemic toxoplasmosis associated with infected cats. *New England Journal of Medicine* **300**: 695–699.

\_\_\_\_\_, A. J. Sulzer, J. E. Ramsey, W. A. Murray, and D. D. Juranek. 1980. *Toxoplasma gondii* isolated from amniotic fluid. *Obstetrics and Gynecology* **55**: 2S–4S.

Thomas, N. J., J. P. Dubey, D. S. Lindsay, R. A. Cole, and C. U. Meteyer. 2007. Protozoal meningoencephalitis in sea otters (*Enhydra lutris*): A histopathological and immunohistochemical study of naturally occurring cases. *Journal of Comparative Pathology* **137**: 102–121.

Vaudaux, J. D., C. Muccioli, E. R. James, C. Silveira, S. L. Magargal, C. Jung, J. P. Dubey, J. L. Jones, M. Z. Doymaz, D. A. Bruckner, et al. 2010. Identification of an atypical strain of *Toxoplasma gondii* as the cause of a waterborne outbreak of toxoplasmosis in Santa Isabel do Ivaí, Brazil. *Journal of Infectious Diseases* **202**: 1226–1233.

Vanwormer, E., H. Fritz, K. Shapiro, J. A. K. Mazet, and P. A. Conrad. 2013. Molecular to modeling: *Toxoplasma gondii* oocysts at the human-animal-environment interface. *Comparative Immunology, Microbiology and Infectious Diseases* **36**: 217–231.

Wallace, G. D. 1969. Serologic and epidemiologic observations on toxoplasmosis on three Pacific Atolls. *American Journal of Epidemiology* **90**: 103–111.

\_\_\_\_\_. 1971a. Experimental transmission of *Toxoplasma gondii* by filth-flies. *American Journal of Tropical Medicine and Hygiene* **20**: 411–413.

\_\_\_\_\_. 1971b Isolation of *Toxoplasma gondii* from the feces of naturally infected cats. *Journal of Infectious Diseases* **124**: 227–228.

\_\_\_\_\_. 1972. Experimental transmission of *Toxoplasma gondii* by cockroaches. *Journal of Infectious Diseases* **126**: 545–547.

Weiland, G., and D. Kühn. 1970. Experimentelle *Toxoplasma*-Infektionen bei der Katze. II. Entwicklungsstadien des Parasiten im Darm. *Berliner und Münchener Tierärztliche Wochenschrift* **83**: 128–132.

Weinman, D., and A. H. Chandler. 1956. Toxoplasmosis in man and swine—an investigation of the possible relationship. *Journal of the American Medical Association* **161**: 229–232.

Witte, H. M., and G. Piekarski. 1970. Die Oocysten-Ausscheidung bei

experimentell infizierten Katzen in Abhangigkeit vom *Toxoplasma*-Stamm.  
*Zeitschrift fur Parasitenkunde* **33**: 358–360.

Woke, P. A., L. Jacobs, F. E. Jones, and M. L. Melton. 1953. Experimental results on possible arthropod transmission of toxoplasmosis. *Journal of Parasitology* **39**: 523–532.

Wolf, A., D. Cowen, and B. H. Paige. 1939a. Toxoplasmic encephalomyelitis. III. A new case of granulomatous encephalomyelitis due to a protozoon. *American Journal of Pathology* **15**: 657–694.

\_\_\_\_\_. \_\_\_\_\_, and \_\_\_\_\_. 1939b. Human toxoplasmosis: Occurrence in infants as an encephalomyelitis verification by transmission to animals. *Science* **89**: 226–227.

Work, K., and W. M. Hutchison. 1969a. A new cystic form of *Toxoplasma gondii*. *Acta Pathologica et Microbiologica Scandinavica* **75**: 191–192.

\_\_\_\_\_. \_\_\_\_\_, and \_\_\_\_\_. 1969b. The new cyst of *Toxoplasma gondii*. *Acta Pathologica et Microbiologica Scandinavica* **77**: 414–424.

Yuan, Z. G., S. J. Luo, J. P. Dubey, D. H. Zhou, Y. P. Zhu, Y. He, X. H. He, X. X. Zhang, and X. Q. Zhu. 2013. Serological evidence of *Toxoplasma gondii* infection in five species of bats in China. *Vector-Borne and Zoonotic Diseases* **13**: 422–424.

Yilmaz, S. M., and S. H. Hopkins. 1972. Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. *Journal of Parasitology* **58**: 938–939.



## **Part III**

# **Host-Parasite Interactions—The Challenge**



# Chapter 11

## Tick Paralysis\*

John L. Todd

*(Communicated to the Royal Society of Canada, May 28, 1914.)*

Although for many years it has been well known by physicians practicing in southern British Columbia that paralysis may appear in children who have been bitten by ticks, it is only a few months since accounts of such an affection as "tick paralysis" appeared in scientific publications (Todd, 1912, 1912a; Temple, 1912). Medicine owes a debt to these practitioners, Corsan, Henderson, Hall, Kingston, Morris, Rose, Shewan and Temple. Their observations have supplied the first records of paralysis produced in children by the bites of ticks.

The first accounts of the disease were merely short descriptions of the symptoms which had been observed by half a score of physicians in a series of more than twenty-five cases. The patients had been seen during a number of years — Temple saw his first case in 1898 — in various places in southern British Columbia and in the neighboring portions of the United States. All of the patients were children ; there were, however, somewhat obscure accounts of instances in which symptoms, resembling those observed in children, had been seen in men who had been bitten by ticks. The history and symptoms were much alike in all of the cases. The usual story was that an active and apparently healthy child suddenly developed a paresis or paralysis of the legs ; neither abnormal temperature nor any other symptom but paralysis was constant. After the tick was discovered and removed the symptoms disappeared within a few hours, with the possible exception of a more or less local reaction, often probably due to a secondary bacterial infection, at the site of the tick's bite. In some of the cases reported the tick was not removed ; in them the paralysis progressively involved the whole body until reflexes and control of the sphincters were lost, and death ensued.

The symptoms of these cases suggest infantile paralysis (acute poliomyelitis) ; but they are probably to be distinguished from cases

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\* I wish to express my indebtedness to all those who have supplied me with the information and material which has made this paper possible; the names of most of them are mentioned in it.

of that disease by the invariably transitory nature of their paralyses. In no instance has "tick paralysis" left permanent disability. It seems impossible to explain these many cases of "tick paralysis" as mere coincidences, in which tick infection has been fortuitously associated with sporadic attacks of a peculiarly mild form of acute poliomyelitis; none of the practitioners who saw these cases have recorded the contemporary existence of definite cases of acute poliomyelitis. Also, the symptoms of these cases of paralysis have little in common with the symptoms of the typhus-like "spotted fever," which is seen in persons bitten by ticks (*Dermacentor venustus*) in some parts of Montana. For these reasons it seems certain that a form of paralysis associated with the bites of ticks occurs in children in western North America and that the infection can be identified with no known disease. Similar cases from Wyoming and Montana have been reported (Bishopp and King, 1913). More recently very similar histories of instances in which paralysis has followed the bites of ticks (probably *Ixodes ricinus* or *I. holocyclus*) have been reported from Australia (Eaton, 1913).

Hadwen (1913) describes the occurrence of paralysis in sheep, which have been bitten by ticks, in British Columbia. He and Nuttall (1914) give references to publications which describe the existence of a paralysis caused by ticks (of a species, *I. pilosus*, other than that which exists in British Columbia) in South African sheep. Hadwen succeeded in producing paralysis in a lamb on which a tick (*D. venustus*) had, experimentally, been allowed to feed. He, with Nuttall (Hadwen and Nuttall, 1913), was successful in producing the same symptoms in a dog. A very complete bibliography of the effects of tick bite can be reached by a search through the papers referred to in this communication. Hadwen, especially, gives a good list of articles on the bites of ticks.

Since the observations contained in the publications mentioned above were published, the cases recorded below have been reported.

Dr. W. J. Knox, Kelowna, B. C., records two cases.

April 23, 1913, a boy of 4 years had pronounced flaccid paralysis from the hips down. He could not stand; his arms were weak; there was hyperesthesia; the pupils were normal; the temperature was 100 F.; the pulse was 112. The first symptom had been a little stiffness of the legs twenty-four hours previously; it progressed until in twelve hours walking was extremely difficult. A tick was found in the left axilla. It was removed by snipping it out with the tissue about its head; the wound was dressed antiseptically. A purgative was given to the child and, under slight stimulation, the pulse quickly became full and steady. In twenty-four hours the child could move his limbs fairly well, and in forty-eight hours he was apparently as well as ever.

April 21, 1914, a girl of 3 years had slight paralysis of the right leg. The temperature was normal. Her condition was said to be better than it had been two hours previously; so rest and a purgative were prescribed. Twelve hours later the child was stuporous. Her legs and arms were entirely par-

alyzed; she could neither articulate nor swallow, and seemed to be sinking very rapidly. The pulse was 130, small and weak. The temperature was 102 F. No tick was found on the child's body, but the hair was clipped short and a small wood tick was found at the base of the skull. It was removed by snipping it out with the tissue surrounding its head. The wound was dressed antiseptically and purgatives and stimulants were given; in six hours the child's temperature was 99.4 F., her pulse 100 and regular and she was conscious and asking for water. In twelve hours she could move her limbs and in five days was running about as well as ever.

Dr. Knox also reports a case of quite another type in an adult.

April 27, 1914, a man of 28 years was seen. He gave a history of having pulled off the body of a tick, leaving the head behind, from the calf of his left leg. At the site of the bite was a large bluish-black area surrounded by much induration. The patient's body was covered with a spotted erythema. He felt very ill, and complained of vertigo and of pains in the back and in the legs. His pulse was 108, his temperature 102.5 F. Sensation was lost in the right leg from 6 inches below the hip-joint; the left leg was paralyzed. The head of the tick was excised and the wound dressed antiseptically. A purgative was given and in twelve hours the temperature had dropped to 99.6 F. and the pulse to 86. In thirty hours the temperature was normal, and the rash and pain were gone; in four days the patient, being well, was discharged.

Dr. Knox records an instance in which a very thin colt, so weak that it could not stand, was covered with ticks; the ticks were removed and within a few days the colt was able to walk and gradually regained its health. (It is possible that this was nothing more than a case of severe "tick worry.")

Dr. Elmer Fessler, St. Regis, Montana, records two cases.

On the morning of May 19, 1914, a 2-year-old girl was found to be unable to walk or stand, although she had slept well and had been quite well on the previous day. She tried to walk several times during the day but she was unable to do so. When the physician first saw her, on the morning of the twentieth, her temperature was 96.5 F., her pulse 120 and her respiration was normal. She could move her legs, but she could not stand, and the leg reflexes were gone. There was considerable loss of function of the arms. The mother said more than on the previous day. No urine had been passed for twenty-four hours. Although the child was somewhat peevish, she took her food as usual. Two ticks (*Dermacentor venustus*) were removed from the nape of the neck; one was a half-engorged female, the other a male. The child was given cathartics. In six hours her hands were moved less clumsily and a little later she tried, unsuccessfully, to stand. At 9 the next morning she could walk and by noon was running about as usual. She has been quite well ever since.

In 1905 a girl of 5 years was seen who was said to have been unable to walk for forty-eight hours. She could not move her legs which were without reflexes. She could move her arms clumsily, but could hold nothing in her hands. A large tick was removed from the base of the skull and on the following day she seemed to be quite well, save for a slight weakness that persisted for some days.

Dr. A. W. Kenning, who practiced in Rossland, southern British Columbia, for sixteen years, mentions two cases.

One, the only fatal case, which he saw, was in a girl of 8. She had general paralysis and when the tick was found on her arm, she was comatose with a rapid pulse and high temperature. The other case was a child of 6 who had general paralysis; the whole body was involved. On removing the tick from the child's head, she recovered as was usual in such cases.

Dr. G. S. Gordon, Vancouver, B. C., has records of one case.

In 1904 (?) a girl of 5 years was seen. The child came of neurotic stock; she seemed well in every particular save that, though she could move her arms and legs while sitting on the floor, she could not walk nor put one leg before the other when she was supported in the erect position. A second practitioner, whose opinion was asked, told the parents to look for "wood ticks." One was found and on its removal the symptoms disappeared.

Dr. R. W. Irving, Kamloops, B. C., records a case in a child.

May 1, 1912, a boy of 8 years was seen. He complained of numbness in his legs and was unable to stand. He seemed rather sleepy and dull; but otherwise was healthy, save that he could only move his legs in an ataxic manner while lying in bed and that at times his arms seemed to become weak. The special senses were not affected nor were the cranial nerves involved. The knee-jerks were not obtainable. There was no ankle clonus, nor Babinski's sign. The sphincters were under control and there were no areas of anesthesia or hyperesthesia. The pulse was normal and the temperature was 99.2 F. A tick was found at the base of the skull and it was removed. Under rest and catharsis the boy, in three or four days, became perfectly well.

Dr. Irving, with Dr. Murphy, saw a case in which definite nervous symptoms in an adult were associated with the presence of a tick.

A normal, well-nourished male of 40 years complained that four days previously he found it difficult to speak and that he was awkward in his movements. He had been perfectly well and said that he had felt as though he were "partially drunk." A day later he fell on attempting to arise in the morning and was unable to balance himself in any way. When the patient was seen there were few constitutional symptoms and his only other complaint was that he felt "a bit seedy." On examination his hands and arms were found to be weak and incoordination was definitely present. The knee-jerks were absent; there was no ankle clonus, Babinski's sign, area of anesthesia or hyperesthesia, and no involvement of cranial nerves nor loss of sphincter control. A tick was found on the upper part of the back and removed. The symptoms commenced to disappear within twenty hours. In three days the man was as well as ever, and in three days more he was married.

These and other cases add a little to the first descriptions of the affection; they show that an elevated temperature, a rapid pulse and respiration and other constitutional conditions may frequently be symptoms. It also seems probable that nervous symptoms may supervene in adults who have been bitten by ticks. Convulsions, sudden stupidity and clumsiness are mentioned as symptoms that may follow tick bite. One physician asserts that in southern British Columbia practitioners, who have been in the country for long, always look for ticks on a child who has a convulsion. One or two physicians mention cases which suggest that spotted fever may not be entirely confined to Montana. There are many records of ulcers and other inflammatory lesions at the site of ticks' bites. Sometimes these lesions are said to be suggestively obstinate to ordinary treatment.

The experiments recorded below were made in the hope of producing, under experimental conditions, paralysis in laboratory animals, by the bites of ticks.

The way in which the experiments were done was practically identical in each instance. The animals, together with control animals, were well cared for. The lambs, when the experiments commenced, were from 4 to 6 weeks old; they were kept in pens with their mothers. The puppies were about the same age; they were kept in individual cages and fed on milk. The ticks were attached either to the nape of the neck or to the loins as is stated. The hair was removed either by slipping and shaving or by epilating powder. The ticks were then placed on the skin beneath a finely perforated porcelain filter cone, which measured about 1 inch in diameter at the base and 1 inch in height. The cone was fastened to the animal by an ample bandage of adhesive plaster, firmly stitched in position. As a rule, this method of attaching the ticks was satisfactory. In only one or two instances did the ticks find their way beneath the edge of the cone and become lost or fixed to the plaster. Paralysis was produced in no experiment.

MONKEY 1.—Aug. 16, 1912. Two ticks (*D. venustus* or *D. andersoni*), sent by Dr. J. B. McClintic, Victor, Mont., were placed on the nape of the neck of a large rhesus monkey; August 21, both ticks, each half engorged and firmly attached, were removed. August 24, three ticks, from the same source, were placed on the nape of the neck; August 31, two engorged ticks were removed; one tick had disappeared. November 18, four ticks, obtained by Dr. Lee Ganson of Odessa, Wash., from "jack rabbits," were placed on the nape of the neck of this monkey; November 30, during the night the monkey pulled off the bandage and destroyed the ticks.

MONKEY 2.—Aug. 16, 1912, three ticks (*D. venustus* or *D. andersoni*), sent by Dr. J. B. McClintic, Victor, Mont., were placed on the nape of the neck of a small rhesus monkey; August 20, the half-engorged ticks were removed. August 24, two ticks, from the same source, were placed on the neck; August 30, one engorged tick was removed; the second was dead.

MONKEY 3.—May 2, 1913, three ticks (not identified) were placed on the nape of the neck of a large rhesus monkey. One of these ticks was that which was removed from the little boy whose case is recorded above by Dr. Knox at Kelowna, B. C. The other two were sent by Dr. Boyce, from a horse, from the same place. May 26, the engorged ticks were removed.

Ten ticks, all of them very probably *D. venustus*, fed on one or the other of these three monkeys. Paralysis appeared in none of the three, although the child from which one of the ticks had been taken was paralyzed.

LAMB 1.—April 20, 1914, two ticks (*D. venustus*) from H. P. Wood, Esq., Florence, Mont., were placed on the neck of a lamb about 3 weeks old. The ticks were collected from cattle in a district where "spotted fever" exists. April 21, the lamb was obviously disinclined to move. April 22, the lamb died. A naked-eye examination at the autopsy revealed nothing beyond four or five superficial, almost petechial, pneumonic areas on the lungs. One of these was sectioned and the lung was found to be congested and collapsed, rather than pneumonic. The stomach and intestines were filled with normally digested food.

LAMB 2.—On April 20, 1914, three female ticks, two *D. venustus* and one *D. albipictus*, collected by Seymour Hawden, D.V.Sci., from horses at Keremeos,

B. C., were placed on the neck of a lamb about 4 weeks old. April 30, the three ticks, none of them engorged, were removed. Four ticks, three male and one female *D. venustus*, sent by H. P. Wood, Esq. (see Lamb 1), were placed on the neck; May 9, the engorged female and three unfed male ticks were removed. May 28, an engorged female *D. venustus*, taken by Dr. Arthur from the skin of a bear, at Nelson, B. C., was placed on the neck; June 5, the tick was removed unfed. June 19 two ticks, *D. venustus*, sent by R. A. Cooley, Esq., Bozeman, Mont., were placed on the neck; June 24, both ticks were removed, unfed.

LAMB 3.—April 24, 1914, four ticks, from H. P. Wood, Esq., Florence, Mont., were placed on the neck; April 30, two of the ticks were lost and one dead, the remaining living tick was not engorged. Four other ticks, two male and two female *D. venustus*, from the same source, were placed on the lamb; May 9, one of the ticks was dead, the three living ones were removed, none were engorged. May 28, one female, *D. venustus*, partially engorged, from Dr. Arthur (see above) was placed on the neck; June 5, the tick was dead; and probably was never attached. June 19, two *D. venustus*, from Bozeman, Mont., were placed on the neck; June 24, one unfed tick was removed, one was missing. June 25, three *D. venustus*, two females and one male, from Bozeman, Mont., were placed on the neck; July 2, one engorged female and one attached male tick was removed.

LAMB 4.—April 24, 1914, one male and one female *D. venustus*, from Florence, Mont., were placed on the neck; April 30, ticks removed; both had attached; one was fully engorged. Four other ticks, one male and three females, from the above source, were placed on the loins; May 9, ticks removed; all had attached; three were engorged. May 28, three partially fed ticks, one male and two female *D. venustus*, from Dr. Arthur (see above) were placed on the neck; June 5, ticks removed; the two female ticks were engorged; the male was not. June 6, a half-engorged female *D. venustus* was placed on the neck. This tick was one of those removed from the case recorded above by Dr. Fessler. June 12, tick removed; probably had not attached. June 19, a male *D. venustus*, also from Dr. Fessler's case, previously fed on Puppy 1, was placed on the neck; June 24, tick dead. June 25, a male and a female *D. venustus* from Bozeman, Mont., were placed on neck; July 2, the engorged female and unfed male ticks were removed.

LAMB 5.—April 30, 1914, a tick (unidentified) from the case described above by Dr. Irving, was placed on the neck; May 9, tick dead; apparently had not attached. May 28, one male and two female *D. venustus* from Dr. Arthur (see above) were placed on the neck; June 5, ticks removed; two were engorged; one not. June 19, two ticks, *D. venustus*, from Bozeman, Mont., were placed on the neck; June 24, ticks removed, only one was half engorged. June 25, four ticks, two male and two female *D. venustus*, from Bozeman, Mont., were placed on the neck; July 2, one female was dead, the remainder being attached, were left in position; July 7, an engorged female and one dead and one still attached male were removed.

LAMB 6.—May 5, 1914, two female *D. venustus*, from Dr. Hawden (see above), were placed on the neck of this lamb; May 16, both ticks, attached but not engorged, were dead. May 28, one partially engorged female *D. venustus* from Dr. Arthur (see above), was placed on the neck; June 5, tick dead, probably had not attached. June 19, one male and one female *D. venustus*, from Bozeman, Mont., were placed on the neck; June 24, both ticks dead; one attached; neither engorged. June 25, one female and one male *D. venustus*, from Bozeman, Mont., were placed on the neck; July 2, male dead, female engorged.

LAMB 7.—May 9, 1914, a female *D. venustus*, obtained from surveyors by Dr. Arthur of Nelson, B. C., was placed on the neck of this lamb. May 16, tick removed, three-fourths engorged. May 28, two partially engorged female *D. venustus*, obtained from Dr. Arthur, at Nelson, B. C., from a bear skin, were

placed on the neck; June 5, both ticks dead, probably never attached. June 19, two ticks, *D. venustus*, from Bozeman, Mont., placed on the neck; June 24, ticks removed, both attached, one half engorged. June 25, two ticks, *D. venustus*, from Bozeman, Mont., were placed on the neck; July 2, one male tick and one attached female removed, both were unfed.

About twenty ticks, almost all of them adult male or female *D. venustus*, fed on one or the other of seven lambs. Six of the lambs remained in perfect health. One of them (Lamb 1) died on the second day after two ticks were placed on it; it is not probable that the ticks were the cause of death, since other ticks from the same source were harmless to similar animals.

GUINEA-PIG 1.—May 6, 1914, a male and a female *D. venustus* sent by H. P. Wood, Esq., Florence, Mont., were placed on the loins; May 16, ticks removed, the female was partially engorged. June 3, dead; the guinea-pig had seemed to be quite well, and temperature was not abnormal until May 16, when daily observations were discontinued. At autopsy, there were no signs of irritation about tick bites; the cause of death was bronchopneumonia.

No sign of paralysis followed the feeding of a single tick on a guinea-pig.

PUPPY 1.—June 6, 1914, a male *D. venustus*, sent by R. A. Cooley, Esq., Bozeman, Mont., was placed on the back of a puppy, about 6 weeks old. This tick was one of those taken by Dr. Fessler from the case recorded above by him; June 12, tick removed, unattached and unfed. June 19, two male *D. venustus*, from Bozeman, Mont., were placed on the back; June 24, two ticks removed, both unfed and dead. June 25, one male and one female *D. venustus*, from Bozeman, Mont., were placed on the loins; July 2, both ticks dead, neither fed.

PUPPY 2.—June 19, 1914, two male *D. venustus*, from Bozeman, Mont., were placed on the loins of a puppy about 8 weeks old; June 24, both ticks removed, unfed and dead.

PUPPY 3.—June 19, 1914, two female *D. venustus*, from Bozeman, Mont., were placed on the loins of a puppy about 8 weeks old; June 24, ticks removed, both engorged.

Paralysis did not appear in a puppy on which two ticks fed.

The ticks used in all of these experiments were identified with the assistance of the key published by Banks (1908). *Dermacentor venustus* and *D. albipictus* were the only ticks received from southern British Columbia, and of these the former was much the more common. In a personal letter, Hadwen wrote that it is difficult to get *D. venustus* to feed under laboratory conditions unless it has been recently collected; the diaries of the above experiments ratify his statement. The ticks used in these experiments were confined in Ehrlemeyer flasks plugged with cotton wool, and were kept in a humid atmosphere at 25 C. Care was taken to give every tick every chance for feeding; several of the ticks used in these experiments had opportunities for feeding on two or more animals. These conditions seemed to agree with some females which laid many fertile eggs; but about half of the females laid only a few eggs and these infertile ones.

In the records of the tick-feeding experiments only those ticks which were definitely engorged are counted as having fed. It is unfortunate that their number is not larger and, especially, that the ticks which had been removed from paralyzed children would not all feed well on the experimental animals. Enough fed, however, to justify the statement that, under the conditions of these experiments, not every bite of a tick (*D. venustus* and *D. albipictus*) is able to cause paralysis in the laboratory animals employed.

It was thought that the paralysis produced by the bites of ticks in children might be caused by some toxin secreted by the ticks. Experiments were therefore designed to ascertain whether an extract capable of producing paralysis in laboratory animals could be obtained from the bodies of ticks.

EXPERIMENT 380.—About 4 c.cm. of larvae of "Texas fever ticks," (*Margaropus* sp.?), obtained from Washington, D. C., through the courtesy of the Bureau of Animal Industry, were dried for six weeks, and were then ground up in 50 c.cm. of a 4 per cent. solution of glycerin in distilled water. The resulting mixture was shaken for one and a half hours and then passed through a Buchner and a Berkefeld filter. The fluid so obtained was inoculated beneath the skin of the rump of two rats and two mice; one rat and one mouse received 2 c.cm., the other rat and mouse 1 c.cm. The two mice died within twenty-four hours; no cause of death was evident. The rat which received 1 c.cm. died ninety-six hours after inoculation; the cause of death was not evident. The second rat died two weeks after inoculation, from bronchopneumonia; neither it nor any of the animals had ever shown any sign of paralysis, or of suppuration at the site of inoculation.

EXPERIMENT 400.—Ten adult *Margaropus annulatus* and about 700 larvae, also obtained from Washington, D. C., were dried, ground up in glycerin and water, shaken and filtered in the same way as was done in Experiment 380. Four c.cm. of the filtrate was inoculated beneath the skin at the back of the neck, of two rats; one received 2 c.cm., the other 4 c.cm. Neither rat developed any sign of paralysis nor was there suppuration at the site of inoculation in either.

EXPERIMENT 531.—About 3.5 c.cm. of dried ticks of all ages and of both sexes were employed. Most of them were adults. With the exception of one or two *D. albipictus*, all were *D. venustus*. These ticks were those, or their progeny, which had been used in the feeding experiments described above. All were ground up in 50 c.cm. of a 4 per cent. solution of glycerin in distilled water, shaken for two and a half hours and then filtered through a Buchner and a Berkefeld filter. Respectively, 2 c.cm., 4 c.cm. and 5 c.cm. of the clear filtrate was inoculated under the skin of the rumps of three young white rats, weighing about 30 gm. each. Paralysis appeared in none of them, although as in the previous experiments, all showed some disinclination to move; probably because of the soreness at the site of inoculation. One rat died four days after the inoculation. There was no sign of suppuration at the wound, and the cause of death was not evident to naked-eye examination, at the autopsy.

EXPERIMENT 532.—Five male, eight female and about 1,500 larval ticks, all living *D. venustus*, which had been used, or were the progeny of those used, in the above feeding experiments, were ground up, shaken and filtered in the same way as in Experiment 531. Respectively, 2 c.cm., 4.5 c.cm. and 6 c.cm. of the clear filtrate obtained was inoculated beneath the skin over the rump

of three young rats, each weighing about 30 gm. All of the rats survived; in none did paralysis appear.

EXPERIMENTS 550-551.—Three boxes, each containing a female *D. venustus*, and young seed ticks from her, were obtained from the Bitter Root Valley through the courtesy of R. A. Cooley, Esq. In two of the boxes the females and many hundreds of seed ticks were dead; in the third box the female and seed ticks were living. All were ground up together in 50 c.cm. of normal saline solution, shaken for two hours and then passed through Buchner and Berkefeld filters. About two-thirds of the whole filtrate obtained was inoculated subcutaneously over the withers of a 4½-months old lamb; 3.5 c.cm. of the filtrate was inoculated subcutaneously over the rump of a young white rat. Paralysis was never observed in either of these animals.

It has been proved (Nuttall and Strickland, 1908) that ticks secrete an anticoagulin which prevents blood from clotting. Observations were, therefore, made to ascertain whether an anticoagulin existed in the filtrate of extracted ticks, which was inoculated into the animals used in Experiments 380, 400, 531 and 532. Blood from a healthy man was drawn up into capillary tubes, of about 1 mm. internal diameter, with one-third, occasionally one-fourth, of its volume of the filtrate to be tested. A control tube was made in every instance, in which a 4 per cent. solution of glycerin was substituted for the filtrate. The tubes were then kept at room temperature, and the time in which the blood of each coagulated was observed. The tick extract used in Experiments 380 and 400 seemed to have no definite power of preventing coagulation, since coagulation usually occurred in both tubes in the same length of time. The tick extracts used in Experiments 532 and 531 had a definite, though slight, power of preventing coagulation. That used in Experiment 532 was definitely more powerful than that used in Experiment 531; with it, coagulation had usually only commenced at the end of three minutes, while it was complete at the end of that time in the control tubes. It is possible that the lack of symptoms obtained by the inoculation of the filtrates, as well as the lack of coagulating power of the filtrates used in Experiments 380, 400 and 531, may be explained in part by the comparatively large quantity of diluent in which the ticks were extracted. It must also be remembered that only in Experiment 532 was the extract made from living ticks.

It can be concluded that an extract of ticks, prepared in the manner described, will not cause paralysis in rats even when it possesses slight power to prevent the coagulation of human blood.

#### SUMMARY

1. Previous publications have proved:

(a) That a paralysis in children may be associated with the bites of ticks in western North America and in Australia.

(b) That a paralysis of sheep has been associated with the bites of ticks in British Columbia and in South Africa.

(c) That the ticks associated with these affections are of more than one sort.

(d) That *Dermacentor venustus* has produced paralysis in lambs and in a puppy in experiments made under laboratory conditions.

(e) That the paralysis following tick-bite is probably an individual and novel condition.

2. The paralysis of children is not infrequently accompanied by elevation of temperature and by other constitutional symptoms; it is possible that symptoms resembling those observed in children sometimes may appear in adults who have been bitten by ticks.

3. Under experimental conditions by no means every tick bite produces paralysis in laboratory animals.

4. A weak extract of ticks will not cause paralysis when injected into white rats, even though it possesses definite power to prevent the coagulation of blood.

#### REFERENCES

- Banks, Nathan. 1908. A Revision of the Ixodoidea or Ticks of the United States. U. S. Bur. Entom., Technical Series No. 15.
- Bishopp, F. C., and King, W. V. 1913. Additional Note on the Biology of the Rocky Mountain Spotted-Fever Tick. Jour. Econ. Entom., 6. Abstr. in Trop. Dis. Bull., 2: 203-6.
- Cleland. 1912. Austral. Med. Gaz., 32: 295-296. Abstr. in Trop. Dis. Bull., 2: 203-6.
- Eaton, E. M. 1913. A Case of Tick Bite Followed by Wide-Spread Transitory Muscular Paralysis. Austral. Med. Gaz., 33: 391-394. Abstr. in Trop. Dis. Bull., 2: 203-6.
- Hadwen, S. 1913. On "Tick-Paralysis" in Sheep and Man, Following Bites of *Dermacentor venustus*. Parasitol., 6: 283-297.
- Hadwen, S., and Nuttall, G. H. F. 1913. Experimental "Tick-Paralysis" in the Dog. Parasitol., 6: 298-301.
- Nuttall, G. H. F. 1914. "Tick Paralysis" in Man and Animals. Parasitol., 7: 95-104.
- Nuttall, G. H. F., and Strickland. 1908. On the Presence of an Anticoagulin in the Salivary Glands and Intestines of *Argas persicus*. Parasitol., 1: 302-310.
- Temple, I. U. 1912. Acute Ascending Paralysis or Tick Paralysis. Med. Sentinel (Portl., Ore.), 20: 507-514.
- Todd, J. L. 1912. Does a Human Tick-Borne Disease Exist in British Columbia? Jour. Can. Med. Assn., n. s., 2: 686. Abstr. in Trop. Dis. Bull., 2: 203-6. Tick Bite in British Columbia. Jour. Can. Med. Assn., n. s., 1912, 2: 148-149. Abstr. in Trop. Dis. Bull., 2: 203-6.



# **Tick paralysis: Some host and tick perspectives**

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Tick paralysis is a form of tick toxicosis that affects host motor neurons and causes an ascending flaccid paralysis of the limbs after one or more individuals of certain species of ticks have fed for a prolonged period (several days) on a host and secreted non-infectious neurotoxins. The paralysis is reversible if the ticks are removed. Some additional physiological manifestations can be caused by these toxins, e.g., increased host respiration rate and cardiovascular anomalies (Estrada-Peña and Mans, 2014). The earliest detailed account of tick paralysis in North America was published 100 years ago in Volume 1 of *The Journal of Parasitology* (Todd, 1914). Although the condition had been known to certain physicians in British Columbia since 1898 (possibly earlier), Todd (1914) documented case reports of several patients from this region, some of whom died (usually when the paralysis reached the diaphragm muscles and breathing was no longer possible). He also identified the causative tick species in British Columbia as *Dermacentor venustus*, now treated as *Dermacentor andersoni*, the Rocky Mountain wood tick. Todd also described experiments he completed by feeding adult ticks on monkeys (species not stated), lambs, puppies, and guinea pigs. Various results were obtained from these experimental efforts, but some clear cases of tick paralysis were reproduced, reinforcing the hypothesis that feeding ticks were responsible for the condition.

Substantiating the role of attached ticks as vehicles of toxicoses, including paralysis, two additional relevant papers were published in Volume 2 of *The Journal of Parasitology*. The first of these documented severe toxic reactions experienced by humans following attachment by the Pajaroello tick (*Ornithodoros coriaceus*) in California and Mexico, and included descriptions of experimental feedings by this tick species on macaques (*Macaca rhesus*), rabbits, and mice including the resulting host lesions (Herms, 1916). The author underlined the severity of these reactions by stating that humans in endemic regions,

...fear this parasite more than they do the rattlesnake, and tell weird tales of this or that man having lost an arm or leg, and in one instance even death having ensued, as the result of a bite by the Pajaroello (Herms, 1916).

In the same journal volume, McCaffrey (1916) provided two additional case reports of tick toxicosis; and one of the tick paralysis reports in humans in British

Columbia stated that the tick that caused the condition was identified as *D. venustus*.

The typical clinical course for tick paralysis, as exemplified by toxin-secreting female *D. andersoni* ticks attached to humans, begins with the loss of leg coordination and difficulty walking, which is similar to the initial symptoms of hind leg immobility in dogs parasitized by toxin-releasing females of *Dermacentor variabilis*, the American dog tick (Gregson, 1973). However, in Karoo paralysis in South Africa, caused by *Ixodes rubicundus*, all four limbs of tetrapods become paralyzed simultaneously (Fourie et al., 1992). In patients parasitized by toxin-secreting *D. andersoni*, complete ataxia of the legs typically develops within 24 hours of the onset of symptoms and within another day complete limb paralysis (tetraplegia) follows. Moreover, speaking becomes difficult, eventually resulting in respiratory paralysis and death (Gregson, 1973). Some additional symptoms may occur as the paralysis ensues, e.g., headache, vertigo, itchiness, leg pain, and, for the Australian paralysis-causing tick *Ixodes holocyclus*, vomiting, nausea, and an extended illness (Stone et al., 1989).

# Synopsis of current knowledge of tick paralysis

A great deal of knowledge has been collected concerning tick paralysis in the last 100 years and several review papers and book chapters have addressed the topic (Stanbury and Huyck, 1945; Gregson, 1973; Gothe et al., 1979; Malik and Farrow, 1991; Gothe, 1999; Mans et al., 2004, 2008; Estrada-Peña and Mans, 2014). During this time period, several important papers published in *The Journal of Parasitology* have contributed to our understanding of the nature, mechanisms, and distribution of tick paralysis. Chronologically, these publications include studies on the possible role of the lone star tick, *Amblyomma americanum*, in North America, causing tick paralysis (Swartzwelder and Seabury, 1947), the clinical pathology of paralysis caused by larvae of the soft tick, *Argas persicus*, in hens (Gothe and Kunze, 1970), isolation of a neurotoxin from the salivary glands of the African hard tick, *Rhipicephalus evertsi evertsi* by Viljoen et al. (1986), and changes in the lipid profile in the salivary glands of *A. americanum* during feeding (Shipley et al., 1993).

At least 73 species of ticks have now been reported to cause various forms and degrees of tick paralysis ([Table 1](#)). This group includes 14 species of soft ticks (Argasidae) and 59 species of hard ticks (Ixodidae). Tick toxicoses without accompanying paralysis, are also caused by various species of argasid ticks such as *O. coriaceus*. The latter tick has emerged as an economically important species not only because its bites can cause severe reactions in humans, but also because it has been shown in California to transmit a causative agent of bovine epizootic abortion in cattle (King et al., 2005). The contrasting importance of argasid and ixodid ticks in causing tick paralysis can be attributed to their different feeding strategies and life cycles. Paralysis-causing toxins secreted by feeding ticks only cause paralysis after the attached tick has fed on its host for several days (typically, 4–8 days, depending on the tick species) (Mans et al., 2004). Each active stage (larvae, nymphs, and adult females) except adult males, of ixodid ticks typically attach to the host for several days, whereas only the larval stages of argasid ticks attach to the host for comparable time periods. Therefore, within the Argasidae, it is typically only larval ticks of neurotoxin-secreting species that can cause tick paralysis.

**Table 1** Tick species reported to cause tick paralysis.\*

Tick species	Region	Main hosts affected
Family Argasidae:		
<i>Argas africolumbae</i> <sup>2</sup>	Africa	Chickens, other birds
<i>Argas arboreus</i> <sup>2</sup>	Africa, Palearctic region	Egrets
<i>Argas miniatus</i> <sup>2</sup>	Nearctic and Neotropical regions	Poultry, other birds
<i>Argas monolakensis</i>	USA (California)	Gulls

<i>Argas persicus</i> <sup>2</sup>	Palearctic (introduced elsewhere)	Poultry
<i>Argas radiatus</i>	North America	Poultry, other birds
<i>Argas reflexus</i>	Palearctic region	Pigeons, other birds
<i>Argas robertsi</i>	Australasia and Asia	Birds
<i>Argas sanchezi</i> <sup>2</sup>	W. North America	Poultry, other birds
<b><i>Argas walkerae</i><sup>2</sup></b>	Southern Africa	Chickens, other birds
<i>Ornithodoros capensis</i>	Worldwide (oceans and coastal)	Seabirds
<i>Ornithodoros lahorensis</i> <sup>2</sup>	Oriental & Palearctic regions	Ungulates
<i>Ornithodoros savignyi</i>	Africa, Asia	Ungulates
<i>Otobius megnini</i>	Nearctic (introduced, other regions)	Ungulates
Family Ixodidae:		
<i>Amblyomma americanum</i>	E. United States	Carnivores, humans
<i>Amblyomma argentiniae</i> <sup>2</sup> , 3	Neotropics	Reptiles
<i>Amblyomma cajennense</i> complex <sup>2</sup>	Neotropical and S. Nearctic	Ungulates
<i>Amblyomma hebraeum</i>	Southern Africa	Humans, sheep, goats
<i>Amblyomma maculatum</i> <sup>2</sup>	S. Nearctic and N. Neotropics	Dogs, Humans
<i>Amblyomma ovale</i> <sup>2</sup>	Neotropics	Humans
<i>Amblyomma rotundatum</i>	Florida, Neotropics	Toads, snakes
<i>Amblyomma variegatum</i>	Africa (introduced to Caribbean)	Ungulates
<i>Dermacentor albipictus</i>	North America	Ungulates
<b><i>Dermacentor andersoni</i><sup>2</sup></b>	W. North America	Humans, dogs, ungulates
<i>Dermacentor auratus</i>	Oriental region	Humans
<i>Dermacentor marginatus</i>	Palearctic region	Humans, ungulates
<i>Dermacentor nuttalli</i>	Palearctic region	Ungulates
<i>Dermacentor occidentalis</i> <sup>2</sup>	W. North America	Ungulates (mainly), dogs, humans
<i>Dermacentor reticulatus</i>	Palearctic region	Sheep

<i>Dermacentor rhinocerinus</i> <sup>2</sup>	Africa	Rabbits
<i>Dermacentor silvarum</i>	Palearctic region	Sheep
<b><i>Dermacentor variabilis</i></b> <sup>2</sup>	North America	Humans, canids
<i>Haemaphysalis chordeilis</i>	North America	Human
<i>Haemaphysalis cinnabarina</i> <sup>4</sup>	Brazil	Ungulate
<i>Haemaphysalis inermis</i>	Palearctic region	Ungulates
<i>Haemaphysalis kutchensis</i> <sup>2</sup>	Oriental region	Rabbits
<i>Haemaphysalis parva</i>	Middle East	Sheep
<i>Haemaphysalis punctata</i> <sup>2</sup>	Oriental and Palearctic regions	Ungulates, birds
<i>Haemaphysalis sulcata</i>	Eurasia	Ungulates
<i>Hyalomma aegyptium</i>	Mediterranean region	Tortoises, sheep
<i>Hyalomma detritum</i>	Oriental and Palearctic regions	Sheep
<i>Hyalomma truncatum</i> <sup>2</sup>	Africa	Sheep, Humans
<i>Ixodes arboricola</i>	Europe	Birds
<b><i>Ixodes brunneus</i></b> <sup>2</sup>	North America	Birds
<i>Ixodes cookei</i>	North America	Humans
<i>Ixodes cornuatus</i>	S. Australia	Dogs, cats, humans
<i>Ixodes crenulatus</i>	Palearctic region	Sheep
<i>Ixodes eudyptidis</i>	New Zealand	Albatrosses, petrels
<i>Ixodes frontalis</i>	Palearctic region	Birds
<i>Ixodes gibbosus</i> <sup>2</sup>	Europe, Middle East	Ungulates
Tick species	Region	Main hosts affected
<i>Ixodes hexagonus</i>	Europe	Humans
<i>Ixodes hirsti</i>	Australia (Tasmania)	Cats
<b><i>Ixodes holocyclus</i></b> <sup>†</sup>	Eastern Australia	Dogs, sheep, humans
<i>Ixodes muris</i>	North America	Dogs, cats
<b><i>Ixodes pacificus</i></b> <sup>†</sup>	W. North America	Dogs, humans
<i>Ixodes redikorzevi</i>	Middle East	Humans

<i>Ixodes ricinus</i>	Europe	Humans, sheep
<i>Ixodes robertsi</i>	Australia	Various mammals
<b><i>Ixodes rubicundus</i><sup>†</sup></b>	Southern Africa	Sheep, rabbits
<i>Ixodes scapularis</i>	E. North America	Humans, Dogs
<i>Ixodes tancitarius</i>	Mexico	Humans
<i>Ixodes tasmani</i>	Australia	Marsupials (mainly koalas)
<i>Rhipicentor nuttalli</i>	Africa	Dogs
<i>Rhipicephalus (Boophilus) annulatus</i>	Europe, Africa, Mexico	Humans
<i>Rhipicephalus bursa</i>	Mediterranean region	Sheep
<i>Rhipicephalus exophthalmus</i> <sup>†</sup>	Southern Africa	Goats & sheep
<b><i>Rhipicephalus evertsi</i><sup>†</sup></b>	Africa	Sheep
<i>Rhipicephalus praetextatus</i>	N. Africa	Humans
<i>Rhipicephalus punctatus</i>	Southern Africa	Ungulates
<i>Rhipicephalus sanguineus</i>	Almost worldwide	Dogs, Humans
<i>Rhipicephalus simus</i>	Africa	Humans
<i>Rhipicephalus tricuspis</i>	Africa	Ungulates
<i>Rhipicephalus warburtoni</i> <sup>†</sup>	Southern Africa	Goats, sheep

\* Tick species of major importance in causing paralysis are in **bold** font.

<sup>†</sup> Paralysis caused by these tick species has been confirmed by experimental induction of paralysis by feeding ticks on hosts under controlled conditions, multiple reports implicating the tick species, or recovery of the host following removal and identification of the tick(s).

<sup>‡</sup> Originally reported as *Amblyomma testudinis*, which is currently treated as a junior synonym of *A. argentineae* by most authors.

<sup>§</sup> *Haemaphysalis cinnabarina* is treated as a junior synonym of *H. chordeilis* by some authors.

An exception to this trend is paralysis in sheep and cattle caused by the argasid *Ornithodoros lahorensis* in parts of eastern Europe and central Asia, where slow-feeding neurotoxin-secreting, third stage nymphs can cause paralysis (Gregson, 1973). Because of their small size and the smaller volumes of secreted toxins, multiple argasid larvae attached to the host at the same time are typically needed in order to cause paralysis. Similarly, for ixodid species with nymphs that cause paralysis, large numbers (typically hundreds) of attached nymphs are needed to induce paralysis in the host, as in the case of Karoo paralysis in South Africa, which mainly effects non-native ungulates, e.g., sheep, goats, and cattle, but also some native ungulate species and (rarely) carnivores (Fourie and Horak, 1987;

Fourie et al., 1992). Overall, however, it is typically the much larger females, which secrete more neurotoxin, of certain species of ixodid ticks that cause paralysis. For example, in Karoo paralysis, caused by *I. rubicundus* (where both nymphs and females can cause paralysis), fewer feeding females can cause paralysis compared to the number of feeding nymphs required to elicit the same outcome (Spickett et al., 1989; Fourie et al., 1992). In fact, for some species, such as *D. andersoni*, just one attached female tick can cause paralysis and resulting death in adult humans, cows, and other large mammals (Gregson, 1973).

In both argasid and ixodid ticks that cause paralysis, the paralytic condition occurs during the rapid engorgement phase of the attached ticks after they have been attached for several days, and when larger volumes of toxin are secreted. Tick paralysis is reversed after the offending ticks have been removed from the host (Gregson, 1973). For most tick species, movement of the limbs is gradually regained fairly rapidly (within hours or 2–3 days), while in paralysis caused by *I. holocyclus*, symptoms peak 48 hours after tick removal and complete recovery may take weeks (Stone et al., 1989). The ability to cause tick paralysis is a heritable trait at least in species such as *D. andersoni*, as shown by Lysyk (2010). Further, not all individuals of a given species or of the same stage or sex are able to induce the condition. Within the range of *D. andersoni* in western North America, for example, there are distinct populations of this tick that either cause, or do not cause, paralysis (Gregson, 1973; Wilkinson, 1985).

Tick paralysis is an almost cosmopolitan phenomenon, with different tick species causing the problem in different regions (Gregson, 1973; Gothe, 1999; Mans et al., 2004, 2008; Estrada-Peña and Mans, 2014) (Table 1). The first cases were apparently recorded in 1824 in Australia and were described as able to, “destroy either man or beast” (Stanbury and Huyck, 1945; Gregson, 1973), but more detailed accounts were recorded in 1904 and 1912, in sheep and humans, respectively, in Australia, then in 1890 in South Africa, and in 1912 in North America (Todd, 1914; Gregson, 1973). Tick paralysis has been recorded in a variety of vertebrate animals, including reptiles (Hanson et al., 2007), birds (Luttrell et al., 1996) and, especially, mammals (Gregson, 1973). Several ixodid species, including *Amblyomma americanum*, *A. hebraicum*, *A. maculatum*, *A. ovale*, *Dermacentor andersoni*, *D. auratus*, *D. occidentalis*, *D. variabilis*, *Hyalomma truncatum*, *Ixodes hexagonus*, *I. holocyclus*, *I. pacificus*, *I. redikorzevi*, *I. ricinus*, *Rhipicephalus praetextatus*, and *R. simus* have been reported to cause paralysis in humans (Table 1) but argasid ticks are not known to induce this condition in humans (Gregson, 1973).

As a general rule, tick paralysis typically manifests in non-native mammals such as sheep, goats, cattle, humans, and domestic dogs and cats (Estrada-Peña and Mans, 2014). However, there are many exceptions to this trend with, for example, American bison and experimentally infected native rodents sometimes being paralyzed by *D. andersoni* in North America (Kohls and Kramis, 1952; Hughes and Philip, 1958), some native ungulate species being affected by *I. rubicundus* in

South Africa (Fourie et al., 1992), native snowshoe hares being paralyzed by *I. pacificus* in western Canada (Scott et al., 2014), and native canids paralyzed presumably by *D. variabilis* in the United States (Davidson et al., 1992; Beyer and Grossman, 1997). Nevertheless, the fact that tick paralysis is much less common in native vertebrates in the natural range of tick species that cause paralysis suggests some form of local evolutionary adaptation or tolerance to these toxins.

# Toxins involved in tick paralysis

The pharmacopeia of proteins secreted by ticks during feeding, generally referred to as the sialome, may range from hundreds to thousands of different proteins from a single species (Francischetti et al., 2009; Karim et al., 2011; Mans et al., 2011; Schwarz et al., 2013). Important host defense systems that include inflammation, pain sensing, blood coagulation, platelet aggregation, and vasoconstriction, are targeted by feeding ticks to ensure uninterrupted blood-flow and escape from the host immune system (Francischetti et al., 2009; Mans et al., 2011). Formation and maintenance of an adequate hematoma from which to feed, through degradation of the extra-cellular matrix and interference with wound healing, are also an integral component of the feeding process. These modulatory mechanisms may have local and systemic effects on healthy, immune-compromised and defense-deficient hosts and may be considered part of general toxicoses, although not strictly the mediators of paralysis (Estrada-Peña and Mans, 2014).

Recently, it was proposed that all ticks should be considered venomous based on similarities in salivary protein families between ticks and other venomous arachnids (Cabezas-Cruz and Valdés, 2014). In this context, most proteins secreted by ticks would be considered as venoms or toxins (Fry et al., 2009), blurring the distinction between defense and immune modulatory proteins and the causative agents of paralysis. These toxins have been shown to be well defined entities that may be purified from salivary and tick homogenates. In *Argas walkerae*, *I. holocyclus*, and *R. evertsi evertsi*, paralysis toxins were shown to be associated with high molecular mass complexes ranging from 40–100 kDa (Masina and Broady, 1999; Viljoen et al., 1986, 1990). Purification of the toxins resulted in low molecular mass toxins (5–11 kDa) in *A. walkerae* and *I. holocyclus*, respectively (Thurn et al., 1992; Maritz et al., 2000). In *R. evertsi evertsi*, a ~20 kDa trimeric complex results in a toxin with a molecular mass of ~68 kDa, whereas the toxins from *D. andersoni* have masses ranging from 36–43 kDa (Lysyk et al., 2009a). To date, holocyclotoxin-1 from *I. holocyclus* is the only paralysis toxin for which molecular data have been reported (Masina and Broady, 1999). This toxin is a small (~6 kDa), disulphide-rich (8-cysteine) peptide:



Holocyclotoxin-1 was reported to exhibit a structural fold, similar to that associated with scorpion neurotoxins (Masina and Broady, 1999; Nicholson et al., 2006). However, since its deposition in Genbank in 2004, no homologs have been retrieved using any of the databases available (Estrada-Peña and Mans, 2014). The major folds in scorpion neurotoxins include the Toxin\_2 fold that targets potassium channels (**Cx5Cx3Cx10Cx4Cx1C**), the knottin fold (**Cx3Cx5–9Cx3Cx9–11Cx1Cx14–19C**) that targets sodium channels, and the cystine-knot fold (**Cx3–7Cx4–6Cx0–5Cx1–4Cx4–10C**) (Pallaghy et al., 1994; Rodríguez de la

Vega and Possani, 2005; Tan et al., 2006). The latter is also found extensively in spider toxins with 65 scorpion and 775 spider toxins in the KNOTTIN database (Smith et al., 2011; Vink et al., 2014). None of these folds have cysteine motifs that correspond with that for holocyclotoxin-1 (**Cx7Cx3Cx3Cx1Cx14Cx2Cx9C**).

Recently, the structure of holocyclotoxin-1 was determined and shown to exhibit a core disulphide bond motif similar to that of the cystine-knot. Surprisingly, the three-dimensional structure is unique from spider and scorpion cystine knot fold; the possibility of convergent evolution of this fold in arachnids was proposed (Vink et al., 2014). Available data would, therefore, suggest that tick paralysis toxins display a variety of molecular sizes that may be linked to different protein folds, with no distinct evolutionary relationship to toxins from other arachnids. This conclusion should not, however, be taken as grounds to classify all salivary proteins as toxins because most of these proteins have a benign effect on the host (Estrada-Peña and Mans, 2014).

## Vaccines protective against tick paralysis

Survival of animals exposed to paralysis ticks can lead to development of immunity against paralysis toxins, as has been observed in cattle and dogs for *I. holocyclus* infestations (Ross, 1935; Oxer, 1948; Doube and Kemp, 1975; Stone et al., 1983). It has been considered that immunity against paralysis caused by *D. andersoni* does not occur (Gregson, 1973), but recently it was shown that immunity may develop in cattle and sheep (Lysyk et al., 2009a, 2009b). Treatment with hyperimmune serum may also inhibit paralysis in dogs, humans, and sheep (Pearn, 1983; Masina and Broady, 1999; Lysyk et al., 2009b). Purified toxic preparations that were inactivated using gluteraldehyde showed significant ability to induce immunity against paralysis; these results indicated that a subunit vaccine may be feasible (Wright et al., 1983; Stone et al., 1986). A recombinant form of holocyclotoxin-1 has also been produced and shown to be effective against paralysis (Masina and Broady, 1999). Since this latter study, however, no new reports on vaccine development have been published in peer-reviewed literature, although interest in vaccine development has not waned, as indicated by a recent patent that describes a family of peptides related to holocyclotoxin-1 (WO2014/018724A1).

# Could tick paralysis be an evolutionary strategy of ticks?

Is tick paralysis an evolutionary vestige acquired by ticks from their arachnid ancestors, or an evolutionary strategy that gives some ticks a fitness advantage, or something else? Phylogenetically, the closest ancestors of ticks are other mites (ticks are large mites), non-toxic ricinuleids (tick-like spiders), and then other arachnids, including the toxic (venomous) spiders and scorpions (Schultz, 1990). Unless ticks retained the trait of toxin secretion via chelicerae from spiders or other venomous arachnids, these phylogenetic relationships do not support the premise that toxin production by ticks is an evolutionary holdover from their ancestors. Further, the molecular structures of tick toxins are not closely related to those of spider (or scorpion) toxins (Mans et al., 2004) and, therefore, they almost certainly evolved independently from venoms of other arachnids. Recently, a close phylogenetic relationship between ticks and pseudoscorpions was proposed, with the ancestor of ticks being a venomous predator of small prey that evolved to feed on larger vertebrates (Cabezas-Cruz and Valdés, 2014). This proposed relationship is not, however, supported by molecular systematics or morphology (Schultz, 1990; Regier et al., 2010; Ovchinnikov and Masta, 2012).

Extreme convergence of toxins within the Arachnida had been proposed (Fry et al., 2009), making inferences regarding toxin relationships between Orders difficult, if not impossible, as indicated by the recent structure of holocyclotoxin-1 (Vink et al., 2014). Molecular phylogenetic analyses by Dobson and Barker (1999) and total evidence data analyses by Klompen et al. (2000) have shown the sister group of the Ixodida (comprising all ticks) to be the Holothyrida, an assemblage of free-living mites that typically feed on body fluids of dead arthropods, a trait that ticks presumably also had before they switched to an ectoparasitic blood-feeding lifestyle (Walter and Proctor, 1998; Mans et al., 2011). Holothyrids are not equipped with oral toxins so, again, acquisition of toxins from their ancestors by paralysis-causing ticks seems a remote possibility at most. Similarly, the non-hematophagous ancestors of ticks would have had no need for oral toxins. These data suggest that toxin secretion by ticks has derived *de novo*, and the various chemical compositions of these toxins strongly suggest that toxin secretion has evolved independently on multiple occasions. Further, toxin production likely evolved when ticks switched to a hematophagous lifestyle, probably when early mammals were diversifying >120 million years ago (Klompen et al., 1996; Mans et al., 2011). At this time, ticks would have also evolved salivary secretions to modulate host defense responses.

So, why are some tick species equipped with oral paralysis-causing toxins? This is difficult to determine because only certain species of ticks secrete toxins. Available evidence suggests that these toxins are synthesized by the ticks themselves during prolonged feeding and that they are not derived from pathogens or other symbiotic organisms in ticks, or from interactions between tick and host

chemicals during the ectoparasitic feeding process (Mans et al., 2004). There are several scenarios that can be envisaged whereby ticks would gain an advantage by temporarily immobilizing their hosts (Gothe, 1984). Host immobility, especially of the limbs, would impair grooming ability so that attached ticks would be difficult or impossible to remove by self-grooming host activities. Further, the fact that host paralysis occurs following 4–8 days of tick attachment coincides with the rapid engorgement phase of feeding ticks, which is when large ectoparasites would be most likely to be noticed and removed by the host.

Tick paralysis is also typically accompanied by both increased host respiration (Gregson, 1973) and increased exhalation of CO<sub>2</sub>, physiological events that could attract more ticks to the host. This attraction would be further enhanced by release of attraction and aggregation pheromones by the ticks themselves. More ticks on the same host individual confers more mating opportunities for adult ticks, which would result in more gravid female ticks and eventually to more egg masses and resulting larvae to perpetuate tick populations. Such aggregation is especially significant for metastriate ticks (comprising all ixodid genera except *Ixodes*) which, for maturation and developmental reasons, can only mate on the host while the female is attached and feeding (Oliver, 1989). However, even for prostriate ticks (species of *Ixodes*), which can mate either on or off the host (Oliver, 1989), finding a mate would be easier on the confines of a host body if a large number of conspecific ticks are parasitizing or crawling on it simultaneously.

Tick paralysis toxins could also cause local anesthesia in hosts, prevent blood clotting at tick feeding sites, or act as feeding stimulants for other attached ticks (Stone et al., 1989). Some tick toxins could, therefore, be by-products of compounds that have evolved to increase feeding efficacy in attached ticks (Estrada-Peña and Mans, 2014). Crause et al. (1993) have suggested a role in regulating tick protein synthesis by the paralysis toxin of *Rhipicephalus evertsi* in Africa. If some tick paralysis toxins have a role in tick physiology, then causing paralysis in the host could represent a by-product of these molecules. In support of this idea, independent origins of tick toxins would correspond with the independent evolution of anti-hemostatic and anti-inflammatory mechanisms in ticks (Mans, 2011). Another contrasting consideration is that tick paralysis with resultant death of the host could act as a density dependent mechanism for regulating the abundance of certain tick species in nature (Estrada-Peña and Mans, 2014).

Conversely, there are also obvious potential disadvantages for ticks that cause host paralysis. If the condition ultimately causes death of the host then any attached ticks are left without an immediate source of blood and would likely perish. Some ticks can mature or lay egg masses if they are not fully engorged and, rarely, some tick species can detach and then re-attach to another host (Oliver, 1989), but this situation would be an atypical, unpredictable, and precarious predicament for a tick. Further, immobilized hosts are more prone to predation than healthy ones, resulting in co-predation of attached ticks, or to death by other means (drowning,

dehydration, etc.), events that would also result in the loss of the host for ticks.

Given these scenarios, it is difficult to state whether tick paralysis represents an overall advantage or disadvantage to tick populations. However, because of the large numbers of ticks that can feed, mate, and develop egg masses from paralyzed hosts, there is likely a small advantage to tick populations, although this advantage probably differs by tick and host species. For example, a paralyzed small bird or small mammal would be more likely to be taken by a predator than would a paralyzed cow or sheep, at least in most parts of the world.

Anecdotally, we also highlight a case of apparent human toxicity to feeding ticks published in *The Journal of Parasitology* in which the tables were turned (Brennan, 1947). Adult and nymphal ixodid ticks (*Amblyomma americanum* and *D. andersoni*) that attached to an adult human male for a few minutes either in nature or under experimental conditions almost invariably died or dropped off the host and became, “paralyzed” (Brennan, 1947). The cause of this apparent toxicity towards ticks could not be determined, but we are aware of similar cases (which were not further investigated) of certain individual humans killing ticks that had attached to them.

## Lessons learned

Perhaps the most important and basic lesson learned from studies of tick paralysis is that the condition is reversible when the offending ticks are removed from the host (although with *I. holocyclus*-induced paralysis in Australia, symptoms can persist and often peak about 2 days after tick removal). One clinical case that illustrates the importance of timely tick removal in tick paralysis cases was documented by Felz et al. (2000) and involved a 6-year-old girl who was admitted to an intensive care unit with ascending paralysis. Cerebellar ataxia, cervical cord compression, and (especially) Guillain–Barré syndrome were all considered as potential diagnoses. The girl's condition rapidly deteriorated. However, during preparation for femoral vein access for emergency plasmapheresis, an engorged female tick (*D. variabilis*) was found on the patient's head beneath her hair. The tick was removed and the girl gradually resumed motor activity; within 24 hours, she was completely mobile and had recovered uneventfully without further medical intervention. If the tick had not been located and removed, the clinical outcome could have been very different.

Manual tick removal from larger livestock animals such as sheep and cattle is not typically practical, especially if large numbers of attached ticks are present. For these animals, and for most pets, acaricidal treatments are usually more reliable and may be administered via dipping, spraying, topical applications, or systemic products. The development of anti-tick vaccines and of vaccines directed against paralysis-causing tick toxins is an active and ongoing field of research that promises to yield significant results in the future. In-depth biochemical analyses of the various toxins secreted by feeding ticks will greatly aid these efforts.

## Literature cited

- Beyer, A. B., and M. Grossman. 1997. Tick paralysis in a red wolf. *Journal of Wildlife Diseases* **33**: 900–902.
- Brennan, J. M. 1947. An instance of the apparent toxicity of man to ticks. *Journal of Parasitology* **33**: 491–494.
- Cabezas-Cruz, A. and J. J. Valdés. 2014. Are ticks venomous animals? *Frontiers in Zoology* **11**: 47. PubMedCentral 4085379
- Crause, J. C., J. A. Verschoor, J. Coetzee, H. C. Hoppe, J. N. Taljaard, R. Gothe, and A. W. Neitz. 1993. The localization of a paralysis toxin in granules and nuclei of prefed female *Rhipicephalus evertsi evertsi* tick salivary gland cells. *Experimental and Applied Acarology* **17**: 357–363.
- Davidson, W. R., V. F. Nettles, L. E. Hayes, E. W. Howerth, and C. E. Couvillion. 1992. Diseases diagnosed in gray foxes (*Urocyon cinereoargenteus*) from the southeastern United States. *Journal of Wildlife Diseases* **28**: 28–33.
- Dobson, S. J., and S. C. Barker. 1999. Phylogeny of the hard ticks (Ixodidae) inferred from 18S rRNA indicates that the genus *Aponomma* is paraphyletic. *Molecular Phylogenetics and Evolution* **11**: 288–295.
- Doube, B. M. and D. H. Kemp. 1975. Paralysis of cattle by *Ixodes holocyclus* Neumann. *Australian Journal of Agricultural Research* **26**: 635–640.
- Estrada-Peña, A., and B. J. Mans. 2014. Tick-induced paralysis and toxicoses, In D. E. Sonenshine & R. M. Roe (eds.), *Biology of ticks*, Vol. **2**. Oxford University Press, Oxford, U.K., p. 313–332.
- Felz, M. W., C. D. Smith, and T. R. Swift. 2000. A six-year-old girl with tick paralysis. *The New England Journal of Medicine* **342**: 90–94.
- Fourie, L. J., and I. G. Horak. 1987. Tick-induced paralysis of springbok. *South African Journal of Wildlife Research* **17**: 131–133.
- Fourie, L. J., I. G. Horak, and J. M. van Zyl. 1992. Seasonal occurrence of Karoo paralysis in Angora goats in relation to the infestation density of female *Ixodes rubicundus*. *Veterinary Parasitology* **41**: 249–254.
- Francischetti, I., A. Sa-Nunes, B. J. Mans, I. M. Santos, and J. M. Ribeiro. 2009. The role of saliva in tick feeding. *Frontiers in Bioscience* **14**: 2051–2088.
- Fry, B. G., K. Roelants, D. E. Champagne, H. Scheib, J. D. Tyndall, G. F. King, T. J. Nevalainen, J. A. Norman, R. J. Lewis, R. S. Norton, C. Renjifo, and R. C. de la Vega. 2009. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. *Annual Review of Genomics and Human Genetics* **10**: 483–511.

- Gothe, R. 1984. Tick paralyses: reasons for appearing during ixodid and argasid feeding. *Current Topics in Vector Research* **2**: 199–223.
- \_\_\_\_\_. 1999. *Zecken Toxikosen*. Heironyms, Munich, Germany, 377 p.
- \_\_\_\_\_, and K. Kunze. 1970. Research on the clinical pathology of *Argas persicus* larvae paralysing hens. *Journal of Parasitology* **56**: 123.
- \_\_\_\_\_, \_\_\_\_\_, and H. Hoogstraal. 1979. The mechanisms of pathogenicity in the tick paralyses. *Journal of Medical Entomology* **16**: 357–369.
- Gregson, J. D. 1973. *Tick paralysis: an appraisal of natural and experimental data*. Canada Department of Agriculture Monograph No. 9, Ottawa. 109 p.
- Hanson, B. A., P. A. Frank, J. W. Mertens, and J. L. Corn. 2007. Tick paralysis of a snake caused by *Amblyomma rotundatum* (Acari: Ixodidae). *Journal of Medical Entomology* **44**: 155–157.
- Herms, W. B. 1916. The Pajaroello tick (*Ornithodoros coriaceus* Koch) with special reference to life history and biting habits. *Journal of Parasitology* **2**: 137–142.
- Hughes, L. E., and C. B. Philip. 1958. Experimental tick paralysis in laboratory animals and native Montana rodents. *Experimental Biology and Medicine* **99**: 316–319.
- Karim, S., P. Singh, and J. M. Ribeiro. 2011. A deep insight into the sialotranscriptome of the gulf coast tick, *Amblyomma maculatum*. *PLoS One* **6**: e28525.
- King, D. P., C.-I. Chen, M. T. Blanchard, B. M. Aldridge, M. Anderson, R. Walker, J. Maas, D. Hanks, M. Hall, and J. L. Stott. 2005. Molecular identification of a novel Deltaproteobacterium as the etiologic agent of bovine epizootic abortion (foothill abortion). *Journal of Clinical Microbiology* **43**: 604–609.
- Klompen, J. S. H., W. C. Black IV, J. E. Keirans, and D. E. Norris. 2000. Systematics and biogeography of hard ticks, a total evidence approach. *Cladistics* **16**: 79–102.
- \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and J. H. Oliver, Jr. 1996. Evolution of ticks. *Annual Review of Entomology* **41**: 141–161.
- Kohls, G. M., and N. J. Kramis. 1952. Tick paralysis in the American buffalo, *Bison bison* (Linn.). *Northwest Science* **26**: 61–64.
- Luttrell, M. P., L. H. Creekmore, and J. W. Mertins. 1996. Avian tick paralysis caused by *Ixodes brunneus* in the southeastern United States. *Journal of Wildlife Diseases* **32**: 133–136.

- Lysyk, T. J. 2010. Tick paralysis caused by *Dermacentor andersoni* (Acari: Ixodidae) is a heritable trait. *Journal of Medical Entomology* **47**: 210–214.
- \_\_\_\_\_, D. M. Veira, and W. Majak. 2009a. Cattle can develop immunity to paralysis caused by *Dermacentor andersoni*. *Journal of Medical Entomology* **46**: 358–366.
- \_\_\_\_\_, \_\_\_\_\_, J. P. Kastelic, and W. Majak. 2009b. Inducing active and passive immunity in sheep to paralysis caused by *Dermacentor andersoni*. *Journal of Medical Entomology* **46**: 1436–1441.
- Malik, R., and B. R. H. Farrow. 1991. *Tick paralysis in North America and Australia*. In *Veterinary clinics of North America: Small animal practice Vol. 21: Tick-transmitted diseases*, J. D. Hoskins (ed.) W. B. Saunders, Philadelphia, Pennsylvania. p. 157–171.
- Mans, B. J. 2011. Evolution of vertebrate hemostatic and inflammatory control mechanisms in blood-feeding arthropods. *Journal of Innate Immunology* **3**: 41–51.
- \_\_\_\_\_, D. de Klerk, R. Pienaar, and A. A. Latif. 2011. *Nuttalliella namaqua*: a living fossil and closest relative to the ancestral tick lineage: implications for the evolution of blood-feeding in ticks. *PLoS One* **6**: e23675.
- \_\_\_\_\_, R. Gothe, and A. W. H. Neitz. 2004. Biochemical perspectives on paralysis and other forms of toxicoses caused by ticks. *Parasitology* **129**: S95–S111.
- \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_. 2008. Tick toxins: perspectives on paralysis and other forms of toxicoses caused by ticks. In *Ticks: biology, disease and control*, A. S. Bowman and P. A. Nuttall (eds.). Cambridge University Press, Cambridge, U.K., p. 108–126.
- Maritz, C., A. I. Louw, R. Gothe, and A.W. Neitz. 2000. Detection and micro-scale isolation of a low molecular mass paralysis toxin from the tick, *Argas (Persicargas) walkerae*. *Experimental and Applied Acarology* **24**: 615–630.
- Masina, S., and K. W. Broady. 1999. Tick paralysis: Development of a vaccine. *International Journal for Parasitology* **29**: 535–541.
- McCaffrey, D. 1916. The effect of tick bites on man. *Journal of Parasitology* **2**: 193–194.
- Nicholson, G. M., A. Graudins, H. I. Wilson, M. Little, and K. W. Broady. 2006. Arachnid toxinology in Australia: From clinical toxicology to potential applications. *Toxicon* **48**: 872–898.
- Oliver, Jr., J. H. 1989. Biology and systematics of ticks (Acari: Ixodida). *Annual Review of Ecology and Systematics* **20**: 397–430.

Ovchinnikov, S., and S. E. Masta. 2012. Pseudoscorpion mitochondria show rearranged genes and genome-wide reductions of RNA gene sizes and inferred structures, yet typical nucleotide composition bias. *BMC Evolutionary Biology* **12**: 31 BioMedCentral 1471–2148/12/31.

Oxer, D. T. 1948. The preparation of canine anti-tick serum. *Australian Veterinary Journal* **24**: 95–96.

Pallaghy, P. K., K. J. Nielsen, D. J. Craik, and R. S. Norton. 1994. A common structural motif incorporating a cystine knot and a triple-stranded beta-sheet in toxic and inhibitory polypeptides. *Protein Science* **3**: 1833–1839.

Pearn, J. H. 1983. Clinical aspects of tick envenomation of humans. *Toxicon* **21** (Suppl. 3): 349–352.

Regier, J. C., J. W. Shultz, A. Zwick, A. Hussey, B. Ball, R. Wetzer, J. W. Martin, and C. W. Cunningham. 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature* **463**: 1079–1083.

Rodríguez de la Vega, R. C., and L. D. Possani. 2005. Overview of scorpion toxins specific for Na<sup>+</sup> channels and related peptides: Biodiversity, structure-function relationships and evolution. *Toxicon* **46**: 831–844.

Ross, I. C. 1935. Tick paralysis: a fatal disease of dogs and other animals in eastern Australia. *Journal of the Council for Scientific and Industrial Research (Australia)* **8**: 8–13.

Schultz, J. W. 1990. Evolutionary morphology and phylogeny of arachnids. *Cladistics* **6**: 1–38.

Schwarz, A., B. M. von Reumont, J. Erhart, A. C. Chagas, J. M. Ribeiro, and M. Kotsyfakis. 2013. De novo *Ixodes ricinus* salivary gland transcriptome analysis using two next-generation sequencing methodologies. *FASEB Journal* **27**: 4745–4756.

Scott, J. D., C. M. Scott, and J. F. Anderson. 2014. Tick paralysis in a snowshoe hare by *Ixodes pacificus* ticks in British Columbia, Canada. *Journal of Veterinary Science and Medicine* **2**: 5–10.

Shipley, M. M., J. W. Dillworth, A. S. Bowman, R. C. Essenberg, and J. R. Sauer. 1993. Changes in lipids of the salivary glands of the lone star tick, *Amblyomma americanum*, during feeding. *Journal of Parasitology* **79**: 834–842.

Smith, J. J., J. M. Hill, M. J. Little, G. M. Nicholson, G. F. King, and P. F. Alewood. 2011. Unique scorpion toxin with a putative ancestral fold provides insight into evolution of the inhibitor cystine knot motif. *Proceedings of the National Academy of Sciences USA* **108**: 10478–10483.

Spickett, A. M., E. G. R. Elliott, H. Heyne, and J. A. Neser. 1989. Paralysis of

laboratory rabbits by nymphae of *Ixodes rubicundus* Neumann, 1904 (Acarina: Ixodidae) and some effects on the life-cycle following feeding under different temperature conditions. *Onderstepoort Journal of Veterinary Research* **56**: 59–62.

Stanbury, J. B., and J. H. Huyck. 1945. Tick paralysis: a critical review. *Medicine* **24**: 219–242.

Stone, B. F., K. C. Binnington, M. Gauci, and J. H. Aylward. 1989. Tick/host interactions for *Ixodes holocyclus*: Role, effects, biosynthesis and nature of its toxic and allergenic oral secretions. *Experimental and Applied Acarology* **7**: 59–69.

Stone, B. F., A. L. Neish, J. J. Morrison, and M. L. Uren. 1986. Toxoid stimulation in dogs of high titres of neutralising antibodies against holocyclotoxin, the paralysing toxin of the Australian paralysis tick *Ixodes holocyclus*. *Australian Veterinary Journal* **63**: 125–127.

Stone, B. F., A. L. Neish, and I. G. Wright. 1983. Tick (*Ixodes holocyclus*) paralysis in the dog—quantitative studies on immunity following artificial infestation with the tick. *Australian Veterinary Journal* **60**: 65–68.

Swartzwelder, J. C., and J. H. Seabury. 1947. Bite of *Amblyomma americanum* associated with possible tick paralysis. *Journal of Parasitology* **33**: 22.

Tan, P. T., A. Veeramani, K. N. Srinivasan, S. Ranganathan, and V. Brusic. 2006. SCORPION2: a database for structure-function analysis of scorpion toxins. *Toxicon* **47**: 356–363.

Thurn, M. J., A. Gooley, and K.W. Broady. 1992. Identification of the neurotoxin from the paralysis tick, *Ixodes holocyclus*. In *Recent advances in toxicology research*, P. Gopalakrishnakone and C. K. Tan (eds.). Venom and Toxin Research Group, National University of Singapore, Singapore, p. 243–256.

Todd, J. L. 1914. Tick paralysis. *Journal of Parasitology* **1**: 55–64.

Viljoen, G. J., J. D. Bezuidenhout, P. T. Oberem, N. M. Vermeulen, L. Visser, R. Gothe, and A. W. H. Neitz. 1986. Isolation of a neurotoxin from the salivary glands of female *Rhipicephalus evertsi evertsi*. *Journal of Parasitology* **72**: 865–874.

Viljoen, G. J., S. Van Wyngaardt, R. Gothe, L. Visser, J. D. Bezuidenhout, and A.W. Neitz. 1990. The detection and isolation of a paralysis toxin present in *Argas (Persicargas) walkerae*. *Onderstepoort Journal of Veterinary Research* **57**: 163–168.

Vink, S., N. L. Daly, N. Steen, D. J Craik, and P. F. Alewood. 2014. Holocyclotoxin-1, a cystine knot toxin from *Ixodes holocyclus*. *Toxicon*. doi: 10.1016/j.toxicon.2014.08.068.

Walter, D. R., and H. C. Proctor. 1998. Feeding behavior and phylogeny: Observations on early derivative Acari. *Experimental and Applied Acarology* **22**: 39–50.

Wilkinson, P. R. 1985. Differences in paralyzing ability and sites of attachment to cattle of Rocky Mountain wood ticks (Acarai: Ixodidae) from three regions of western Canada. *Journal of Medical Entomology* **22**: 28–31.

Wright, I. G., B. F. Stone, and A. L. Neish. 1983. Tick (*Ixodes holocyclus*) paralysis in the dog—induction of immunity by injection of toxin. *Australian Veterinary Journal* **60**: 69–70.



# Chapter 12

## Effect of Low Temperatures on Trophozoites of *Giardia Muris*<sup>\*</sup>

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In recent years, due to the difficulty often encountered in the propagation of parasitic protozoa, several individuals investigated the effects of low temperatures on these organisms; they hoped to utilize such techniques as a means of prolonged preservation of the living organisms. The first successful experiments were done by Coggesshall (1939). Manwell (1943) and other workers made additional contributions to the low temperature work on malarial parasites.

Polge, Smith and Parkes (1949) discovered that spermatozoa would remain alive and viable after storage at -76°C, if the semen was stored in 10 percent glycerol. This discovery soon led to some attempts to apply this technique to various protozoans. Fulton and Smith (1953) found that *Entamoeba histolytica* trophozoites survived at -79°C after slow cooling in buffered horse serum—Ringers medium containing either 5 or 10 percent glycerol, for periods of 7 and 50 days, respectively.

Levine and his co-workers have recently reported some interesting work on the survival of *Trichomonas foetus* in glycerol. Levine and Marquardt (1954) reported the survival of this parasite at freezing temperatures. Joyner (1954) reported no survival of this same parasite in glycerol-treated semen. Levine and Marquardt (1955) cultured *T. foetus* in CPLM medium (Johnson and Trussell 1943) to which was added 5 or 10 percent glycerol. This mixture was then frozen at temperatures of -20°C or -76°C. They froze the parasites slowly in some experiments and rapidly in others. Their results showed that *T. foetus*, if frozen slowly, would survive freezing for periods up to 64 days. They did not survive quick freezing. They also conducted experiments in which they substituted compounds related to glycerol for the glycerol. None of the compounds utilized were as effective as glycerol.

### MATERIALS AND METHODS

The experimental techniques used in these experiments were similar to those employed by Levine and his co-workers. The flagellate studied was *Giardia muris* from the small intestines of mice. The initial difficulty encountered was due to the fact that there was no known medium which would grow and maintain any species of *Giardia*. A survival medium, which had previously been devised by the author, and in which the trophozoites remain alive and motile for approximately 3 days, was employed. This medium was composed of the following: Tyrode's solution, 50 ml; ascorbic acid, 0.02 g; L-cysteine HC1, 0.1 g; and Pangestin, a Difco preparation of pancreatic enzymes, 0.05 g. Tween 80, a surface active agent, was sometimes added to the Tyrode's as 1 percent of this solution. The pH was adjusted to 7.2 with NaOH. Glycerol was added in amounts ranging from 11 to 50 percent. Ethylene glycol was substituted for glycerol in one experiment.

The trophozoites used in this series of experiments were collected directly from the intestine of a heavily infected mouse. No attempt was made to sterilize the inoculum. The contents of the small intestine were mixed with the survival medium and the trophozoites checked for motility. The results were calculated on the basis of 100 percent motility. Motility of the flagella was used as the criterion for determining if the parasites survived freezing. The first 100 trophozoites, or the total number per slide if less than 100 were present, were checked for motility and the percentage of organisms living determined from this information.

The trophozoites, after collection from the host and mixing with the media, were placed in either flat-bottomed 22-by 122-mm or round-bottomed 16- by 125-mm screw-capped vials and mixed with definite quantities of glycerol. These tubes were then subjected to low temperatures. Several temperatures were used: (1) The first temperature was 8°C; (2) the second, -20°C; (3) the majority of the tubes were stored at -38°C ± 5°C, either wrapped in cotton or placed in the freezer without insulation; (4) a few tubes were stored at -70°C. All of these were quick-frozen in a dry ice-alcohol bath.

When the tubes were removed from the cold for examination, they were allowed to stand at room temperature for approximately 1 hour. After this interval the medium warmed and the trophozoites were motile. The tubes were then sampled and checked, as previously described, to determine the number of *Giardia* alive. If a considerable number of living trophozoites were noted in a particu-

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lar tube, the tube was sometimes refrozen to see if the parasite could tolerate this procedure.

#### RESULTS

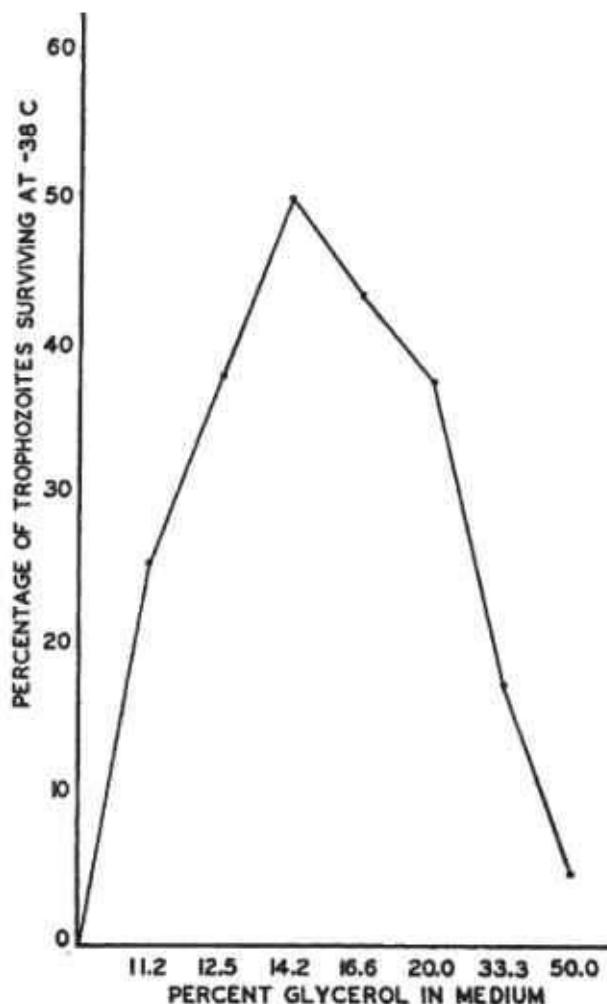
The results of this experiment indicated that *G. muris* and *T. foetus* react to cold in the presence of glycerol in a somewhat similar manner. No living trophozoites were observed after 24 hours exposure at 8 C. At -20 C, -38 C and -70 C the parasites would survive at least 24 hours if glycerol were employed in the medium. A total of 118 control tubes, without glycerol, were checked throughout the course of the experiments. No living or dead trophozoites, at any of the temperatures employed, were ever observed in the control tubes. The cells had lysed and disappeared after 24 hours. A total of 330 tubes containing glycerol were examined. One hundred and thirty-eight or 41.8 percent of these tubes contained living *Giardia* trophozoites.

At -20 C, 18 tubes were used containing 33.3 percent glycerol and 18 with 50 percent glycerol. The results showed a 15.3 percent average survival per 100 organisms per tube containing 33.3 percent glycerol and no survival in the tubes containing 50 percent glycerol.

Many more samples were exposed to -38 C than -20 C. This temperature appeared, in some of the initial work, to be the most favorable for the survival of *G. muris*. Utilizing this temperature, additional factors that might influence the survival of *Giardia muris* in the cold were examined. Figure 1 shows the effect of various glycerol concentrations on the survival of the trophozoites at -38 C. A concentration of 14.2 percent glycerol gave the best results. As the concentration was increased or decreased the number of living organisms present decreased. The rate of cooling and the presence of Tween 80 in the medium also appeared to influence the survival of *G. muris* at -38 C.

Slow freezing in cotton insulated tubes resulted in a higher survival rate than did quick freezing, though the survival rate is low under both conditions. These results agree in general with the results obtained by Levine and Marquardt (1955) with the exception that they reported no survival of *T. foetus* after thawing and refreezing. It was also noted that if the basal medium contained 1 percent by volume of Tween 80 and glycerol was added, the number of living trophozoites present after freezing and thawing was greater than when the Tween 80 was not present in the medium.

A few tubes of inoculated medium containing 5 different concentrations of glycerol were exposed to -70 C. The samples were cooled rapidly to freezing by immersing the tubes in a dry ice-alcohol bath. All of the tubes contained Tween 80. There was no survival at the higher concentrations of 80, 66.6, and 50 percent glycerol. In lower concentrations of 33.3 and 20 percent glycerol, survival was good. The extremely rapid freezing of the tubes at these temperatures did not kill the trophozoites. In fact the limited number of tubes used, four in each case, showed an average of over 50 percent survival, 53 percent for the tubes with 33.3 percent glycerol, and 69 percent for the tubes contain-



ing 20 percent glycerol. As only a small number of tubes were used, these figures may be high, but the results do show that the trophozoites of *G. muris* will survive quick freezing at a low temperature.

An attempt was made to substitute ethylene glycol for the glycerol. There was no survival in any of the 20 tubes containing concentrations of ethylene glycol ranging from 10 to 50 per-

cent.

Several tubes were also checked to determine if a normal saline solution could be substituted for the basal survival medium. Survival in normal saline was zero; in Tyrode's solution, which is actually a part of the basal survival medium, the average survival was 5.1 percent.

In all experiments, where fairly large numbers of tubes were examined under the same conditions, a tremendous variation was noted in the number of living organisms. The range was from 0 to 97 percent survival. Levine (1959) noted this same situation in his work with *T. foetus*.

The length of time *G. muris* will survive at low temperatures has not been determined past 27 days for unthawed samples and 40 days for refrozen samples. The number of tubes examined was small and the number of surviving organisms was also small, but *G. muris* will survive prolonged exposure to low temperatures.

#### DISCUSSION

An explanation of the action of glycerol was proposed by Lovelock (1953, 1954) from information derived from work on hemolysis of red blood cells. He stated that glycerol functions as a "salt buffer." If the temperature is lowered, more water is withdrawn and the salt concentration of the cell increases. If glycerol is added to the salt solution, the concentration of salt in equilibrium with ice is lowered for any temperature during freezing or thawing. As a result the cells remain intact in the presence of glycerol, while without it the cells will lyse.

Lovelock (1954a) listed four characteristics that a substance should possess in order to protect cells during freezing and thawing: (1) Low molecular weight, (2) miscibility with salt solutions, (3) non-toxicity, and (4) ability to permeate the cell freely. A surface activating agent such as Tween 80 could increase the permeability of the cell wall and thus increase the protective ability of glycerol for cells exposed to sub-zero temperatures.

The concentration of glycerol used should be approximately 14 percent of the total volume of the medium for maximum efficiency when freezing *G. muris*. Increasing or decreasing the concentration decreases its ability to protect the trophozoites at low temperatures.

In all of the tubes checked for trophozoites no cysts were ever seen. The trophozoites either

persisted in that form or it lysed completely. Cyst formation apparently is not dependent upon an adverse temperature situation. Temperature may play no part in cyst formation.

Insulation of the tubes at the time they are frozen aids in the survival of the parasites. *G. muris* will, however, survive quick freezing in a dry ice-alcohol bath. The tubes were, in this case, allowed to stand for about 30 minutes before immersion in the dry ice-alcohol bath to allow time for the glycerol to penetrate the cell.

#### SUMMARY

*Giardia muris* can survive cooling to low temperatures and subsequent thawing in the presence of glycerol. Retarding the rate of freezing by utilizing cotton as the insulating material appears to facilitate the survival of *G. muris* at -38°C, as does the addition of Tween 80 to the medium. The trophozoites will survive quick freezing at -70°C. The parasite can survive in the frozen state for 40 days and can survive thawing and refreezing.

#### LITERATURE CITED

- COGGESHALL, L. T. 1939 Preservation of viable malaria parasites in the frozen state. Proc. Soc. Exp. Biol. Med. 42: 499-501.
- FULTON, J. D. AND SMITH, A. U. 1953. Preservation of *Entamoeba histolytica* at -79°C in the presence of glycerol. Ann. Trop. Med. Parasit. 47: 240-246.
- JOHNSON, F. AND TRUSSELL, R. E. 1943 Experimental basis for the chemotherapy of *Trichomonas vaginalis* infestations. Proc. Soc. Exp. Biol. Med. 54: 245-249.
- JOYNER, L. P. 1954. The elimination of *Trichomonas foetus* from infected semen by storage in the presence of glycerol. Vet. Rec. 66: 727-730.
- LEVINE, N. D. AND MARQUARDT, W. C. 1954. The effects of glycerol on survival of *Tritrichomonas foetus* at freezing temperatures. J. Prot. 1 (supp): 4.
- AND MARQUARDT, W. C. 1955. The effect of glycerol and related compounds on survival of *Tritrichomonas foetus* at freezing temperatures. J. Prot. 2: 100-107.
- LEVINE, N. D., McCaul, W. E. AND MIZELLE, M. 1959. The relation of the stage of the population growth curve to the survival of *Tritrichomonas foetus* upon freezing in the presence of glycerol. J. Prot. 6: 116-120.
- LOVELOCK, J. E. 1953a. The haemolysis of human red blood cells by freezing and thawing. Biochem. Biophys. Acta. 10: 414-426.
- 1953b. The mechanism of the protective action of glycerol against haemolysis by freezing and thawing. Biochem. Biophys. Acta
- MANWELL, R. D. 1943. The low temperature freezing of malaria parasites. Am. J. Trop. Med. 23: 123-131.
- POLGE, C., SMITH, A. U. AND PARKES, A. S. 1949. Revival of spermatozoa after nitrification and dehydration at low temperatures. Nature 164: 166.





# ***Giardia intestinalis* biochemistry and regulation: An evolutionary tale**

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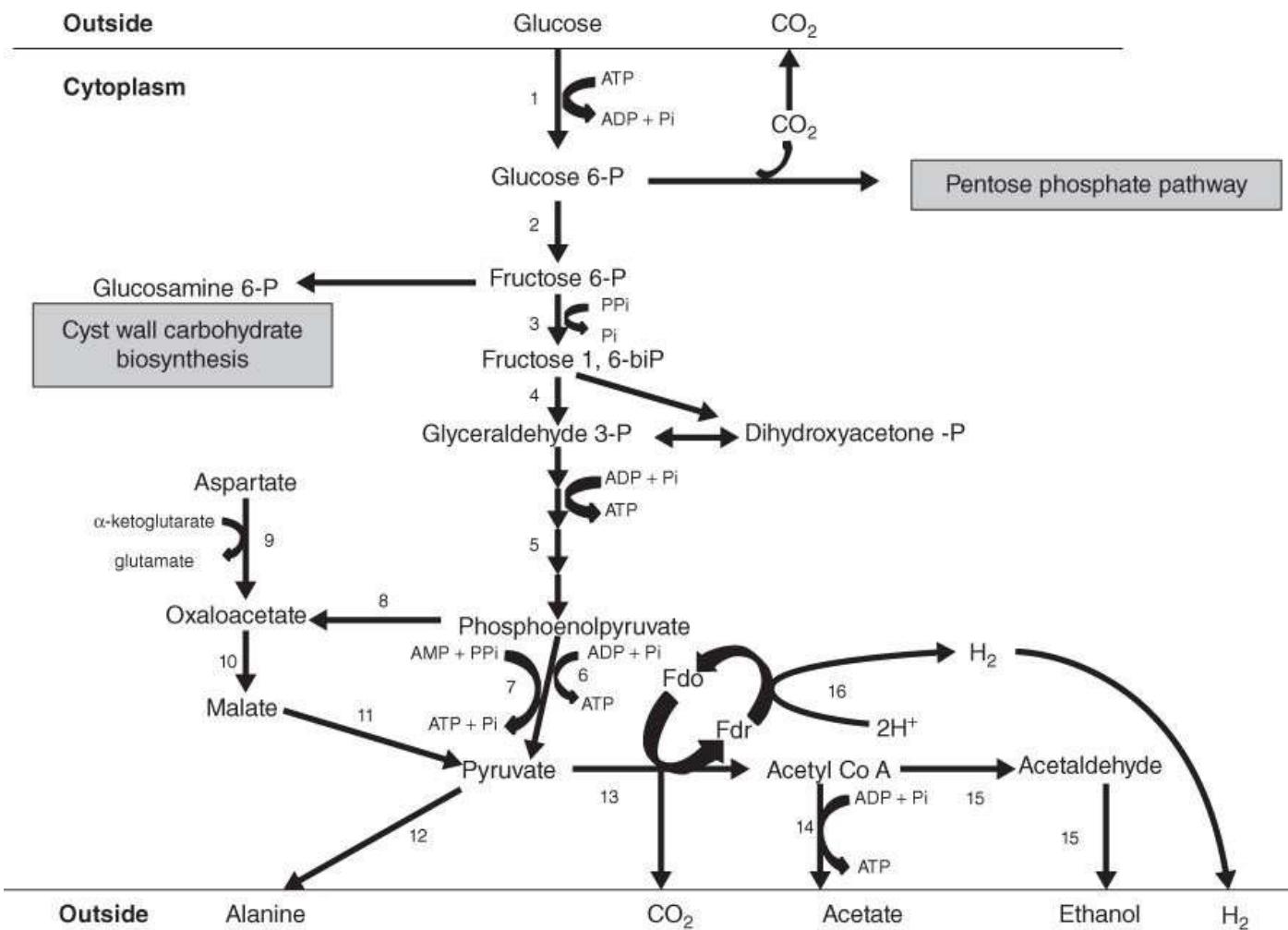
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## **Four decades of *Giardia intestinalis* research**

Since *Giardia intestinalis* (syn. *G. lamblia*, *G. duodenalis*) was cultivated axenically in the 1970s (Meyer, 1970; Fortess and Meyer, 1976), much has been learned, not only regarding the general biology of this organism, but also about its biochemistry, cell biology, and pathogenicity. Here, we discuss our present understanding of how the *G. intestinalis* carbohydrate and energy metabolism has evolved. In addition, we review how integrated “omics” technologies, i.e., proteomics and metabolomics, have and will guide our understanding of the *G. intestinalis* survival and adaptation to *in situ* changes, as well as how these technologies provide alternative approaches for the identification of new chemotherapeutic targets and their modes of action.

# Glucose metabolism in growing (non-encysting) trophozoites

*Giardia intestinalis*, a simple eukaryote and aerotolerant anaerobe containing few organelles, notably lacks typical Golgi apparatus and mitochondria. Metabolically, the trophozoites lack cytochrome-mediated oxidative phosphorylation. Initial studies on *G. intestinalis* metabolism used standard biochemical approaches, namely, enzyme assays and radiolabeling. It is clear from these earlier studies that when grown in axenic culture, *G. intestinalis* uses glucose as its sole sugar for energy production by substrate level phosphorylation (Lindmark, 1980; Jarroll et al., 1981; Schofield, et al., 1991) (Fig. 1). However, there is a pronounced difference in glucose utilization in cysts and encysting trophozoites compared to that in non-encysting (growing) trophozoites (Paget et al., 1993, 1998). When *G. intestinalis* trophozoites are encysting, glucose stimulates the oxygen uptake rate (a measure of metabolism) initially, but that stimulation drops precipitously by 12 h into encystment. Oxygen uptake rates approximately double (from  $4.9 \text{ } \mu\text{M O}_2 \text{ min}^{-1} \text{ } 10^{-6} \text{ cells}$ ) during the first 5 h into encystment. This increase is followed by a marked decrease to  $2.3 \text{ } \mu\text{M O}_2 \text{ min}^{-1} \text{ } 10^{-6}$  by 12 h into encystment. By 50 h, oxygen uptake was  $0.7 \text{ } \mu\text{M O}_2 \text{ min}^{-1} \text{ } 10^{-6}$  cells. Glucose stimulated oxygen uptake by 89% in trophozoites, but did not stimulate demonstrable oxygen uptake in cells after 12 h into encystment. Deoxy-D-glucose uptake dropped by more than an order of magnitude in encysting cells compared to non-encysting cells. In contrast, aspartate and arginine uptake remained relatively constant regardless of whether cells were encysting or not. This set of observations indicates that there is a change in *G. intestinalis*'s ability to transport glucose during cyst formation; a similar change in the parasite's ability to transport amino acids was not observed after 40 h into encystation.



**Figure 1** *Giardia* glucose metabolism in trophozoites. 1. hexokinase; 2. glucose phosphate isomerase; 3. pyrophosphate-dependent phosphofructokinase; 4. fructose biphosphate aldolase; 5. glycolytic enzymes- (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase); 6. pyruvate kinase; 7. Pyruvate; orthophosphate dikinase; 8. Phosphoenolpyruvate carboxyphosphotransferase (GTP dependent); 9. aspartate aminotransferase; 10. malate dehydrogenase; 11. malate dehydrogenase (decarboxylating); 12. Alanine aminotransferase; 13. pyruvate: ferredoxin oxidoreductase; 14. acetyl-CoA synthetase; 15. primary alcohol dehydrogenase (NAD); 16. hydrogenase. Abbreviations used; oxidized ferredoxin (Fdo), reduced ferredoxin (Fdr)

In *G. intestinalis*, the enzymes responsible for glucose metabolism and energy production are cytosolic, with acetate, ethanol, alanine, carbon dioxide, and hydrogen as end-products of this metabolism (Fig. 1). The balance of these end products in growth medium depends in part, upon oxygen tension and glucose levels in the medium (Lindmark, 1980; Paget et al., 1990; Schofield et al., 1990–1992; Lloyd et al., 2002).

Not all of *G. intestinalis*'s glycolytic enzymes are typically eukaryotic. For example, the catabolism of fructose 6-phosphate to fructose 1, 6-bisP is reversibly catalyzed by a pyrophosphate-dependent phosphofructokinase, which is not regulated (Mertens, 1990, 1993; Phillips and Li, 1995). Other examples include adenylate

kinase, which in combination with pyrophosphate-dependent pyruvate phosphate dikinase, sequentially converts two ADPs to ATP + AMP (kinase) and subsequently phosphoenolpyruvate (PEP) plus AMP and Pi to pyruvate + ATP (dikinase), generating two ATP molecules in the process (Hrdy et al., 1993; Mowatt et al., 1994; Bruderer et al., 1996; Hiltbold et al., 1996).

In *G. intestinalis*, pyruvate from glycolysis is converted to acetyl-CoA with the concomitant reduction of ferredoxin by the oxygen sensitive pyruvate:ferredoxin oxidoreductase (PFOR) (Lindmark, 1980). Pyruvate, the preferred substrate of PFOR, donates electrons to *G. intestinalis* ferredoxin, and although it is oxygen sensitive, the *G. intestinalis* PFOR is more stable than PFORs from other protozoans (Sanchez, 1998).

Reduced ferredoxin also can be oxidized by hydrogenase producing molecular hydrogen (Lloyd et al., 2002). *Giardia intestinalis*'s hydrogenase is a truncated, but typical, iron hydrogenase that is oxygen sensitive and inhibited by carbon monoxide and the drug metronidazole (Lloyd et al., 2002). The *G. intestinalis* hydrogenase gene sequence lacks an N-terminus motif that would direct it to an organelle; thus, the positioning of this enzyme in the cell is unclear.

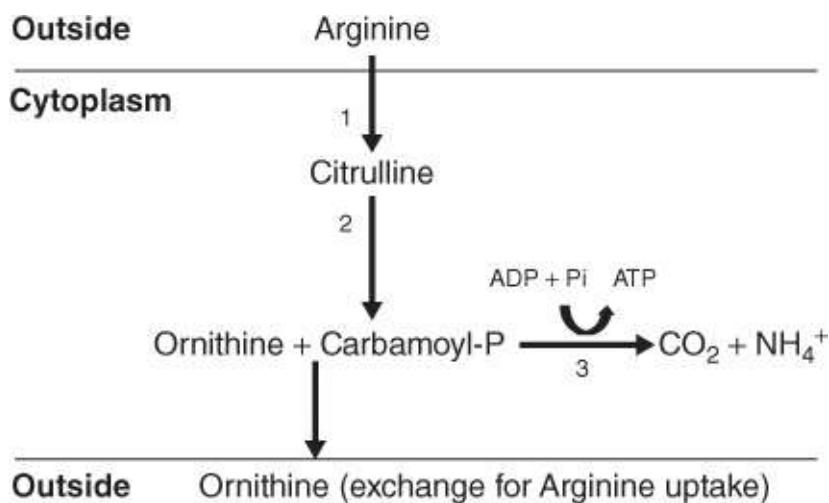
Acetyl-CoA is converted to acetate, ATP, and Co-A by acetyl-CoA synthetase (Sanchez et al., 2000); alternatively acetyl-CoA is converted to acetaldehyde and then to ethanol by a bifunctional aldehyde dehydrogenase (Sanchez, 1998).

Alanine is produced from pyruvate by the combined action of alanine aminotransferase (Paget et al., 1990; Schofield et al., 1992). Small changes in oxygen concentrations affect pyruvate metabolism. Anaerobically, alanine is the major product and a small amount of hydrogen is produced. With the addition of  $< 0.25 \mu\text{M O}_2$ , ethanol production is stimulated, but alanine and hydrogen production are inhibited. At  $> 50 \mu\text{M O}_2$  concentrations, alanine production stops, and acetate and  $\text{CO}_2$  become the major products (Paget et al., 1990). Edwards et al. (1989) identified the *G. intestinalis* acetyl Co-A synthase gene in *Escherichia coli* and showed that acetyl-CoA and adenine nucleotides are the synthase's preferred substrates. Its aldehyde dehydrogenase is a bifunctional enzyme that catalyzes the irreversible conversion of acetyl-Co-A to acetaldehyde and CoA; NAD, but not NADP, acts as a cofactor. The second activity, a primary alcohol dehydrogenase, is also  $\text{NAD}^+$  specific (Sanchez, 1998).

Jarroll et al.'s (1981) observation of  $^{14}\text{CO}_2$  production from [ $1-^{14}\text{C}$ ] glucose suggested that *G. intestinalis* possessed a functional pentose phosphate pathway (PPP). This conclusion is supported by the presence of glucose-6-phosphate dehydrogenase activity and ribose 5-isomerase genes in *G. intestinalis* as shown by Lindmark (1980) and Esteve et al. (2007). The PPP has been little studied in this protozoan; therefore, whether it is a classical PPP or some modified form is unknown.

## Arginine dihydrolase pathway (ADIHP)

Another potential source of energy in *G. intestinalis* is the arginine dihydrolase pathway (Schofield et al., 1990; Edwards et al., 1992); arginine deiminase, catabolic ornithine transcarbamylase, carbamate kinase, and ornithine decarboxylase were detected in *G. intestinalis* extracts, indicating the presence of an active ADiHP pathway that converts arginine to ornithine by way of citrulline, with carbamoyl-P being converted to  $\text{NH}_4^+$  and the generation of ATP by substrate-level phosphorylation (Fig. 2). This pathway is most probably a significant source of energy for the cell *in situ*.



**Figure 2** Arginine dihydrolase pathway in *Giardia* trophozoites. 1. arginine deiminase 2. ornithine transcarbamoylase 3. carbamate kinase.

This pathway is likely to be very important during encystment. It is clear that when *G. intestinalis* encysts, the parasites divert some glucose from energy metabolism toward cyst wall synthesis (Macechko et al., 1992). Also during encystment, it is likely that another energy source must be used. It is probable that ADiHP is the origin of this energy and this pathway becomes vital as encysting trophozoites shift from glucose catabolism to cyst wall synthesis (Paget et al., 1998).

# Mitosomes and the evolution of *Giardia*

For the past 30 years *Giardia* has been involved in one of the most intriguing question in biology, that of the evolution of the eukaryotic cell. *Giardia* has long been thought of as a true “early” eukaryote as electron microscopy performed during the 1980s showed that *Giardia* lacked a Golgi apparatus and mitochondria (Coggins and Schaefer, 1986; Sogayar and Gregório, 1986). Indeed the first universal tree based on small–subunit ribosomal RNA showed the relationships between the various types of cellular forms (Woese et al., 1990) and in this tree *Giardia* was one of the three amitochondrial protozoa classed as Archezoa. These organisms were thought to have divided away from the main eukaryotic lineage prior to the mitochondrial endosymbiotic event and this idea was further supported by the work of Sogin (Sogin and Silberman, 1998; Simpson et al., 2002). It was exciting to think that this parasite was possibly an intermediate in the development of the eukaryotic cell, and indeed like many other researchers, we used this detail in many grant applications to highlight the importance of the research (mostly, this didn't work!). However, the first chinks in this story started to appear in 1995, when Clark and Roger (1995) identified mitochondrial Hsp60 in *Entamoeba histolytica*, and then in 1999, Roger reported Hsp 60 in other members of the Archezoa. Although these data would seem to suggest that indeed ancestors of these organisms had harbored mitochondria, it was proposed that lateral gene transfer had occurred and this was not related to the presence of mitochondria. However, it is interesting to note that in 1986 Sogayar and Gregório showed the presence of double-membrane bodies that were  $0.2\text{ }\mu\text{m}$  in size. Were these the first observation of mitosomes?

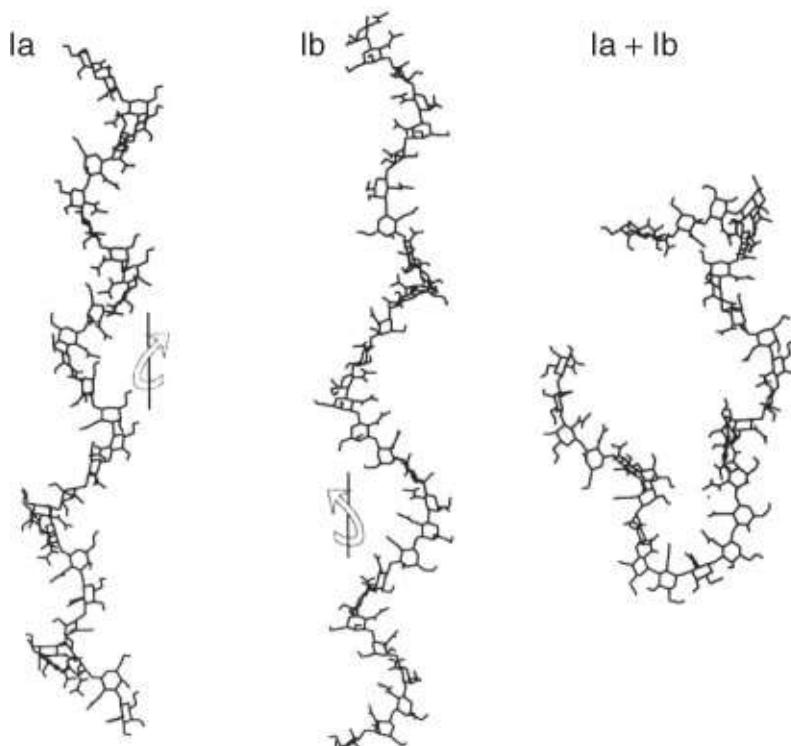
The complete analysis of the *G. intestinalis* genome generated significant new information related to the evolution of this protozoan (McArthur et al., 2000). The identification of genes ostensibly of mitochondrial origin (mitochondrial-like chaperonins) generated a major shift in our understanding of the organism. Soon after the McArthur et al. (2000) work, components of the iron sulfur clusters assembly system (characteristic of mitochondria) were identified (Tachezy et al., 2001). At the same time, a remnant organelle, called a mitosome, was revealed in *G. intestinalis* by Tovar et al. (2003).

Mitosomes occur in other anaerobic protists, where these structures are also believed to be related to mitochondria. The function of the mitosome in *G. intestinalis*, as well as in other organisms, is unclear; however, it is obviously a severely reduced organelle. Detailed genome searches have failed to identify any elements of the mitochondrial electron transport chain in mitosomes (Embley and Martin, 2006). This organelle may be more similar to hydrogenosomes than to mitochondria (Lloyd et al., 2002). Support for this assertion is the presence of a membrane potential in the mitosome and the production of hydrogen by *G. intestinalis*. However, recent work has shown that neither PFOR nor hydrogenase is located in the mitosome (Emelyanov and Goldberg, 2011; Jedelský et al., 2011).

This story has highlighted the limitations of phylogeny especially with respect to ancient lineages, however it also questions what the unifying characteristics of the various forms of “mitochondria” are (including hydrogenosomes and mitosomes). Finally, we must never forget that indeed both aspects of the story may be correct —*Giardia* may indeed be an ancient eukaryote, however, reductive evolution could have pared down the functions of this cell to make it the ultimate parasite.

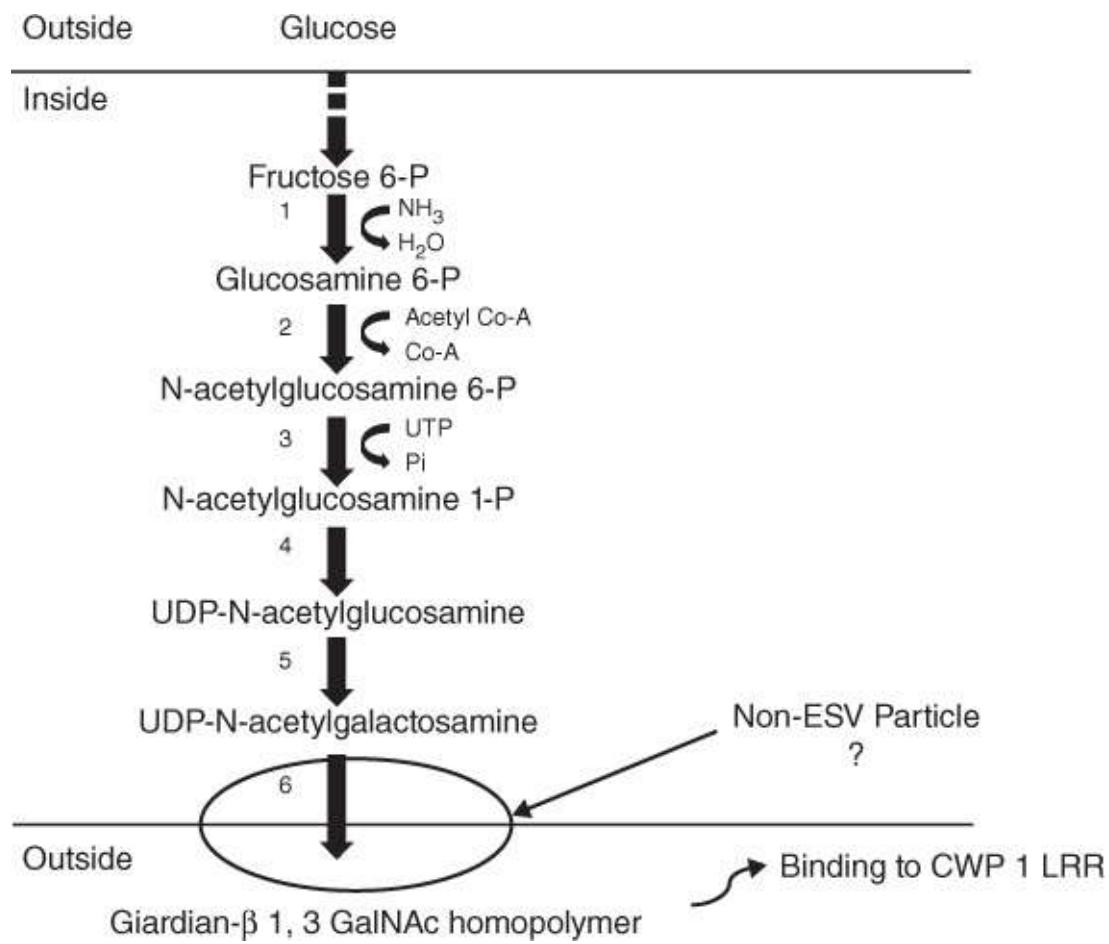
# Glucose conversion to giardan during encystment

The cyst wall of *G. intestinalis* is constructed primarily of a  $\beta$  1, 3 N-acetyl-D-galactosamine (UDP-GalNAc) polymer (2-acetamido-2-deoxy-D-galactan), now known as *giardan* and believed to be unique to this species ([Fig. 3](#)) (Gerwig et al., 2002; Sener et al., 2009) and secondarily of three major cyst wall specific proteins referred to as CWP 1, 2, and 3 (Chatterjee et al., 2010).



[Figure 3](#) Model polysaccharide chains constructed from disaccharide and linkage information randomly selected from appropriate conformational regions. All polysaccharides contain 30 monosaccharide residues. The polymer constructed from region Ia forms a right-handed (clockwise) helix, whereas the polymer built from region Ib has a left-handed helical conformation. The polysaccharide assembled from regions Ia and Ib (4:1) shows a random coil conformation. (Gerwig et al., 2002. The *Giardia intestinalis* filamentous cyst wall contains a novel  $\beta(1\rightarrow 3)$ -N-acetyl-D-galactosamine polymer: A structural and conformational study. *Glycobiology* **12**: 499–505. Reproduced with permission of Oxford University Press).

During encystment, glucose is diverted, at least partially, from energy metabolism into giardan synthesis by five inducible, cytosolic enzymes (Macechko et al., 1992) and one inducible, particle-associated enzyme termed cyst wall synthase (Cws), (Karr and Jarroll, 2004) ([Fig. 4](#)). Cws appears to be a  $\beta$  1, 3 UDP-GalNAc transferase (Karr and Jarroll, 2004).



**Figure 4** Giardan synthesis pathway. 1. glucosamine 6-P deaminase; 2. glucosamine 6-P N-acetylase; 3. phosphoacetylglucosamine mutase; 4. and UDP-N-acetylglucosamine pyrophosphorylase; 5. UDP-N-acetylglucosamine 4'-epimerase; 6. cyst wall synthase ( $\beta$  1, 3 GalNAc transferase).

Giardan evidently binds with leucine-rich regions of CWP1 but not to the cysteine-rich regions of CWP1, and it appears on the surface of the encysting trophozoite while CWP 2 and 3 are still in unexpressed ESVs (Chatterjee et al., 2010; Samuelson and Robbins, 2011). Also, giardan evidently is formed in a specific set of vesicles (Karr and Jarroll, 2004; Chatterjee et al., 2010) (Fig. 4), and that its production requires fructose 6-P conversion to glucosamine 6-P by glucosamine 6-P deaminase (isomerase) (Steimle et al., 1997). van Keulen et al. (1998) identified two genes, gnp1 and gnp2, encoding Gnp in *G. intestinalis* but only gnp1 is expressed. The transcript for gnp1 appeared at around 6 h after cells were induced to encyst by exposure to bile. Using a different nomenclature for these same genes, Knodler et al. (1999) showed that these genes have distinct patterns of expression. A shorter antisense transcript was detected during encystment. There is transcriptional control in gnp1; in mature cysts the induced deaminase is removed by an ubiquitin-mediated pathway (Lopez et al., 2002).

Glucosamine N-acetylase converts glucosamine 6-P to N-acetylglucosamine 6-P has been cloned and sequenced in *G. intestinalis*. This acetylase is transcriptionally regulated (Lopez et al., 2003). Phosphoacetylglucosamine mutase, which converts N-acetylglucosamine 6-P to N-acetylglucosamine 1-P, has been partially purified and characterized; its activity increases 12-fold during

encystment and requires  $Mg^{2+}$ , glucose 1, 6-bisP, and diethyldithiocarbamate (hydroxyquinoline may substitute) (Lindmark and Schmidt, pers. comm.). Lopez et al. (2003) showed that this mutase is also transcriptionally regulated.

Evidently, two UDP-N-acetylglucosamine (UDP-GlcNAc) pyrophosphorylase activities occur in encysting trophozoites. Typically, this pyrophosphorylase converts N-acetylglucosamine 1-P to UDP-GlcNAc, and in *G. intestinalis* it has been characterized as inducible (iUpp) and transcriptionally regulated (Lopez et al., 2003; Mok et al., 2005; Mok and Edwards, 2005; Şener et al., 2009). Lopez et al. (2003) showed that iUpp regulation is transcriptional. Bulik et al. (2000) reported a constitutive pyrophosphorylase (cUpp) stimulated by GlcN-6-P anabolically toward UDP-GalNAc synthesis. The gene encoding cUpp has not yet been cloned; it may not be a typical Upp but rather another enzyme capable of behaving as an Upp. Şener et al. (2004) demonstrated that both enzyme activities are present based on the observation that the enzymes have different pH optima.

UDP-N-acetyl glucosamine 4' epimerase is transcriptionally regulated (Lopez et al., 2003) and converts UDP-GlcNAc to UDP-N-acetylgalactosamine (UDP-GalNAc). Conversion of UDP-GalNAc to UDP-GlcNAc is favored *in vitro*; an excess of UDP-GlcNAc is required to drive the reaction towards the synthesis of UDP-GalNAc (Lopez et al., 2007). Unlike most other members of this epimerase family in different organisms, *G. intestinalis*'s epimerase only catalyzes the interconversion of UDP-GlcNAc and UDP-GalNAc (Macechko et al., 1992; Ishiyama et al., 2004). A recent report suggests that an endoplasmic reticulum associated epimerase may be responsible for synthesizing the UDP-GalNAc (Wampfler et al., 2014).

UDP-GalNAc synthesized in the cytoplasm is merged into the giardan portion of the cyst wall. Evidently, incorporation is stimulated by an inducible, particle-associated transferase known as Cws (Karr and Jarroll, 2004). Partially purified Cws exhibits absolute substrate specificity for UDP-GalNAc, plus a requirement for divalent cations (Karr and Jarroll, 2004). Midlej et al. (2013) showed that the particles with which Cws likely is associated are different from the lysosome-like and encystment-specific vesicles.

# Nitric oxide regulation of metabolic shifting in encystment

Until 2002, *G. intestinalis* was not known to produce hydrogen. The absence of this process from the inferred metabolic map of *G. intestinalis* was based on the genome published in 2007 (Morrison et al., 2007). However, another gas, nitric oxide (NO), was suggested to be produced in *G. intestinalis* by a NO synthase (Harris et al., 2006).

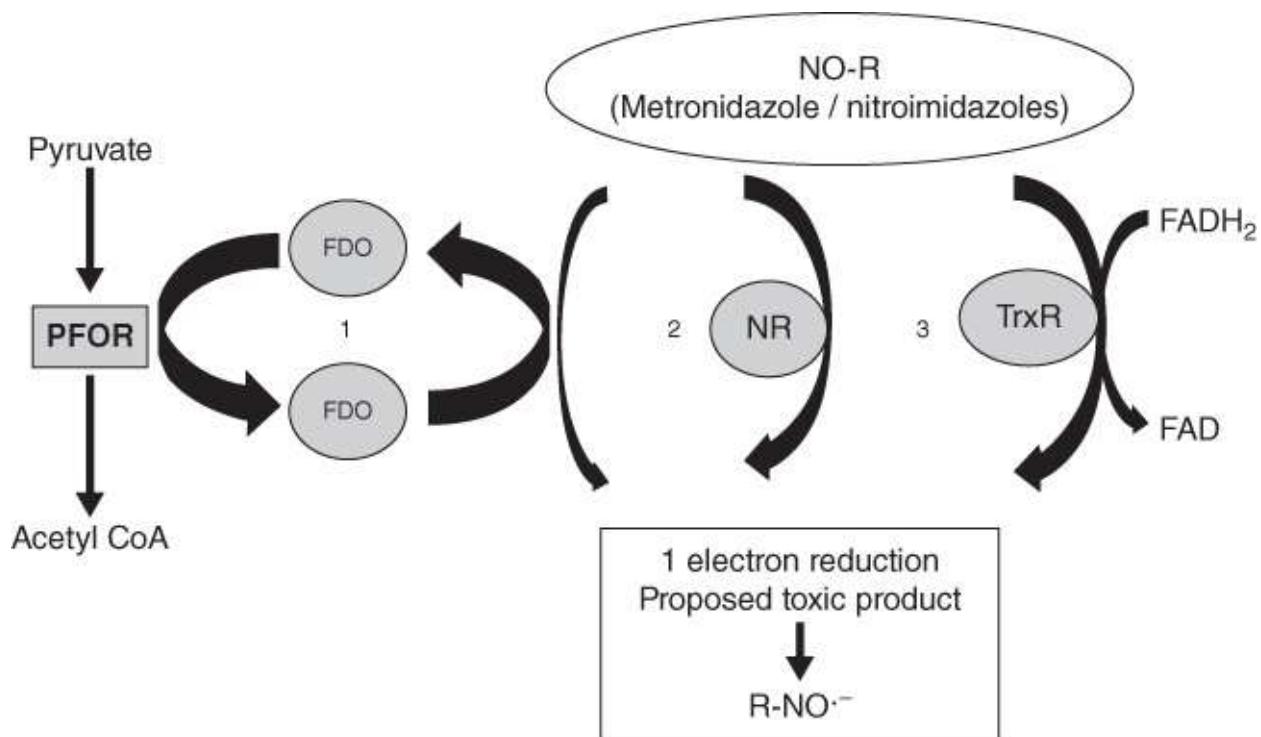
As previously noted, metabolism in encysting trophozoites undergoes a shift towards increased use of arginine for energy production and carbohydrate synthesis. The manner in which this process is achieved is unclear. However, such a shift would require either a highly integrated series of regulators or possibly one global regulator. Thinking about the regulation of the pathways changed when Harris et al. (2006) observed that *G. intestinalis* synthesizes NO, a ubiquitous signaling molecule that is involved in many cellular and tissue processes, including vasodilatation in humans and control of enzyme activity (Mohr et al., 1996; Derakhshan et al., 2007). NO causes protein modification of cysteine residues by means of S-nitrosation, or the term more often used, S-nitrosylation, with a subsequent nitrosothiol formation (S-NO) (Derakhshan et al., 2007).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the enzymes known to be modified by NO in humans (Mohr et al., 1996, 1999). GAPDH is a pleiotropic enzyme with various functions outside of its role in glycolysis (Sirover, 1999). Modification of this enzyme by NO at its catalytic cysteine inhibits the enzyme's activity and can allow it to bind other substrates (Hara and Snyder, 2006; Mannick, 2007). GAPDH can be nitrosylated because the catalytic cysteine is more acidic than cysteines at other locations in the enzyme (Mohr et al., 1996). Cysteine can become more acidic when it is in the presence of basic amino acids either as part of a protein's tertiary or even primary structure. The consensus sequence, X Y C Z in which X = G,S,T,C,Y,N,Q, plus Y = K,R,H,D,E, and Z = D,E, was proposed to be nitrosylated at the cysteine site (Stamler et al., 1997). In *G. intestinalis*, this putative consensus sequence is in several key enzymes, including but not limited to, GAPDH, phosphogluconate dehydrogenase, glycogen synthase, and glucosamine 6-phosphate deaminase (Gnp). NO signaling could be involved in the switch from glycolysis during the trophozoite growth phase to cyst wall synthesis during encystment (van Keulen, pers. comm.). *In vitro* nitrosylation of the recombinant Gnp (deaminase/isomerase) shows that its  $K_m$  is lowered significantly, suggesting that Gnp's affinity for fructose-6-P increases upon its nitrosylation. Screening Gnp's cysteine residues by site-directed mutation indicated that three of them, positions 113, 156, and 230, may be the targeted residues. In contrast, GAPDH activity in *G. intestinalis* is decreased significantly following nitrosylation (van Keulen, pers. comm.). So far, our evidence is not conclusive enough to convince us that this NO based regulation is the “global” regulator in *G. intestinalis*; however, this simple molecule does have the potential

to function in this way.

# Carbohydrate metabolism and nitroimidazoles

Giardiasis is treated most commonly with metronidazole (MTZ), a 5-nitroimidazole drug, in addition to tinidazole, which has also been approved to treat this infection in the U.S.A. (Leitsch et al., 2012). MTZ, a pro-drug, is reduced by ferredoxin that is linked to PFOR, a key enzyme associated with glucose metabolism in *G. intestinalis* (Fig. 5) (Townson et al., 1996; Upcroft and Upcroft, 2001). The two pro-drugs undergo a series of reduction steps to the highly active nitroradical anions, which are believed to be the active forms of the drugs. In another protozoan parasite, *Trichomonas vaginalis*, the MTZ reduction product reacts with a variety of cell components leading to cell death (Yarlett et al., 1987).



**Figure 5** Mechanism(s) for the reduction and activation of metronidazole and other nitroimidazoles in *Giardia*. Metronidazole and nitroimidazoles (NO-R) are pro-drugs activated by cellular reduction. It is suspected that the nitro radical anion species R-NO<sup>-·</sup> is responsible for toxicity. Three mechanisms of drug reduction are proposed; 1. Interaction of drug with reduced ferredoxin (Fdr) linked to pyruvate: ferredoxin oxidoreductase activity (PFOR), 2. The action of a nitroreductase (NR) and 3. Via an FADH<sub>2</sub> linked thioredoxin reductase (TrxR).

More recently, another mechanism of drug reduction has been proposed for *G. intestinalis*, one that involves the direct reduction of nitroimidazoles by a nitroreductase (Müller et al., 2007). MTZ resistance in the clinical environment is now well-established, and it would seem that resistance is multifactorial but may include reduced activity of PFOR along with decreased NADPH oxidase/flavin reducing activity (Leitsch et al., 2011).

MTZ sensitivity is reduced significantly in cysts as compared with trophozoites. MTZ inhibited oxygen uptake by 77% in trophozoites, but there was no detectable

inhibition of oxygen uptake 8 h after trophozoites were transferred to encystment medium (Paget et al., 1998). The MTZ resistance may be due to a change in metabolic flux separate from the PFOR pathway. Oxygen uptake by non-induced cysts increased exponentially during the 30 min following the induction of excystation. Likewise, MTZ sensitivity returned within 15 min after the induction of excystation, and full sensitivity had returned by 30 min into excystation (Paget et al., 1998).

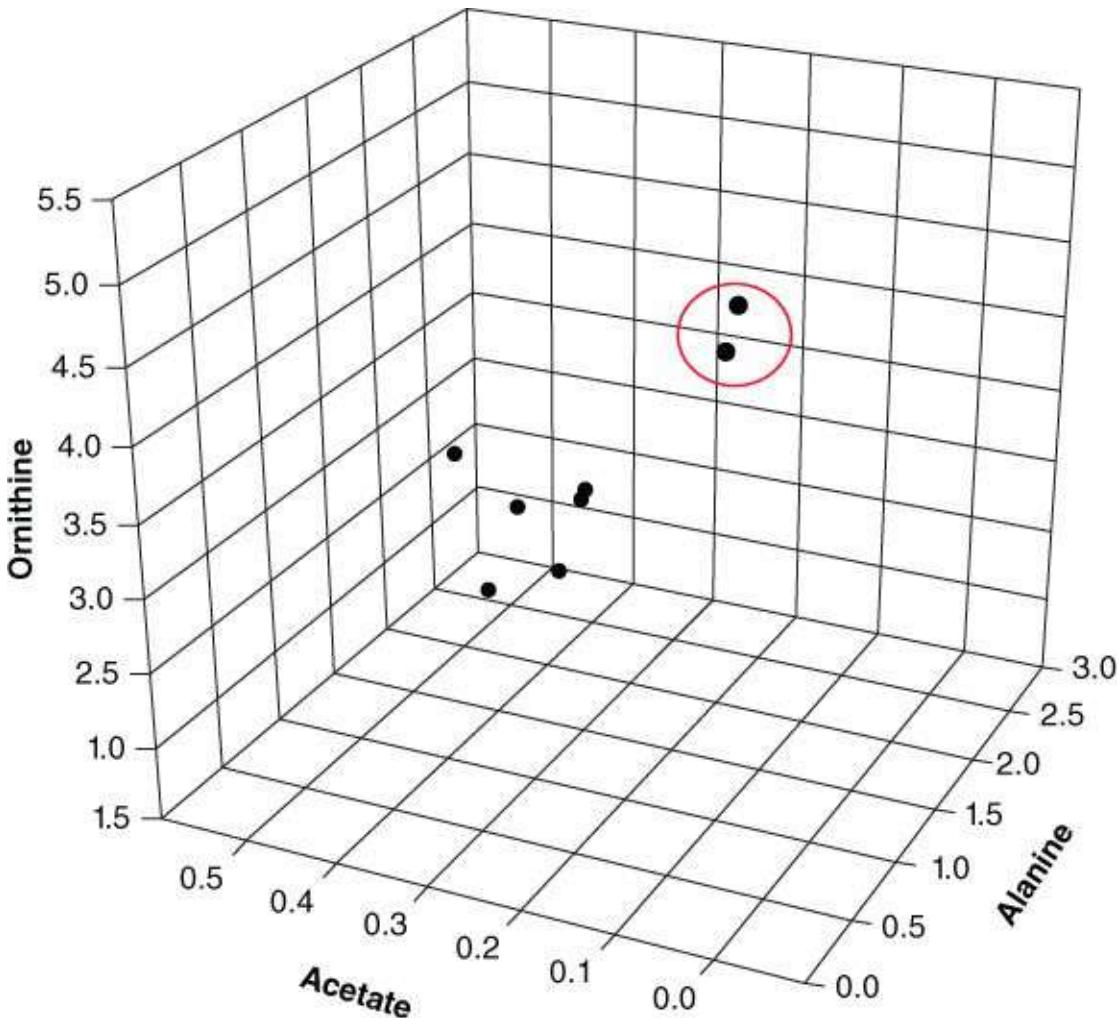
## Where next?

With the adoption of “-omic” technologies, there have been significant strides in our understanding of *G. intestinalis* structure (Cacciò and Ryan, 2008; Monis et al., 2009) and aspects of cell biology, including cyst wall formation (Kim et al., 2009; Han and Collins, 2012; Thompson and Monis, 2012; Faso et al., 2013; Wampfler et al., 2014); however, there are still major deficiencies in our knowledge. The preceding portion of this chapter demonstrates that *G. intestinalis* metabolic analysis has focused on specific pathways and, although some NMR-based analyses of metabolic end products have been performed previously (Paget et al., 1990), to date there are no studies on metabolic profiles or metabolic flux analyses in *G. intestinalis*. Interestingly, the closest to an overview of metabolism has come from genome and proteome mining (Morrison et al., 2007; Han and Collins, 2012). Metabolic pathway reconstruction based on genome and proteome data will not identify changes during a parasite's life cycle. Linking transcriptomics into the analysis can help. However, the environment of a cell and nutrient availability also constrain the metabolome; this type of regulation is not accessible except via direct measurement of metabolites.

Metabolomics is an analytical approach to biochemical analysis that generates a detailed profile of low-molecular-weight metabolites in a biological system (Creek et al., 2012; Lakshmanan et al., 2012; Paget et al., 2013). Techniques used for metabolomics can include GC-MS, LC-MS, and NMR spectroscopy and the data generated not only give definitive identification of compounds but also their concentrations. The power of this approach is that it can be used to study the changes in cells in response to drugs or physiological change and to compare variation at isolate or genotype level, but the approach is not without limitation. Problems include analysis of the massive data sets generated from even simple experiments, and variation within, and between, samples. Most of these problems can be overcome with appropriate replicates and controls, but it is important to use power calculations to identify numbers of replicates required for each data set.

We have been working and developing appropriate robust methodologies to compare metabolism among isolates, genotypes, and in cells under stress. One area that has generated interesting data is comparative study of key metabolite levels between MTZ-resistant and MTZ-sensitive *G. intestinalis* isolates. [Figure 6](#) is a scatter plot showing concentrations of acetate, alanine, and ornithine, end products of energy-related metabolism in *G. intestinalis*. Acetate and alanine levels indicate flux through glycolysis and ornithine levels relate to activity of the ADiHP. These levels were determined in medium after 24 h of incubation with trophozoites from eight isolates, two of which were MTZ-resistant (these were clinical isolates and MICs determined by terminal subculture after incubation with various concentrations of MTZ). The three metabolic end products were identified and assayed using GC-MS/MS. The two resistant isolates clearly do not group with the other six MTZ-sensitive isolates and both would seem to export

higher levels of ornithine and lower levels of acetate and alanine into the medium. Such observations suggest that these isolates have reduced glycolytic activity, but elevated ADiHP activity. ATP levels in MTZ-resistant cells are not significantly different from those in drug sensitive isolates (data not shown), suggesting that decreased activity through glycolysis and concomitant ATP production is balanced by increasing ATP production by the ADiHP. These observations support the work of Leitsch et al. (2011) who identified reduced activity of PFOR as being a factor in MTZ resistance.



**Figure 6** Graphic representation of alanine, ornithine and acetate concentrations from *Giardia* trophozoites metabolism as determined by GC-MS/MS analysis of growth medium after 8hr incubation. Each data point was compiled from a minimum of five independent replicates. Each spot represents one isolate and the two spots circled are results obtained from two metronidazole resistant isolates (determined by MIC). The data from these two are significantly different ( $P = 0.01$ ). Alanine and acetate are products of glucose fermentation in *Giardia* and ornithine is produced via the arginine dihydrolase pathway. Values presented are in  $\text{nmol h}^{-1} 10^{-6}$  cells.

Clearly, the application and use of metabolomics coupled with other global technologies and traditional biochemical techniques will help us better understand how the *G. intestinalis* metabolome functions, how it varies among isolates and genotypes and how it responds to stress. These types of studies are

likely to impact on the development of new methods or drugs for control and this information may give greater insights into pathogenicity. Research into *G. intestinalis* has not yet reached its centenary; however, over the last 40 years we have moved a long way on the path to understanding this enigmatic parasite, but, as always, there is more work to be done.

The “omics revolution” has altered the way we look at biological systems and like other cell systems it has had a major impact on our understanding of *Giardia* cell biology however we caution that there are situations, such as those discussed here in this chapter, where important new information would have been missed had we relied entirely on “omics.” We would have missed the fairly insoluble  $\beta$ 1, 3 GalNAc polysaccharide and continued to believe that the *G. intestinalis* cyst wall is chitin instead of giardan. Furthermore, we would have missed the accompanying cyst wall synthase that synthesizes giardan since it has no known similarity with other polysaccharide synthesizing enzymes. Even with the “omics” techniques being employed, hydrogenase in *G. intestinalis* was missed until it was discovered by traditional biochemical methodology. Thus, we emphasize that there is no real substitute for asking the important questions and then applying the appropriate techniques to try and answer them rather than simply applying techniques blindly assuming that all the answers have been found or will be found.

## Acknowledgments

This contribution is dedicated to Professor Ernest A. Meyer, the first person to grow *G. intestinalis* axenically *in vitro*, my (ELJ) first postdoctoral advisor, mentor, and friend, and to the late Professor Donald G. Lindmark, my (ELJ) second postdoctoral advisor and friend. We shall be forever indebted to both of these outstanding scientists for years of productive accomplishments and lively discussions on our favorite topic—*Giardia*.

## Literature cited

- Bruderer, T., C. Wehrli, and P. Köhler. 1996. Cloning and characterization of the gene encoding pyruvate phosphate dikinase from *Giardia duodenalis*. *Molecular and Biochemical Parasitology* **77**: 225–233.
- Bulik, D., P. van Ophem, J. Manning, Z. Shen, D. Newburg, and E. Jarroll. 2000. UDP-N-acetylglucosamine pyrophosphorylase: A key enzyme in encysting *Giardia* is allosterically regulated. *Journal of Biological Chemistry* **275**: 14722–14728.
- Cacciò, S. M. and U. Ryan. 2008. Molecular epidemiology of giardiasis. *Molecular and Biochemical Parasitology* **160**: 75–80.
- Chatterjee, A., A. Carpentieri, D. Ratner, E. Bullitt, C. Costello, P. Robbins, and J. Samuelson. 2010. *Giardia* cyst wall protein 1 is a lectin that binds to curled fibrils of the GalNAc homopolymer. *PLoS Pathogens* **6**: e1001059.
- Clark, C. G. and A. J. Roger. 1995. Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. *Proceedings of the National Academy of Science USA* **92**: 6518–6521.
- Coggins, J. R. and F. W. Schaefer, 3rd. 1986. *Giardia muris*: ultrastructural analysis of *in vitro* excystation. *Experimental Parasitol.* **51**: 219–228.
- Creek, D. J., A. Chokkathukalam, A. Jankevics, K. Burgess, R. Breitling, and M. Barrett. 2012. Stable isotope-assisted metabolomics for network-wide metabolic pathway elucidation. *Analytical Chemistry* **84**: 8442–8447.
- Derakhshan, B., G. Hao, and S. Gross. 2007. Balancing reactivity against selectivity: The evolution of protein S-nitrosylation as an effector of cell signaling by nitric oxide. *Cardiovascular Research* **75**: 210–219.
- Edwards, M. R., F. Gilroy, B. Jimenez, and W. O'Sullivan. 1989. Alanine is a major end product of metabolism by *Giardia lamblia*: A proton nuclear magnetic resonance study. *Molecular and Biochemical Parasitology* **37**: 19–26.
- \_\_\_\_\_, P. Schofield, W. O'Sullivan, and M. Costello. 1992. Arginine metabolism during culture of *Giardia intestinalis*. *Molecular and Biochemical Parasitology* **53**: 97–103.
- Embley, T. M., and W. Martin 2006. Eukaryotic evolution changes and challenges. *Nature* **440**: 623–630.
- Emelyanov, V. V., and A. Goldberg. 2011. Fermentation enzymes of *Giardia intestinalis*, pyruvate:ferredoxin oxidoreductase and hydrogenase, do not localize to its mitosomes. *Microbiology* **157**: 1602–1611.
- Esteve, M., D. Maugeri, A. Stern, P. Beluardi, and J. Cazzulo. 2007. The pentose phosphate pathway in *Trypanosoma cruzi*: A potential target for the

chemotherapy of Chagas' disease. *Annals of the Brazilian Academy of Sciences* **79**: 649–663.

Faso, C., S. Bischof, and A. Hehl. 2013. The proteome landscape of *Giardia lamblia* encystation. *PLoS One* **8**: e83207.

Fortess, E., and E. Meyer. 1976. Isolation and axenic cultivation of *Giardia* trophozoites from the guinea pig. *Journal of Parasitology* **62**: 689.

Gerwig, G., J. van Kuik, B. Leeflang, J. Kamerling, J. Vliegenthart, C. Karr, and E. Jarroll. 2002. Conformational studies of the  $\beta$  (1–3)-N-acetyl-D-galactosamine polymer of the *Giardia lamblia* filamentous cyst wall. *Glycobiology* **12**: 1–7.

Harris, K. M., B. Goldberg, G. Biagini, and D. Lloyd. 2006. *Trichomonas vaginalis* and *Giardia intestinalis* produce nitric oxide and display NO-synthase activity. *Journal of Eukaryotic Microbiology* **53**: S182–S183.

Han, J. and L. Collins. 2012. Reconstruction of sugar metabolic pathways of *Giardia lamblia*. *International Journal of Proteomics* **2012**: 980829.

Hara, M. R. and S. Snyder. 2006. Nitric oxide-GAPDH-Siah: A novel cell death cascade. *Cellular and Molecular Neurobiology* **26**: 527–538.

Hiltbold, A., R. Thomas, and P. Köhler. 1999. Purification and characterization of recombinant pyruvate phosphate dikinase from *Giardia*. *Molecular and Biochemical Parasitology* **104**: 157–169.

Hrdy, I., E. Mertens, and E. Nohynkova. 1993. *Giardia intestinalis*: Detection and characterization of a pyruvate phosphate dikinase. *Experimental Parasitology* **76**: 438–441.

Ishiyama, N., C. Creuzenet, J. Lam, and A. Berghuis. 2004. Crystal structure of WbpP, a genuine UDP-N-acetylglucosamine 4-epimerase from *Pseudomonas aeruginosa*. *Journal of Biological Chemistry* **279**: 22635–22642.

Jarroll, E. L., P. Muller, E. Meyer, and S. Morse. 1981. Lipid and carbohydrate metabolism of *Giardia lamblia*. *Molecular and Biochemical Parasitology* **2**: 187–196.

Jedelský, P. L., P. Doležal, P. Rada, J. Pyrih, O. Smíd, I. Hrdý, M. Sedinová, M. Marcinčíková, L. Voleman, A. Perry, et al. 2011. The minimal proteome in the reduced mitochondrion of the parasitic protist *Giardia intestinalis*. *PLoS One* **62**: e17285.

Karr, C. and E. Jarroll. 2004. Cyst wall synthase: N-acetylgalactosaminyl-transferase activity is induced to form the novel GalNAc polysaccharide in the *Giardia* cyst wall. *Microbiology* **150**: 1237–1243.

Kim, J., S. Bae, M. Sung, K. Lee, and S. Park. 2009. Comparative proteomic

analysis of trophozoites versus cysts of *Giardia lamblia*. *Parasitology Research* **104**: 475–479.

Knodler, L., S. Svärd, J. Silberman, B. Davids, and F. Gillin. 1999. Developmental gene regulation in *Giardia lamblia*: first evidence for an encystation-specific promoter and differential 5' mRNA processing. *Molecular Microbiology* **34**: 327–340.

Lakshmanan, V., K. Rhee, W. Wang, Y. Yu, K. Khafizov, A. Fiser, P. Wu, O. Ndir, S. Mboup, D. Ndiaye, and J. Daily. 2012. Metabolomic analysis of patient plasma yields evidence of plant-like α-linolenic acid metabolism in *Plasmodium falciparum*. *Journal of Infectious Disease* **206**: 238–248.

Leitsch, D., A. Burgess, L. Dunn, K. Krauer, K. Tan, M. Duchêne, P. Upcroft, L. Eckmann, and J. Upcroft. 2011. Pyruvate:ferredoxin oxidoreductase and thioredoxin reductase are involved in 5-nitroimidazole activation while flavin metabolism is linked to 5-nitroimidazole resistance in *Giardia lamblia*. *Journal of Antimicrobial Chemotherapy* **66**: 1756–1765.

\_\_\_\_\_, S. Schlosser, A. Burgess, and M. Duchêne. 2012. Nitroimidazole drugs vary in their mode of action in the human parasite *Giardia lamblia*. *International Journal of Parasitology Drugs and Drug Resistance* **2**: 166–170.

Lindmark, D. G. 1980. Energy metabolism of the anaerobic protozoon *Giardia lamblia*. *Molecular and Biochemical Parasitology* **1**: 1–12.

Lloyd, D., J. Ralphs, and J. C. Harris. 2002. Hydrogen production in *Giardia intestinalis*, a eukaryote with no hydrogenosomes. *Trends in Parasitology* **18**: 155–156.

Lopez, A., M. Hossain, and H. van Keulen. 2002. *Giardia intestinalis* glucosamine 6-phosphate isomerase: The key enzyme to encystment appears to be controlled by ubiquitin attachment. *Journal of Eukaryotic Microbiology* **49**: 134–136.

\_\_\_\_\_, K. Şener, E. Jarroll, and H. van Keulen. 2003. Transcription regulation is demonstrated for five key enzymes in *Giardia intestinalis* cyst wall polysaccharide biosynthesis. *Molecular and Biochemical Parasitology* **128**: 51–57.

\_\_\_\_\_, \_\_\_\_\_, J. Trosien, E. Jarroll, and H. van Keulen. 2007. UDP-N-acetylglucosamine 4'-epimerase from the intestinal protozoan *Giardia intestinalis* lacks UDP-glucose 4'-epimerase activity. *Journal of Eukaryotic Microbiology* **54**: 154–160.

Macechko, P. T., P. Steimle, D. Lindmark, S. Erlandsen, and E. Jarroll. 1992. Galactosamine synthesizing enzymes are induced when *Giardia* encyst. *Molecular and Biochemical Parasitology* **56**: 301–310.

- Mannick, J. B. 2007. Regulation of apoptosis by protein S-nitrosylation. *Amino Acids* **32**: 523–526.
- McArthur, A. G., H. Morrison, J. Nixon, N. Passamaneck, U. Kim, G. Hinkle, M. Crocker, M. Holder, R. Farr, C. Reich, G. Olsen, et al. 2000. The *Giardia* genome project database. *FEMS Microbiology Letter* **189**: 271–273.
- Mertens, E. 1990. Occurrence of pyrophosphate: fructose 6-phosphate 1-phosphotransferase in *Giardia lamblia* trophozoites. *Molecular and Biochemical Parasitology* **40**: 147–149.
- \_\_\_\_\_, 1993. ATP versus pyrophosphate: Glycolysis revisited in parasitic protists. *Parasitology Today* **9**: 122–126.
- Meyer, E. A. 1970. Isolation and axenic cultivation of *Giardia* trophozoites from the rabbit, chinchilla, and cat. *Experimental Parasitology* **27**: 179–183.
- Midlej, V., I. Meinig, W. deSouza, and M. Benchimol. 2013. A new set of carbohydrate-positive vesicle in encysting *Giardia lamblia*. *Protist* **164**: 261–271.
- Mohr, S., H. Hallak, A. de Boitte, E. Lapetina, and B. Brüne. 1999. Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. *Journal of Biological Chemistry* **274**: 9427–9430.
- Mohr, S., J. Stamler, and B. Brüne. 1996. Posttranslational modification of glyceraldehyde-3-phosphate dehydrogenase by S-nitrosylation and subsequent NADH attachment. *Journal of Biological Chemistry* **271**: 4209–4214.
- Mok, M., E. Tay, E. Sekyere, W. Glenn, A. Bagnara, and M. Edwards. 2005. *Giardia intestinalis*: Molecular characterization of UDP-N-acetylglucosamine pyrophosphorylase. *Gene* **357**: 73–82.
- \_\_\_\_\_, and M. Edwards. 2005. Kinetic and physical characterization of the inducible UDP-N-acetylglucosamine pyrophosphorylase from *Giardia intestinalis*. *Journal of Biological Chemistry* **280**: 39363–39372.
- Monis, P. T., S. Caccio, and R. Thompson. 2009. Variation in *Giardia*: Towards a taxonomic revision of the genus. *Trends in Parasitology* **25**: 93–100.
- Morrison, H. G., A. McArthur, F. Gillin, S. Aley, R. Adam, G. Olsen, A. Best, W. Cande, F. Chen, M. Cipriano, et al. 2007. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. *Science* **317**: 1921–1926.
- Mowatt, M., E. C. Weinbach, T. C. Howard, and T. E. Nash, 1994. Complementation of an *Escherichia coli* glycolysis mutant by *Giardia lamblia* triose phosphate isomerase. *Experimental Parasitology* **78**: 85–92.
- Müller, J., J. Wastling, S. Sanderson, N. Müller, and A. Hemphill. 2007. A novel *Giardia lamblia* nitroreductase, GLNR1, interacts with nitazoxanide and other

- thiazolides. *Antimicrobial Agents and Chemotherapy* **51**: 1979–1986.
- Paget, T., N. Haroune, S. Bagchi, and E. Jarroll. 2013. Metabolomics and protozoan parasites. *Acta Parasitologica* **58**: 127–131.
- \_\_\_\_\_, M. Kelly, E. Jarroll, D. Lindmark, and D. Lloyd. 1993. The effects of oxygen on fermentation in *Giardia lamblia*. *Molecular and Biochemical Parasitology* **57**: 65–72.
- \_\_\_\_\_, P. T. Macechko, and E. Jarroll. 1998. Metabolic changes in *Giardia intestinalis* during differentiation. *Journal of Parasitology* **84**: 222–226.
- \_\_\_\_\_, M. Raynor, D. Shipp, and D. Lloyd. 1990. *Giardia lamblia* produces alanine anaerobically but not in the presence of oxygen. *Molecular and Biochemical Parasitology* **42**: 63–67.
- Phillips, N. F. and Z. Li. 1995. Kinetic mechanism of pyrophosphate-dependent phosphofructokinase from *Giardia lamblia*. *Molecular and Biochemical Parasitology* **73**: 43–51.
- Roger, A. J. 1999. Reconstructing early events in eukaryotic evolution. *American Naturalist* **154**: S146–S163.
- Samuelson, J. and P. Robbins. 2011. A simple fibril and lectin model for cyst walls of *Entamoeba* and perhaps *Giardia*. *Trends in Parasitology* **27**: 17–22.
- Sánchez, L. B. 1998. Aldehyde dehydrogenase (CoA-acetylating) and the mechanism of ethanol formation in the amitochondriate protist, *Giardia lamblia*. *Archives of Biochemistry and Biophysics* **354**: 57–64.
- Sánchez, L. B., Y. Galperin, and M. Müller. 2000. Acetyl-CoA synthetase from the amitochondriate eukaryote *Giardia lamblia* belongs to the newly recognized superfamily of acyl-CoA synthetases (nucleoside diphosphate-forming). *Journal of Biological Chemistry* **275**: 5794–5803.
- Schofield, P. J., M. Costello, M. Edwards, and W. O'Sullivan. 1990. The arginine dihydrolase pathway is present in *Giardia intestinalis*. *International Journal for Parasitology* **20**: 697–699.
- \_\_\_\_\_, M. Edwards, and P. Kranz. 1991. Glucose metabolism in *Giardia intestinalis*. *Molecular and Biochemical Parasitology* **45**: 39–47.
- \_\_\_\_\_, \_\_\_, J. Matthews, and J. Wilson. 1992. The pathway of arginine catabolism in *Giardia intestinalis*. *Molecular and Biochemical Parasitology* **51**: 29–36.
- Şener, K., Z. Shen, D. Newburg, and E. Jarroll. 2004. Amino sugar phosphate levels change during formation of the *Giardia* cyst wall. *Microbiology* **150**: 1225–1230.

- \_\_\_\_\_, H. van Keulen, and E. Jarroll. 2009. Giardan: Structure, synthesis, regulation and inhibition. In *Giardia and Cryptosporidium from molecules to disease*, G. Ortega-Pierres, S. Cacciò, R. Fayer, T. Mank, H. Smith, and R.C. Thompson (eds.) CABI Press, Cambridge, U.K., p. 382–397.
- Simpson, A. G., A. J. Roger, J. D. Silberman, D. D. Leipe, V. P. Edgcomb, L. S. Jermiin, D. J. Patterson, and M. L. Sogin. 2002. Evolutionary history of “early-diverging” eukaryotes: the excavate taxon Carpeditemonas is a close relative of *Giardia*. *Molecular Biology and Evolution* **19**: 1782–1791.
- Sogayar, M. I. and E. A. Gregório. 1986. Cytoplasmic inclusions in *Giardia*: an electron microscopy study. *Annals of Tropical Medicine and Parasitology* **80**: 49–52.
- Sogin, M. L. and J. D. Silberman. 1998. Evolution of the protists and protistan parasites from the perspective of molecular systematics. *International Journal for Parasitology* **28**: 11–20.
- Sirover, M. A. 1999. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. *Biochimica Biophysica Acta* **1432**: 159–184.
- Stamler, J. S., E. Toone, S. Lipton, and N. Sucher. 1997. (S)NO signals: Translocation, regulation, and a consensus motif. *Neuron* **18**: 691–696.
- Steimle, P., D. Lindmark, and E. Jarroll. 1997. Purification and characterization of glucosamine 6-phosphate isomerase from encysting *Giardia*. *Molecular and Biochemical Parasitology* **84**: 149–150.
- Tachezy, J., L. Sánchez, and M. Müller. 2001. Mitochondrial type iron-sulfur cluster assembly in the amitochondriate eukaryotes *Trichomonas vaginalis* and *Giardia intestinalis*, as indicated by the phylogeny of IscS. *Molecular Biology Evolution* **18**: 1919–28.
- Thompson, R. C., and P. Monis. 2012. *Giardia*—from genome to proteome. *Advances in Parasitology* **78**: 57–95.
- Townson, S. M., J. Upcroft, and P. Upcroft. 1996. Characterization and purification of pyruvate:ferredoxin oxidoreductase from *Giardia duodenalis*. *Molecular and Biochemical Parasitology* **79**: 183–193.
- Tovar, J., G. León-Avila, L. Sánchez, R. Sutak, J. Tachezy, M. van der Giezen, M. Hernández, M. Müller, and J. Lucocq. 2003. Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature* **13**: 172–176.
- Upcroft, P. and J. Upcroft. 2001. Drug targets and mechanisms of resistance in the anaerobic protozoa. *Clinical Microbiology Review* **14**: 150–164.
- van Keulen, H., P. Steimle, D. Bulik, R. Boroviak, and E. Jarroll. 1998. Cloning of

two putative *Giardia lamblia* glucosamine 6-phosphate isomerase genes only one of which is transcriptionally active during encystment. *Journal of Eukaryotic Microbiology* **45**: 637–642.

Wampfler, P. B., V. Tosevski, P. Nanni, C. Spycher, and A. Hehl. 2014. Proteomics of secretory and endocytic organelles in *Giardia lamblia*. *PLoS One* **9**: e94089.

Woese, C. R., O. Kandler, and M. L. Wheelis. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Science U S A* **87**: 4576–4579.

Yarlett, N., C. Rowlands, J. Evans, N. C. Yarlett, and D. Lloyd. 1987.

Nitroimidazole and oxygen derived radicals detected by electron spin resonance in hydrogenosomal and cytosolic fractions from *Trichomonas vaginalis*. *Molecular and Biochemical Parasitology* **24**: 255–261.



# **Chapter 13**

## **Development of an Acquired resistance In rabbits By repeated Infection with an Intestinal Nematode, *Trichostrongylus Calcaratus Ransom, 1911.*\***

Merritt P. Sarles

In a short paper dealing with reinfection of the domestic rabbit with a gut nematode, *Trichostrongylus calcaratus*, under natural conditions, Stoll (1932) presented evidence that in some cases at least this host illustrated an effect he had demonstrated earlier (1929) with domestic sheep and a stomach worm, *Haemonchus contortus*; namely, an initial development of parasitism followed by an apparent host response with discharge of worms. The present article deals with a further exploration of this reaction in a small group of rabbits kept under laboratory conditions precluding natural reinfection, but given definite increasing doses of *T. calcaratus* larvae at regular intervals, and followed by frequent counts of the number of parasite eggs in the feces in order to determine the ultimate effect on both hosts and parasites.

Investigation of acquired immunities to parasitic nematodes is comparatively recent. Sandground (1928) found an immunity in dogs and cats to a second infection of *Strongyloides stercoralis*, an intestinal parasite of man, but regarded it as probably due to the fact that the parasite was in an abnormal host. Stoll (1929) emphasized the importance of studying animals exposed to repeated infection since "the usual hosts of nematode parasites are not infected just once in their lives, but receive more or less constant increments of additional parasites, at least seasonally." He demonstrated that sheep exposed to such reinfection with *H. contortus* developed a resistance, or self-cure and protection, and stated that this phenomenon, if of general occurrence among other hosts and parasites, would necessitate a revision of the generally accepted ideas concerning helminthic infections. The advance abstract of the present paper (Sarles, 1930) indicated that the phenomenon was not limited to sheep, and predicted that it could accordingly be expected to occur with other hosts and worms when appropriately studied. This statement has been substantiated by subsequent reports of acquired resistance to parasitic nematodes, many of which may be interpreted on the basis of self-cure and protection.

McCoy (1931a) studying *Ancylostoma caninum* in dogs and cats demonstrated what he interpreted as an "immunity reaction" following repeated infection. Casual evidence of increased resistance in dogs

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after previous infection with hookworms had been reported earlier by Payne (1923), Herrick (1928), and Sarles (1929), using *A. caninum*, and by Fülleborn (1926), using *Uncinaria stenocephala*.

An acquired resistance in rats following infection with *Nippostrongylus muris* has been reported by Schwartz, Alicata and Lucke (1931), Africa (1931), and Chandler (1932). Rats also acquire a relative immunity following infection with *Trichinella spiralis*, as shown by McCoy (1931b). An acquired resistance in chickens to infection with *Ascaridia lineata* has been reported by Graham, Ackert, and Jones (1932).

#### MATERIALS AND METHODS

The parasite used in this study, *Trichostrongylus calcaratus* Ransom (1911) is one of the bursate nematodes with the following life history: entrance into the host as a filariform larva; growth to adult stage in a relatively localized area of the gastro-intestinal tract (in this case principally the jejunum); mating of males and females and production of large numbers of eggs into the lumen of the gut from whence they escape into the feces; embryonation and hatching of the eggs in the dung, and development into the infective larval stage after a week or two under favorable moisture and temperature conditions. Stoll (1932) has shown that infections of *T. calcaratus* may result from the larva penetrating through the skin, or by ingestion.

The fact should be emphasized that there is no multiplication of the worms within the host. One egg develops into one larva, which after developing to the infective stage may parasitize a host and become one adult male or female worm. Hosts which harbor large numbers of worms have, therefore, necessarily received large numbers of larvae. Under natural conditions the number of eggs shed by the individual female worms gradually permit an extensive number of infective larvae to develop and reinfect the host. The numerous eggs given off into the gut by the adult female worms permit the experimenter to follow the degree and fate of the infestations by the simple means of egg counting the feces.

Inasmuch as rabbits from our laboratory stock occasionally show light infestations of *T. calcaratus* and *Obeliscoides cuniculi* (a stomach worm), we obtain them when they are weaned at 6 weeks of age, and only use for experimentation those which show no worm eggs in their feces during a subsequent period of at least 3 weeks. Such animals can be reasonably assumed to have never been infected with the two above species. The 7 rabbits reported in this paper showed only coccidial oocysts upon weekly examination of their feces by flotation during the 3 to 6 week period which elapsed from the time that they were weaned until their first infection. They were kept in metal cages

throughout the experiment, on one-half inch mesh wire screens fastened over the top of a metal pan. The screens were scrubbed daily, and the rabbits were transferred each week to a clean set of cages which had been sterilized in boiling water. In one rabbit (No. 110) small numbers of the eggs of the rabbit oxyurid, *Passalurus ambiguus*, were noted at two different periods during the experiment. It does not seem probable that the oxyurid or coccidial infection had any appreciable influence on the outcome of the experiment. A control rabbit remained uninfected throughout the experiment indicating that no infections were being picked up from food or in cage transfers.

Screens supporting the rabbits allowed the fecal pellets to fall on moistened paper towels in the 3 inch deep pan below, from which they were collected daily and their consistency noted. The 24 hour specimen was weighed, a portion taken for cultures and the remainder either used immediately for making up egg count flasks or kept under refrigeration until used. Two egg count flasks were made up using 10 grams of pellets to 300 cc. of diluent: a water flask for flotation counts and a sodium hydroxide flask for dilution egg counts. The manipulation and counting of these flasks were in strict accordance with the steps recently outlined and discussed in detail by Stoll (1930). All counts were made by the author, the collection and tubing of specimens being done by an experienced technician under supervision. The one pipette used for all the dilution egg counts, together with all flasks, etc., were calibrated against Bureau of Standards certified pipettes. The flotation method was used to detect the first appearance of eggs in the feces and for counts under 200 eggs per gram, whereas the dilution counting technic was utilized for all larger counts. The flotation counts were carefully established and checked by counting 3 covers from each of 2 tubes, i. e. the eggs from one gram of feces were enumerated. The dilution egg counts may also be regarded as being highly reliable since they were based on the counting of 2 to 9 large drop (0.15 cc.) slides from each flask, with an average of 4.5 slides, utilizing sufficient slides to reach the 5 per cent limit (Stoll, 1930) on 78 of the total series of 91 flasks and the 10 per cent limit on 11 other flasks. It is not feasible to present in this paper the results of a statistical analysis of the egg count data secured beyond the statement that both of the counting methods employed were demonstrably satisfactory for enumerating *T. calcaratus* ova in rabbit dung.

Originally it had been planned to make daily counts on all of the rabbits but this was soon found impracticable, and the number was reduced to 2 or 3 a week, except in the case of Rabbit 103, which was followed daily from the 14th to the 139th day. In the final weeks of the experiment counts were not made oftener than once a week.

The larvae used for the first 2 infections were cultured from one of the animals earlier reported by Stoll (1932). Larvae for later infections were cultured from the feces of the 6 rabbits of the present experiment, and from 4 other originally "clean" rabbits infected from them. High and uniform infectivity of the larvae, and purity of the parasitic species throughout the study were proven by the nature of the infestations produced in 12 other rabbits infected with larvae from the same cultures used in this experiment, and by the recovery of only *T. calcaratus* from these animals at autopsy.

To secure infective larvae, fecal pellets from infected rabbits were left in a Petri dish, moist chamber, or pasteboard carton, at room temperature for 10 days and then isolated in the Baermann apparatus (Cort, et al, 1922). The larvae were then washed several times in tap water and counted under the microscope, using a 40 mm. objective and a 10 $\times$  ocular. Doses up to 400 larvae were counted direct, but larger doses were done by dilution, by counting two or more one-half cc. samples from a larval suspension of 10, 25, or 50 cc. The degree of accuracy of these dilution counts was ascertained by the method of Scott (1928) for each of the 3 dilutions that were used. For suspensions of 10 cc., which were used to count doses of 400 to 4,000 larvae, the coefficient of variation of the average of two counts was  $12.5 \pm 1.2$  per cent; on suspensions of 25 cc. and 50 cc., which were used to count doses of 1,000 to 21,000 larvae, the coefficient of variation was  $8.5 \pm 0.8$  per cent.

Mouth infections were given by holding the rabbit's jaws open and pipetting into the back of the mouth a few drops of water containing the larvae. The rabbit swallowed readily in most instances, with only occasional slight loss of fluid. The skin infections were made by clipping the hair as closely as possible over an area 3 to 4 cm. in diameter, placing the larvae on this area in a thin film of water and allowing the water to evaporate slowly (15 to 30 minutes), while the animal was held in one position. Each successive cutaneous infection was made on a previously unused area of the body.

The ages of the rabbits when first infected November 16, 1929, were three months for Rabbits 103 and 104, which were litter mates of the control, and two and one-half months for Rabbits 110, 111, 112 and 113, also litter mates. The second series of infections were given when the animals were 21 weeks older.

The rabbits were kept on a uniform diet consisting of a daily ration of one-half pint grain mixture, one-fourth pint moistened beet pulp, and 4 to 8 ounces of green cabbage, fed in door cups each morning, with water given freely. On this diet, which appeared to be adequate, the rabbits produced formed fecal pellets that crushed easily in the egg

count flasks and contained very little coarse vegetable débris. Such dung has been found optimum for convenience and accuracy of egg counting and well suited for the culturing of *T. calcaratus* larvae.

It was planned to build up infestations in the 6 rabbits by giving weekly doses of increasing numbers of larvae; 3 animals to be infected by mouth, and 3 by skin. Partly for convenience, and partly to simulate the assault of an increasing number of parasites, such as hosts might be exposed to when on limited areas out-of-doors, each succeeding dose was to be the sum of all earlier ones. As a matter of fact, the requirements for infective larvae outstripped our ability to culture them at first, when we were dependent upon a single animal for cultures, and also towards the end of the initial series of infections when the practically simultaneous loss of worms by 5 of the 6 rabbits was reflected in a rapidly decreasing yield of larvae from cultures. Consequently it was impossible to maintain the weekly doses during the first two months of the experiment, and some of the final doses were not increased in size to the degree planned. With these exceptions the plan of weekly additive doses was closely followed, as may be seen from Figures 1-3 and 6-8. This method of infection is a process by which, substantially, the experimenter assists in developing, with a parasite which does not naturally multiply in its host, total infestations of a size comparable to what might have obtained by a parasite multiplying *in vivo*. As it was known that this parasite has the potentiality of being fatal for its host when present in large numbers, such a building up of the worm burden provided a method of forcing a host response.

#### RESULTS

The course of the infestation produced in each of the 6 rabbits by the doses of larvae which it received are shown in Figures 1-3 and 6-8, which are graphs of the eggs per day counts listed in Tables 1 and 2. (The infections showed the same course in terms of eggs per gram counts as used by Stoll (1932), but the counts were somewhat more variable from day to day.) In these figures the crosses indicate the number of eggs which were passed on each day that a count was made (except in the case of Rabbit 103 where they represent 3-day moving averages of daily counts) while the open circles on the base line show that an examination was negative. At the top of the graphs are listed the number of larvae given at each successive dose. The arrows indicate the size of these doses and when given, and also constitute a theoretical curve of expected egg production based on the number of eggs which appeared following the first dose of larvae. The first dose given to the 3 orally infected rabbits produced average egg counts for the 3rd week after infection of 23, 32 and 28 eggs per day per larvae, with

TABLE I.—*Eggs Per Day Counts (in Thousands)*

Days after first inf.	Rabbits			Rabbits			Rabbits			Rabbits				
	104	110	111	112	113	104	110	111	112	113	110	111	112	113
3	N	N	N	N	N	44	0.3	3	0.2	1	111	—	51	N
10	N	N	N	N	N	47	—	4	—	—	114	—	86	N
13	N	0.1	N	N	N	48	—	3	—	—	121	1	15	N
14	N	0.1	N	N	N	49	—	—	—	5	128	8	7	N
15	N	0.2	N	0.2	N	50	—	—	—	3	135	N	11	0.7
16	0.3	0.7	0.1	0.5	N	51	0.3	3	0.5	8	142	N	10	4
17	0.1	1	0.1	1	N	54	0.5	2	0.4	5	149	N	22	4
18	0.2	1	0.3	1	N	58	0.6	4	0.3	4	156	N	9	1
19	0.7	1	0.3	4	N	61	0.2	3	0.3	10	158	N	13	2
20	0.5	2	0.1	2	N	65	0.4	10	0.5	15	160	N	17	0.5
21	0.1	4	0.1	1	N	67	0.5	10	0.9	13	163	N	7	0.2
22	0.2	2	0.1	0.4	N	69	0.8	13	0.4	20	165	N	14	N
23	0.3	4	0.6	4	N	72	0.6	23	1	62	167	N	14	N
24	0.5	3	3	2	N	74	0.5	21	3	81	170-1	N	7	N
25	0.1	—	0.5	2	N	76	0.4	21	1	50	177-8	N	16	7
26	0.5	3	0.4	2	0.1	79	2	45	1	69	191-2	N	17	N
27	0.1	3	1	1	0.1	81	1	16	1	74	212-3	N	0.8	18
30	0.4	4	0.4	2	0.1	83	3	22	1	79	219	7	—	1
31	0.2	2	0.5	2	0.5	86	7	12	2	5	226-7	N	15	16
33	0.1	2	0.6	2	1	88	—	17	—	N	254-5	—	—	282
34	0.2	1	0.3	1	0.5	90	9	0.1	2	0.2	10	—	18	N
36	0.6	2	0.4	3	0.1	93	24	N	2	0.2	15	—	18	N
37	0.5	3	1	1	0.7	97	24	—	4	—	34	1	31	N
40	0.4	5	1	3	0.7	100	10	N	5	—	31	—	15	N
41	0.4	1	1	2	1	104	2	N	0.1	—	11	15	—	N
43	0.3	1	1	1	0.4	107	N	N	N	—	—	—	—	N

N = Negative for eggs.



a mean of 28, which was the factor used for plotting the dosage arrows for all oral infections. The first dose given the 3 cutaneously infected rabbits yielded for the same period 6, 4, and 0 eggs per day, with a mean of 3.3, which was the factor used for plotting the dosage arrows for all skin infections. As it takes approximately 2 weeks for the larvae to develop into adult worms and produce eggs, and another week for the count to reach its maximum, each arrow represents the increase in the egg count which might be expected during the 3rd week after that infection if the rabbit were as susceptible to all doses as it was to the first one. The factor used for the oral infections is a very conserva-

TABLE 2.—*Daily Egg Counts on Rabbit 103 (in Thousands)*

Days	EPD	Days	EPD	Days	EPD	Days	EPD	Days	EPD
3	N	40	4	68	62	96	63	124	87
10	N	41	1	69	58	97	66	125	90
11-13	N	42	2	70	62	98	34	126	65
14	N	43	3	71	82	99	75	127	100
15	1	44	2	72	132	100	51	128	100
16	1	45	1	73	114	101	92	129	74
17	0.1	46	2	74	109	102	41	130	103
18	1	47	3	75	96	103	21	131	136
19	1	48	1	76	65	104	27	132	69
20	1	49	3	77	96	105	17	133	72
21	1	50	2	78	93	106	44	134	79
22	0.04	51	1	79	94	107	17	135	70
23	3	52	3	80	162	108	53	136	69
24	1	53	3	81	103	109	73	137	226
25	0.1	54	4	82	189	110	66	138	52
26	1	55	7	83	139	111	117	139	43
27	1	56	3	84	101	112	40	142	73
28	1	57	3	85	105	113	45	144	9
29	1	58	7	86	101	114	70	146	0
30	0.4	59	7	87	84	115	66	149	0
31	0.3	60	4	88	75	116	59	151	0
32	1	61	8	89	72	117	25	153	0
33	1	62	11	90	77	118	37	156-7	0
34	1	63	8	91	90	119	35	163-4	0
35	1	64	28	92	101	120	36	170-1	0
36	2	65	26	93	112	121	73	177-8	0
37	1	66	107	94	93	122	87	191-2	0
38-39	1	67	63	95	41	123	111	212-3	0
								219	0

EPD = eggs per day. N = negative for eggs.

tive one when compared with that of 43 eggs per day per larva produced by the single infection of 2,500 larvae given to Rabbit 116. Accordingly the failure of the egg counts to increase with the theoretical curve is somewhat minimized.

*Rabbit 103.* Figure 1 shows the egg counts (as moving 3-day averages of daily counts) which resulted from a series of 18 oral infections. The changes in the total number of eggs passed per day are seen to occur in weekly periods or cycles starting on or a day or two before the 14th day after each infection (which is the period required for the larvae of *T. calcaratus* to develop into adult worms and produce eggs). The first 8 doses of larvae produced rises in the egg count



roughly proportional to the number of larvae given, but then, despite the giving of larger and larger doses, the count fell off precipitously in the 13th to 15th week. A second rise in the count occurred in the 16th week (evidently induced by the large 12th dose of 26,000 larvae) but was followed by a drop in the 17th week. The egg count mounted a third time in the 18th and 19th weeks, in evident response to the

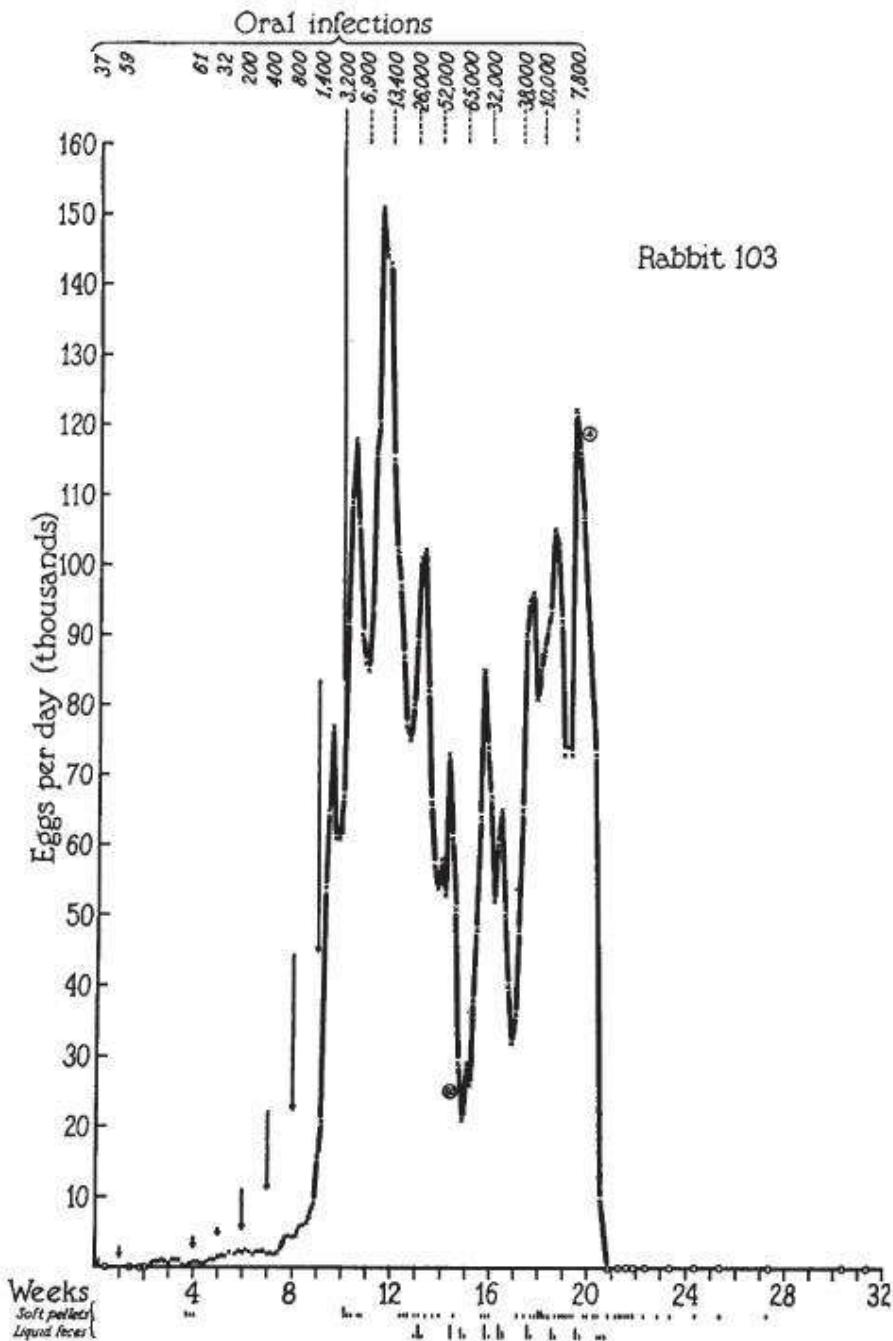


Fig. 1. Infestation record of Rabbit 103, showing initial susceptibility to infection, incomplete elimination of the infection followed by breaking through of the large doses of larvae, and finally complete elimination of the infection. Numbers in circles indicate adult worms recovered from feces. At bottom of chart are shown the association of passage of soft pellets with infection, and liquid feces with decrease in the egg count.

large 14th and 15th infections of 65,000 and 32,000 larvae, but dropped sharply to a negative in spite of two additional infections, and was consistently negative on 10 examinations during the following 11 weeks.



The reduced scale graph of this same infection (Figure 4) shows the contrast between the actual curve of egg production and the curve which would have been expected had the rabbit remained as susceptible to all the doses of larvae as it was to the first one. We interpret these egg counts as indicating that the rabbit was initially susceptible but that repeated infections developed a resistance which threw off the greater part of the worms. Some of the larger doses temporarily broke through this developing resistance. However, the rabbit not only failed to show a proportional increase in amount of infection when assaulted repeatedly with very large numbers of infective larvae, but finally completely rid itself of the infection.

The failure of the huge doses of larvae to produce comparable rises in Rabbit 103's egg count may be partly explained by the fact that a large proportion of the larvae passed directly through the animal during the two days following these infections. Thus 62 per cent of the 13th dose of larvae was recovered, as counted by dilution, 30 per cent of 14th dose, 18 per cent of the 15th dose, and 21 per cent of the 16th dose. (Passage of infective larvae through the alimentary tract has been previously observed by Stoll (1929) when large doses of *Haemonchus contortus* larvae were fed to sheep.)

Rabbit 110. Figure 2 shows that the number of eggs passed by

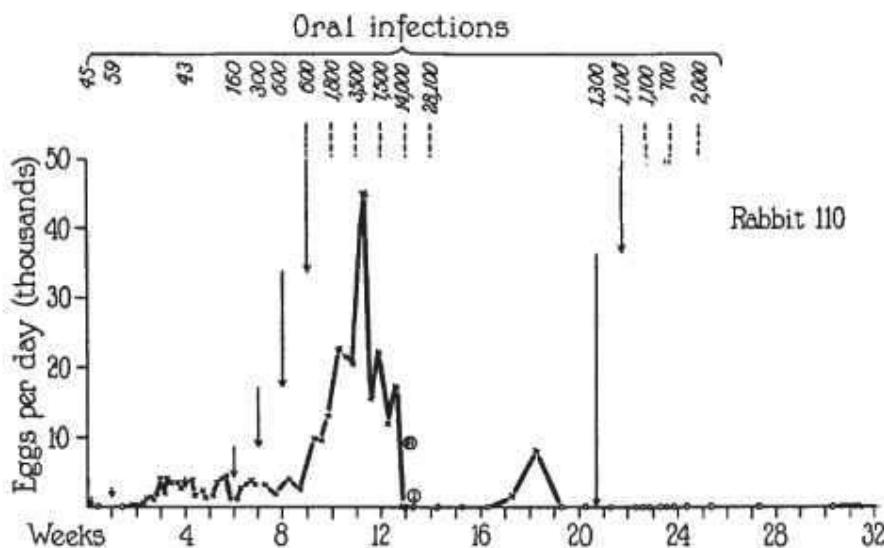


Fig. 2. Infestation record of Rabbit 110, showing initial susceptibility, occurrence of crisis and self-cure, and resistance to a second series of infections.

this rabbit increased with the number of larvae given for the first seven infections but then dropped off to a negative despite the larger and larger doses. Evidently a few worms from some of the last of these oral doses temporarily broke through the resistance which the animal had developed and produced a small egg count in the 18th and 19th weeks. A second series of 5 small oral doses, given to test the animal's resistance, produced only a delayed and barely detectable count. The chart of this infection at a reduced scale (see Figure 4) shows



the enormous disproportion between the amount of infection which the animal should have taken had it been uniformly susceptible and the amount which it actually acquired and then eliminated.

*Rabbit 112.* In this orally infected animal (Figure 3) only the

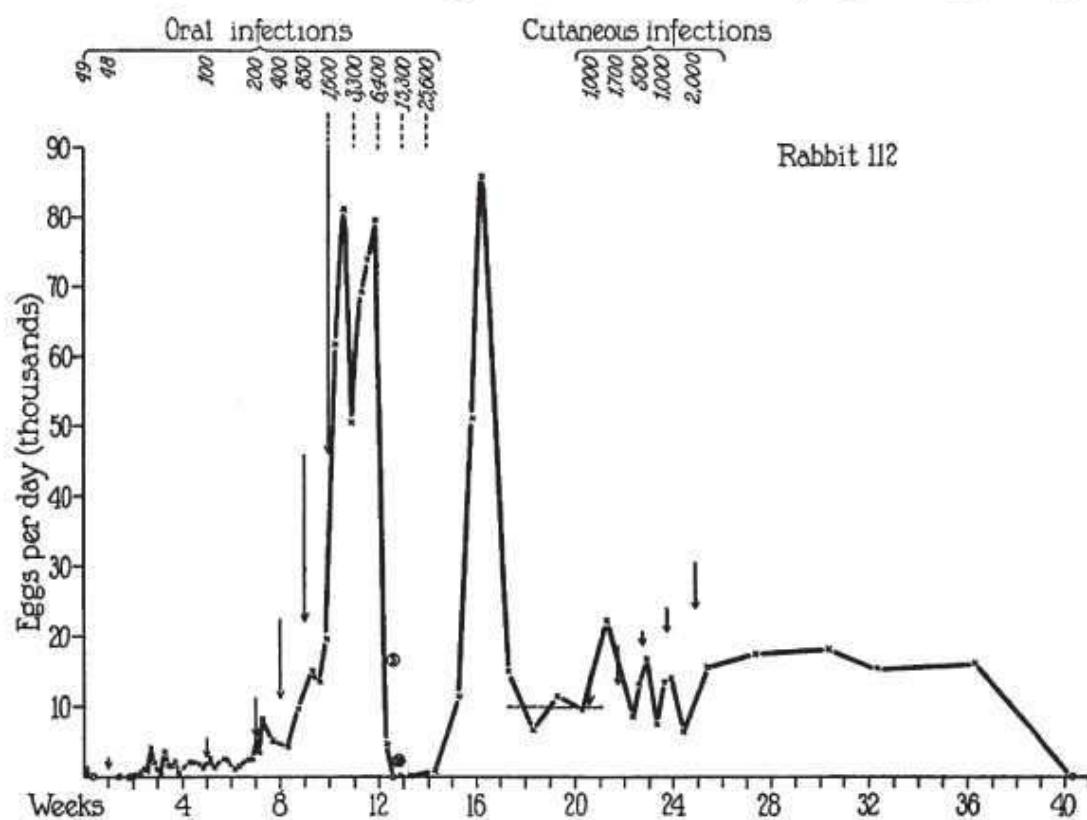


Fig. 3. Infestation record of Rabbit 112, showing initial susceptibility, self-cure, breaking through of heavy doses leaving a residual infection, relative immunity to a test series of skin infections, and ultimate elimination of the infection.

first six doses of larvae added increments to the egg count which followed the larval curve, the count dropping to a negative in the 13th week after infection. A small count reappeared, however, and evidently the large 10th and 11th doses both broke through the rabbit's newly developed resistance to build up a high count in the 16th to 17th week. This secondary peak of infection was short lived but it left a residual infection (shown in Figure 3 by the dotted line at the 18th to 21st weeks) of about 10,000 eggs per day, upon which were superimposed 5 weekly doses by skin of between 500-2,000 larvae each. Apparently the count decreased during the time of these infections instead of increasing, showing that the animal was resistant to superimposed infection. At the termination of these test doses the infection established itself at a slightly higher level than before and remained there for some time. When the animal was examined a final time it had become negative, and no worms were found in the intestinal tract at autopsy six days later. The chart plotted at a reduced scale (see Figure 4) to show the complete theoretical curve of expected egg production



illustrates the failure of the host to become parasitized by more than a very small fraction of the larvae which it received.

The gradual increase in the number of eggs passed in the dung of the 3 rabbits given an initial series of increasing infections by mouth, and the later abrupt drop in the egg count may be contrasted with the egg counts of Rabbit 116 (see Figure 5). This animal received a single

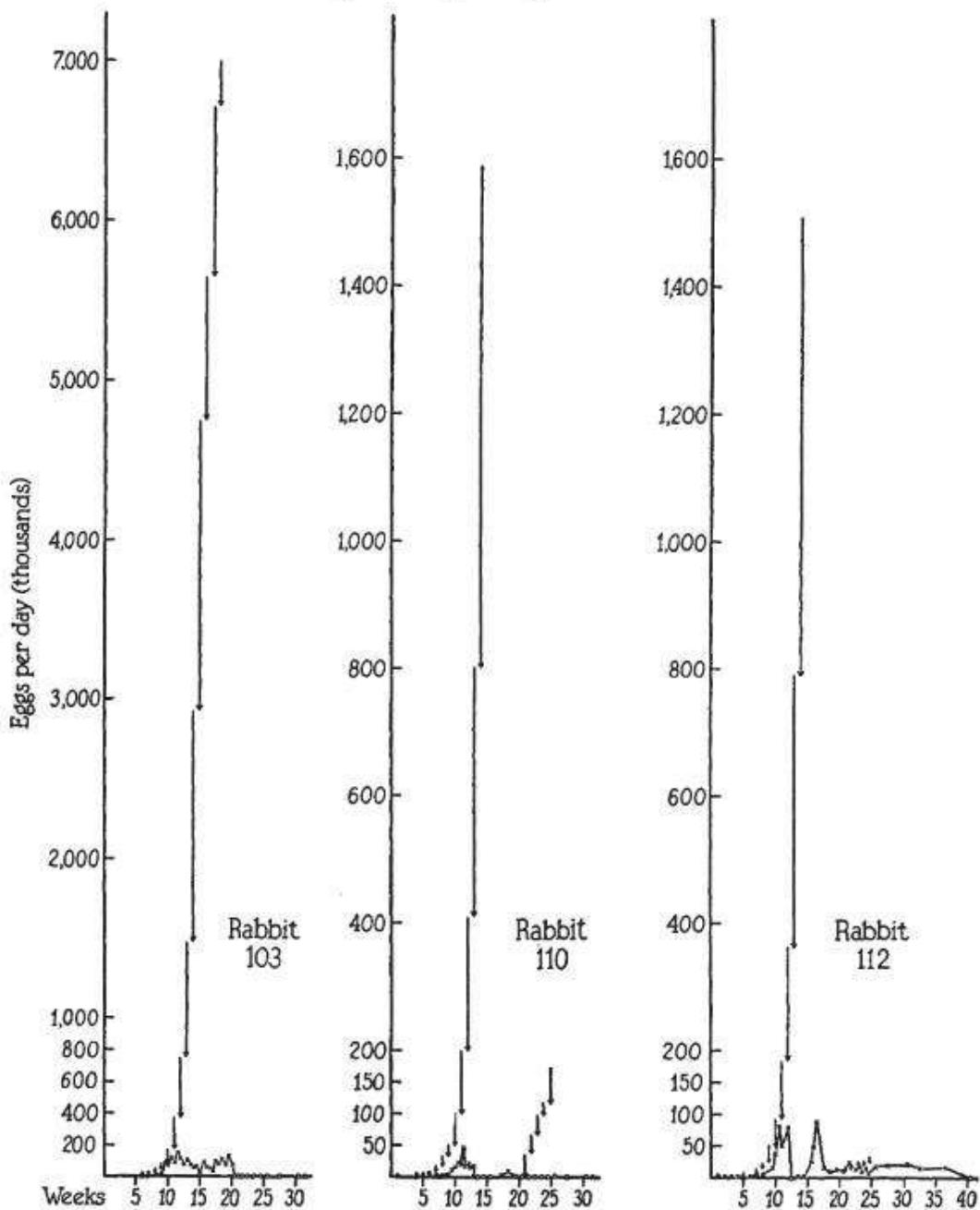


Fig. 4. Reduced scale graphs of the infections shown in Figures 1-3 contrasting the small size of the actual egg counts with the theoretical counts, indicated by the arrows, which would have resulted if the rabbits had remained as susceptible to all doses of larvae as they were to the first dose.

dose of 2,500 *T. calcaratus* larvae by mouth at the time when a dose of approximately the same size and of the same larvae was given to Rabbits 103, 110 and 112, as one of a series of doses, at 10 weeks after their first infection. In Rabbit 116, which had been found repeatedly negative for worm eggs from the time it was weaned until infected at four and one-half months of age, eggs appeared first on the 13th day



and the count mounted very rapidly during the 3rd and 4th weeks to a peak of 108,000 eggs per day, and then declined gradually over a period of nearly a year, becoming permanently negative 49 weeks after infection. (When the decreasing egg counts of this animal were plotted on arith-logarithmic paper they approximated a straight line, from which the smooth curve  $Y = 156,000 (.86)^x$  of Figure 5 was fitted graphically, indicating that the infection was being lost at a constant rate.) This sharp rise in the egg count, and the following gradual decline are typical for single infections of this size. In the other 3 rabbits

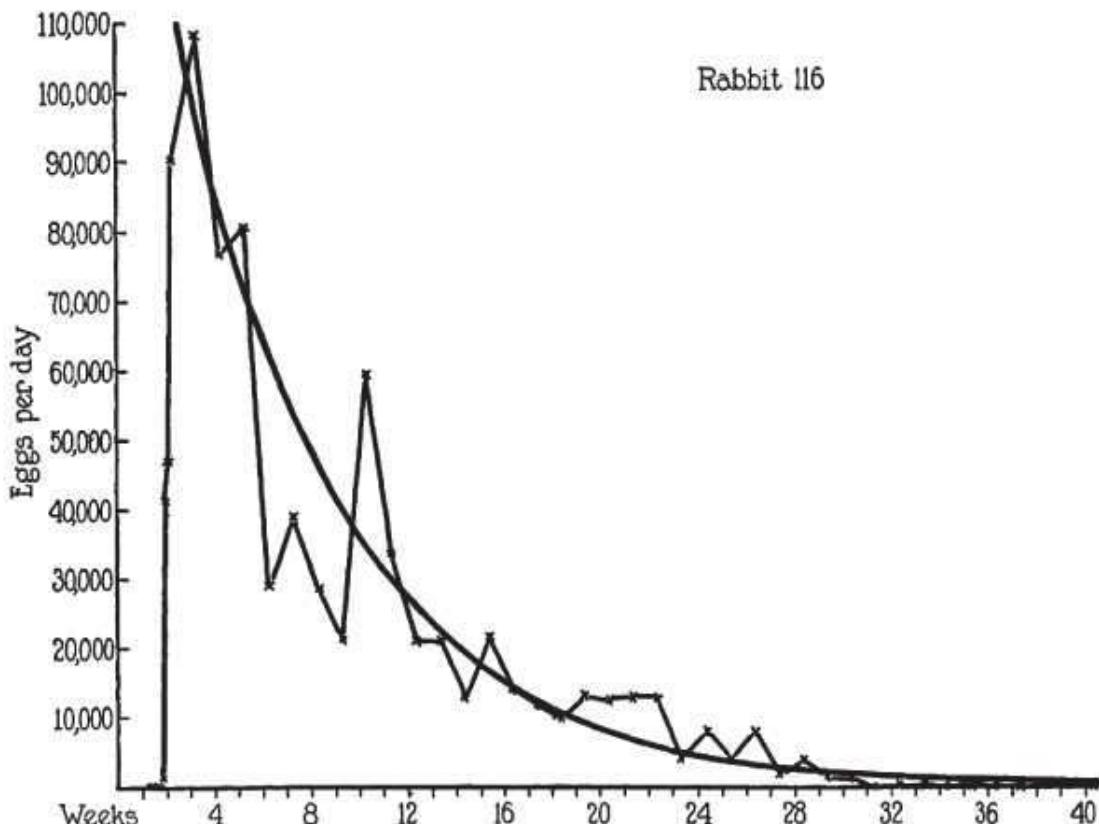


Fig. 5. Infestation produced by giving a previously uninfected rabbit a single oral infection of 2500 *T. calcaratus* larvae. Contrast the constant rate of loss of this infestation with the abrupt elimination of infestation in the 6 repeatedly infected rabbits.

which received roughly the same doses, as only one of the smaller doses in their initial series of infections, there was no increase in count that could be attributed to this infection, and, on the contrary, the change in egg count which occurred in the 13th week, when eggs from this infection should have been expected to appear, was in every case a decrease, as may be seen in Figures 1, 2 and 3. The *whole series* of doses fed to Rabbits 103, 110 and 112 produced only in Rabbit 103 a count as high as that occasioned by the single dose given to Rabbit 116.

*Rabbit 104.* This animal received 11 infections by skin (Figure 6), but due to a shortage of larvae it and the other two infected by skin received no doses of larvae for 5 weeks after the first two doses. There was very little increase in the small count arising from the first two infections until the sixth infection was given. This dose and the next



one were followed by proportional increases in the egg count, which then dropped sharply to a negative in spite of three final infections of large numbers of larvae. The animal was autopsied the day after it became negative, and only 17 adult *T. calcaratus* were found although the rabbit had received a total of 38,000 larvae. If the animal had remained uniformly susceptible throughout the experiment the egg count would have reached the height shown by the arrows.

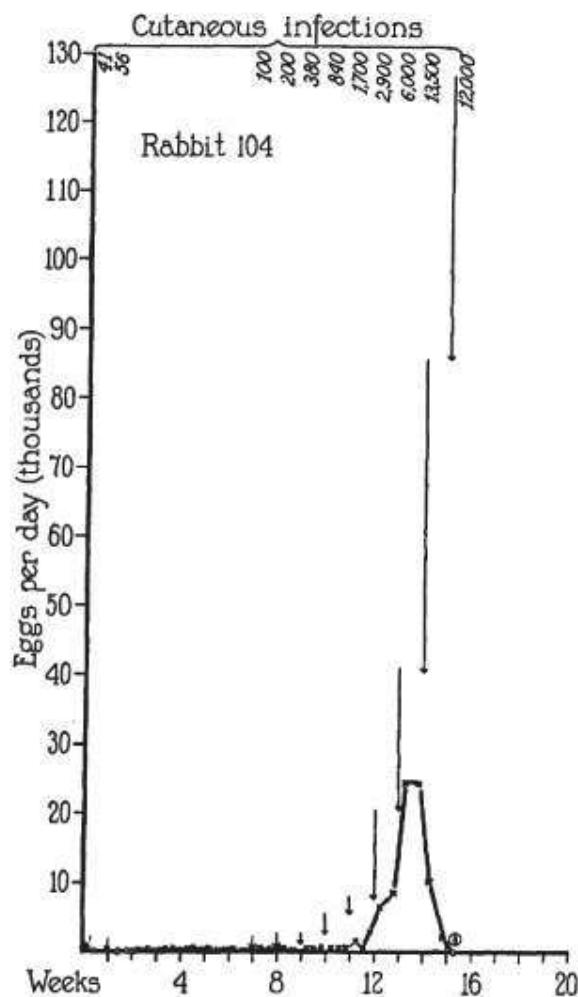


Fig. 6. Infestation record of Rabbit 104, showing occurrence of self-cure following repeated cutaneous infection.

*Rabbit III.* The number of worms colonizing the intestines of this animal, as indicated by the egg count (Figure 7), increased with the number of larvae given at first, then lagged behind, and finally dropped to a negative in the 16th week. The two final doses of the first series were evidently responsible for the slight temporary increase in the count during the 17th and 18th weeks. Five weekly doses of larvae given *orally* as a crucial test of this animal's resistance produced only a delayed and barely detectable egg count, whereas in a previously uninfected animal the count shown by the arrows would have been expected. This rabbit was the least susceptible to repeated infection of any of the 6 animals and demonstrated conclusively that a resistance acquired against skin infection also provides effective protection against mouth infection.



*Rabbit 113.* The first skin infection given this animal apparently did not induce any detectable infection, but the second to eighth doses inclusive produced proportional egg count increases (Figure 8). In contrast to this, the final three doses were followed by a rapid decrease in the count to a negative. Some of the larvae of these last three infections were probably responsible for the small and delayed count which appeared in the 20th, 21st and 22nd weeks. The resistance which had thus manifested itself was tested by a second series of 5 small weekly doses of larvae also given by skin. The egg count decreased while

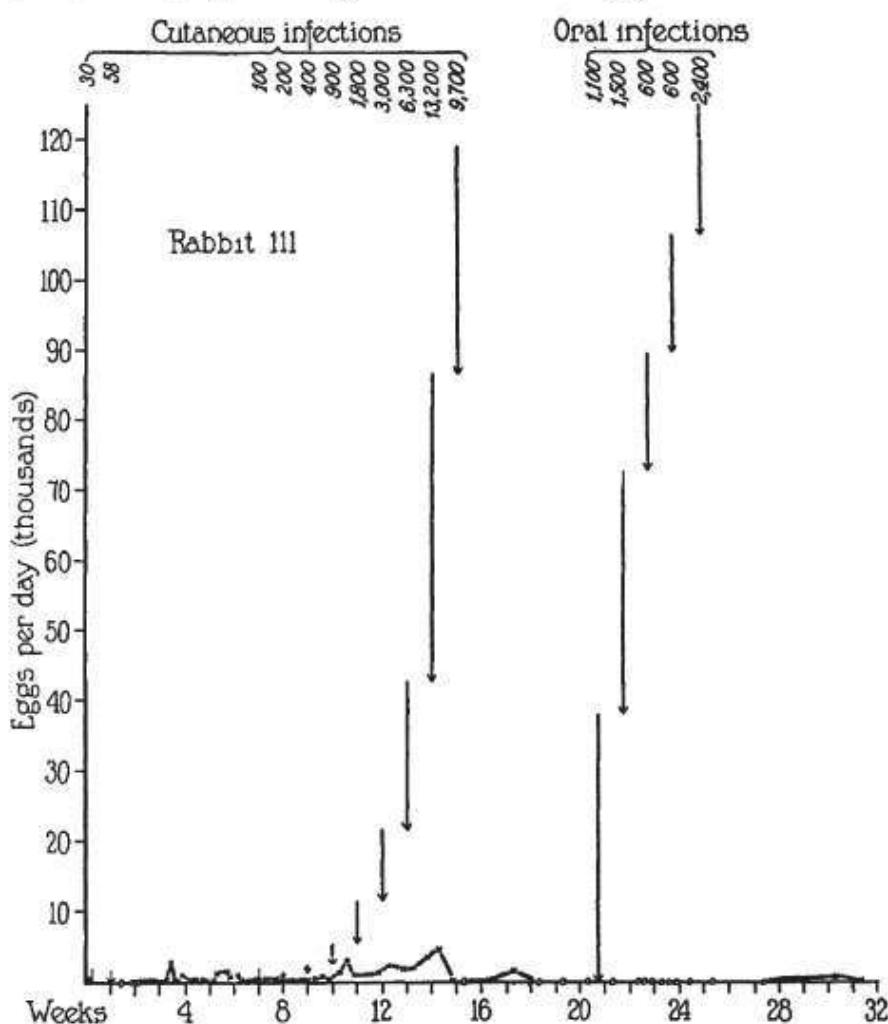


Fig. 7. Infestation record of Rabbit 111, showing early development of resistance to cutaneous infection and resulting refractoriness to oral infection.

these were being given. The two transient increases which occurred following this second series of "test" doses may be interpreted as the breaking through of an infection followed by reestablishment of the animal's resistance.

*Worms recovered from feces.* Five of the 6 repeatedly infected rabbits passed adult worms in their dung at the time when the sharp drop occurred in their egg counts, proving that the decrease in the count was actually associated with loss of parasites. The worms were recovered by screening the crushed feces through screens of 20, 40 and 60 meshes to the inch. The total number of worms calculated to



have been passed by each rabbit on each day that worms were recovered is shown by the numbers in circles in Figures 1 to 3 and Figures 6 to 8. These worms, recovered by the occasional screening of a comparatively small portion of the total feces passed by the rabbits, obviously represent only part of the total number of parasites which must have been eliminated to account for the great decreases in egg counts which occurred at the time the worms were found. That this passage of worms is not merely evidence of a normal turnover in the parasitic population of an animal exposed to repeated infection is shown by failure to find worms immediately before and after these sudden drops

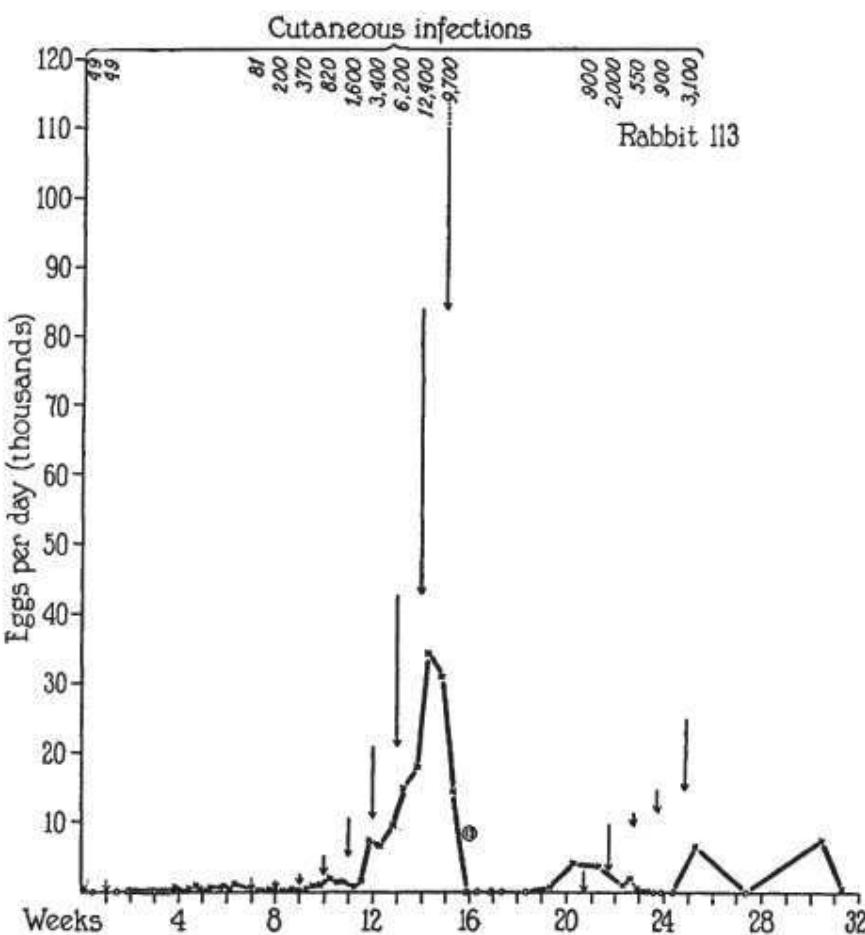


Fig. 8. Infestation record of Rabbit 113 illustrating the acquirement of resistance to superinfection, occurrence of self-cure, and relative resistance to a second series of infections.

in the egg count, and by the more detailed data concerning Rabbit 103. At least 10 per cent of the dung passed each day by this rabbit from the 88th through the 101st day after its first infection was screened for worms but none were found. For this interval the egg count averaged 74,000 eggs per day. But when the egg count dropped on the 104th day to 26,500 eggs per day on a 102 gram specimen, of which 31 grams were liquid, 6 worms were recovered from 10 grams of the mixture, giving an estimated number of 60 worms in the total specimen.

*Passage of soft pellets and liquid feces.* Parasitic infection and the development of self-cure and resistance in the rabbits were asso-

ciated with the passage of soft pellets and liquid feces. This is best shown by daily information secured on Rabbit 103. At the bottom of Figure 1 are indicated the days on which feces of either of these two consistencies were found in addition to the usual formed pellets. The short bars indicate that a small amount of the day's fecal specimen was of the designated form, the large ones that a relatively large proportion was passed. Before infection, and throughout the first 10 weeks after infection, the animal passed firm fecal pellets almost exclusively, but from the 10th week on masses of soft pellets occurred very frequently and diarrheic feces were observed every week from the 14th through the 21st. The worms which were recovered by screening Rabbit 103's feces were all from liquid specimens. There is not as detailed a record for the other five rabbits, but they, too, show a correlation of soft pellets and diarrheic feces with infection and loss of worms. This change in fecal consistency was not caused by diet, since it did not occur in the control rabbit, nor can it be laid to coccidial infection, as the animals had ceased to pass oocysts. Stoll (1929) observed a similar passage of soft pellets by sheep at the height of *Haemonchus contortus* infections.

*Skin lesions.* The only other clinical sign of the rabbits' reaction to repeated infection (their weight curves were not significantly different from that of the uninfected control) was the appearance of small nodules at the site of infection following some of the larger doses given by skin. Rabbit 104 and Rabbit 111 developed nodules after the 7th, 9th, 10th, and 11th skin infection, and Rabbit 113 showed them after the 9th and 10th doses of the first series, and after the 3rd and 4th test doses. They appeared within a few days after infection as papules of 2 to 5 mm. in diameter. They were firm, movable, yellow in the center, circumscribed with a narrow red zone, and blanched on pressure. They decreased in size for 2 or 3 weeks, becoming dry and white, and finally sloughed away leaving a pigmented spot in the skin.

#### DISCUSSION

Although the most striking characteristic of the infections produced in the 6 rabbits given increasing weekly doses of larvae may seem at first to be their dissimilarity, careful study of Figures 1 to 3 and Figures 6 to 8 will show that the general course of the infection was the same in each animal and that the differences were merely a matter of degree. All 6 were initially susceptible as shown by the establishment of an infection and its increase with the increase in the doses of larvae. All became resistant to superinfection after 6 to 8 doses of larvae, and this phase of their immunity development was uniformly followed by an abrupt drop in their egg counts associated with the passage of

worms in their feces. In each animal this process of self-cure was followed by the temporary establishment of a new infestation resulting from the thousands of larvae which were being given at the culmination of the first series of doses. These secondary peaks of infection were in turn more or less completely reduced and the resistance reestablished as shown by the rabbits' relative resistance to a second series of doses. The increase of the egg count to a maximum occurred at the same time in all of the animals infected by the same method, although later in those infected by skin (first peak of infection at the 14-15th week) then in those infected by mouth (first peak of infection at the 11-12th week) because of the five week period when no larvae were given to the former group. The first sudden decrease in the egg count curves, indicating the occurrence of self-cure, also took place at roughly the same time in all rabbits of each group, occurring in the 12-15th week in the orally infected animals and in the 15-16th week in the cutaneously infected ones.

Obvious differences between the infections of the 6 repeatedly infected rabbits are shown in the general shape of the egg count graphs, and in the size of the egg counts. Since the number of larvae received by each rabbit was not the same the latter are better compared in terms of the egg-larva ratio, i. e. by a ratio obtained by dividing the egg count produced by a particular dose or group of doses by the number of larvae which had been given a sufficient time previously (at least two weeks) to have played a possible part. Table 3 gives such egg-larva ratios for each animal and also summarizes other comparative data. It will be seen from this table that infections given by skin took longer to appear than those given by mouth. The egg-larva ratio calculated for the total ascending portion of the egg count curves, or period of initial susceptibility, was 41 for oral infections and only 4.4 for cutaneous infections. The egg-larva ratio for Rabbit 116, given a single dose of 2,500 larvae, was 43. Thus during the early phase of the infection repeated doses of larvae were equally as effective in establishing worms in the three orally infected rabbits as a large dose of larvae given all at once to another animal.

The egg-larva ratios for individual animals during this same period show that Rabbit 110 was less susceptible to infection than the other two orally infected rabbits and Rabbit 111 less susceptible than the other two infected cutaneously. It is of interest that the later behavior of the rabbits is in accordance with their comparative susceptibility classification. Thus Rabbits 103 and 112 were more susceptible, on the basis of these ratios, than Rabbit 110, and in both the heaviest doses of the initial series of infections broke through their partially established resistance and succeeded in producing large infestations

which were only thrown off after some time, whereas in Rabbit 110, the heavy doses of larvae succeeded only in reestablishing a small infection which was rapidly and completely eliminated. Likewise, among the skin infections, Rabbit 111, which had the lowest ratio and appeared to become resistant very early, showed a slightly and more transient breaking through of the largest doses of larvae than the more susceptible (by ratio) Rabbit 113, and also proved less susceptible to the test infections.

The egg-larva ratio may also be used to contrast the large total number of larvae which the rabbits received in their initial series of doses with the small amount of infection which was left at the time of the test doses. As Table 3 shows, 4 of the 6 rabbits had a zero ratio at this time, being negative for worm eggs, and the other two had ratio of below 0.2 eggs per day per larvae. The most striking case is that of Rabbit 103. This animal had been highly susceptible initially as shown by a ratio of 50 eggs per day per larva for the first 8 infections, totalling 3,000 larvae, but its ratio became zero in the 3rd week after its last infection of 7,800 larvae, although it had received a total of 257,000 larvae.

The purpose of the second series of doses which were given to 4 of the rabbits was to test the resistance to infection which the animals were believed to have acquired as a result of exposure to their initial series of infections and also in 2 rabbits to test for immunity against infection by the alternate method from that previously used. The degree to which the rabbits were susceptible to this second series of doses is shown in Table 3, in terms of egg-larvae ratios based on the maximum increase which was observed in the egg counts. The five test doses given orally to Rabbit 110, which had become negative following a previous series of oral doses, gave a ratio of only 0.02. A similar series of oral doses given to Rabbit 111, which was negative as the result of an initial series of cutaneous doses, gave an egg-larva ratio of only 0.1. These two factors show that both these animals were relatively resistant to infection, since they represent the establishment of an insignificant egg count when compared with the average ratio of 41 for animals susceptible to oral infection. In Rabbit 113, which had eliminated most of its original infestation produced by cutaneous infection, the egg-larva ratio for the maximum increase in egg count produced by the test doses given cutaneously was 0.4. For Rabbit 112, carrying a residual infestation of uncertain level from an initial series of oral doses, the egg count decreased during the time that the test doses were being given by skin, but increased later to a new plateau of infection, which, if brought about solely by the test doses, would give a ratio of 1.3. As the ratio for animals susceptible to infection by skin

TABLE 3.—Comparative Data Concerning Infestations of Rabbits Repeatedly Infected with *Trichostrongylus calcaratus*

Rabbit No.	Infected	Initial Series of Infections					Test Infections					
		1st Eggs (Days)	1st Peak (EPD)	No. doses involved	Total larvae given	Egg-larva ratio	Residual count (EPD)	Total larvae given	Egg-larva ratio	Infected	Total larvae given	Max. increase (EPD)
103	by mouth	15	151,000	8	3,000	50	0	257,000	0	.....	.....	...
110	" "	13	45,000	7	1,800	25	0	57,000	0	by mouth	6,200	100
112	" "	15	81,000	6	1,650	49	10,000	54,000	0.2	by skin	6,250	0.02
Average for mouth infections		14.3	94,000			41						1.3
104	by skin	16	24,000	7	3,300	7.3	0	38,000	0	.....	.....	...
111	" "	16	5,000	8	6,500	0.8	0	36,000	0	by mouth	6,200	0.1
113	" "	26	34,000	8	6,600	5.1	4,000	35,000	0.1	by skin	7,450	0.4
Average for skin infections		19.3	21,000			4.4						

EPD = Eggs per day.

was 4.4, both rabbits were relatively resistant by this test, as the increase brought about by the test doses was very slight in the case of Rabbit 113, and small, even if solely due to the test doses, in Rabbit 112. Of course any ratio above zero resulting from the test doses, although small in size when compared with the ratio for susceptible animals, is significant in that it demonstrates that the resistance of the rabbits was relative, not absolute. The resistance displayed by Rabbits 111 and 112 to infection by the alternative method from that to which they had previously become resistant is most marked in the case of Rabbit 111, but sufficiently definite in both animals to demonstrate that the protection is not limited to infection by the route used to produce the resistance.

The increasing age of the rabbits during the experiment is not considered to have played any significant part in their development of a resistance to infection since we have failed so far to demonstrate the presence of any marked age resistance in rabbits to infection with *T. calcaratus*.

The occurrence of self-cure and protection in rabbits exposed to repeated infection with *T. calcaratus*, as reported in the present paper has also been observed in a second series of rabbits given larger increasing doses of larvae, as well as in several groups of rabbits exposed to repeated infection out-of-doors.

The exact mechanism of the phenomenon has not been determined but the following points support the idea that this resistance is primarily a host reaction: the passage of soft pellets and diarrheic feces concomitantly with the loss of worms; the appearance of nodules in the skin after repeated cutaneous infection; the refractoriness to infection of rabbits which were originally susceptible to infection but have lost their worms; and the occurrence of self-cure in rabbits carrying infestations of greatly different magnitudes. The last two points would appear to furnish adequate proof that self-cure and resistance in rabbits is not related to mere parasitic overcrowding, since rabbits may be immune to infection although carrying no worms, and can discharge their worms although only few are present. The process appears then to be clearly analogous if not homologous to that observed by Stoll (1929) in sheep exposed to repeated infection with a stomach worm, *Haemonchus contortus*.

#### SUMMARY

Weekly increasing doses of the infective larvae of an intestinal nematode, *Trichostrongylus calcaratus*, were given to laboratory rabbits by mouth and by skin, and the resulting infections followed by frequent egg counts.

All of the rabbits were susceptible to infection at first as shown by increases in the egg counts roughly proportional to the number of larvae given, but after 6 to 8 weekly doses of larvae they became refractory to superimposed infection and finally discharged the worms which they did harbor. This self-cure manifested itself by sharp drops in the egg counts associated with passage of soft pellets and liquid feces containing adult worms.

The rabbits as tested by a later second series of infections were found to possess a high degree of acquired resistance. Those previously infected by mouth were resistant to later infection by mouth and by skin, and those infected initially by skin were also resistant to infection by either method.

In contrast to the sudden loss of infection from rabbits given repeated doses of larvae, the infection produced in a rabbit given a single dose of larvae showed a constant rate of loss over a period of 49 weeks, as determined by egg counts.

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#### REFERENCES CITED

- Africa, C. M. 1931.—Studies on the host relations of *Nippostrongylus muris*, with special reference to age resistance and acquired immunity. *Jour. Parasit.*, 18:1-13.
- Chandler, A. C. 1932.—Susceptibility and resistance to helminthic infections. *Jour. Parasit.*, 18:135-152.
- Cort, W. W., Ackert, J. E., Augustine, D. L., and Payne, F. K. 1922.—Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil. *Amer. Jour. Hyg.*, 2:1-16.
- Fülleborn, F. 1926.—Über das Verhalten der Hakenwurmlarven bei der Infektion per os. *Arch. Schiffs- Trop.-Hyg.*, 30:638-653.
- Graham, G. L., Ackert, J. E., and Jones, R. W. 1932.—Studies on an acquired resistance of chickens to the nematode, *Ascaridia lineata* (Schneider). *Amer. Jour. Hyg.*, 15:726-740.
- Herrick, C. A. 1928.—A quantitative study of infections with *Ancylostoma caninum* in dogs. *Amer. Jour. Hyg.*, 8:125-157.
- McCoy, O. R. 1931a.—Immunity reactions of the dog against hookworm (*Ancylostoma caninum*) under conditions of repeated infection. *Amer. Jour. Hyg.*, 14:268-303.
- 1931b.—Immunity of rats to reinfection with *Trichinella spiralis*. *Amer. Jour. Hyg.*, 14:484-494.
- Payne, F. K. 1923.—Investigations on the control of hookworm disease, XXXI. The relation of the physiological age of hookworm larvae to their ability to infect the human host. *Amer. Jour. Hyg.*, 3:584-597.
- Ransom, B. H. 1911.—Two new species of parasitic nematodes. *Proceed. U. S. Nat. Museum*, 41:363-369.

- Sandground, J. H. 1928.—Some studies on susceptibility, resistance, and acquired immunity to infection with *Strongyloides stercoralis* (Nematoda) in dogs and cats. Amer. Jour. Hyg., 8:507-538.
- Sarles, M. P. 1929.—The length of life and rate of loss of the dog hookworm, *Ancylostoma caninum*. Amer. Jour. Hyg., 10:667-682.
- 1930.—The occurrence of self-cure and protection in rabbits receiving repeated infections of *Trichostrongylus calcaratus* Ransom. Jour. Parasit., 17:114.
- Schwartz, B., Alicata, J. E., and Lucke, J. T. 1931.—Resistance of rats to super-infection with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes. Jour. Wash. Acad. Sc., 21:259-261.
- Scott, J. A. 1928.—An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Amer. Jour. Hyg., 8:158-204.
- Stoll, N. R. 1929.—Studies with the strongyloid nematode, *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. Amer. Jour. Hyg., 10:384-418.
- 1930.—On methods of counting nematode ova in sheep dung. Parasit., 22:116-136.
- 1932.—Note on re-infection under "natural" conditions with a gut nematode of the rabbit. Jour. Parasit., 19:54-60.



# The early history of immunoparasitology in the United States

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In the latter part of the 1800s, scientists and clinicians in the U.S.A. realized that European biologists were leaps and bounds ahead of them in understanding immunity to infectious diseases. A small group of extraordinary Americans would change that. Before discussing who they were and what they did, however, one must set the stage for the events that led the Americans on the road to leadership in immunology and infectious diseases.

The long-term campaign dealing with infectious diseases started many centuries ago in the Middle East, where it was common for people to acquire wet chancre sores on their skin that, when healed, left deep, ugly, scars. Such disfiguring lesions would have had a significant negative impact on young women who commonly had only their faces exposed to the bites of insects, which carried the infectious organisms that caused the sores. At some point, it was determined that such a lesion would occur only once in their lifetime. The practice then developed for young people (and perhaps especially young girls) to be purposely exposed (often on the buttocks) to puss from someone with an active wound. A lesion would form on the inoculated area, but would spare, for example, the face of young girls from unsightly disfigurement (Khamesipour et al., 2005). The ingenuity of this procedure may also have had an economic aspect to it since it was important in those times to have daughters wed and facial deformities could have been a significant deterrent to marriage. We know now these infections were caused by parasitic protozoans, *Leishmania* spp. and that the protective procedure was likely the first attempt at intervention to stimulate immunity to a parasite (Cox, 1996).

The next advance to understanding immunity to infectious agents came from the work of Edward Jenner (1749–1823), the son of a vicar in Berkeley, England. Even as a child, he was drawn to the natural sciences. He was orphaned at age five and lived with his older brother until, at the age of 13, he was apprenticed to a physician; he later went on to study medicine in London with some of the leaders of surgery. It was during this time he repeatedly heard that milkmaids were resistant to smallpox because of their earlier exposure to cowpox. After his medical training, he returned to his childhood village to practice medicine. Like the women of the Middle East centuries before him, Jenner had a keen sense of observation. Over the years, he noticed that during epidemics of smallpox, milkmaids on local farms seemed resistant to smallpox, confirming the anecdotal reports he had heard earlier. He also observed that these smallpox-resistant milkmaids had been exposed to cowpox. He finally decided that there was a

connection, i.e., prior exposure to cowpox made one resistant to smallpox (Reidel, 2005).

In an experiment that today would be considered entirely unethical, Jenner proposed to the parents of a young boy, James Phipps, that they allow Jenner to expose the youngster to cowpox and then later to give him an inoculation of smallpox. A young milkmaid named Sarah Nelms, had an active infection of cowpox and was the source for exposing young James. After about 9 days, Jenner then infected the boy with smallpox. The boy did not develop the infection, and Jenner went on to endorse this preventative treatment in England (Reidel, 2005). For generations, this method was used as a smallpox vaccine and is essentially the technique that resulted in the eradication of the horrible disease. It should be emphasized that neither the Middle Easterners nor Jenner had any idea of the causative agents of the diseases with which they were dealing, and that they had no concept of the nature of immunity. They only knew that their methods worked.

It would be nearly a century after Jenner's experiment that Louis Pasteur (1822–1895) in Paris and Robert Koch (1843–1910) in Berlin would introduce the idea of the “germ theory” for infectious diseases (Sakula, 1983). Koch meticulously developed methods to isolate and identify infectious organisms and is famous for discovering the bacteria responsible for tuberculosis, anthrax, and other diseases. Koch's postulates laid the foundation for clearly and accurately recognizing disease-causing bacteria. Pasteur was first to employ attenuated infectious agents, including *Bacillus anthracis*, chicken cholera, and the rabies virus for use as vaccines. He also developed the process of pasteurization (which saved the struggling wine and beer industries). He rescued the silk industry by identifying the parasite that killed silkworms. Of all of the accomplishments, however, his prophylactic treatment of rabies was hailed as his most brilliant discovery. This enhanced his fame and led to the establishment of the Pasteur Institute in Paris. Soon, scientists from around the world were working with Pasteur with many more important discoveries yet to be made. Similarly, Koch attracted extraordinarily creative and productive colleagues to work with him in Berlin (Koch; [Nobelprize.org](#)).

Following the ground-breaking discoveries by Pasteur and Koch, an amazing collection of scientists further extended earlier observations on infectious diseases and set the stage for understanding some aspects of how immunity was ultimately manifested. The primary players in expanding concepts on immunology included Paul Ehrlich (1854–1915; [Nobelprize.org](#)), Emil von Behring (1854–1917; [Nobelprize.org](#)), Ilya Mechnikov (1845–1916; [Nobelprize.org](#)), and George Nuttall (1862–1937; Graham-Smith and Keilin, 1939). These scientists had very different perspectives from each other, but generally worked in different, but nonetheless related, areas. They knew each other personally and had, at one time or another, worked in the laboratories of either Pasteur or Koch. Although both Pasteur and Koch had demonstrated the methods for developing immunity/resistance to pathogenic organisms, they had no idea what was responsible for immunity. This

facet of the developing discipline of immunology would be left to Ehrlich, von Behring, Mechnikov, and Nuttall (see [Fig. 1](#)).



**Figure 1** (A) Emil von Behring, (B) Paul Ehrlich, (C) Ilya Mechnikov, (D) George Nuttall, and (E) Almroth Wright.

Paul Ehrlich received his M.D. degree for developing aniline dyes in the study of animal tissues. He determined that various components of cells would stain differently, depending on dyes classified as neutral, basic, or acidic. Particularly striking were his stains for the different leucocytes in blood. For his studies on the identification of mast cells, he employed the Gram method for staining bacteria (Ehrlich; [Nobelprize.org](http://www.nobelprize.org)).

After posts at several universities, Pasteur invited Ehrlich to become one of his assistants, and there he began his studies on immunology. Importantly, this work included quantifying of serum titers of hemolysins by serial dilution analysis. The ability to quantify the levels of hemolysins led to his appointment to the Institute for Therapeutic Sera in Berlin, of which he eventually became the director. As Ehrlich's work was progressing, von Behring and others found that the broth in which *Corynebacterium diphtheriae* (the causative agent of diphtheria) was cultured and contained a toxin that, when injected into animals, caused the same

symptoms as the disease itself. von Behring generated antisera to the toxin and found that mixing the antisera with the toxin (forming a toxin-antitoxin complex), could inactivate the toxin, and could be used to induce immunity to diphtheria. Also, the antisera could be used successfully to treat patients who already had contracted the generally fatal disease. It should be noted here that, at the time, antibodies, as molecular entities, were unknown. They were eventually identified because of their ability to agglutinate bacteria and foreign erythrocytes. The antitoxin antibodies, when injected into infected patients, neutralized the toxins and allowed the patients to overcome the disease.

Since Ehrlich discovered how to quantify the relative concentration of antibodies in serum by titration, he was able to determine the kinetics of the antibody response following injection of antigens into animals. He could then demonstrate primary and secondary immune responses and later discovered the placental transfer of antibodies from mothers to fetuses. His contributions were recognized with a Nobel Prize in Physiology and Medicine in 1908, which he shared with Ilya Mechnikov.

Emil von Behring came from a poor family and could not afford medical school. In due course, however, he received his medical degree (1878) free from a military university, but was required to spend some years in military service. He was intensely interested in infectious diseases. With the discovery of toxins, he focused on ways to use them to prevent disease. He first employed iodoform, which was effective in inactivating toxins. After some years of independent research, he moved in 1888 to work with Robert Koch. When the latter transferred to the Institute for Infectious Diseases in 1889, von Behring accompanied him. The next year, Paul Ehrlich joined the Institute (Ehrlich; [Nobelprize.org](http://Nobelprize.org)).

von Behring's work in showing the effectiveness of toxin-antitoxin therapy brought him great fame but, consequently, also problems in his relationship with Koch, presumably because Koch became envious of the attention von Behring was receiving . Eventually, von Behring, with some investors, started the first biotech company (Behringwerke, which still exists) where he continued his research. The company brought great wealth to von Behring. He built an estate and continued some of his research there, in some cases using his own farm animals for his studies. Based on the successes von Behring had with immunization with toxin-antitoxin mixtures, he was awarded the First Nobel Prize in Physiology or Medicine in 1901.

Ilya Mechnikov was a brilliant student and went on to become a renowned zoologist with his main interests on invertebrates. He was, early in his career, plagued with serious bouts of depression and on two occasions attempted suicide. Following his second suicide attempt, he resigned his professorship at the University of Odessa and, with his second wife, went to Messina. There, he built a functional laboratory and continued his studies on starfish larvae. These organisms are essentially transparent when observed using light microscopy. He

became particularly interested in the motile cells (hemocytes) observed in the body cavity (hemocoel) of the larvae. Curious as to their function, he pierced some larvae with small thorns and observed them the next morning. Using his microscope, he saw that the migratory cells had encapsulated the tips of the thorns. He surmised that these cells, in some way, provided protection from this foreign assault. Later, after returning to the University of Odessa and working with water fleas (*Daphnia*), he harvested the hemolymph (containing hemocytes) from *Daphnia* and placed pathogenic yeast in the hemolymph. He observed that hemocytes engulfed and killed the yeast cells. This observation, along with his having seen similar appearing white blood cells in vertebrate blood, led him to propose that immunity is in some way related to these cells. He termed these cells “phagocytes” and proposed that immunity was due to phagocytosis. In 1888, Mechnikov moved to work with Pasteur in Paris and remained there through the rest of his career (Mechnikov; [Nobelprize.org](https://www.nobelprize.org)). For his work on phagocytes and the cellular basis of immunity, Mechnikov shared the Nobel Prize with Ehrlich in 1908.

Not surprisingly, there developed controversy and skepticism regarding the concept of the cellular basis of immunity. The controversy was heightened by the work of George Nuttall, who was born in California where his father was a physician. He spent much of his formative years in Europe (England, France, Germany, and Switzerland) when his father moved the family there. His ability to speak several languages was beneficial for the work he would pursue later in his life when he would return to Europe to do research. As with others mentioned above, Nuttall was fascinated by the natural sciences (Graham-Smith and Keilin, 1939).

In 1884, Nuttall earned the M.D. degree on return to California and then spent a year at Johns Hopkins University where he worked with William Henry Welch, the eminent pathologist. In 1886, he went to Germany where, over the next 4 years, he developed an interest in parasitology, which would later become the main focus of his research. In 1888, Nuttall discovered that antisera from animals surviving anthrax possessed strong antibacterial activity. He also showed that if this antiserum was heated at 55°C for 30 minutes, the antibacterial activity was lost. These observations were the basis for the concept of a “humoral basis of immunity,” which contrasted with Mechnikov’s notion of a “cellular basis of immunity.” It was initially believed by many that humoral immunity was the main source of immune resistance and that Mechnikov’s phagocytes were basically “garbage collectors” to clean up the dead bacteria killed by the antisera (Graham-Smith and Keilin, 1939).

While Nuttall made numerous and important discoveries in immunology, he contributed significantly in other areas as well. In 1889, he was invited to take a position at Cambridge University, where he founded the *Journal of Hygiene*, which he edited until his death. With Patrick Manson, he helped to create a Diploma in Tropical Medicine and Hygiene at Cambridge. In 1908, he founded the

journal *Parasitology*. Importantly, he also established, and obtained the funding for, the Molteno Institute for Research in Parasitology at Cambridge in 1921.

The controversy between cellular versus humoral based immunity ended with both points of view being essentially correct. Almroth Wright is probably best known as Alexander Fleming's professor and mentor, and for his development of anti-typhoid fever inoculations. During World War I, this treatment was administered to millions of British troops and is credited with saving the lives of enormous numbers of wounded soldiers who otherwise might have died of infection (Allison, 1974). Wright also performed experiments showing that antisera to bacteria coated the organisms and enhanced phagocytosis by macrophages. He termed this phenomenon as "opsonization" and the coating antisera as containing "opsonins." In effect, Wright demonstrated that both Mechnikov and Nuttall were correct. He showed that phagocytes not only played a prominent role in immunity by engulfing bacteria, but that antisera could both kill bacteria directly and also opsonize living bacteria, making them more readily phagocytosed by macrophages (Wright and Douglas, 1903; Allison, 1974).

Almost all vaccine development, immunization, and the rudimentary understanding of how the immune system functioned were completed by investigators in Europe during the latter part of the nineteenth and early twentieth Century. These advances, however, were well recognized by American scientists. Pasteur, Koch, Ehrlich, von Behring, Mechnikov, Nuttall, and Wright produced the knowledge on which the field of immunology and, its subdiscipline, immunoparasitology, would be built. Among those Americans focusing on the work in Europe were Theobald Smith and William Henry Welch, both prominent and renowned pathologists.

Theobald Smith (1859–1934) was a brilliant student in mathematics at Cornell, which is what his professors assumed he would continue with further study. Instead, Smith enrolled in medical school. He received his medical degree at the age of 25 from the Albany Medical College. He realized, however, that 2 years of medical school did not prepare him to practice medicine and he did not want to intern with a country physician to become competent as a physician. He decided, therefore, to return to Cornell for graduate study and concentrated on histology (Zinsser, 1936).

While Smith was engaged in his studies, Smith's mentor, Professor Simon Henry Gage was contacted by Dr. Daniel E. Salmon, the head of the Bureau of Animal Industry in Washington, D.C. He inquired if Gage knew of someone who could help the Bureau deal with the increasing problems of infectious diseases in domestic animals, chiefly cattle and swine. Gage recommended Smith who, just one year out of medical school, accepted the position and moved to Washington in 1884. Smith knew very little regarding pathology and even less about bacteriology. Moreover, he had no books or journals to which he could turn to learn the basics of bacteriology. He did, however, have the papers of Pasteur and Koch and set

about learning from these icons in the field. The work of Pasteur and Koch taught Smith well and within a year he had adopted their methods of pure cultures and introduced these methods to other laboratories in the U.S.A. Much of his early work at the Bureau of Animal Industry dealt with swine plague and hog cholera (Zinsser, 1936).

One of the greatest problems facing the Bureau of Animal Industry was Texas Cattle Fever. Large numbers of cattle in Texas and other regions in the southwest were dying of this disease. The cattlemen themselves had already determined that somehow the disease was associated with ticks and a number of studies had already been done in an effort to link ticks to the disease, but without success. It was left to Smith and his co-workers to definitively demonstrate that ticks were the vectors of the disease and that the etiologic agent was a protozoan identified as *Babesia bigemina* (Zinsser, 1936; Dolman, 1969). This brilliant research was the first evidence that ticks could transmit an infectious disease. It was also the first complete life cycle of transmission involving an arthropod as a vector for parasitic disease (Zinsser, 1936).

Smith next turned his attention to blackhead disease of turkeys, which were dying in large numbers from the disease in North America. After performing many necropsies on turkeys with disease, Smith discovered the infectious agent was a pleiomorphic protozoan, later classified as *Histomonas meleagridis*. He attempted to directly infect birds with the parasite, but it did not work. However, he found that if eggs of the nematode, *Heterakis gallinae*, from infected turkeys were transferred to uninfected turkeys, the infection would develop. The nematode eggs, as it turned out were infected with the parasite (a relatively rare case of hyperparasitism), which was necessary for an infection to occur with subsequent development of “Turkey blackhead disease” (Zinsser, 1936).

In 1895, Smith was recruited by the Massachusetts Department of Public Health and Harvard, and he moved to Boston. His charge at the Department of Public Health was to supervise the production of smallpox vaccine and toxin-antitoxin preparations for tetanus and diphtheria. For the latter disease especially, he was able to greatly increase the potency of the toxin-antitoxin treatments by enhancing the production of antitoxin antibodies in horses. From 1895–1896, 1,724 doses of the diphtheria toxin-antitoxin biologics were distributed. This increased to 40,211 in 1901–1902, and it is estimated that more than 10,000 lives were saved. It was during these studies that Smith learned that guinea pigs, when given a second injection of normal horse serum, would develop a form of immune-mediated hypersensitivity with some of the guinea pigs dying of anaphylaxis—known then as the “Theobald Smith phenomenon” (Niemi, 2009).

Smith made other major contributions to immunology and parasitology, including the use of killed bacteria for prophylactic immunizations, a method to measure fecal coliforms in water supplies, and the means to control water levels in rivers to inhibit the production of mosquito larval development as a way of reducing

malaria transmission.

When Smith began his scientific research, few, if any, studies could be performed on infectious diseases in humans. Accordingly, Smith began using experimental animals as models of human disease, thereby creating the field of “comparative pathology.” He provided the support and documentation for using experimental animals, which opened many new fields of research and led to significant new insights on the basic biology of infectious diseases and other ailments of humans. Because of Smith’s compelling support for using experimental animals, important research, which was unable to be done in humans, could be accomplished. He essentially legitimized animal research on human diseases, with many investigators using the comparative pathology approach in their work. During his career, Smith published 300 papers in multiple areas of biomedical sciences (Dolman, 1969; Niemi, 2009).

Perhaps no one played a greater role in the advancement of medicine and biomedical sciences than William Henry Welch. Welch came from a family of physicians (father, grandfather and uncles). He grew up with strong moral values inculcated from a family that honored education and discipline (Flexner, 1942).

Welch entered Yale College in 1866, when there were only eight full professors in the faculty. He was very interested in the classics, especially Greek, which he hoped to teach after college. While at Yale, and because of his leadership qualities, his intellectual prowess and academic accomplishments, he was elected to the secret honor society, Skull and Bones. Coincidentally, a fellow member of Skull and Bones was Donald C. Gilman, who would play a significant role in Welch's subsequent contributions to Johns Hopkins University and medicine in America.

On graduating from Yale, Welch diligently sought a position teaching classics but none developed. He then turned to his family tradition and decided to go to medical school. Because he felt some inadequacies in his academic background and to prepare himself better for medical school, he first went to the Sheffield School of Science for a year of study. He then enrolled in the College of Physicians and Surgeons in New York, which, at the time, had no scientific laboratories or admission requirements for students. The only exam was the final, taken at the end of the 2-year program. Welch described it as the easiest test he had ever taken (Silverman, 2011).

On the advice of one of his professors (Abraham Jacobi), Welch, like many young, ambitious medical graduates, went to Europe in April 1876 for additional training, mostly in Germany. He visited several laboratories but was especially influenced by Carl Ludwig, considered at the time as the best experimental physiologist in the world. Ludwig conscientiously followed the scientific method, avoided speculating, and stuck to the facts when reaching a conclusion. Welch was also strongly influenced by visits to the laboratory of Julius Cohnheim, the prominent experimental pathologist.

In February 1878, Welch left Germany with great appreciation of the revolution in

medicine he observed there and dedicated himself to bringing that revolution to America. He was, thereafter, committed to scientific investigation.

When he arrived back in America, however, there were no positions in pathology available to him so he took an unpaid lectureship in pathology at the College of Physicians and Surgeons in New York. Through a college classmate, Welch later obtained an appointment at Bellevue Medical College. At Bellevue, he managed to acquire three small rooms, six old microscopes, and all of \$25 to equip his laboratory! He is credited with establishing the first research and teaching laboratory at an American medical school. Soon, students from several medical schools in New York flocked to his laboratory to learn from him (Silverman, 2011).

In 1867, Johns Hopkins, a wealthy Baltimore businessman, gave a large portion of his fortune to establish a university, which was to include a hospital and medical school. It took several years, however, for Johns Hopkins University to be built, staffed, and become functional. In 1884, the hospital at Johns Hopkins was ready to open, and they needed a pathologist. Dr. John S. Billings, who had briefly met Welch in Germany, was searching for the right candidate. At much the same time, the trustees of Johns Hopkins had sent an emissary to German to find such a person. When the emissary returned, he reported that the Germans said, "Find Welch. We have no one bigger here" (*Time Magazine*, 1930). Billings visited Welch's lab at Bellevue and observed his teaching and the enthusiasm of his students. He reported back to the President of Johns Hopkins, Dr. Donald C. Gilman (Welch's Skull and Bones classmate) that he thought Welch was the man for the Chair in Pathology. Welch was hired for the position, finally realizing his dream of 1877. Based on his experience in Europe and his work at Bellevue, Welch knew that bacteriology, physiology, pathology, and immunology should be at the forefront of American medicine on infectious diseases and he set about vigorously advocating these areas of study at Johns Hopkins.

When he arrived at Hopkins, Welch established his laboratory, which would come to be known as the "Pathological." His work flourished there in numerous areas of study, including diseases of the kidneys, pathology of fever, bacteriology of diphtheria, and the cause of hog cholera (Silverman, 2011).

Welch was innovative in both his research and his teaching. He was instrumental in helping to establish the Johns Hopkins School of Medicine, including among his accomplishments (along with President Gilman's policies) the creation of faculty chairs in gynecology, medicine, surgery, and other disciplines. For the first time, those students who wished to be accepted in the medical school were required to have background courses in biology, chemistry, and physics, plus a reading knowledge of French and German. Shortly thereafter, the requirements for admission to the medical school would include a college degree. Welch was soon named Dean of the new medical school (Flexner, 1942).

Welch was also keenly aware of the scarcity of programs of study in the areas of public health and hygiene. In 1916, with the support of the Rockefeller

Foundation, the School of Hygiene and Public Health of Johns Hopkins was established, the first of its kind in the world, with Welch as its first Director. Welch's vision of the new school was that it would not be staffed only with physicians, but also with scholars who knew basic science and could simultaneously address fundamental problems dealing with public health and hygiene. President Gilman's goal was that the school would be a guild of scholars. The founding of the School of Hygiene and Public Health and, particularly the Department of Medical Zoology, was the foundation for which studies on the immunology of parasitic infections was built in the U.S.A.

Welch appointed Robert W. Hegner to be director of the Department of Medical Zoology, even though Hegner worked almost exclusively on free-living protozoans (Rozenboom, 1990). Hegner, having been a professor at the University of Michigan for several years was, at the time of his appointment in Medical Zoology, a Johnston Fellow at Johns Hopkins in the Department of Zoology.

Some indication of the esteem Welch garnered from his work is illustrated in the following:

On April 8, 1930, a special tribute was held in Washington, DC, to celebrate the 80th birthday and honor the achievements of William Henry Welch. No physician before or after Dr. Welch has been recognized so widely or honored in such an extraordinary manner. Sixteen hundred of his friends and associates assembled with President Herbert Hoover. The event was disseminated around the country and the world by radio broadcast.

Additional celebrations occurred in Norfolk, Connecticut, Dr. Welch's birthplace; Yale University, his alma mater; Columbia College of Physicians and surgeons, where he graduated from medical school; Rockefeller Institute; Princeton University; the University of Pennsylvania; Western Reserve University; Vanderbilt University; Minnesota University; and Stanford University. Further celebrations were held by the American Social Hygiene Association in New York City, Portland, Oregon, and Cleveland, Ohio. Around the world, celebrants gathered at the League of Nations in Geneva, Switzerland; the Pasteur Institute in Paris, France; the London School of Hygiene in London, England; the Peiping Union Medical College in Beijing, China; the Kitasato Institute in Tokyo, Japan; the Robert Koch Prussian Institute in Berlin, Germany; and medical societies in Leipzig, Copenhagen, and Dublin (Silverman, 2011).

Hegner (see [Fig. 2](#)) was extremely insightful in his choices of faculty members for his new department. Some of these deserve particular mention. He recruited and hired William Walter Cort who, by the time of his appointment, was already a recognized and productive parasitologist. William H. Taliaferro, on the other hand, had just finished his Ph.D. in the Department of Zoology and would be responsible for teaching parasitology, of which, by his own admission, he had practically no experience. Indeed, Taliaferro had only seen one parasite in his life

and that was *Trypanosoma lewisi* (Taliaferro, 1968). Francis Root was to handle medical entomology but, as with Taliaferro, he had no experience in that field. Hegner, Cort, Taliaferro, and Root would make major, if not historic, contributions to parasitology.



**Figure 2** Robert Hegner (*Journal of Parasitology* **23**:1 (1937). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

During his career, Hegner made his own significant contributions to the field of parasitology, especially in regards to the effect of the host's diet on intestinal protozoa (Hegner, 1937). He showed that a diet rich in proteins, especially casein, and low in carbohydrates could dramatically decrease the number of protozoan parasites in the gut. A great deal of his work also focused on avian malaria, host specificity, and many other areas of parasitology. He published more than 150 papers and books. His books included *Introduction to Zoology*, 1910, *College Zoology*, 1912–1942 (five editions), *Diagnosis of Protozoa and Worms Parasitic in Man* (with W. W. Cort), 1921, *Outline of Medical Zoology* (with W. W. Cort and F. M. Root), 1921, *Human Protozoology* (with W. H. Taliaferro), 1924, *Host-Parasite Relations between Man and His Intestinal Protozoa*, 1927, *Problems and Methods of Research in Protozoology* (with J. Andrews), 1930, *Parasitology*, 2nd Edition (with F. M. Root, D. L. Augustine, and C. G. Huff), 1938, and *Big Fleas Have Little Fleas*, 1938. He is credited with introducing the term “host-parasite relations.” He also wrote a compelling article on how important medical zoology was to advancements in public health and hygiene, which most likely influenced perspectives of the American Medical Association and its constituent

members (Hegner, 1920)

Cort (see [Fig. 3](#)) received his bachelor's degree from Colorado College and his Ph.D. (1914) from the University of Illinois with his Ph.D. advisor being Henry Baldwin Ward, who is considered the "Father of Parasitology" in the United States. Prior to joining the Department of Medical Zoology at Hopkins, Cort had spent 3 years at the University of California at Berkeley where he had developed an intense interest in hookworms, especially among the gold miners. He would continue his interest in hookworms for his professional life and made huge contributions to understanding this parasite. During his many years at Johns Hopkins, Cort mentored numerous graduate students, one of whom was Normal Stoll, who himself would contribute hugely to the field of parasitology and immunology.



**Figure 3** William Walter Cort (*Journal of Parasitology* **39**:4 (1953). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

During the early part of Cort's career, the hookworm control program in the southern United States, through the auspices of the International Health Board (funded by the Rockefeller Foundation), had made great strides in alleviating the people of that area of the debilitating effects of hookworms. Because of the successes of hookworm control in the U.S.A., many thought enough information was known regarding the biology, ecology, and behavior of hookworms that success was assured for control programs in undeveloped countries. Cort thought otherwise and he set about critically determining what was not known about

hookworms and devised plans to find answers to unsolved problems. How long could larvae survive without infecting a host? Were larvae dispersed by farm animals? What types of vegetation aided in the dispersal of larvae? And many other critical questions as well.

To address these types of questions, it was necessary to go to places where hookworms were affecting large populations of people. This led him to various parts of the world including Trinidad, Puerto Rico, Panama, and an extensive field study in China. Norman Stoll, his graduate student, accompanied him on most of these field studies.

It was during the trip to Puerto Rico in 1922 that Stoll refined a technique to isolate and quantify hookworm larvae (Stoll, 1923) and, importantly, to later use the technique to measure the numbers of eggs in fecal samples. The “Stoll dilution egg-counting technique” was used for decades by a multitude of investigators in numerous studies on intestinal helminths, measuring eggs per gram (epg) in studies of human and experimental animal infections (Stoll, 1923).

On leaving Johns Hopkins, Stoll moved to the Rockefeller Institute, which then was under the direction of Theobald Smith. At the Institute, Stoll did not train graduate students since at that time it was strictly oriented toward research and was not an academic institution. However, many of Cort's students would continue their work at Rockefeller with Stoll.

Before Stoll's egg-counting technique became available, one would infect animals with helminths, but the only way to know the worm burden, or any resistance to the infection, required killing the animals to collect the worms and determine worm burdens. This approach, of course, generally required large numbers of animals. The egg-counting technique allowed investigators to estimate worm burdens by relating the number of eggs to worm pairs. It allowed quantification of numbers of eggs produced by worms as well as the onset of fecundity. One could also determine indirectly the relative levels of resistance by the host, the effect of self-cure on resistance to re-infection, and the efficacy of various immunological manipulations and drug effectiveness during the course of an infection.

When the name, Norman R. Stoll ([Fig. 4](#)), is mentioned, any student of parasitology will immediately think of his classic paper, *This Wormy World*, delivered as his presidential address at the 1946 meeting of the American Society of Parasitologists (Stoll, 1947). It is arguably the most cited paper in the history of parasitology.



**Figure 4** Norman R. Stoll (*Journal of Parasitology* 33:1 (1947). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Cort also spent many summers teaching parasitology at the University of Michigan's field station at Douglas Lake. It was there that he discovered “swimmer's itch” was caused by cercariae of a digenetic trematode (Cort, 1928)

William H. Taliaferro completed his Ph.D. in Zoology at Hopkins shortly after returning from military service in 1919. He accepted a Johnston Fellowship in Medical Zoology but then was offered a position as an Assistant Professor and gave up the fellowship to become a faculty member. Taliaferro's doctoral research examined sensory structures in planaria. He had no background in medical zoology, but nonetheless accepted the position to teach parasitology. He, therefore, began learning as much as he could about parasites, taught courses in parasitology, and began a research program focused on *Trypanosoma lewisi* in rats. He became fascinated with this parasite and throughout his career kept returning to study aspects of this host-parasite relationship.

Taliaferro (Fig. 5) became so knowledgeable about protozoans that he and Hegner produced a lengthy and detailed book titled *Human Protozoology*, which was published in 1924, just five years after he completed his Ph.D. Taliaferro was a rising star at Hopkins, but when an opportunity arose for him to take a position at the University of Chicago in parasitology, he jumped at it. Before leaving Hopkins, he married Lucy Graves, and she completed the D.Sc. with Hegner studying bird malaria (Taliaferro, 1925). Lucy Graves-Taliaferro was critical to the research the two of them would do. She would work as an unpaid research assistant with Taliaferro for the next 50 years.



**Figure 5** William H. Taliaferro (*Journal of Parasitology* 20.3 (1934). Reproduced with permission from *The Journal of Parasitology*, Allen Press Publishing Services.)

Theobald Smith did not agree that hosts could develop immunological resistance to parasites and preferred that the term immunity be reserved for bacterial infections for which immunity could be readily demonstrated. Smith preferred, in regards to parasites, that “resistance” or “self-cure,” not immunity, be used. It was left to Stoll to clearly demonstrate that self-cure and resistance are the outcomes of immunity against helminth parasites. He reached these conclusions on studies of sheep and calves infected with *Haemonchus contortus* (Stoll, 1929). Animals that self-cured of the infection also had much greater resistance to re-infection. He also showed that sheep immunized by intraperitoneal or subcutaneous injection of *H. contortus* larvae had significantly less serious infections when challenged orally with the parasite (Stoll, 1929, 1958).

Merritt P. Sarles worked with Cort at the Princeton, New Jersey lab in the Rockefeller Institute. There, along with Cort, Sarles showed that one could induce immune resistance in rabbits against the nematode, *Trichostrongylus calcaratus* by repeated injections of the parasite. (Sarles, 1932) This work, along with Stoll's experiments with *H. contortus*, left no doubt that hosts infected with intestinal nematodes develop protective immune responses.

Asa Crawford Chandler ([Fig. 6](#)) received his Ph.D. from the University of California in 1914 and taught at the Oregon State Agricultural College for 4 years before moving to the Rice Institute (later Rice University) as one of three professors in the Department of Biology. Except for research trips to India (1924–1927) and Egypt (1952) and military service in World War I, he would remain at Rice until his sudden and unexpected death in 1958 while attending a conference in Europe on tropical medicine and malaria.



**Figure 6** Asa C. Chandler. (Courtesy of Woodson Research Center Fondren Library, Rice University. Reproduced with permission.)

From early in his tenure at Rice, Chandler had an intense interest in nematode parasites and the resistance hosts developed against them. His work focused primarily on the role of metabolic products of these parasites in inducing immune resistance (Chandler, 1958). Working initially on *Nippostrongylus muris* in rats, he expanded his research to include immunity against metabolic products of numerous other parasites. His research was particularly important because it led other investigators to examine the possible roles of anti-metabolite immunity in many host-parasite systems, including schistosomiasis, trichinellosis, and dog hookworm (Chandler, 1932). While preparing this historical review of immunoparasitology relative to *The Journal of Parasitology*, it was unfortunate

that no biographies of Asa Chandler could be located; not even a copy of his *curriculum vitae* could be found.

While Cort, Stoll, Sarles, and Chandler were on the early forefront of studying the immunology of helminth infections, the person who provided the major impetus for immunoparasitology of both protozoan and helminth parasites, was William Hay Taliaferro. In many ways, he can be considered the “Father of Immunoparasitology” in the U.S.A. He co-wrote the book *Human Protozoology* with Hegner (1924) and, importantly, he wrote the first major work on *The Immunology of Parasitic Infections* in 1929. This massive tome of 414 pages focused mostly on the use of immunological methods (hemolysis, precipitins, agglutination, and complement fixation) for the diagnosis of parasitic diseases. Because so little was known about basic immune mechanisms at the time, only a few pages were devoted to acquired immunity to protozoan and metazoan parasites. Nonetheless, the book covered the then current state of the art on the immunology of parasitic infections. Although Taliaferro insisted that parasites could induce immune resistance, notwithstanding Theobald Smith's view that they did not, the book was dedicated to Smith in acknowledgement of the latter's many contributions to parasitology, immunology, and comparative pathology.

Taliaferro received the major recognition for work done in his lab, but much of his success was due to the efforts of his wife, Lucy. As mentioned earlier, she worked with Taliaferro as an unpaid assistant for more than 50 years. She ran the laboratory, supervised the technical staff, played a major role in training of graduate students, assisted in data collection and analysis, and in manuscript preparation. Lucy was an exceptional scientist in her own right, co-authored many papers with William Taliaferro and some papers on which her husband was not a co-author.

Taliaferro left Johns Hopkins in 1924 and spent the next 36 years at the University of Chicago, first as a professor, then head of the department, and later as Dean of the Division of Biology (including medicine). He directed the research of many Ph.D. and Masters students, some post-doctoral students, and had several visiting scholars in his lab. Many of his students and post-docs made important discoveries in their careers as well (Taliaferro, 1968).

He did most of his basic work on parasites during the first 20 years of his career, turning thereafter to more basic immunological studies. His first papers on parasites appeared in 1921 and concerned the life cycle stages of *Trypanosoma lewisi* in rats (Taliaferro, 1921a, 1921b). Following that first excursion into parasitology, he published 93 papers on host-parasite relationships over his career. Sixty-one of these papers, book chapters, and books were on immunity to parasites, the majority of which (54) were published between 1921 and 1950. Taliaferro did not focus on just a few parasite models to study; his range was wide, including work on six species of *Plasmodium*, three species of trypanosomes, *Schistosoma mansoni*, several amoebas, and various nematodes (Taliaferro,

1968).

He became more and more interested in immunology as he became increasingly aware that greater knowledge of how the immune system works would lead to a much better understanding of the host-parasite relationship. His advances in immunology proved to be critical for other investigators in their research on immunoparasitology. He did extensive research on the effects of radiation on the immune response and the diversity of antibodies during immune responses. However, he never could get away from studying parasites and his last journal article was on *Trypanosoma lewisi*, the organism that initially prompted his interest in parasitology (Taliaferro and D'Alesandro, 1971).

The years from 1920s into the 1950s saw major advances in understanding the immunology of host-parasite relationships. Many young investigators were influenced and attracted to the field by the work of Chandler, Hegner, Cort, Stoll, Sarles, and the Taliaferros. In April 1958, Chandler assembled some of the leading scientists involved with immunity to parasites for a conference to help inaugurate the new Biology building (The M. D. Anderson Biological Laboratories) at the Rice Institute (later Rice University) in Houston, Texas. Many of the participants were the pioneers of immunoparasitology and/or innate mechanisms of immunity to parasites. Those who spoke at the meeting included Chandler (the role of immune responses to parasite excretory/secretory products), Paul F. Russell (malaria), Cecil A. Hoare (amebiasis), Clark P. Read (physiological resistance), Clay G. Huff (haemosporidians), Leslie A. Stauber (leishmaniasis), Robert M. Lewert (helminth larvae), William H. Taliaferro (antibody synthesis), Jose Oliver Gonzalez and Enrique Koppisch (immunopathology of ascariasis), Irving G. Kagan (immunology of schistosomiasis), and Norman R. Stoll (vaccination against *Haemonchus*) (see Rice Institute Pamphlet 45(1): 1–208)

The field of immunoparasitology moved more rapidly after the 1950s. In 1959, the National Institutes of Health established the Laboratory of Parasitic Diseases in Bethesda and much of the work performed there focused on immunoparasitology. Prior to the 1970s there were few scientific meetings where there was an emphasis on immunoparasitology. In the years that followed, various societies provided sessions for presentation of papers on immunoparasitology, including the American Society of Parasitologists, the American Society of Tropical Medicine and Hygiene, the American Association of Immunologists, and smaller focused meetings such as the Fancy Gap Immunoparasitology Workshop (1974–present) and the Woods Hole Immunoparasitology Meeting (1997–present).

Research over the past 120 years or so has provided incredible insights into how the immune system functions. We have learned about the multitude of cells involved in immune responses, and how sub-populations of lymphocytes and mononuclear phagocytes have specific, and often overlapping, effector and immunoregulatory functions. Based on this wealth of knowledge, vaccines against a number of viruses and bacteria have been produced. Today, there are numerous

studies ongoing to develop vaccines against parasites of humans, including schistosomiasis, malaria, leishmaniasis, and Chagas' disease, to name just a few. Notwithstanding huge efforts by many laboratories around the world to develop vaccines effective in preventing parasitic diseases in humans have, to date, been unsuccessful. Unfortunately, funding for research on parasitic diseases has been, and remains, grossly insufficient, especially considering the massive devastation these diseases cause throughout the world, and particularly in developing countries. With parasites, we are dealing with very clever critters that, over the millennia, have evolved immune avoidance and escape mechanisms that are exceedingly difficult to overcome. Hopefully, immunoparasitologists will find chinks in the armor of these extraordinary organisms, which may, one day, result in effective vaccines.

# **Contributions to the American Society of Parasitologists of some of these pioneers**

**Asa Chandler:** Vice President, 1928—President, 1945—Council Member, 1928–1932 and 1935–1939.

**William W. Cort:** Founding member of the American Society of Parasitologists—President, 1930—Secretary-Treasurer, 1925–1928—Council Member 1930–1934 and 1934–1938—Editor of *The Journal of Parasitology*, 1932–1937 and 1948.

**Robert Hegner:** Chair of the Committee to establish the American Society of Parasitologists and a founding member—Vice President, 1928—President, 1936—Council Member, 1924–1927.

**Norman Stoll:** President, 1946—Secretary-Treasurer, 1929–1932—Council Member, 1932–1944—Editor, *The Journal of Parasitology*, 1937–1943.

**William H. Taliaferro:** Vice President, 1932—President, 1933—Council Member, 1927–1931.

## Literature cited

- Allison, V. D. 1974. Personal reflections of Sir Almroth Wright and Sir Alexander Fleming. *The Ulster Medical Journal* **43**(2): 89–98.
- Chandler, A. C. 1932. Susceptibility and resistance to helminthic infections. *Journal of Parasitology* **28**: 135–152.
- Chandler, A. C. 1958. Introductory remarks. *Rice Institute Pamphlet* **45**(1): 4–9.
- Cort, W. W. 1928. Schistosome dermatitis in the United States (Michigan). *Journal of the American Medical Association* **90**(13): 1027–1029.
- Cox, F. E. G. 1996. Old World leishmaniasis. In *The Wellcome Trust illustrated history of tropical diseases*, F. E. G. Cox (ed.) The Wellcome Trust, London, U.K. p. 207–217.
- Dolman, C. E. 1969. Theobald Smith, 1859–1934, *Life and Work*. N. Y. State *Journal of Medicine* **69**: 2801–2816.
- MLA style: “Paul Ehrlich—Biographical”. *Nobelprize.org*. Nobel Media AB 2013. Web. 17 June 2013a.  
[www.nobelprize.org/nobel\\_prizes/medicine/laureates/1908/ehrlich-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1908/ehrlich-bio.html) (accessed July 31, 2015).
- Flexner, S. 1942. William Henry Welch. National Academy of Sciences: *Biographical Memoirs Vol. XXII (12th memoir)*: 215–231.
- Graham-Smith, G. S. and D. Keilin. 1939. *Obituary Notices: Fellows of the Royal Society* **2**: 492–499.
- Hegner, R. W. 1937. Parasite relations to host modifications. *Journal of Parasitology* **23**: 1–12.
- Hegner, R. W. 1920. The relation of medical zoology to public health problems. *The Journal of the American Medical Association* **75**(24): 1607–1610.
- Khamesipour, A., Y. Dowlati, A. Asilian, R. Hashemi-Fesharki, A. Javadi, S. Noasin, and F. Modabber. 2005. Leishmanization: Use of an old method for evaluation of candidate vaccines against leishmaniasis. *Vaccine* **23**: 3642–3648.
- MLA style: “Robert Koch—Biographical”. *Nobelprize.org*. Nobel Media AB 2013. Web. 11 Mar 2014.  
[www.nobelprize.org/nobel\\_prizes/medicine/laureates/1905/koch-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1905/koch-bio.html) (accessed July 31, 2015).
- MLA style: “Ilya Mechnikov—Biographical”. *Nobelprize.org*. Nobel Media AB 2013. Web. 17 Jun 2013b.  
[www.nobelprize.org/nobel\\_prizes/medicine/laureates/1908/mechnikov-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1908/mechnikov-bio.html)

(accessed July 31, 2015).

Niemi, S. M. 2009. *Theobald Smith: Brief life of a pioneering comparative pathologist*. *Harvard Magazine, July-August*: 28–29.

Reidel, S. 2005. Edward Jenner and the history of smallpox and vaccination. *Baylor University Medical Center (Proceeding)* **18**: 21–25.

Rozenboom, L. E. 1990. Medical Zoology at the Johns Hopkins University School of Hygiene and Public Health (A History). Publisher: Johns Hopkins Department of Immunology & Infectious Diseases, 141 p. ASIN: Boo135MRDY.

Sakula, A. 1983. Robert Koch: Centenary of the discovery of the tubercle bacillus, 1882. *Canadian Veterinary Journal* **24**: 127–131.

Sarles, M. P. 1932. Development of an acquired resistance in rabbits by repeated infection with an intestinal nematode *Trichostrongylus calcaratus* RANSOM, 1911. *Journal of Parasitology* **19**: 61–82.

MLA style: “Emil von Behring—Biographical”. *Nobelprize.org*. Nobel Media AB 2013. Web. 17 Jun 2013c.

[www.nobelprize.org/nobel\\_prizes/medicine/laureates/1901/behring-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1901/behring-bio.html)  
(accessed July 31, 2015).

Silverman, B. D. 2011. William Henry Welch (185–1934): the road to Johns Hopkins. *Baylor University Medical Center (Proceedings)* **24**(3): 236–242.

Stoll, N. R. 1923. Investigations on the control of hookworm. XV. *An effective method of counting hookworm eggs in feces*. *American Journal of Hygiene* **3**: 59–70.

Stoll, N. R. 1929. Studies with the strongyloid nematode, *Haemonchus contortus*, 1. *Acquired resistance of hosts under natural reinfection conditions out-of-doors*. *American Journal of Hygiene* **10**: 384–418.

Stoll, N. R. 1947. This wormy world. *Journal of Parasitology* **33**: 1–18.

Stoll, N. R. 1958. The induction of self-cure and protection, with special reference to experimental vaccination against *Haemonchus*. *Rice Institute Pamphlet* **45**(1): 184–208.

Taliaferro. W. H. 1921a. Variation and inheritance in size in *Trypanosoma lewisi*. 1. Life cycle in the rat and a study in size and variation in “pure line” infections. *Proceedings of the National Academy of Sciences* **7**: 138–143.

Taliaferro. W. H. 1921b. Variation and inheritance in size in *Trypanosoma lewisi*. 2. The effects of growing “pure lines” in different vertebrate and invertebrate hosts and a study of size and variation in infections occurring in nature. *Proceedings of the National Academy of Sciences* **7**: 163–168.

Taliaferro, Lucy Graves. 1925. Periodicity of reproduction, infection and resistance in bird malaria. *Proceedings of the National Academy of Sciences* **11**: 348–352.

Taliaferro, W. H. 1968. Prefactory Chapter: The lure of the unknown. *Annual Review of Microbiology* **22**: 1–15.

Taliaferro, W. H. and P. D'Alesandro. 1971. *Trypanosoma lewisi* infection in the rat: Effect of adenine. *Proceedings of the National Academy of Sciences* **68**: 1–5.

*Time Magazine*. 1930 (April 14, 1930). “Medicine: Patriarch's Party.” **15**(5): 34–35.

Wright, A. E. and S. R. Douglas. 1903. An experimental investigation of the role of the blood fluids in connection with phagocytosis. *Proceedings of the Royal Society of London* **72**: 357–370.

Zinsser, H. 1936. Theobald Smith. National Academy of Sciences: Biographical Memoirs Vol. **XVII (12th memoir)**: 1–45



# **Chapter 14**

## **Some Practical Principles of Anthelmintic Medication**

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## INTRODUCTION

It is entirely appropriate that a memorial number of the Journal of Parasitology in honor of Dr. B. H. Ransom should contain a paper on anthelmintics. Dr. Ransom had done some work on anthelmintics at one time and a paper on the action of anthelmintics on parasites located outside of the alimentary canal was published by Ransom and Hall in 1912. While the subject was never one of his major projects, he was keenly interested in it and came to an early appreciation of the fact that our knowledge of these drugs was very inadequate and that much of the literature was a hodge-podge of formulae with little in it of proved value. For this reason he decided to inaugurate in the Zoological Division a project for the study of anthelmintics and in 1915 this work was begun with the present writer in charge of the project and the late W. D. Foster collaborating. Since Mr. Foster's death, Dr. J. E. Shillinger has collaborated on this project. The general plan of work along the line of what we have called critical testing was settled in conference by Dr. Ransom and the writer, and has been followed since with but little modification.

The results of the anthelmintic project have justified Dr. Ransom's judgment in inaugurating it. The method of critical testing has enabled us to use training-camp methods in determining the values of the anthelmintic weapons for use in the warfare on worms, has added new weapons, and has taught us more precise strategy and tactics; even the failures in our experimental attacks have shown us what not to do and thereby effected widespread savings in the broad field of practice. The facts ascertained have had immediate application in veterinary medicine and have in some instances been transferred successfully to the field of human medicine. As the originator of this project and as collaborator in planning it, Dr. Ransom deserves much credit for the benefits which have followed from it.

It is hardly overstating the case to say that when Dr. Ransom inaugurated the anthelmintic project he was opening up a new era in anthelmintic medication. For centuries anthelmintics by the score had been used on the basis of an uncritical empiricism. For a half century there had been an epoch of critical clinical consideration of anthelmintics, especially of those intended to remove hookworms from man, and there was a concomitant refinement of laboratory procedure in judging the

effects of anthelmintics. But critical testing opened a third phase of development with the application of the modern experimental method to anthelmintic medication and not only enabled us to obtain accurate information as to our drugs, but also to begin the correlation of the chemical and physical properties with the anthelmintic efficacy and safety, and to lay down certain broad principles in regard to these correlations and in regard to anthelmintic medication in general.

This paper represents an attempt to formulate certain broad principles which appear to be true and of value in the present state of our knowledge. In a previous paper by Hall in 1918 something of the same sort was attempted. That paper was written after two or three years of critical testing, and most of the critical testing which has been done up to the present time was done after that paper was written. It is now in order to recast the conclusions in that paper.

#### FACTORS IN ANTHELMINTIC MEDICATION

The essential factors in anthelmintic medication have been stated alliteratively by Hall in 1925 as the six P's: 1. Practitioner (physician or veterinarian). 2. Patient. 3. Parasite. 4. Parasiticide (anthelmintic). 5. Purgative. 6. Prophylaxis. The issue is joined when a practitioner confronts a supposedly parasitized patient. The outcome depends on the intelligent correlation of the six factors involved. None of these factors should be slighted, and in considering the tentative principles of anthelmintic medication they are grouped under these factors to emphasize the importance of such grouping.

#### PRINCIPLES OF ANTHELMINTIC MEDICATION

1. *Practitioner*.—The practitioner must be a person of adequate training, experience and judgment if anthelmintic medication is to be successful. He must know all the other factors. An injudicious selection of an anthelmintic may cause serious results or make the treatment unsuccessful; a failure to recognize a contra-indication for treatment may be fatal. While the importance of the personal equation seems self-evident, it is actually one that is often overlooked. One occasionally finds a writer complaining of a lack of efficacy or safety in a drug which others find effective and safe. The difference in their results evidently may follow from the obviously different factor, the individual practitioner, but it is a rare thing to find a writer suggesting that the fault may be his. The existence of a practitioner is justified by his exercise of good independent individual judgment since it is impossible for the specialist to cover in writing, as in a paper of this sort, all possible considerations.

In this connection it should be said that training in parasitology is much neglected in American medical and veterinary colleges, and that

the practitioner today is handicapped by this lack of training. This situation may be remedied in time; in the meanwhile the cautious acquirement of experience and the exercise of good judgment will serve here as elsewhere: "We learn by doing." Before using anthelmintics the practitioner should acquaint himself with the few that are of known value, learn their indications and contraindications, assure himself that the indications for treatment are present and the contraindications absent in his patient before he administers treatment, and then make sure that he obtains prompt purgation after treatment; this is in the interest of "safety first." A knowledge of the parasite and its habits, and of the dosage of the drug, will then be an aid to efficiency.

2. *Patient*.—In deciding on the drug and the size of the dose, the practitioner must carefully consider the patient—age, size, sex, food and other habits, and general condition—especially as regards the presence of certain pathological conditions discussed under the heading of *Parasiticide*.

The period of fasting before treatment may be stated in general terms, but if special conditions indicate the advisability of altering this period one way or another, it should be altered accordingly. In general, human patients can be given a light meal in the evening and treated the next morning 3 or 4 hours before the morning meal; nothing is gained, as a rule, by a longer fast, there is a decided loss in many cases in that the digestive tract is in an abnormal and usually sensitive condition as a result of unusual fasting, and there is likely to be greater weakness and more disposition to headache on the part of the patient. Dogs and cats also should be fasted only overnight. Swine require more prolonged fasting—at least 24 hours—to make an anthelmintic effective. Sheep and other ruminants require only an overnight fast in most cases, the prolonged fasting of sheep in connection with the South African copper sulphate and sodium arsenite powder being exceptional; it is out of the question and unnecessary to clear the rumen by fasting in any event. Horses require prolonged fasting, 36 hours, in connection with anthelmintics to destroy worms in the large intestine, but 12 to 24 hours is apparently sufficient in treating for stomach worms, bots, and ascarids.

The nature of the food appears to be important. Alcohol is contraindicated in connection with anthelmintics in general, and acute or chronic alcoholism is a contraindication for anthelmintic medication, especially with carbon tetrachloride. Bozzolo reported good results from the use of wine in connection with thymol, but it is possible that the alcoholic solution of the thymol in the stomach, with the subsequent precipitation of the thymol in a finely divided state as the alcohol was diluted or absorbed, might lead to increased anthelmintic efficacy without, however, lessening the danger from the alcohol. Hall in 1920 has shown experimentally the inadvisability of using fluid extracts of anthel-

mintic drugs. Carbohydrates are apparently indicated with such drugs as are most likely to cause some injury to the liver, as chloroform, carbon tetrachloride, and tetrachlorethylene. Fats, oils (with the exception of castor oil), cream and related substances are contraindicated. Whether they increase the total absorption or the rapidity of absorption, or cause localized absorption, or act in some entirely different way is not sufficiently known; such oils as olive oil tend to remain in the stomach and in connection with a depressant and constipating drug, such as chenopodium, there may be a serious resultant damage to the stomach. Protein foods occupy an intermediate position between the carbohydrates and the fats; they are not harmful and may be desirable, at least with certain drugs. Antidiabetic diet has been recommended in connection with treatments for human pinworm infestation.

3. *Parasite*.—A knowledge of the parasite, especially of its habits, including its location and life history, is essential to sound procedure in anthelmintic medication. If a patient is subject to constant reinfection with a parasite having a life history which includes the wandering of the larvae through the body tissues outside of the digestive tract for days, weeks or months, it may happen that an anthelmintic treatment which removes all the worms from the lumen of the digestive tract will be followed by recurrent infestation as these larvae return to the digestive tract, even though the patient is removed from the sources of infection at the time of treatment. To prevent this would require removal of the patient from all sources of infection for an adequate period before treatment. What might be regarded as a successful treatment for ascarids might nevertheless leave ascarid eggs continuing to appear in the feces as a result of the existence of a female ascarid in the gall duct and out of reach of the drug. The location of the whipworm in the cecum is such that it is difficult to reach it by the common methods of treatment, even though experience indicates that this worm is relatively susceptible to comparatively weak anthelmintics; it is possible that relatively feeble anthelmintics in large doses or repeated small doses may be used successfully to reach and remove this worm. It is unnecessary to enlarge on the necessity for a knowledge of parasites on the part of the practitioner, as it seems sufficiently clear that a lack of knowledge as to enemy strength, habits, and disposition of forces must be a handicap to any commander directing an attack on that enemy.

4. *Parasiticide* (Poison!).—It is well to keep in mind that all anthelmintics are poisons, intended to poison the worms and administered in such doses as will accomplish this with a minimum of risk to the patient. In general they should not be used on the assumption that a patient is infested or even on clinical evidence to that effect, at least in private practice; it is too simple a matter to make a laboratory diagnosis and proceed with certainty. In field work where one is dealing

with thousands of persons with a high incidence of infection and with the necessity of conserving funds for treatment of the sick rather than laboratory examinations to eliminate a small percentage of uninfected individuals, mass treatment on the basis of probabilities becomes justified, at least in many cases.

In choosing an anthelmintic, one must keep in mind that these drugs are rather selective in their action. Santonin is effective against ascarids, but can be given over long periods of time without affecting hookworms or tapeworms. Chenopodium is apparently more effective against the hookworm *Ancylostoma duodenale* than is carbon tetrachloride, but carbon tetrachloride is more effective against the hookworms *Necator americanus* and *Ancylostoma caninum* than is chenopodium. Chenopodium is much more effective against *Ascaris lumbricoides* than is carbon tetrachloride, but carbon tetrachloride is almost as effective as chenopodium against the dog ascarids. In spite of the fact that chenopodium is so widely effective against ascarids in general and so superior in this respect to carbon tetrachloride, chenopodium is apparently inferior to carbon tetrachloride in removing ascarids from horses. Male fern destroys the common liver fluke, *Fasciola hepatica*, but not the lancet fluke, *Dicrocoelium dendriticum*. Tartar emetic or emetine will kill blood flukes but not *Filaria bancrofti*. These findings should warn us against too great generalities as to the value of these drugs whenever any one factor, such as the worm or host, is changed in any problem. When we know how anthelmintics affect worms, a thing concerning which we know practically nothing, we *may* be able to predict results in problems with a new factor—or we *may not*.

The older books state that an anthelmintic is a relatively insoluble drug intended to poison the worm and that purgatives should be given after the drug has had time to act in order to sweep it out of the digestive tract before it can be absorbed. This is perhaps closer to the description of an ideal anthelmintic than to a statement of known fact. So far as we are aware, the known anthelmintics are actually absorbed in the digestive tract to an unpleasantly large extent. Seidell in 1915 found that one-half to two-thirds of the thymol administered was destroyed or temporarily fixed in the body. Of the even more insoluble carbon tetrachloride, Wells in 1925 finds that practically all of small doses is absorbed. By solubility is meant here water-solubility. In spite of these findings, there is some likelihood that there is a correlation between the efficacy and safety of an anthelmintic and its water solubility. Hall and Shillinger in 1925 have pointed out the increasing efficacy against hookworms and the increasing safety of the patient in the series  $\text{CHCl}_3$ ,  $\text{CCl}_4$  and  $\text{C}_2\text{Cl}_4$ , and Hall and Cram in 1925 have shown the entire inefficacy and apparently entire safety of the theoretically

valuable, but entirely insoluble, compound  $C_2Cl_6$ , and have discussed the apparent relation of solubility and anthelmintic efficacy. It is quite probable that there is an optimum solubility of anthelmintics, a point at which they display maximum efficacy in destroying worms with a maximum safety consistent with that efficacy.

We are just beginning to obtain some data which may make it possible to definitely correlate anthelmintic efficacy with chemical composition. Caius and Mhaskar in 1923, after an extensive series of investigations of the drugs used for the removal of human hookworms, found the four most effective drugs to be for the most part rather unrelated substances—chenopodium, carbon tetrachloride, thymol, and betanaphthol. They regarded the efficacy in each particular case as correlated with the chemical composition as follows: That of chenopodium with the dioxide character of its effective constituent, ascaridol; that of carbon tetrachloride with its halogen content; that of thymol and betanaphthol with their free phenolic hydroxyl group. In the Zoological Division we have taken one group of halogen compounds, the chlorinated hydrocarbons, and found an apparent correlation between the anthelmintic efficacy and the chlorine content in such substances as  $C_2H_4Cl_2$ ,  $CHCl_3$ ,  $CCl_4$ , and  $C_2Cl_4$ . This correlation, as already noted, appears to be modified by the factor of water solubility, since the theoretically valuable  $C_2Cl_6$  proved of no value, apparently because of its practically entire insolubility. Similar experiments with other halogen compounds are indicated, as well as those with compounds containing free phenolic hydroxyl groups and with dioxides.

The distinction between vermicide and vermifuge appears to be entirely academic and of little or no practical value. In the first place it is of no importance whether a worm is passed alive or dead, uninjured or sick and poisoned; a worm removed is a worm destroyed. At present we do not know how anthelmintics affect worms. They may cause muscular incoordination, as santonin apparently does in ascarids, but we do not know why santonin does not cause this in hookworms or tapeworms if it causes it in ascarids. They may dissolve fatty structures, as might be the case with carbon tetrachloride which is highly destructive to hookworms, but this is pure theory and affords no explanation as to why carbon tetrachloride should not affect dog tapeworms in the same manner. They may depress the nervous system of the worm and cause a relaxation of the hold or induce a degree of narcosis, but until we know more about the subject this is pure speculation. If the anthelmintic is purgative it may bring the worms out promptly, but if it is not, a purgative will remove them promptly and peristalsis will remove them reasonably soon in any case. In some cases a time factor should be considered, but as a rule drugs do not need much time to act in destroying worms. In many cases the purgative may be given

with the drug; in some cases a delay may be indicated owing to a possible need for time for the anthelmintic to act or because of an interaction between the purgative and the anthelmintic or for some other unknown reason; this matter should be given further consideration. The matter of anthelmintic efficacy should always be weighed in with the patient's safety, and prompt purgation is protective.

The contraindications for treatment depend on the patient and the drug. It is essential that the practitioner know the status of the patient and the effect of the drug on the host as well as on the worm. The general contraindications for anthelmintic medication, with a resultant need for omitting treatment or diminishing the therapeutic dose, are as follows: Extreme youth or old age, febrile or debilitating conditions, gastroenteritis, gross helminthiasis, alcoholism, high fatty diet, and such conditions as gastric stasis or pronounced constipation. Special contraindications depend on special effects of the anthelmintic on the patient and some of these are as follows:

Chenopodium is decidedly irritating, depressant, and constipating, and the special contraindications for this drug include gastroenteritis, pronounced weakness, gastric stasis, and constipation. Carbon tetrachloride has a rather constant effect on the liver in the production of acute yellow necrosis, and is contraindicated in cases of icterus or other hepatic derangements. Betanaphthol is said to cause destruction of red blood cells and is contraindicated in malaria and similar conditions. Chenopodium, thymol, and betanaphthol appear to be unsafe during pregnancy, but carbon tetrachloride appears to be quite safe in pregnancy and also in cases of kala azar and some pyrexias. A special contraindication for carbon tetrachloride is gross ascariasis, particularly with young and feeble patients, as this drug appears to cause a clumping of the ascarids with resultant intestinal obstruction fatal to a patient with an atonic intestine incapable of eliminating these masses; there is the further possibility that the drug dissolves certain constituents from these worms which may cause a toxic condition following its absorption by such patients. In ascariasis accompanied by gastroenteritis santonin is the drug of choice as it is not irritant.

This is by no means an exhaustive consideration of these drugs, but merely indicates something of what the practitioner must know about his drugs and his patient before administering treatment. The writer's limited experience with anthelmintics for the destruction of worms outside of the digestive tract has led to no special conclusions of interest here. The factors in this field differ, in the absence of the action of digestive fluids, and the matter of absorption by the blood, from those in anthelmintic treatment for worms in the alimentary canal, and the subject of anthelmintics begins to border on the field of chemotherapy for destruction of haematozoon parasites.

5. *Purgative* (Protection).—As noted above, prompt purgation is protective. Whether this is because of its elimination of the unabsorbed anthelmintic, which does not appear to be the general case, or because it distributes the anthelmintic along the digestive tract and thus prevents localized absorption and local injury, or whether it slows absorption and thus eases off the shock on the patient, is not definitely known, but clinical and experimental evidence all points to the value of prompt purgation. The purgative should be given at the earliest possible moment consistent with efficacy, or even sooner if safety requires it. Many anthelmintics, such as areca nut, arecoline hydrobromide, and kamala are purgative. Many combinations of anthelmintics and purgatives, such as chenopodium and castor oil, carbon tetrachloride and magnesium sulphate, and santonin and calomel, are effective for practical purposes. Whenever possible it is advisable to use the combinations, even though a diminution of efficacy necessitates repeated treatment; the idea of safety, especially in treating human beings, must be kept uppermost. We have shown that dogs will survive lethal doses of chenopodium or male fern when given with large doses of castor oil. In this connection it should be said that small doses of purgatives are decidedly contraindicated; always give plenty, especially with such constipating drugs as chenopodium. We sometimes see reports of deaths following the use of anthelmintics in which purgation was not obtained for 24 to 30 hours. There is usually a failure on the part of the practitioner in such cases. If early purgation is not obtained by the routine procedure, extraordinary measures are indicated. One must resort to purgation by duodenal tube if purgatives are vomited; use enemas, abdominal massage or any other measures known to medical science to cause evacuation of the bowels. Constipated patients should be given a preliminary purgative the evening before treatment.

In connection with the subject of purgation it may be said that it appears to be common practice to suppose that all worms removed will pass in 24 hours after treatment, and results are sometimes judged on this basis. Actually worms may pass for days, the time depending on the worm species, the drug, and the anatomy and condition of the host. In dogs the majority of worms come away in 24 hours, and in the case of tapeworms of the genus *Tænia*, all such worms come away in this period in our experience, although this might not always be the case. We found that dog ascarids came away as follows: The first 24 hours, 82.7%; the second, 7.7%; the third, 4.3%; the fourth, 3.1%; the fifth, 1.5%; the sixth, 0%; the seventh, 0.5%. Hookworms came away as follows: The first 24 hours, 74.1%; the second, 15.7%; the third, 7.4%; the fourth, 2.8%. Whipworms came away as follows: The first 24 hours, 57.6%; the second, 15.2%; the third, 18.2%; the fourth,

10%. *Dipylidium* came away as follows: The first 24 hours, 91%; the second, 7.4%; the third, 0%; the fourth, 1.6%.

6. *Prophylaxis*.—The practitioner has not completed his task nor fulfilled his duty to his client until he has informed him as to the probable source of his trouble and the means for preventing a recurrence. He must know the life histories of the parasites involved—that ascariasis involves coprophagy and a lack of sanitation or personal hygiene somewhere; that hookworm infestation probably means skin exposure to infective larvae with concomitant lack of sanitation along the line; that trichinosis implies the eating of improperly cooked or inadequately processed pork or pork material; that infestation with certain other worms (*Dipylidium*, spirurids, and acanthocephalids) involves the ingestion of such small intermediate hosts as insects; and that filariasis is indicative of attack by infective mosquitoes.

It is not only necessary to tell this to a patient but it is necessary also to keep such information before the public as much as possible, if we are to do our duty along the lines of preventive medicine. But at this point we leave anthelmintic medication and enter another field.



# **Chemotherapy of helminth infections: A centennial reflection**

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It was a century of therapeutic abundance; it was a century of therapeutic innovation; but most all, it was a century of therapeutic empiricism. Human health and, especially, animal husbandry benefited from the introduction of a great many agents for the control of parasitic worms, all of them arising from tradition, experience, or the trial-and-error method of testing compounds to see if they work. Most of these “anthelmintic” agents were used against roundworms, and several chemical classes rose to prominence, even dominance, on the strength of their improved safety and breadth of spectrum in the control of nematode parasites. Included in this group were the benzimidazoles, imidoazthiazoles, tetrahydropyrimidines, organophosphates, and macrocyclic lactones. For flukes and tapeworms, too, drugs of great importance (praziquantel and triclabendazole, for example) became available. In addition there were anthelmintic compounds that had specialty uses or that, for one reason or another, did not stimulate the development of multiple derivatives to compete in the animal-health marketplace or to vie for position in human medicine. Taken together with a few odds and ends carried over from earlier times, at least 130 anthelmintics (sold under more than 500 trade names) were in use in the twentieth century. Most of them were used only in veterinary medicine, but many were used also in human medicine.

The anthelmintics of the early twentieth century were far from satisfactory (Ransom and Hall, 1912; Grove, 1990). Some old herbal anthelmintics such as thymol, tobacco, chenopodium, and male-fern extract were still in use; but Ehrich's new “chemotherapy” had opened a door that could not be shut. In 1918, Christopherson reported the efficacy of potassium antimony tartrate in both the urinary and the intestinal type of schistosomiasis (Campbell, 1986; Grove, 1990). In 1919, Ransom initiated an anthelmintic project in the U.S. Bureau of Animal Industry, whence carbon tetrachloride emerged as a treatment for roundworm infections in 1921, to be followed by tetrachlorethylene in 1925 (Hall, 1921; Hall and Shillinger, 1925).

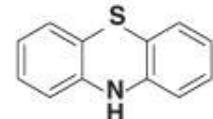
In the following year *The Journal of Parasitology* recognized the growing significance of chemical anthelmintics by publishing an article about them (Hall, 1926). It was an article of a type now rarely seen, namely, a discourse on the principles of anthelmintic use. A great deal of attention was devoted to matters of patient diet, the need for adjunctive laxative therapy, and the need for both medical and veterinary practitioners to understand helminth biology, especially in relation to disease prevention. Hall's paper recognized that the therapeutic effects of chemical anthelmintics depended on their pharmacological properties, which in

turn depended on their chemical composition. It may seem absurd that this relationship had to be pointed out in a scientific journal in 1926. In medieval times, it was perfectly natural for Chaucer to make a distinction between the “substance” and the “accident” of a thing; but it was only at the dawn of the nineteenth century that it was realized (following the teachings of Dalton and others) that the “accidents,” the physical characteristics, of a substance depended on the atoms inside. Now, a century later, Hall and his contemporaries could take a step farther and attribute pharmacological characteristics to the atoms—and those atoms could be rearranged to form molecules of almost infinite variety.

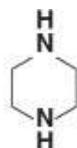
Evidently carbon tetrachloride got off to a good start; within a year, Rockefeller scientists in Fiji treated 50,000 patients “with brilliant results” (Silverman, 1941). Maurice Hall's introduction of carbon tetrachloride and tetrachloroethylene as anthelmintics for human and veterinary medicine were major events, coming as they did at a time when chemical anthelmintics were rarities. Inquiry then was focused on whether carbon tetrachloride was too dangerous for community-based treatment of hookworm infection in children (it was) and whether tetrachloroethylene was significantly safer (it was, but it had a way to go). At least since the days of Paracelsus some five centuries earlier, it had been understood, and widely forgotten, that the essential difference between a medicine and a poison is dosage. The distinction is hard to manage in practice, and Hall struggled with it in the 1920s. Christopherson's introduction of organic antimony (previously) led to many decades of trying (with fair success) to balance efficacy and toxicity in treating schistosomiasis. In the 1960s, the cyanine dye, dithiazanine was used against the more intractable of the intestinal nematode infections and was associated with several human fatalities before being withdrawn from the market (Abadie and Samuels, 1965; Grove, 1990].

The year 2014 is the one-hundredth anniversary of the formal closing of the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease (Ettling, 1981; Elman et al., 2014). The endeavor relied on education and thymol (neither being entirely reliable) and it was plagued by sociological and political obstacles—as are today's global effort to control soil-transmitted helminthiases.

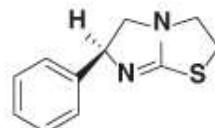
This article, being an essay rather than a literature review or a compendium of drugs, provides a somewhat capricious selection of references to the (English language) literature. The chemical structures of some significant anti-nematodal agents of the twentieth century are illustrated in [Fig. 1](#), while those of some anti-trematodal and anti-cestodal drugs are shown in [Fig. 2](#). More complete assemblages of anthelmintic structural diagrams and chemical data may be found in Fisher (1986), Mrozik (1986), and Andrews and Bonse (1986).



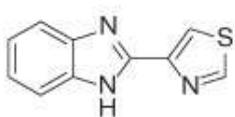
PHENOTHIAZINE



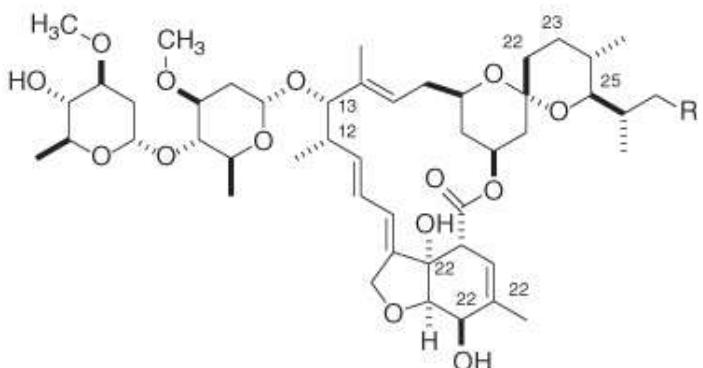
PIPERAZINE



Levamisole



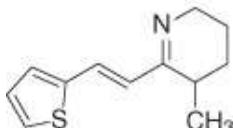
Thiabendazole



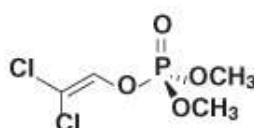
IVERMECTIN

R = H, avermectin B1b

R = CH<sub>3</sub>, avermectin B1a

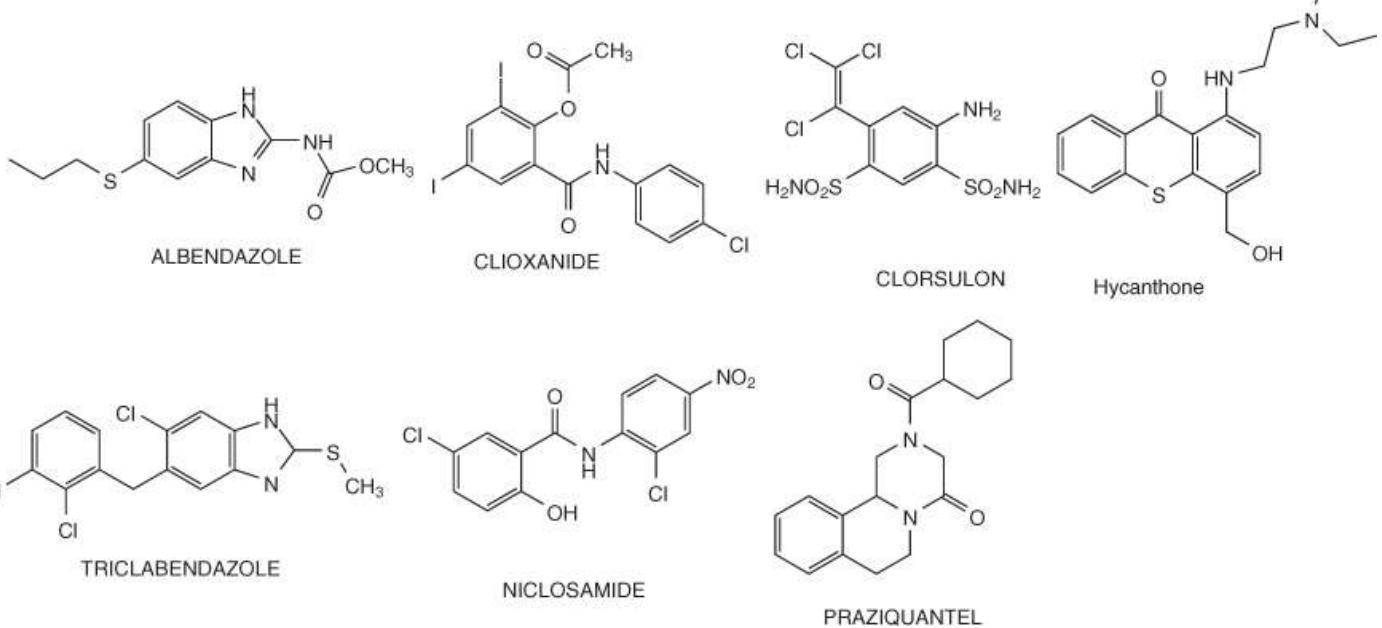


Pyrantel



Dichlorvos

**Figure 1** Some anthelmintics used in the twentieth century for treatment of nematode infections.



**Figure 2** Some anthelmintics used in the twentieth century for treatment of trematode and cestode infections.

## Stocking the armamentarium

In the 1930s and 1940s, new anthelmintics appeared in modest number, some of them emerging from serendipitous observations of side-effects noted in unrelated fields of inquiry, and some of them found as a result of searches undertaken in the face of imminent military operations. These compounds included hycanthone for schistosomiasis; phenothiazine and piperazine for intestinal nematodiasis; and diethylcarbamazine and thiacetarsamide for extra-intestinal nematodiasis. For cestodes, there were but a few old herbal remedies.

It was in the second half of the twentieth century that chemical anthelmintics appeared in substantial number. To a large extent this advance was the result of commercial incentives to develop drugs to control nematodes in livestock. The era of benzimidazole anthelmintics began with the introduction of thiabendazole in 1961 (Brown et al., 1961). For use in treating nematode infections in livestock, thiabendazole had two main advantages over the drugs then current. It had a broad efficacy-spectrum that included juvenile stages. And it was safe—not merely in preventing losses arising from accident or misuse, but in preventing the temporary “setback” in weight-gain that had until then been considered a necessary cost of “worming” livestock.

Thiabendazole set a new standard; and the benzimidazoles, including many analogs and pro-drugs, came to dominate the anthelmintic segment of the animal-health market for at least three decades (see special feature on the benzimidazoles in *Parasitology Today* **6(4)**: 1990). These compounds were joined by two other major broad-spectrum anthelmintic classes—the imidazothiazoles (tetramisole and levamisole) and, later, the macrocyclic lactones. Ivermectin and other macrocyclic lactones became the new leaders in the animal health market because they brought a new order of potency and spectrum against nematodes, as well as efficacy against certain parasitic arthropods, and highly desirable pharmacodynamic properties. Comprehensive reviews of the macrocyclic lactones have been published (Vercruyse and Rew, 2002; Gonzalez-Canga, 2012). Many other important anthelmintics were introduced in this period, and enjoyed widespread use in both veterinary and human medicine. For nematode infections this group included: organophosphates, the tetrahydropyrimidines (pyrantel and oxantel), and bephenium; for trematode infections: bithionol, niridazole, rafoxanide, clorsulon, triclabendazole, and praziquantel; for cestode infections: niclosamide, mebendazole, and praziquantel.

# Learning how medications work

Research conducted on anthelmintic medicines expanded the existing body of knowledge on the basic physiological and biochemical processes of helminths. It was ever thus. At the beginning of the Common Era, the great Roman encyclopedist Celsus stated this feature very precisely:

It was afterwards ... when the remedies had already been discovered that people began to discuss the reasons for them: the Art of Medicine [L. *medicamentum*] was not a discovery following upon reason, but after the discovery of the remedy, the reason for it was sought out (Celsus, 30 C.E.).

It does not follow that it will be forever thus. (The foregoing does not, of course, imply that physiological and biochemical knowledge of helminths was derived only from the study of anthelmintics.)

As the efficacy of each successive twentieth century anthelmintic was reported, its mechanism of action was sought out in greater and greater depth, as made possible by the rapidly accruing developments in chemistry, biochemistry and molecular biology. When Hall published his paper in *The Journal of Parasitology* in 1926, questions were being asked about structure-activity relationships and about physiological mode of action. The surmises of Hall and his contemporaries appear both quaint and prescient in the light of later advances. In regard to mode-of action, for example, Hall (1926) wrote:

At present we do not know how anthelmintics affect worms. They may cause muscular incoordination, as santonin apparently does in ascarids, but we do not know why santonin does not cause this in hookworms or tapeworms, if it does cause it in ascarids. They may dissolve fatty structures, as might be the case with carbon tetrachloride which is highly destructive to hookworms, but this is pure theory and affords no explanation as to why carbon tetrachloride should not affect dog tapeworms in the same manner. They may depress the nervous system of the worm and cause a relaxation of the hold or induce a degree of narcosis, but until we know more about the subject this is pure speculation.

In contrast to such observational and barely-physiological considerations were the biochemical studies of subsequent decades, showing, for example, that benzimidazoles inhibit tubulin polymerization thus blocking formation of intracellular microtubules; that organic phosphates inhibit acetyl cholinesterase in the nervous system of nematodes and thereby cause depolarization of nerve-cell membranes with consequent blockade of signal transmission; that levamisole and pyrantel depolarize nerve-cell membranes by cholinergic agonist action, thus again disrupting nerve transmission; and, that macrocyclic lactones block nerve transmission by binding to receptor subunits in the glutamate-gated ion channels of neuronal cell-membranes, thereby hyperpolarizing the cells and paralyzing the worms.

Modes of action were also elucidated for anti-trematode and anti-cestode agents. The salicylanilides were reported to act as uncouplers of oxidative phosphorylation in flukes, while clorsulon was found to inhibit trematode glycolytic enzymes (at levels much lower than those required for inhibition of the corresponding enzymes of mammals) and praziquantel was seen to disrupt the parasites' integumental integrity and muscle condition. The mode-of-action of triclabendazole remained in doubt at century's end, but seemed to hinge on the binding of its metabolite(s) to a site on the tubulin molecule different from the binding site of the common anti-nematode benzimidazoles (Fairweather, 2005). In tapeworms, too, praziquantel evidently functioned to disrupt the integument, although not in the same way as in flukes, and the benzimidazoles were reported to act, in part, through blockade of tubulin polymerization as in nematodes. By the end of the century, the structure-activity relationship of almost every significant anthelmintic had been explored and the biochemical mode of action had been described. Reviews of the subject included those by Bennett and Thompson (1986), Rew and Fetterer (1986), and Vanden Bossche (1986). The topic was recently updated (Robertson et al., 2012).

## Discovery and evaluation

A few of the twentieth century's important anthelmintics were found without being sought—that is, they were found serendipitously. Piperazine, for example, began clinical life as an agent for suppressing the agonies of gout. Treated patients may not have passed a more restful night but they did pass large worms—and piperazine went on to become a very important anthelmintic (used especially against ascarids) in both human and veterinary medicine. More often, however, anthelmintics were found because they were sought. An empirical search presumes a method of trial-and-error testing—in short, an assay. The process of devising and exploiting a superior assay for a particular purpose is the central scientific challenge of the empirical endeavor. Progress in this area, though often painfully slow, was made throughout the past century.

Mere immersion of a helminth in a test solution, with short-term observation of its fate, is a form of *in vitro* assay that goes back as least as far as Redi's studies in the mid-seventeenth century (Campbell, 1986). The merits and demerits of such simplicity hardly need mention here. In the *in vitro* assays of the second half of the twentieth century, test organisms included larval (juvenile) trichostrongylid parasites; adult *Caenorhabditis elegans* free-living nematodes (Simkin and Coles, 1980); nematode microfilariae (Hawking, 1963); excysted trematode metacercariae (Keiser, 2010; Panic et al., 2013); and strobilar and cystic cestodes (Webbe, 1986).

The great advantages of *in vitro* assays are economy of test substance and laboratory resources (enabling the testing of a large number, and wide diversity, of test substances). Parasites that are dead can usually, and sensibly, be pronounced dead upon simple visual inspection. It is often desirable to have objective and automated methods of recording lethal effects as well as sublethal effects such as loss of motility and subtle morphological alterations. The advantage of such refinement must be weighed against possible loss of efficiency. *In vitro* assays based not on whole organisms but on isolated biochemical processes and molecular entities usually have even greater advantages in scale, and also have the advantage of much more sophisticated, defined and sensitive targets. Although the outstanding characteristic of *in vitro* assays is their “high throughput,” they also enjoy a high “hit rate” (percentage of “actives” recorded)—much higher than that found in whole-animal *in vivo* tests. Typically the rate of conversion from *in vitro* hit to *in vivo* active has been low. This rate does not need to be high—failure is the default option. The *in vitro* trichostrongylid-juvenile assay, successful in mid-century in the introduction of the benzimidazoles (see next) would still be successful early in the twenty-first century when this assay revealed the nematocidal activity of amino-acetonitrile derivatives, leading to the development of monepantel (Kaminsky and Rufener, 2013).

In an *in vivo*, whole-animal assay, test substances are administered to helminth-infected host animals. Assays of this kind have the very serious, sometimes

prohibitive, reciprocal disadvantages—a need for large amounts of test substance, expensive operational resources, weightier ethical concerns, and so on. Often such assays involve laborious, therefore costly, procedures for evaluating efficacy. In a rat-*Fasciola hepatica* assay, for example, searching for very immature fluke in liver tissue can be undesirably time-consuming. It is sometimes possible, as in that case, to devise a biochemical alternative (Campbell and Barry, 1970); but whereas biochemical methods may give a more objective read-out, they will not necessarily be more efficient than counting (or estimating) the number worms or making a visual assessment of host tissue damage (Luttermoser, 1954; Campbell et al., 1978).

*In vivo* assays have, on the other hand, some incomparable advantages over *in vitro* ones (Campbell, 1983). Observation of an *in vivo* “active” is in itself a hint of a Therapeutic Index greater than unity. Even more important, a test substance has some potential (to a degree dependent on the unknown and infinitely complex pharmacological circumstance of the moment) of reaching not just a single biochemical process, but all of the biochemical processes the worm possesses (including those not yet discovered).

Because of the economic importance of nematodes in ruminants, attempts were made to establish consistent infections of those worms in common laboratory mammals (including mice, rats, rabbits and guinea pigs) but it was an uncommon laboratory animal that provided a major breakthrough. As reported by Leland and confirmed by Kates and Thompson, the gerbil, *Meriones unguiculatus*, proved to be a very useful host for *Trichostrongylus* spp. (Leland, 1963; Kates and Thompson, 1967). Kates and Thompson noted that gerbils offer not only the advantage of small body-mass but also of docility. The gerbil (*Meriones* spp.) would go on to achieve popularity in assay operations, commonly in the phenotypically distinct variety known as jird.

Throughout the second half of the twentieth century, *in vivo* assays were used to discover the activity of the major anthelmintic classes of the period. In the case of the benzimidazoles, anthelmintic activity of the class was detected in John Egerton's mouse-*Heligmosomoides* assay (adapted from Baker, 1954) and also in his adaptation of Jack Tiner's (unpublished) trichostrongylid-juvenile-*in vitro* assay (the latter being acknowledged in the initial announcement of the discovery, Brown et al., 1961). It is the author's recollection that the lead compound (2-phenyl benzimidazole) was found active more or less simultaneously and independently in the *in vitro* and *in vivo* assays.

The discovery of levamisole is rich in lessons for the experimental chemotherapist (Raeymaekers et al., 1966; Campbell, 2005). Empirical screening was done in parasitized chickens and led to discovery of a compound that removed worms from birds, but not from mammals. Activity against worms in mammals was the main objective, and the chickens obligingly excreted a metabolite that was active in mammals, thereby enabling humans to develop the metabolite into tetramisole

and then levamisole.

In the case of the macrocyclic lactones, the anthelmintic activity was detected in an assay developed by John Egerton and Joe DiNetta for the screening of microbial fermentation products (Egerton et al., 1979; Stapley and Woodruff, 1982). There had previously been little, if any, *in vivo* screening of fermentation products for activity against worm infections. In retrospect it can be seen that there had been a methodological impasse in helminthology that had not been the case in bacteriology. What was needed was an efficient way to screen fermentations for anthelmintic activity. The new assay was based on the dietary administration to individual mice of an unknown amount of an unknown substance that might not be there. More specifically, freeze-dried fermentation broths of isolated but unidentified microbes were fed, in an arbitrarily set quantity, to mice that had been infected with *Heligmosomoides bakeri* and that would be examined at necropsy for the presence of infection. Loss of infection would suggest that the microbe in question contained, or had released, a substance and that the substance, whatever it was, had anthelmintic activity *in vivo*. The assay might be seen as taking empiricism to an extreme. In all three of these cases of anthelmintic discovery, chance played an important part; but none was completely serendipitous.

In the last few decades of the century, certain aspects of assay technology were markedly improved. In assays using worm-infected laboratory animals, for example, there had long been the question of the optimum size of the test groups for a specific assay system. Small group size is desirable for reasons of test-material economy, housing-and-maintenance economy, and animal welfare consideration. In using a mouse model for testing substances against *Schistosoma mansoni*, Standen used 10 mice per group (Standen, 1953), the present author used 5 or 10 per group (Campbell et al., 1978) and Kikuth and Gonnert used 2 per group (Kikuth and Gonnert, 1948). Ostlind reduced it to one mouse per group. He later applied the principle to other anthelmintic assays without loss of assay utility (Ostlind et al., 2013). Use of the one-mouse group was a conspicuous feature of the assay used for the discovery of ivermectin (see previously). This group size would appear to be both the irreducible minimum, and the optimum. The essential element in this singular approach is the establishment of infections with almost 100% reliability. An assay that does not have such a uniformly high infection rate does not need more animals; it needs more work.

For reasons stated previously it is highly desirable that the host animal in an *in vivo* assay be of small size. In practical terms this requirement generally means that the species of choice is the mouse, *Mus musculus*. White laboratory mice were commercially available in the period under discussion, which was not the case in the 1870s when Robert Koch was obliged to depend on his daughter's surplus pet mice (Brock, 1999). For the most part, mice do not permit mature infections of the trichostrongylid nematodes that are clinically and economically important pathogens of ruminants. It is in that context that one can appreciate why reliance

on active immunosuppression of host animals constituted a profound change in assay methodology in the second half of the twentieth century.

Enhancement of experimental helminth infection through immunosuppression was reported in mid-century (Coker, 1955). John R. Egerton pioneered the use of the technique for assay purposes in the late 1950s by developing a screening assay in which cortisone-injected mice were infected with *Haemonchus contortus* (see Ostlind et al., 2013). It soon became common practice to use corticosteroids to overcome host-parasite incompatibility, and the use of immunosuppressives became an essential element in several anthelmintic “screening” assays. Ostlind et al. (2013) used immunosuppressives in testing graded dietary levels of ivermectin against dual infections of *H. polygyrus (bakeri)* and *H. contortus*, and concluded that the sensitivity of the mouse-*Haemonchus* model rivaled that reported in the literature for the gerbil-*Trichostrongylus* model, while offering advantages in terms of animal maintenance costs (Ostlind et al., 2013).

The advantage of immunosuppression was not confined to the trichostrongylids. At a time when I and others were struggling to establish patent infections of the whipworm *Trichuris muris* in the common strains of laboratory mice, a young technician was transferred to my lab. He had come from a lab in which endocrinologists were routinely using hypophysectomized rats in their research. Being smart and eager to make a good impression, he asked what effect hypophysectomy would have on a helminth infection. I had (and it now occurs to me that I still have) no idea of the answer, but I thought it would be simpler and more likely to yield an interesting result if we took the opposite approach and tried to elevate rather than lower the corticosteroid level in our parasitized mice. We treated the mice with cortisone and got bountiful whipworm infections (Campbell and Collette, 1962). *Trichuris muris* does not seem to have been used in primary anthelmintic screening, but is believed to have been a significant factor in the identification of oxantel as a compound with marked activity against *Trichuris* spp. *In vivo* assays for filarial infections were (and are) particularly needed. A major breakthrough occurred in mid-century with the development of assays based on infections of *Litosomoides carinii* in cotton rats (Hawking and Sewell, 1848; Hawking, 1963). Much later, the ferret, *Mustela putorius furo*, was shown by Campbell et al. (1979) to be a suitable laboratory host for *Dirofilaria immitis* and *Brugia pahangi*. Neither of these host-parasite systems can be said to be convenient for primary screening operations.

For evaluation of anthelmintics in large animals, and indeed for other types of nematological work, another methodological innovation yielded practical advantages. To gain regulatory approval and market strength it had become necessary to evaluate candidate drugs against numerous species and strains of parasites. In sheep and cattle, it is often necessary, or at least *de rigueur*, to test drugs against at least a dozen nematode species, and many infra-specific strains that exhibit special characteristics, especially in relation to drug resistance. The serial passage of these organisms in sheep and cattle entails enormous

expenditures associated with large-animal facilities and laboratory technical resources. In the context of needed parasite control, the most important group of helminths in domestic animals is the superfamily Strongyloidea, and for this group a simple technique was found to enable investigators to preserve and store the parasites outside their host animals. If infective strongyle juveniles are treated so as to cause loss of their outer sheath, they can be cryopreserved for years by simply putting them in water in vials and then placing the vials in the vapor phase of a liquid-nitrogen container (Campbell et al., 1972; Rew and Campbell, 1983).

Reviews of anthelmintic assay methodology include those of Standen (1963), Coles (1990), Johnson et al. (1999), and Frankhauser et al. (2012). Standen's review, considering that he wrote at a time when accessing scientific literature was more difficult than it is now, stands as a work of extraordinary scholarship. I remember being received courteously by the eminent Dr. Standen in the 1950s when, as a mere beginner in the anthelmintic field, I visited him in his London office. Later I found that one of the pleasures of industrial research is the *camaraderie* that develops among practitioners of the art. The scientists of competing companies can enjoy socializing at conferences and chatting about shared scientific interests without stooping to unseemly prying. Perhaps there is a lesson here for anyone contemplating industrial employment.

## Consequence and strategy

The twentieth century's plethora of chemical anthelmintics brought manifold improvement in human health and, more abundantly, in animal health. The benefits were not only in health *per se*, but also in agricultural economics, livestock management and the overall production of food and fiber to support expanding human populations.

In the animal health arena, the consequences of introducing potent broad-spectrum anthelmintics in mid-century can be noted here only briefly. Most of them were good—the obvious ones being disease control, and the prevention of disease and ill-thrift. A few were good in less obvious ways. In New South Wales, for example, treatment of sheep led to an increase not only in the quantity of wool produced but also in the quality. Worming also caused a reduction in the number of sheep that could be raised on a given piece of land—which was good, because healthier (less wormy) animals could be raised to maturity faster than others, and productivity over a grazing season could thereby be enhanced (Anderson et al., 1976).

An important consequence also arose from advances in pharmaceutical technology. As recently as mid-century, treatment of large domestic animals typically involved the use of a large steel balling-gun to insert a bolus of drug into the throat of the animal. (The author and fellow grad-students had used the old-fashioned balling gun in all seasons to support our professor's contention that even a famously superior dairy farm could be made more profitable by periodic anthelmintic treatment of its showpiece herd.) Thanks to the high potency of the newer anthelmintics, the cumbersome balling-gun procedure was supplanted by the use of commercial preparations of the drug in paste, solution or micelle form, administered into the mouth by plastic syringe, or given by parenteral injection, or by application to skin.

Even more dramatic was the burgeoning use of “slow release” technology to deliver antiparasitic agents. With the macrocyclic lactones, efficacy against endo- and ecto-parasites was obtained by using a device consisting of a drug-laden polymer encased in a material that permits slow release of drug (Anderson and Laby, 1979; Soll et al., 1990). Such devices are typically delivered to the reticulum or rumen of livestock, and are held there by plastic wings that open upon entry, or by incorporation of iron filings to prevent flotation, or by other means. The lipophilicity of macrocyclic lactones was in several instances sufficient to confer a natural persistence of efficacy by slow release of drug from the lipid tissue of treated animals. The pharmacologic properties of the macrocyclic lactones also permitted the development of long-lasting injectable preparations (reviewed by Hennessy and Alvinerie, 2002). Progressive increase in the potency of anthelmintics is celebrated by those who develop the drugs, but it is in the area of pharmaceutical technology that the real value of these drugs is borne out.

The efficacy of new broad-spectrum drugs against larval nematodes provided a

novel research tool as well as a novel therapeutic weapon. It became possible, for example, to terminate heavy experimental *Ascaris suum* infections in rats prior to the destructive liver and lung phases, and to thereby confer almost total protective immunity against reinfection (Campbell and Timinsky, 1965). Other anthelmintic drugs, too, were exploited as research tools; and, it is worth keeping in mind that chemical substances generally have multiple biological actions, and that candidate drugs that fail to reach clinical or commercial utility may prove valuable in basic research.

A mid-century change that should be kept in mind by commentators of the future is the increase in governmental regulations of drugs. In the United States of America, for example, Federal laws of 1906, and 1938, dealt with drug purity and truthful advertising and general safety; but requirements for proof of efficacy did not become law until 1962. Since then, requirements for the introduction of new drugs evolved throughout the world to become much more comprehensive and rigorous. In the United States, the introduction of a new veterinary antiparasitic agent requires the sponsor (would-be seller) to submit a New Animal Drug Application to the Office of New Animal Drug Evaluation, of the Center for Veterinary Medicine, of the Food and Drug Administration, of the Department of Agriculture. The New Animal Drug Application must present evidence of the safety of the antiparasitic agent for the target animal species; of the efficacy of the drug under field conditions against the parasites for which a claim is being made (including particular life cycle stages of the parasite, with consideration given to resistance status of strains of the parasite); of food-safety, if the target animal is a source of human food (including determination of distribution, depletion, and safety of the drug and its metabolites in the tissues of the target animal); of environmental safety of the drug under field usage; of the safety of the drug for manufacturing and dispensing personnel; of the acceptability of the proposed manufacturing materials and methods, including quality of chemicals; of the acceptability of the wording on the proposed product label. If the evidence is deemed sufficiently strong, the Application is approved and marketing may proceed. For an antiparasitic agent intended for use in humans, extensive trials in humans must be undertaken in a series of regulated phases. Before the first phase can begin, the sponsor must submit an Investigational New Drug application to the Food and Drug Administration. The application must provide pharmacological and toxicological data from trials in animals and data from any prior trials known to have been done in humans; details of proposed manufacturing methods and materials and managerial control; and protocols of proposed clinical trials, including ethical responsibilities and commitments.

The consequence of greatest concern was the emergence of wide-spread drug resistance. This problem was, and still is, of global concern in human and veterinary medicine, but is especially serious in the treatment of livestock, where parasitic worms are exposed to anthelmintic drugs with great frequency. The study of biochemical and genetic mechanisms underlying resistance, and of

possible methods of blocking it, became a scientific sub-discipline in itself. Among its achievements, as the century drew to a close, was discovery that benzimidazole resistance in *Haemonchus contortus* was correlated with specific structural variations in the genetic determinants of the beta-tubulin that is the target of benzimidazole action, and that variations in genes are also implicated in resistance to macrocyclic lactones (Lubega et al., 1994; Prichard, 2002).

Resistance has been devastating, too, in the case of flukes. Triclabendazole quickly rose to dominate the fascioliasis market in the latter part of the century, mainly on the strength of its superior activity against immature flukes, but entered the twenty-first century as exemplar of a superior drug facing widespread resistance after widespread use (Fairweather, 2005).

It was because of drug resistance that another important development occurred. The epidemiological aspects of mass treatment programs were scrutinized with new intensity in the hope of finding ways to minimize the emergence and spread of resistance under field conditions. In regard to livestock production, the essential tactics were to design diagnostic, managerial and treatment protocols that would reduce resistance by reducing the exposure of helminths to anti-helminth drugs. In areas of *Haemonchus contortus* endemicity, for example, simple eye inspection was promoted as a means of identifying sheep with severe anemia, and those individuals could then be treated for hemonchosis. In that way, treatment, and thus selective pressure, could be applied to the most heavily infected animals rather than to the entire flock.

The same strategy was approached through the use of tactics such as using fecal worm egg counts to monitor the infection status of individuals and groups, with a view to using drugs only where most needed, as well as using egg hatching-rate to monitor existing drug resistance so as to match animal populations, and their parasites, with the drug most likely to succeed. However, such methods are less sensitive than DNA-based methods mentioned previously and therefore less likely to detect incipient resistance (Prichard, 2002).

In human medicine, mass drug therapy was undertaken late in the twentieth century when several anthelmintics were considered safe enough for such use. The available tools were much superior to those of the hookworm campaign mentioned previously. Mass treatment was adopted for the control of “neglected” helminthic diseases, including soil-transmitted nematode diseases, onchocerciasis, lymphatic filariasis, and schistosomiasis. In some of these programs, notably the onchocerciasis program, success, though difficult to attain, exceeded all expectations. In other cases, evaluation of long-term success was not yet feasible at century's end. The complications and controversies surrounding these programs, especially in relation to soil-transmitted helminths, have recently been discussed in the pages of *The Journal of Parasitology* (Loker, 2013; Hawden, 2014) and elsewhere (e.g., Hotez, 2012; Gallo et al., 2013).

Changes subsequent to, if not entirely consequent to, the common use of broad-

spectrum anthelmintics included a greater degree of collaborative research and planning between industrial and non-industrial laboratories and the increased involvement of non-governmental organizations. The new level of sharing was an accompaniment to an expanding awareness of the magnitude of global health problems, taken together with the increasingly evident interdependence of nations and greater mobility of people. Non-governmental organizations and philanthropic foundations played major roles in instigating programs that use modern therapies and public health methods to attempt parasite control on a wide scale (see Caffrey, 2012; Hotez, 2012; Gallo et al., 2013; Loker, 2013; Hawden, 2014).

Market forces brought about a very different departure from tradition. Sale of anthelmintic drugs to the dairy industry is often, and rightly, curtailed by concern about the excretion of drug or metabolites in milk destined for human consumption. In the case of the lipophilic drug ivermectin, candidate derivatives were administered to dairy cattle and post-treatment milk excretion was measured. By screening candidates in this way, a macrocyclic lactone with minimal excretion in milk was identified, and the compound (eprinomectin) was shown to have retained excellent antiparasitic activity (Shoop et al., 1996).

## Inconstant context

By the end of the twentieth century, competitive pressure had induced drug developers to adopt a “see you and raise you” approach, in the assumption that a new anthelmintic must match the potency-spectrum-safety qualities of the current leader and add something new by way of competitive edge. In practical chemotherapy, however, there is always the specter of drug-resistance or undiscovered liability of some other kind. As suggested by Geary, there are market segments in which new anthelmintics could be very useful without exceeding, or even matching, the standard set by the macrocyclic lactone “endecticides” (Geary, 2002). Structures with new chemotherapeutic profiles do continue to be valuable in the animal health market. The anthelmintic activity of paraherquamide was reported late in the century (Ostlind et al., 1990). A derivative, derquantel, was developed and was introduced commercially in the new century (in combination with a macrocyclic lactone for greater breath of spectrum) (Woods et al., 2012). The new century also saw the introduction of monepantel (see next).

For most of the twentieth century, the divide between “empirical” and “rational” methods of drug discovery coincided with the divide between industrial and non-industrial institutions. That distinction was natural, indeed virtually inevitable. In industry there were legal constraints (matters of patent, confidentiality and potential collusion) but in any case the scientists and governing bodies of academic laboratories were not inclined to invest in costly screening operations that were out of keeping with the mission and traditions of universities. The empirical versus rational divide was accentuated by differences in professional objectives and rewards in industrial versus academic settings in regard to the

finding of leads and the finding of drugs. The concept that academia traditionally found leads and industry developed them into marketable drugs is, at least in the antiparasitic context, largely a myth. It would be more accurate to say that industry found drugs, and academia found leads—leads being derived from biochemical study of the drugs and from unrelated biochemical research. This “division of labor” was entirely consistent with the goals and traditions of both parties.

As the old century gave way to the new, a profound change took place. New approaches to anthelmintic discovery were proposed, including collaborative research and collaborative funding by non-industrial partners, new disease models, new consideration of leads from other scientific disciplines. Academic scientists, using the new tools of the new age, were finding new leads. Large “libraries” of novel chemical structures were screened in high-throughput biochemical assays. Many pharmaceutical companies reduced or abandoned their traditional anthelmintic screening of compounds directly against parasites (*in vitro* or *in vivo*). Tradition was not totally defunct, for early in the new century, monepantel was discovered by direct primary screening against parasites *in vitro* and secondary screening against parasites *in vivo* (Kaminsky and Rufener, 2013). Nevertheless, a major shift in discovery strategy had occurred. Pursuit of multiple approaches is theoretically ideal, but as long as resources are limited (forever?), judgments will have to be made as to their allocation among competing budgetary interests. Those end-of-century changes in the context of drug discovery were a *mise en scène* for the twenty-first century, and will do much to heighten the drama of anthelmintic discovery in the coming seasons. Pertinent commentaries include those of Caffrey (2012), de Hastos (2012), Geary (2002, 2012), and Geary and Thompson (2003).

## **Overview, with lessons from a bygone century**

Over great swaths of the world, common worm diseases of people have been brought under control by management of the environment. That has been one of the unintended consequences of civilization. Other great stretches of the Earth, having not been so thoroughly paved over, have remained plagued by parasites, and medication is still depended upon to keep the worms at bay. Worm control has recently become integrated with large-scale public health schemes that are by no means limited to parasitic disease. Animal husbandry remains a constant battleground, but the worms and their hosts are held by humans in a shaky truce. The chemotherapeutic habits of the past century may have to be revised as human temperaments and global temperatures change, but they have in the meantime changed the world for the better.

The age-old search for antiparasitic medications provided lessons of its own, some of them being peculiar to the empirical method. In screening substances for activity we should rely on practical common sense. Diversity should trump selectivity. We should aspire to the testing of everything, and work back from there to select assay input as indiscriminately as resources and common sense will allow. That usually means accepting a huge restriction in input. In an *in vivo* assay, an “active” is a treasure. A novel bioactive substance may be valuable as a potential therapeutic agent. It may be useful as a research tool. If it is very toxic it may be useful as a toxin. Whatever its biological action, the underlying biochemical action should be examined to the extent warranted by circumstance.

Basic research has continued to become more basic, approaching, it can be hoped, the point at which the distinction between drug and vaccine becomes moot. The absence of parasitic disease is more desirable than the absence of parasites. Humans are more likely to accept that premise of behalf of their livestock than on their own behalf. Still, new insights may lead to a world in which chemotherapists are obsolete. That outcome—the absence of disease, not the unemployment—is a condition worth striving for.

As the old century gave way to the new, innovative approaches to drug discovery and development proliferated in extraordinary profusion. Ideas for mechanism-based assays abounded. Boundaries were blurred between *in-vitro* and *in-vivo* assays, between screening and design, between industry and academia between public and private sectors. The therapeutic past is a prologue from which we can learn; the present is full of promise; and the suspense is unbearable!

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## Literature cited

- Abadie, S. H., and M. Samuels. 1965. A fatality associated with dithiazanine iodide therapy. *Journal of the American Medical Association [JAMA]* **192**: 326–327.
- Anderson, N., R. S. Morris, and I. K. Taggart. 1976. An economic analysis of two schemes for the anthelmintic control of helminthiasis in weaned lambs. *Australian Veterinary Journal* **52**: 174–180.
- Anderson, N., and R. H. Laby. 1979. Activity against *Ostertagia ostertagi* of low doses of oxfendazole. *Australian Veterinary Journal*. **55**: 244–246.
- Andrews, P., and G. Bonse. 1986. Chemistry of anticestodal agents. In: Campbell, W. C. and R. S. Rew (eds.) *Chemotherapy of Parasitic Infections*. Plenum Press, New York, p. 447–456.
- Baker, N. F. 1954. Trichostrongylidosis—the mouse as an experimental animal. Proceedings 91st Annual Meeting of the American Veterinary Medical Association, Seattle. p. 185–191.
- Bennett J. L., and D. P. Thompson. 1986. Mode of action of antitrematodal agents. In W. C. Campbell and R. S. Rew (eds.). *Chemotherapy of parasitic infections*. Plenum Press, New York. pp. 427–443.
- Brock, T. D. 1999. *Robert Koch: a life in medicine and bacteriology*. ASM Press, Washington D.C. p. 321.
- Brown, H. D., A. R. Matzuk, I. R. Ilves, L. H. Peterson, S. A. Harris, L. H. Sarett, J. R. Egerton, J. J. Yakstis, W. C. Campbell, and A. C. Cuckler. 1961. Antiparasitic drugs. IV. 2-(4`thiazolyl)-benzimidazole, a new anthelmintic. *J. Amer. Chem. Soc.* **83**: 1764.
- Caffrey, C. R. 2012. Preface. In *Parasitic helminths: Targets, screens, drugs and vaccines*. C. R. Caffrey (ed.). Wiley-Blackwell, Oxford, U.K. 540 p.
- Campbell, W. C. 1983. Progress and prospects in the chemotherapy of nematode infections of man and other animals. *Journal of Nematology* **15**: 608–615.
- \_\_\_\_\_. 1986. Historical introduction. In W. C. Campbell and R. S. Rew (eds.). *Chemotherapy of parasitic infections*. Plenum Press, New York. p. 3–21.
- \_\_\_\_\_. 2005. Serendipity and new drugs for infectious diseases. *ILAR Journal*. **46**: 352–356.
- \_\_\_\_\_, and J. V. Collette. 1962. Effect of cortisone upon infection with *Trichuris muris* in albino mice. *Journal of Parasitology* **48**: 933–934.
- \_\_\_\_\_, and S. F. Timinski. 1965. Immunization of rats against *Ascaris suum* by means of non-pulmonary larval infections. *Journal of Parasitology* **51**:

\_\_\_\_\_, and T. A. Barry. 1970. A biochemical method for the detection of anthelmintic activity against liver fluke (*Fasciola hepatica*). *Journal of Parasitology* **56**: 325–331.

\_\_\_\_\_, L. S. Blair, and J. R. Egerton. 1972. Motility and infectivity of *Haemonchus contortus* larvae after freezing. *Veterinary Record* **91**: 13.

\_\_\_\_\_, E. Bartels and A. C. Cuckler. 1978. A method for detecting chemotherapeutic activity against *Schistosoma mansoni* in mice. *Journal of Parasitology* **64**: 69–77.

\_\_\_\_\_, L. S. Blair, and J. W. McCall. 1979. *Brugia pahangi* and *Dirofilaria immitis*: experimental infections in the ferret, *Mustela putorius furo*. *Experimental Parasitology* **47**: 327–332.

Celsus, A. C. 30 C.E. *De Medicina. Prooemium 36*. In (W. G. Spencer, Translator) Harvard University Press (Loeb Classical Library) Cambridge. Massachusetts, Vol. **1**, 1937. p. 19–21.

Coker, C. M. 1955. Effects of cortisone on *Trichinella spiralis* infections in nonimmunized mice. *Journal of Parasitology* **41**: 498–504.

Coles, G. C. 1990. Recent advances in laboratory models for evaluation of helminth chemotherapy. *British Veterinary Journal* **146**: 113–119.

de Hastos, E. L., and T. Nguyen. 2012. Anthelmintic drugs: tools for the long road from discovery to product. In C. R. Caffrey (ed.). *Parasitic helminths: Targets, screens, drugs and vaccine*. Wiley-Blackwell, Oxford, U.K. p. 219–232.

Egerton, J. R., D. A. Ostlind, L. S. Blair, C. H. Eary, D. Suhayda, S. Cifelli, R. F. Riek, and W. C. Campbell. 1979. Avermectins, a new family of potent anthelmintic agents: efficacy of the B<sub>1</sub>a component. *Antimicrobial Agents and Chemotherapy* **15**: 372–378.

Elman C., R. A. McGuire, and B. Wittman. 2014. Extending public health: the Rockefeller Sanitary Commission and hookworm in the American South. *American Journal of Public Health* **104**: 47–58.

Ettling, J. 1981. *The germ of laziness*. Harvard University Press, Cambridge. 263 p.

Fairweather, I. 2005. Triclabendazole: new skills to unravel an old(ish) enigma. *Journal of Helminthology* **79**: 227–234.

Fisher, M. H. Chemistry of antinematodal agents. 1986. In W. C. Campbell and R. S. Rew (eds.). *Chemotherapy of parasitic infections*. Plenum Press, New York, NY. p. 239–266.

Frankhauser, R., L. R. Cozzie, B. Nare, K. Powell, A. E. Sluder, and L. G. Hammerland. 2012. Use of rodent models in the discovery of novel anthelmintics. In C. R. Caffrey (ed.) *Parasitic helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. 181–199.

Gallo, K., A. Mikhailov, M. B. Hailemeskal, K. M. Koproc, P. Babazim, and D. Addiss. 2013. Contributions of non-governmental organizations to WHO targets for control of soil-transmitted helminthiases. *American Journal of Tropical Medicine and Hygiene* **89**: 1186–1189.

Geary, T. G. 2002. Macrocytic lactones as antiparasitic agent in the future. In J. Vercruyse, and R. S. Rew (eds.) *Macrocytic lactones in antiparasitic therapy*. CABI Publishing, Wallingford, U.K. p. 413–423.

\_\_\_\_\_, 2012. Mechanism-based screening strategies for anthelmintic screening. In C. R. Caffrey (ed.) *Parasitic helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. 123–134.

\_\_\_\_\_, and D. P. Thompson. 2003. Development of antiparasitic drugs in the 21st century. *Veterinary Parasitology* **115**: 167–184.

Grove, D. I. 1990. *A history of human helminthology*. CABI Publishing, Wallingford, U.K. 848 p.

Gonzalez-Canga, A. 2012. Macrocytic lactones in antiparasitic therapy. *Current Pharmaceutical Biotechnology*. **13**: 851–1119.

Hall, M. C. 1921. The use of carbon tetrachlorid (sic) for the removal of hookworms. *Journal of the American Medical Association* **77**: 1641–1643.

\_\_\_\_\_. 1926. Some practical principles of anthelmintic medication. *Journal of Parasitology* **13**: 16–24.

\_\_\_\_\_, and J. B. Schillinger. 1925. Tetrachlcorethylene, a new anthelmintic. *American Journal of Tropical Medicine* **5**: 229–237.

Hawden, J. M. 2014, Controlling soil-transmitted helminths: time to think inside the box? *Journal of Parasitology* **100**: 166–188.

Hawking, F. 1963. Chemotherapy of filariasis. In R. J. Schnitzer and F. Hawking (eds.) *Experimental chemotherapy*. Academic Press. New York, N.Y. p. 701–892.

\_\_\_\_\_, and P. Sewell. 1948. The maintenance of a filarial infection (*Litomosoides carinii*) for chemotherapeutic investigations. *British Journal of Pharmacology* **3**: 285–296.

Hennessy, D. R., and Alvinerie, M. R. 2002. Pharmokinetics of the macrocyclic lactones: Conventional wisdom and new paradigms. In J. Vercruyse and R. S. Rew (eds.) *Macrocytic lactones in antiparasitic therapy*. CABI Publishing,

Wallingford, U.K. p. 125–140.

Hotez, P. 2012. Preface. In C. R. Caffrey (ed.) *Parasitic helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. v–vi.

Johnson, S. S., E. M. Thomas, and T. G. Geary. 1999. Intestinal worm infections. Chapter 106 In O. Zak and M. A. Sande (eds.) *Handbook of animal models of infection*. Academic Press, San Diego. p. 885–896.

Kaminsky, R. and L. Rufener. 2013. Monepantel: from discovery to mode-of-action. In C. R. Caffrey (ed.) *Parasitic helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. 283–296.

Kates, K. C., and D. E. Thompson. 1967. Activity of three anthelmintics against mixed infections of two *Trichostrongylus* species in gerbils, sheep and goats. *Proceedings of the Helminthological Society of Washington* **34**: 228–236.

Keiser, J. 2010. *In vitro* and *in vivo* trematode models for chemotherapeutic studies. *Parasitology* **137**: 589–603.

Kikuth, W., and R. Gonnert. 1948. Experimental studies on the therapy of schistosomiasis. *Annals of Tropical Medicine and Parasitology* **42**: 256–267.

Leland, S. E. 1963. Preliminary evaluation of *Trichostrongylus axei* in the mongolian gerbil as a screening system for anthelmintics of domestic animals. *Journal of Parasitology* **49**: 15–18.

Loker, E. S. 2013. This de-wormed world? *Journal of Parasitology* **99**: 933–942

Lubega, G. W., R. D. Klein, T. G. Geary, and R. K. Prichard. 1994. *Haemonchus contortus*: the role of two beta-tubulin gene subfamilies in the resistance to benzimidazole anthelmintics. *Biochemical Pharmacology* **47**: 1705–1715.

Luttermoser, G. W. 1954. Studies on the chemotherapy of experimental schistosomiasis I. A method of detecting schistosomicidal activity based on response of *Schistosoma mansoni* infections in mice to Fuadin therapy. *Journal of Parasitology* **40**: 130–137.

Mrozik, H. 1986. Chemistry of antitrematodal agents. In W. C. Campbell and R. S. Rew (eds.) *Chemotherapy of parasitic infections*. Plenum Press, New York, N.Y. p. 365–383.

Ostlind, D. A., W. G. Mickle, D. V. Ewanciw, F. J. Andriuli, W. C. Campbell, S. Hernandez, S. Mochales, and E. Munguira. 1990. Efficacy of paraherquamide against immature *Trichostrongylus colubriformis* in the gerbil (*Meriones unguiculatus*). *Research in Veterinary Science* **48**: 260–261.

\_\_\_\_\_, W. G. Mickle, S. Smith, D. V. Ewanchiw, and S. Cifelli. 2013. Efficacy of ivermectin versus dual infections of *Haemonchus contortus* and

*Heligmosomoides polygyrus* in the mouse. *Journal of Parasitology* **99**: 168–169.

Mrozik, H. 1986. Chemistry of antitrematodal agents. In W. C. Campbell and R. S. Rew (eds.) *Chemotherapy of parasitic infections*. Plenum Press, New York, N.Y. p. 365–383.

Panic, G., K. Ingram, and J. Keiser. 2013. Development of an *in vitro* drug sensitivity assay based on newly excysted larvae of *Echinostoma caproni*. *Parasit Vectors* **6**: 237. doi: 10.1186/1756-3305-6-237.

Prichard, R. K. 2002. Resistance against macrocyclic lactones. In J. Vercruyse, and R. S. Rew (eds.) *Macrocyclic lactones in antiparasitic therapy*. CABI Publishing, Wallingford, U.K. p. 163–182.

Raeymakers, A. H. M., F.T.N. Allewijn, J. Vandenberk, P. J.A. Demden, T.T.T. Offenwert, and P.A.G. Janssen. 1966. Novel broad-spectrum anthelmintics. Tetramisole and related derivatives of 6-aryl-imidazo [2,1,b] thiazole. *Journal of Medicinal Chemistry* **9**: 545–549.

Ransom, B. H., and M. C. Hall. 1912. *The action of anthelmintics on parasites located outside of the alimentary canal*. *Bulletin of the Bureau of Animal Industry*. No. 153. U.S. Dept. of Agriculture. Washington, D.C. [E-book].

Rew, R. S., and W. C. Campbell. 1983. Infectivity of *Haemonchus contortus* in sheep after freezing for ten years over liquid nitrogen. *Journal of Parasitology* **69**: 251–252.

\_\_\_\_ and R. H. Fetterer. 1986. Mode of action of antinematodal agents. In W. C. Campbell and R. S. Rew (eds.) *Chemotherapy of parasitic infections*. Plenum Press, New York, N.Y. p. 321–337.

Robertson, A. P., S. K. Buxton, S. Puttachary, S. M. Williamson, A. J. Wolstenholme, C. Neveu, J. Cabaret, C. Charvet, and R. J. Martin. 2012. Antinematodal Drugs—Modes of Action and Resistance. In C. R. Caffrey (ed.) *Parasitic Helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. 297–308.

Silverman, M. 1941. *Magic in a bottle*. MacMillan Company, New York, N.Y. 332 p.

Shoop, W. L., J. R. Egerton, C. H. Eary, H. W. Haines, B. F. Michael, H. Mrozik, et al. 1996. Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. *International Journal for Parasitology* **26**: 1237–1242.

Simkin, K. G., and G. C. Coles. 1981. The use of *Caenorhabditis elegans* for anthelmintic screening. *Journal of Chemical Technology and Biotechnology* **31**: 66–69.

Soll, M. D., G. W. Benz, I. H. Carmichael, and S. J. Gross. 1990. Efficacy of

ivermectin delivered from an intraruminal sustained-release bolus against natural infestations of five African tick species on cattle. *Veterinary Parasitology* **37**: 285–296.

Standen, O. D. 1953. Experimental schistosomiasis III. *Chemotherapy and mode of drug action*. *Annals of Tropical Medicine and Parasitology* **46**: 26–45.

Standen, O. D. 1963. Chemotherapy of helminthic infections. In R. J. Schnitzer and F. Hawking (eds.) *Experimental chemotherapy*. Academic Press. New York, N.Y. p. 701–892.

Stapley, E. O., and H. B. Woodruff. 1982. Avermectins, antiparasitic lactones produced by *Streptomyces avermitilis* isolated from a soil in Japan. In *Trends in antibiotic resistance*. H. Umezawa, A. L. Demain, T. Hata, and C. R. Hutchinson. (eds.) Japan Antibiotics Research Association. Tokyo. p. 154–170.

Vanden Bossche, H. 1986. Mode of action of anticestodal Agents. In W. C. Campbell and R. S. Rew (eds). *Chemotherapy of parasitic infections*. Plenum Press, New York, N.Y. p. 495–503.

Vercruyssse, J., and R. S. Rew. 2002. *Macrocyclic lactones in antiparasitic therapy*. CABI Publishing, Wallingford, U.K. 412 p.

Webbe, G. 1986. Cestode infections of man. In W. C. Campbell and R. S. Rew (eds.) *Chemotherapy of parasitic infections*. Plenum Press, New York, N.Y. p. 457–477.

Woods, D. J., S. J. Maeder, A. P. Robertson, R. J. Martin, T. G. Geary, D. P. Thompson, S. S. Johnson, and G. A. Conder. 2012. Discovery, mode of action, and commercialization of derquantel. In C. R. Caffrey (ed.) *Parasitic helminths: Targets, screens, drugs and vaccines*. Wiley-Blackwell, Oxford, U.K. p. 297–308.



# **Chapter 15**

## **Anthelmintic drug discovery: Into the future**

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As the great New York Yankee catcher Yogi Berra is reputed to have observed, "It's tough to make predictions, especially about the future." The economic and health benefits of chemotherapeutic control of parasitic infections of human and veterinary importance have been enormous and are undeniable, but many challenges remain to be overcome in the continuing effort to discover new agents with improved performance (efficacy, safety, and convenience), especially in the face of widespread drug resistance in many parasites in many hosts. This review offers some perspective on the factors that will influence drug discovery for these infections in the next decades. Time will truly tell how insightful these considerations are.

In the United States, for example, interest and investment in antiparasitic drug discovery and development was initially driven in the early-to-mid-twentieth century by concerns for human health, e.g., the Rockefeller Sanitary Commission's campaign versus hookworm and efforts to maintain the health of military personnel in war zones that were endemic for many parasites (especially malaria). Improvements in sanitation, housing, and public health reduced the incentive for western governments to support antiparasitic drug discovery programs for human pathogens (except in the military context), and the realization that antiparasite chemotherapy provided economic value in veterinary practice (both livestock and companion animals) shifted the major discovery portfolios to animal health indications. During this period (roughly 1950–1995), the World Health Organization, through the Tropical Diseases Research arm, provided the largest non-military funding source for research into the chemotherapy of human parasitic diseases. However, investments from non-government organizations, spurred by the Bill and Melinda Gates Foundation, the Wellcome Trust, the Drugs for Neglected Diseases Initiative, among others, changed the landscape again by devoting resources to the discovery, development, and deployment of drugs for human tropical diseases, including malaria and systemic helminthiases. This shift was accompanied by (but unrelated to) a dramatic consolidation in the animal health industry, which has noticeably reduced the global investment into

antiparasitic drug discovery for veterinary indications (Geary et al., 2004; Woods et al., 2007). While it is possible that there will continue to be adaptation of veterinary drugs for human use, the “worm” appears to be turning, and a model that incorporates simultaneous development in both domains may become the standard. In any case, the question remains: where do we go from here?

Drug discovery in the parasite realm has obviously evolved over the past century. We can confidently predict that processes in this area will continue to evolve, and almost certainly at a faster pace. Whether the new strategies will deliver the quality and quantity of drugs provided in the past remains to be seen.

# The future of drug discovery for parasitologists

It is perhaps surprisingly difficult to pin down the current cost of bringing a new drug to market. Estimates from the early 2000s are that it took up to 10–15 years and \$1.2 billion to develop a drug (Pharmaceutical Research and Manufacturers of America, 2013). A more recent estimate is \$5 billion, with a success rate of only 5% for compounds introduced into development (Herper, 2013). Clearly, the road to developing a new drug is long and arduous. It should be noted that so few new antiparasitic drugs have been introduced in the past 20 years that it is not possible to determine if the costs of discovery and development of such drugs are lower, higher, or the same as those for drugs intended for indications common in countries in which patients are better able to pay for the medicines. It is unsurprising that companies are generally more willing to risk investments in compounds that can lead to more lucrative products than antiparasitic drugs. In that context, should we expect this process to become more efficient and so less expensive in the future in the realm of antiparasite chemotherapy?

Where do parasitologists fit into this scheme of drug discovery and how can we contribute to finding new drugs to treat parasitic diseases that have devastating impacts on millions of lives? Answers to these questions must be based on an understanding of the sources of current and past antiparasitic drugs, the opportunities presented by new technologies, and gaps that impede progress.

Three general strategies have been employed for the discovery of antiparasitic drugs. The most successful paradigm in history has been to treat animals infected with parasites with experimental compounds and measure consequent changes in parasite burdens after necropsy or in biofluid samples (see Geary and Thompson, 2003). Hosts were definitive for the parasite species used, but models of human or veterinary relevance were almost always employed. This paradigm delivered at least the prototype of almost every important antiparasitic drug in use today. However, screens in infected animals tend to be labor-, time-, and compound-intensive and have largely been abandoned in favor of more economically appealing procedures. As an alternative, parasites (or model organisms) in culture were adopted as a screening strategy, measuring viability or behavior as an endpoint (so-called “phenotypic” screens). This strategy identified valuable new antiparasitic drugs or drug candidates, beginning perhaps with the benzimidazole anthelmintics. Interestingly, recent progress in analyzing viability and behavior with robotics in small volumes (“high content screening”) has made this option much more feasible from a modest to high-throughput perspective (e.g., Nwaka et al., 2011). However, it is important to recognize that tens of millions of test compound-whole organism exposures have been carried out in industrial and academic settings, and the rate of discovery of new drugs has been very low from these assays, compared to those using infected animals. Perhaps this situation will be reversed as the fruits of more recently developed phenotypic screens with parasites of human importance proceed through development. Prospects for

success would be much enhanced by cooperation among the various groups pursuing this strategy; development of on-line databanks that display positive and negative screening data would focus efforts on the most promising compounds by revealing promiscuous actors, and facilitate the identification of new opportunities and decrease redundancy. This type of computational platform needs parasitologists for validation and annotation and is an excellent example of new horizons for students in this field.

The third wave of screens was based on advances in molecular biology and material handling platforms, and energized a massive strategic switch in the pharmaceutical industry in the 1990s from phenotypic or whole organism screens to very high-throughput mechanism-based screens, which identify compounds that affect specific protein targets rather than a biological endpoint. Although little investment has been devoted to mechanism-based screens in parasitology compared to other areas (see Geary, 2012a), it cannot be denied that no compound discovered in a mechanism-based screen has progressed to the clinic. Too few data are available to allow an in-depth analysis of why mechanism-based approaches have not yet succeeded in parasitology (Crowther et al., 2014). Many challenges have limited the ability to conduct mechanism-based screens for antiparasitic drugs, including difficulty in obtaining functional expression of some parasite proteins in assay-friendly formats and extrapolating activity in protein-based assays to whole-organism screens. It remains to be seen if the current dearth of hits derived from these strains reflects poor choice of targets, insufficient investment or a fundamental flaw in the strategy. We believe that the concept remains valid, but that more work, more wisely chosen targets, and the development of more predictive whole organism and infected animal models are needed for success. This approach clearly remains fertile ground for parasitology in the future. Learning more about the fundamental biology, biochemistry, and physiology of parasites can reasonably be expected to lead to more effective drug discovery efforts (Thompson et al., 1996; Geary et al., 1999).

Based on expertise and interest, we focus our considerations of future prospects in antiparasitic drug discovery on anthelmintics. Current programs in anthelmintic discovery operate in both industrial and academic settings; the former focus primarily on discovery of new drugs for veterinary indications, especially gastrointestinal nematodes of livestock animals and the canine heartworm, *Dirofilaria immitis* (Woods et al., 2007, 2011; Woods and Knauer, 2010). In contrast, discovery efforts in academic settings focus more on parasites of human importance, especially schistosomes and filarial nematodes (Caffrey, 2012). Historically, anthelmintics for human use were adapted from approved veterinary drugs (with the exception of diethylcarbamazine, DEC; Geary and Mackenzie, 2011). This situation is likely to change as consolidation in the animal health pharmaceutical sector continues and efforts to discover drugs for neglected tropical diseases (NTDs) accelerate in academic and other non-industry labs.

Like all drug discovery efforts, anthelmintic discovery is at its heart an iterative

process, beginning with screening large compound libraries (diversity collections or scaffold-specific collections) using either high-throughput mechanism-based or phenotypic screens. Because helminth cell lines that express target ion channels or receptors in formats suitable for high-throughput screening remain unavailable, phenotypic screens in this area employ whole organisms. Active compounds (“hits”) in these screens are initially identified by meeting a defined level of activity or efficacy, defined as percentage activation or inhibition of a protein function or percentage change in whole organism behavior or viability, respectively. Hits are further characterized in whole organism screens, with prioritization for further study based on a combination of chemical quality, efficacy, potency, and safety (mammalian cell cytotoxicity is a very preliminary starting point, but must be followed by toxicity tests in animals to obtain reliable estimates of safety). A subset of hits with desirable characteristics is then submitted to secondary screens in infected animal models. Pharmacokinetic properties, i.e., adsorption, distribution, metabolism, and excretion (ADME) and toxicity studies are conducted in parallel in these models to determine if any hit should be considered a “lead,” suitable for in-depth investment in medicinal chemistry and pharmacology. This process is an iterative one in which each step entails go/no-go decisions before a compound is entered into clinical trials to determine dose ranges and preliminary assessment of safety and efficacy (Pink et al., 2005). As each step requires progressively more investment, it is essential to employ quality checks to prevent wasting resources on hopeless compounds. Such “false positive” hits consume considerable resources to discount, but are eventually discarded as they fail to meet crucial go/no-go criteria (see Baell and Walters, 2014).

A more important goal is to limit cases of false negatives, i.e., failing to detect compounds with real value. A presumed benefit of mechanism-based approaches is that the incidence of false negatives is very low; the initial assay detects the ability of a compound to interact with a target in the absence of permeability issues of any sort that could limit the interaction. Some hits in these screens could in theory fail to reach necessary concentrations inside a whole organism (e.g., Thompson et al., 1993; Ho et al., 1994; Burns et al., 2014; Zhou et al., 2014) and so would not be detected in a phenotypic screen. Based on affinity and chemical quality, such hits could warrant additional research to determine why they are inactive in a phenotypic assay, potentially leading to the synthesis of compounds with improved pharmacology. Balancing this is the fact that phenotypic, but not mechanism-based, strategies offer the chance of serendipity, i.e., finding compounds that are effective through unknown or unanticipated mechanisms. Consideration of these two strategies and the roles they will play in the future is the major aim of this paper.

# Phenotypic screens

In the absence of a specific drug target, screening live worms has its advantage since in most cases, the targets are not known *a priori* and compounds can be effective on one or many targets in a living worm (Eggert, 2013; Barrett and Croft, 2014). Screening living worms *in vitro* with compounds nonetheless has its challenges. To design a robust, medium- to high-throughput screen requires knowledge of the basic biology of the parasite, and this area is one in which parasitologists are essential contributors.

Several factors must be taken into consideration when assaying worms *in vitro*, including the length of time for the assay (from hours to days), which life history stages can be maintained in culture (larval hatching, larvae vs adults), the size of the worms (think adult *Ascaris* sp. or adult *Dirofilaria* sp.), which species are available and amenable to *in vitro* assays, and the assessment of efficacy (readouts, e.g., physiological assessments for viability or phenotypic changes such as motility).

## Current methods

How can one assess drug efficacy in worms and how sensitive and quantitative are the methods?

With the aid of a microscope, the human eye is very good at making assessments of worm viability, e.g., intestinal content movement, cuticle tanning, whole worm motility, but this method of evaluation is inherently subjective and time-consuming. A biochemical technique for assessing worm viability is the MTT assay, which is a colorimetric method used to assess cell viability. Cellular oxidoreductases convert the tetrazolium dye 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to formazan, which yields a purple color when solubilized. The intensity of color reflects viability, and the readout can be made in a subjective manner or, more quantitatively, with a spectrophotometer. This method is often used in conjunction with microscopy, but is also time consuming and not suitable for high-throughput screening. Other recent screening assays developed for single parameter read-outs include the use of Alamar Blue (Mansour and Bickle, 2010), isothermal microcalorimetry (Manneck et al., 2011), and fluorescence (Peak et al., 2010).

## New tools: Video imaging and computer software engineering

From work stemming largely from the community of scientists using *Caenorhabditis elegans* as a model organism, a number of assays were developed that utilized an imaged-based system and computer software technology to track worm movements in various assays (Geng et al., 2004; Ramot et al., 2008; O'Reilly et al., 2014). The literature is vast and rapidly expanding, and it is not our intention to provide a comprehensive review of all the worm assays (for a review of automated imaging systems for whole worm assays used in anthelmintic

screening, see Paveley and Bickle, 2013). Here, we cite a few notable articles that highlight the progression of technologies.

Buckingham and Sattelle (2009) used an algorithm and imaging system to measure the effects of levamisole on *Haemonchus contortus*, which displayed contortions while swimming after exposure to the drug. Later, Smout et al. (2010) developed a high throughput assay using the cell monitoring device xCELLigence to assess efficacy of anthelmintics on *H. contortus* eggs and larvae, larval *Strongyloides ratti*, and adults of *Ancylostoma caninum* and *Schistosoma mansoni* in an automated system. For larger worms (>1 mm in length), a low cost system (<US \$3,000) was developed that records and quantifies individual worm movements on an entire 24-well plate (Marcellino et al., 2012). Image analysis to record time- and concentration-dependent multi-parametric responses of schistosomes has been developed (Singh et al., 2009, 2012; Lee et al., 2012; Paveley et al., 2012; Rojo-Arreola et al., 2014). Singh and colleagues have also developed the “QDREC” server that uses a combination of biological imaging and supervised machine learning to automatically measure the quantal response characteristics of macroparasites in phenotypic drug screening (Asarnow et al., 2014). Importantly, QDREC is not computationally complex to operate and requires only a camera to be connected to a microscope and computer together making it ideal for laboratories with modest means to pursue quantitative multi-parametric drug discovery.

## Microfluidics and a lab-on-a-chip

Recently, innovative tools have taken advantage of microfluidics to assess worm movement. Microfluidics combines the study of fluid behaviors in microchannels and the technology of manufacturing of devices such as lab-on-a-chip to manipulate very small (nanoliter to picoliter) volumes of fluids inside very small channels (micrometer sized). Microfluidic technology is precise and automated and, therefore, applicable to drug discovery for cost-efficient and ultra-high-throughput assays using biological materials that are limited in supply (Dittrich and Manz, 2006; Whitesides, 2006). In addition to drug discovery, this innovative technology platform is also of burgeoning use in point-of-care diagnostics and drug delivery for therapeutics.

Because microfluidic devices employ small amounts of reagents and analytes, only small amounts of compounds need to be synthesized. These systems may be particularly amenable for small worms and assessing drug effects on various phenotypes including neurophysiological and behavioral responses (Carr et al., 2011; Chung et al., 2011; Lockery et al., 2012; Lycke et al., 2013).

New technology has not only revolutionized the “lab-on-a-chip” concept, but has also recently “democratized” the use of the microscope: the “Foldscope” ([www.foldscope.com](http://www.foldscope.com); Cybulski et al., 2014) is a novel scientific tool that is accessible everywhere. The Foldscope is cheap, is made of paper, and can be

assembled in minutes. It has no mechanical moving parts, is the size and shape of a letter-sized envelope, robust, is lightweight, and can be used for bright-field and multi-fluorescence or as a projection microscope. With the advent of the Foldscope, it seems highly likely that an ultra-low-cost and higher throughput screening method for microscopic helminths can be run in endemic areas, providing more opportunities for parasitologists around the world.

## Parasite cultures for drug discovery and stem cell technology

Because most parasitic worms have complex life histories and are often host-specific, few helminth species are available for *in vitro* screening. For those that are available, only certain stages can be used in drug screening assays. We owe much of what we know about culturing worms to authors whose papers were first published in *The Journal of Parasitology*. To list a few: for schistosomes, Paul F. Basch (1981a, 1981b, Basch and Humbert, 1981) regarding his multi-year effort to define culture conditions suitable for schistosomes, and Fred A. Lewis et al. (1986); for filariid nematodes, Lawrence R. Ash and John M. Riley (1970), John W. McCall et al. (1973), David Abraham et al. (1987), Nancy Wisnewski and Paul P. Weinstein (1993), C. L. Smillie et al. (1994), and J. A. Yates et al. (1994). These papers have contributed greatly to our ability to screen drugs against helminths *in vitro*. It should be noted that these papers were published 20–40 years ago, and without these basic studies on the development of culture methods, we would essentially have to start *de novo*, investigating media, growth conditions, etc. All of this is to say that the field of parasite cultivation is like an oyster. This area of research can provide pearls for a parasitologist (even a gold mine), e.g., by exploring new ways to cultivate life history stages *in vitro*, developing methods to assess viability and developing methods that can provide target parasite species in mass quantities to study cell biology, biochemical pathways, and mechanisms of pathogenesis all remain major goals of our field.

Although the ability to culture target parasites for at least a few days or weeks now permits us to perform large-scale anthelmintic discovery projects, these efforts remain limited by the need to maintain infected animals as sources of parasites, adding greatly to the costs of screening. Another limitation is that most anthelmintic therapy is targeted at adult stages or larvae derived from vectors; these are typically available in very limited numbers, and researchers often are forced to use more readily available stages as surrogates, e.g., eggs or L<sub>3</sub> larvae of GI nematodes, microfilariae, or the schistosomulum stage of schistosomes. A very important goal for parasitology researchers is to define how well non-target stages mimic the proteomic/transcriptomic landscape of target stages. If gene expression and protein levels differ markedly among stages, the specter of false negatives in screens employing non-target stages may off-set the advantages of convenience and cost, forcing a reconsideration of the strategy for phenotypic screens for new anthelmintics.

The use of the free-living model nematode *C. elegans* for the discovery of

anthelmintics began more than 30 years ago; this species' ease of use makes it an ideal subject for relatively high-throughput screens for compounds that alter survival, reproduction and behavior (Geary and Thompson, 2001). We estimate that tens of millions of compounds, either in pure synthetic form or in fermentation broths, were phenotypically screened through use of *C. elegans*. Surprisingly, no compound discovered in a *C. elegans* screen has been commercialized or even entered into advanced development, as far as is known. Indeed, this failure was one of the motivating factors to develop mechanism-based strategies (Geary, 2012a). Recent research has provided a possible explanation for this failure, namely, that *C. elegans* has evidently evolved the ability to limit accumulation of xenobiotics to a remarkable degree, preventing the consequences of exposure (Burns et al., 2010). This phenomenon was observed with compounds that are highly bioavailable in mammals and does not seem to extend to adult stages of parasitic nematodes (Thompson et al., 1993). The implications of this situation include the fact that, insofar as the vast majority of phenotypic anthelmintic screening has been done with *C. elegans*, we can conclude that we have actually sampled very little of chemical space for this purpose, and phenotypic screens employing parasites may be expected to have much better outcomes. The implications also indicate that research into the mechanisms by which *C. elegans* limits exposure to xenobiotics should be a high priority; such an effort may lead to important insights into drug resistance mechanisms in parasites. Finally, it would be highly valuable to investigate the utility of permeability mutants of *C. elegans* for screening (e.g., Kage-Nakadai et al., 2010), an approach that might overcome the poor accumulation phenotype of wild-type strains.

Recent developments are particularly exciting in the area of parasite stem cell biology. Germinal cells of digenetic trematodes and cestodes give these parasitic helminths the ability to reproduce asexually. A major breakthrough in the cultivation of cells from parasitic helminths is the *in vitro* system developed for *Echinococcus multilocularis* in which primary germinal cells are capable of generating metacestode vesicles under laboratory conditions (Spiliotis et al., 2008; Brehm, 2010; Koziol et al., 2014). Neoblast-like proliferating somatic cells were identified from adult *S. mansoni* (Collins et al., 2013; Wang et al., 2013) that are similar to planarian neoblasts, the cells that give free-living flatworms the ability to regenerate injured tissue and develop into a new worm from a single cell. Short-term (~7 days) primary cell cultures derived from *C. elegans* and *Brugia malayi* offer hope for developing immortal cell lines from parasitic nematodes (Higazi et al., 2004; Zhang et al., 2011). These lines, if developed, could be used to screen for compounds that selectively kill helminth vs. mammalian cells. Alternatively, they could be developed into recombinant expression systems to permit the heterologous expression of helminth ion channels and receptors for high-throughput, cell-based targeted screens. It is difficult to fund research on these kinds of studies, but developments in this area would revolutionize our ability to understand helminth biology, and are worthy goals for parasitologists in

the future.

Future explorations for parasitologists may entail combining the technologies of microfluidics, micro-engineering, and parasite cell culture. Just as “organs-on-a-chip” and “humans-on-a-chip” have been developed for use in drug discovery as alternatives to animal and clinical studies (Selimović et al., 2013; Luni et al., 2014), “parasites-on-a-chip” may offer similar advantages. It may not be too long before parasitologists develop novel platforms of fabricated organ-like structures from worms to simulate physiological features and mechanics of entire worms on which drugs can be screened and the fundamental nature of parasites can be studied. It is difficult to even imagine what new technologies will be developed in the next decades, but it is easy to see that this is the dawning of another great age in parasitology research.

# Mechanism-based screens

The switch to mechanism-based, high-throughput screening strategies was driven by cost concerns (labor, time, reagents, and chemical amounts) as well as frustration with the lack of success of phenotypic anthelmintic screens (Geary, 2012a). The strategy was based on the concept that investment of chemistry resources (the rate-limiting component of drug discovery) could be made with more confidence for compounds with a known mechanism of action than with an unknown target (Geary et al., 2009; Geary, 2012a). In theory, the strategy should work better for chemotherapeutic indications than for more complex clinical syndromes such as diabetes, mental illnesses or obesity, which are unlikely to be attributable to malfunctions in single proteins.

However, reality often poorly supports theory, and many attempts to identify new anthelmintics through mechanism-based screens have not returned the hoped-for results. What have we learned from these exercises and what does the future hold in this area?

## Rethinking the “one gene-one drug” paradigm

Identifying single genes important to maintaining disease states and against which drugs can be designed has been the cornerstone of drug discovery and development programs for the last 30 years. Although successful in yielding some important drugs, a growing body of opinion holds that a re-think of the “one gene, one drug, one disease” paradigm (Zimmermann et al., 2007; Hopkins, 2008; Xie et al., 2012) is required if drug development is to be sustained. For every success story, e.g., statins versus HMG-CoA reductase to treat hypercholesterolemia and odanacatib versus cathepsin K to treat osteoporosis, the drive to achieve single-target specificity has resulted more often in increasingly more expensive failures due to suboptimal modulation of disease states (Butcher et al., 2004; Hopkins, 2008). Although compatible with entrenched medicinal chemistry design and appealing to sometimes parochial leadership paradigms, a continued focus on discovery of drugs that interfere with single targets is both scientifically and financially questionable in the long run.

In hyper-complex biological systems, it is rare for drugs to bind to just one protein. Off-target interactions are often involved and the literature is replete with retrospective analysis of marketed drugs, initially designed for single target specificity, subsequently being shown to interact with more than one protein and in ways relevant to: (1) achieving the therapeutic outcome, (2) generating unwanted side effects, and (3) demonstrating additional therapeutic indications (Hopkins et al., 2006; Hopkins, 2008; Xie et al., 2012). For example, a number of protein kinase inhibitors, originally designed with single-specificity in mind, e.g., imatinib versus BCR-ABL, modulate several targets that account for a diversity of anti-cancer benefits (Ghoreschi et al., 2009; Knight et al., 2010; Chamrad et al., 2013). Even for the statins, there is growing evidence that off-target binding

contributes to a cardiovascular benefit beyond the original goal of lowering circulating cholesterol via the “specific” targeting of HMG-CoA reductase (Lim et al., 2014). For anti-infectives, the goal of single-target specificity is nearly always met by an organism’s ability to compensate via resistance, as is now patently obvious in the animal health sector for anthelmintics, e.g., benzimidazole and macrocyclic lactone classes, a problem of real concern regarding the sustainable control of trematode and soil-transmitted nematode infections in humans.

To improve pharmacological action and limit the development of resistance to single chemical entities (Zimmermann et al., 2007), current clinical practice, including in the treatment of cancer, diabetes, and infectious diseases, calls for combining drugs. AIDS tri-therapy (two reverse transcriptase inhibitors and one protease inhibitor) is a case for which drug combinations are now at the state-of-the-art. A similar situation is standard practice for the treatment of tuberculosis. For NTDs, the problem of resistance together with the constrained economic environment has led to the predominance of combinations of established drugs in the late preclinical and clinical development phases of the major product development partnerships, including the Medicines for Malaria Venture and the DNDi. This strategy is exemplified by the WHO-driven requirement that artemisinin derivatives be ONLY administered in combinations for the treatment of falciparum malaria. For veterinary anthelmintics, the value of drug combinations, even involving single agents to which resistance is established, has been repeatedly shown (see Bartram et al., 2012; Leathwick, 2013). Yet, combining, or strategically rotating drugs, or both, requires careful end-user (farmer) management and implementation, and repeated shortcomings in this regard have facilitated the spread of drug resistance. In addition, the development of new combination drugs is not trivial, as each component and the combination have to be demonstrated as safe and effective (Geary et al., 2012a).

## **“Worm bottlenecks” and the emergence of new tools and opportunities for drug development**

At first blush, the cycle of anthelmintic development followed by drug resistance in veterinary medicine could be seen as a fountain of products from which new drugs might be translated. The reality is, however, that few veterinary drugs translate quickly to human medicine, for many reasons, including lack of an economic incentive to drive registration for human use and concerns about spectrum of efficacy and toxicity. This situation is compounded by the enormous costs involved, exacerbated in the post-blockbuster drug era of consolidation within the pharmaceutical industry, which has resulted in the spinning off or down-sizing of animal health businesses and the concomitant loss of parasitology expertise and capital to invest in new products.

The consequences of these difficulties are that the control of helminth infections in humans relies on a handful of drugs developed long ago by the animal health sector that target a limited set of structural proteins or ion channels (Caffrey,

2012). Furthermore, these compounds are deployed as single doses in mass drug administrative (MDA) programs and are intended to alleviate morbidity and not necessarily cure individuals or eliminate parasites from the population. For example, albendazole, used in standard mass drug administration treatment for GI nematode infections, displays highly variable efficacy against the three main GI nematodes, i.e., *A. lumbricoides*, hookworm, and *Trichuris trichiura*, being essentially sub-curative against the latter two in the regimens employed in the field (Keiser and Utzinger, 2008). One must question the long-term sustainability of this “make do” deployment of what are likely sub-curative monotherapies of drugs for which resistance is now very well established in veterinary settings (Geary et al., 2011; Kaplan and Vidyashankar, 2012) and is an emerging concern in human parasites (Geary et al., 2010; Osei-Atweneboana et al., 2011; Vercruyse et al., 2011; Geary, 2012b; Diawara et al., 2013; Hawdon, 2014).

Encouragingly, over the last 10 years, the development of new tools and technologies (see previously) in academia, supported by both public and private sector funding (and in-kind support), has begun to fill the gap remaining after the condensation and re-organization of the animal health industry in generating new drug targets and candidate anthelmintics. At a fundamental level, drug target identification is facilitated by a range of powerful and “democratic,” i.e., fully public, *in silico* tools, such as relational and interrogable databases that present genomic, transcriptomic, and functional data to aid the selection of putative targets. Sites such as [Nematode.net](#), [Nematodes.org](#), and [Wormbase.org](#) offer a plethora of “mining” tools with which new targets can be identified for experimental and chemical validation. Other databases with a more medical focus and covering a larger spectrum of pathogen species, like [TDRtargets.org](#) and [EuPAthDB.org](#), also offer a range of intuitive tools with which intersectional and union-based queries can be developed to prioritize potential targets. Orthogonal phenotypic response data derived from heritable or non-heritable genetic manipulations in tractable model organisms such as *C. elegans* continue to provide valuable information relating to a putative target's “worthiness.” Although these interrogable sites (Aguero et al., 2008; Caffrey et al., 2009; Crowther et al., 2010) center on the identification of single potentially “druggable targets” based on uniqueness to the organism or shared “essentiality” across organisms (see discussion previously), they are nonetheless an important step forward allowing the broader community access to new ideas and strategies.

These resources will continue to grow in depth and value for the foreseeable future as the cost of whole genome sequencing falls and proteomic and RNA sequencing platforms become more sensitive and accessible. For example, emerging localized heritable drug resistance events can be quickly detected to provide an early warning to veterinary and medical practitioners and policy makers. In addition, quantitative phenotypic metrics (Mansour and Bickle, 2010; Lee et al., 2012; Asarnow et al., 2014; Rojo-Arreola et al., 2014) generated in response to chemical or genetic modulation of proteins and targets will provide an additional layer of

orthogonal information and develop to the point where phenotypic response “signatures” comprising multi-parametric data can be used to associate and cluster different chemistries to the same target(s). Excellent progress to be sure, but fundamental hard-to-crack bottlenecks remain in how drug development moves beyond the discovery phase, not least the problems of worm acquisition and culture, and the lack of small animal infection models as noted elsewhere in this review.

Academia (via single investigator initiated programs) has traditionally been strong in identifying and validating targets for drug discovery. This strength has been augmented over the last 10 years with the establishment of specific academic screening centers and/or consortia (e.g., the Broad Institute, the NIH TRND program, the UCSF Small Molecule Discovery Center) to prosecute the actual business of small molecule screening and lead optimization, including for the NTDs. Importantly, this expanded academic activity has caught the attention of the pharmaceutical industry, which, under its own pressures to develop and maintain drug pipelines, has more willingly collaborated with academia to pursue drug discovery in all major therapeutic areas. In addition, many large companies have portals through which requests for know-how and material support can be made, and many companies offer seed funding mechanisms through universities to develop novel paradigms for drug (small molecule and protein-based) development. For NTDs, malaria and TB, a key player in transducing academic-industry partnerships over the last 3 years has been The World Intellectual Property Organization (WIPO) Re:Search consortium ([www.wipo.int/research/](http://www.wipo.int/research/)) through which academia supplies the intellectual heft in the identification, validation and/or screening of targets (or whole organisms) and industry makes available chemical libraries, screening resources and expertise to accelerate the selection and development of small molecules. As the consortium administrator, BIO Ventures for Global Health (BVGH) helps forge these collaborations. The WIPO Re:Search membership now includes large and small pharmaceutical companies, universities, non-profit research institutions and product development partnerships. Of the current 60 research agreements between consortium members (as of July 2014), 13 focus on anthelmintic discovery. Key principles under WIPO Re:Search membership are that members will provide a royalty-free license for any product developed for use in the least developed countries and that members will provide royalty-free licenses for malaria, TB, and NTD R&D.

## Polypharmacology as a drug development strategy

In some medical fields, e.g., anti-cancer therapy, the idea of combination therapy (see previously) has evolved still further and is based on improved understanding of appropriate, high-value protein target networks or pathways. The rationale is that modulating the activity of multiple targets can achieve an optimal therapeutic effect by overloading the system's (cell, tissue, or organism) ability to invoke

compensatory mechanisms (Zimmermann et al., 2007; Hopkins, 2008). The modalities now being explored include the design of single drugs that: (1) interfere with a restricted set of molecular targets (“selective polypharmacology”; Morphy et al., 2004; Paolini et al., 2006; Hopkins, 2008; (2) modulate one target to facilitate activity at a second target; and (3) bind to separate positions on a target to increase pharmacological action (Zimmermann et al., 2007). Complementary to these ideas is the re-tooling of “promiscuous” drugs to restrict binding to a more limited number of targets, thus better controlling for toxicity as a result of off-target (bystander) effects (Fernandez et al., 2009; Liu et al., 2013). Another approach is the methodical combination of chemical substituents or structural elements into one entity that provides the required multi-target action. This can be the conjugation of two active pharmacophores, e.g., trioquaquines that combine a 1,2,4-trioxane and a 4-aminoquinoline (Portela et al., 2012) or trioxaquantels that combine a 1,2,4-trioxane with praziquantel (Laurent et al., 2009) to more sophisticated drugs that possess various degrees of overlapping elements essential for recognition of different targets (see Morphy et al., 2004); examples of the latter approach are available for enzymes, including proteases (Morphy et al., 2004; Choy et al., 2013) and peptidergic G protein-coupled receptors (GPCRs) (Morphy et al., 2004).

An example of a polypharmacology strategy for anthelmintic discovery is found in a high-throughput, yeast-based screen for non-peptide agonists or antagonists of nematode peptidergic GPCRs; the goal was to specifically discover promiscuous ligands, which acted on multiple GPCRs in the FMRFamide-related peptide family to enhance spectrum and limiting the development of receptor-mediated drug resistance (Geary et al., 2012b). A greater appreciation for the value of compounds that act on multiple related targets could focus discovery efforts on drugs that “resist resistance.”

## **Looking ahead from behind: The lessons learned**

The past informs us that our parochial arrogance holds us back from recognizing that there is not only one right way to approach complex issues such as drug discovery. Each successful case has its own history. Serendipity, a keen eye, an agile mind and a willingness to make data-based decisions are all characteristics of successful drug discoverers. For scientists devoted to drug discovery, the most important outcome is to find a good candidate. Many paths to that end exist.

It is also clear that teams of scientists must work together in this endeavor of rare successes. Assay science and medicinal chemistry are always important; for the discovery of antiparasitic drugs, talented parasitologists are an undeniable requirement for such a team. Detailed knowledge of host-parasite models, of real-world parasitology, and of the deep biology of parasites contributes to the choice of targets and how they are evaluated; this knowledge and experience is also crucial for properly valuing new compounds against the landscape of existing drugs (and resistance to them).

It is clear that we have not invested enough on research into basic parasite biology. Consequently, we know too little about how parasites work as organisms and about how they interact on a molecular level with their hosts to “design” new agents. In this light, one could argue that parasites in culture offer an incomplete set of targets, since the physiological processes employed by parasites to establish a successful infection in the host (virulence factors) cannot be addressed in a petri dish. *C'est la vie*; drug discovery parasitologists must lead the way in making the best choices possible about targets and leads given the available data and tools, and proceed as quickly as possible to a decision about any of them. We do not envision a world without parasites of humans or domestic animals in the foreseeable future. Parasitologists are needed to ensure that the current slow pace of progress in the discovery of new drugs to limit the consequences of parasitism is accelerated.

A final perspective is appropriate. An area of basic research that would completely revolutionize our understanding of host–parasite relationships and provide an unprecedented ability to discover drugs is the development of egg-to-egg culture systems for parasitic helminths. As noted in this review, we are limited by the availability of the optimal life cycle stages of these parasites for drug discovery and by the difficulty of assessing drug action at the host-parasite interface. An egg-to-egg culture system would enable facile functional genomics in these organisms to allow us to validate drug targets in parasitic species and to identify virulence factors that are essential for parasitism. Although it may not be easy to fund such studies in the current fiscal climate, this is only one example of fundamental research that demands attention from enterprising parasitologists who want to change the world.

## Literature cited

- Abraham, D., M. Mok, M. Mika-Grieve, and R. B. Grieve. 1987. *In vitro* culture of *Dirofilaria immitis* third- and fourth-stage larvae under defined conditions. *Journal of Parasitology* **73**: 377–383.
- Aguero, F., B. Al-Lazikani, M. Aslett, M. Berriman, F. S. Buckner, R. K. Campbell, S. Carmona, I. M. Carruthers, A. W. Chan, F. Chen et al. 2008. Genomic-scale prioritization of drug targets: the TDR Targets database. *Nature Reviews Drug Discovery* **7**: 900–907.
- Asarnow D., L. Rojo-Arreola, B. M. Suzuki, C. R. Caffrey, and R. Singh. 2014. The QDREC Webserver: Determining dose-response characteristics of complex macroparasites in phenotypic drug screens. *Bioinformatics* doi: 10.1093/bioinformatics/btu831. First published online: December 24, 2014.
- Ash, L. R., and J. M. Riley. 1970. Development of *Brugia pahangi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. *Journal of Parasitology* **59**: 962–968.
- Baell, J., and M. A. Walters. 2014. Chemistry: chemical con artists foil drug discovery. *Nature* **513**: 481–483.
- Barrett, M. P., and S. L. Croft. 2014. Emerging paradigms in anti-infective drug design. *Parasitology* **141**: 1–7.
- Bartram, D. J., D. M. Leathwick, M. A. Taylor, T. Geurden, and S. J. Maeder. 2012. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. *Veterinary Parasitology* **186**: 151–158.
- Basch, P. F. 1981a. Cultivation of *Schistosoma mansoni* *in vitro*. I. Establishment of cultures from cercariae and development until pairing. *Journal of Parasitology* **67**: 179–185.
- \_\_\_\_\_. 1981b. Cultivation of *Schistosoma mansoni* *in vitro*. II. Production of infertile eggs by worm pairs cultured from cercariae. *Journal of Parasitology* **67**: 186–190.
- \_\_\_\_\_, and R. Humbert. 1981. Cultivation of *Schistosoma mansoni* *in vitro*. III. Implantation of cultured worms into mouse mesenteric veins. *Journal of Parasitology* **67**: 191–195.
- Brehm, K. 2010. *Echinococcus multilocularis* as an experimental model in stem cell research and molecular host-parasite interaction. *Parasitology* **137**: 537–555.
- Buckingham, S. D., and D. B. Sattelle. 2009. Fast, automated measurement of nematode swimming (thrashing) without morphometry. *BMC Neuroscience* **10**: 84–90.

- Burns, A. R., I. M. Wallace, J. Wildenhain, M. Tyers, G. Giaever, G. G. Bader, C. Nislow, S. R. Cutler, and P. J. Roy. 2010. A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nature Chemical Biology* **6**: 549–557.
- Butcher, E.C., E.L. Berg, and E.J. Kunkel. 2004. Systems biology in drug discovery. *Nature Biotechnology* **22**: 1253–1259.
- Caffrey, C. R. 2012. *Parasitic helminths: targets, screens, drugs and vaccines. Drug discovery in infectious diseases*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 516 p.
- Caffrey, C. R., A. Rohwer, F. Oellien, R. J. Marhöfer, S. Braschi, G. Oliveira, J. H. McKerrow, and P. M. Selzer. 2009. A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen, *Schistosoma mansoni*. *PLoS One* **4**: e4413.
- Carr, J. A., A. Parashara, R. Gibson, A. P. Robertson, R. J. Martin, and S. Pandey. 2011. A microfluidic platform for high-sensitivity, real-time drug screening on *C. elegans* and parasitic nematodes. *Lab on a Chip* **11**: 2385–2396.
- Chamrad, I., U. Rix, A. Stukalov, M. Gridling, K. Parapatics, A. C. Müller, S. Altiok, J. Colinge, G. Superti-Furga, E. B. Haura, et al. 2013. A miniaturized chemical proteomic approach for target profiling of clinical kinase inhibitors in tumor biopsies. *Journal of Proteome Research* **12**: 4005–4017.
- Choy, J. W, C. Bryant, C. M. Calvet, P. S. Doyle, S. S. Gunatilleke, S. S. Leung, K. K. Ang, S. Chen, J. Gut, J. A. Osés-Prieto, et al. 2013. Chemical-biological characterization of a cruzain inhibitor reveals a second target and a mammalian off-target. *Beilstein Journal of Organic Chemistry* **9**: 15–25.
- Chung, K., M. Zhan, J. Srinivasan, P. W. Sternberg, E. Gong, F. C. Schroeder, and H. Lu. 2011. Microfluidic chamber arrays for whole-organism behavior-based chemical screening. *Lab on a Chip* **11**: 3689–3697.
- Collins, J. J., B. Wang, B. G. Lambrus, M. Tharp, H. Iyer, and P. A. Newmark. 2013. Adult somatic stem cells in the human parasite, *Schistosoma mansoni*. *Nature* **494**: 476–479.
- Crowther, G. J., D. Shanmugam, S. J. Carmona, M. A. Doyle, C. Hertz-Fowler, M. Berriman, S. Nwaka, S. A. Ralph, D. S. Roos, W. C. Van Voorhis, et al. 2010. Identification of attractive drug targets in neglected-disease pathogens using an in silico approach. *PLoS Neglected Tropical Diseases* **4**: e804.
- \_\_\_\_\_, M. L. Booker, M. He, T. Li, S. Raverdy, J. F. Novelli, P. He, N. R. Dale, A. M. Fife, R. H. Barker Jr., et al. 2014. Cofactor-independent phosphoglycerate mutase from nematodes has limited druggability, as revealed by two high-throughput screens. *PLoS Neglected Tropical Diseases* **8**: e2628.

- Cybulski, J. S., J. Clements, and M. Prakash. 2014. Foldscope: Origami-based paper microscope. *PLoS One* **9**: e98781.
- Diawara, A., C. M. Halpenny, T. S. Churcher, C. Mwandawiro, J. Kihara, R. M. Kaplan, T. G. Streit, Y. Idaghdour, M. E. Scott, M. G. Basanez, et al. 2013. Association between response to albendazole treatment and  $\beta$ -tubulin genotype frequencies in soil-transmitted helminths. *PLoS Neglected Tropical Diseases* **7**: e2247.
- Dittrich, P. S., and A. Manz. 2006. Lab-on-a-chip: microfluidics in drug discovery. *Nature Reviews Drug Discovery* **5**: 210–218.
- Eggert, U. S. 2013. The why and how of phenotypic small-molecule screens. *Nature Chemical Biology* **9**: 206–209.
- Fernandez, A., A. Crespo, and A. Tiwari. 2009. Is there a case for selectively promiscuous anticancer drugs? *Drug Discovery Today* **14**: 1–5.
- Geary, T. G. 2012a. Mechanism-based screening strategies for anthelmintic discovery. In *Parasitic helminths: targets, screens, drugs and vaccines*, C. R. Caffrey (ed.) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 123–134.
- \_\_\_\_\_. 2012b. Are new anthelmintics needed to eliminate human helminthiases? *Current Opinion in Infectious Diseases* **25**: 709–717.
- \_\_\_\_\_, and D. P. Thompson. 2001. *Caenorhabditis elegans*: how good a model for veterinary parasites? *Veterinary Parasitology* **101**: 371–386.
- \_\_\_\_\_, and \_\_\_\_\_. 2003. Development of antiparasitic drugs in the 21st century. *Veterinary Parasitology* **115**: 167–184.
- \_\_\_\_\_, \_\_\_\_\_, and R. D. Klein. 1999. Mechanism-based screening: discovery of the next generation of anthelmintics depends upon more basic research. *International Journal for Parasitology* **29**: 105–112.
- \_\_\_\_\_, and C.D. Mackenzie. 2011. Progress and challenges in the discovery of macrofilaricidal drugs. *Expert Review of Anti-infective Therapy* **9**: 681–695.
- \_\_\_\_\_, G. A. Conder, and B. Bishop. 2004. The changing landscape of antiparasitic drug discovery in veterinary medicine. *Trends in Parasitology* **20**: 449–455.
- \_\_\_\_\_, K. Woo, J. S. McCarthy, C. D. Mackenzie, J. Horton, R. K. Prichard, N. R. de Silva, P. L. Olliaro, J. K. Lazdins-Helds, D.A. Engels et al. 2010. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *International Journal for Parasitology* **40**: 1–13.
- \_\_\_\_\_, D. J. Woods, T. Williams, and S. Nwaka. 2009. Target identification

- and mechanism- based screening for anthelmintics: Application of veterinary antiparasitic research programmes to search for new antiparasitic drugs for human indications. *In Drug discovery in infectious diseases*, P. M. Selzer (ed.) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 1–16.
- \_\_\_\_\_, C. Bourguinat, and R. K. Prichard. 2011. Evidence for macrocyclic lactone resistance in *Dirofilaria immitis*. *Topics in Companion Animal Medicine* **26**: 186–192.
- \_\_\_\_\_, B. C. Hosking, P. J. Skuce, G. von Samson-Himmelstjerna, S. Maeder, P. Holdsworth, W. Pomroy, and J. Vercruyse. 2012a. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guideline: Broad-spectrum anthelmintic combination products targeting nematode infections of ruminants and horses. *Veterinary Parasitology* **190**: 306–316.
- \_\_\_\_\_, K. Chibale, B. Abegaz, K. Andrae-Marobela, and E. Ubalijoro. 2012b. New ways forward for drug discovery for Neglected Tropical Diseases: local screening of African natural products. *Trends in Parasitology* **28**: 176–181.
- Geng, W., P. Cosman, C. C. Berry, Z. Feng, and W. R. Schafer. 2004. Automatic tracking, feature extraction and classification of *C. elegans* phenotypes. *IEEE Transactions of Biomedical Engineering* **51**: 1811–1820.
- Ghoreschi, K., A. Laurence, and J. J. O'Shea. 2009. Selectivity and therapeutic inhibition of kinases: to be or not to be? *Nature Immunology* **10**: 356–360.
- Hawdon, J. M. 2014. Controlling soil-transmitted helminths: time to think inside the box? *Journal of Parasitology*, **100**: 166–188.
- Herper, M. 2013. *Forbes Magazine*, “Pharma & Healthcare.” 11 August.
- Higazi, T., L. Shu, and T. R. Unnasch. 2004. Development and transfection of short-term primary cell cultures from *Brugia malayi*. *Molecular and Biochemical Parasitology* **137**: 345–348.
- Ho, N. F. H., S. M. Sims, T. J. Vidmar, J. S. Day, C. L. Barsuhn, E. M. Thomas, T. G. Geary, and D. P. Thompson. 1994. Theoretical perspectives on anthelmintic drug discovery: Interplay of transport kinetics, physicochemical properties and in vitro activities of anthelmintic drugs. *Journal of Pharmaceutical Science* **83**: 1052–1059.
- Hopkins, A. L. 2008. Network pharmacology: the next paradigm in drug discovery. *Nature Chemical Biology* **4**: 682–690.
- \_\_\_\_\_, J. S. Mason, and J. P. Overington. 2006. Can we rationally design promiscuous drugs? *Current Opinion in Structural Biology* **16**: 127–136.
- Kage-Nakadai, E., H. Kobuna, M. Kimura, K. Gengyo-Ando, T. Inoue, H. Arai, and S. Mitani. 2010. Two very long chain fatty acid acyl-CoA synthetase genes, *acs-20*

and *acs-22*, have roles in the cuticle surface barrier in *Caenorhabditis elegans*. *PLoS One* **5**: e8857.

Kaplan, R. M., and A. N. Vidyashankar. 2012. An inconvenient truth: Global worming and anthelmintic resistance. *Veterinary Parasitology* **186**: 70–78.

Keiser, J., and J. Utzinger. 2008. Efficacy of current drugs against soil-transmitted helminth infections: Systematic review and meta-analysis. *Journal of the American Medical Association* **299**: 1937–1948.

Knight, Z. A., H. Lin, and K. M. Shokat. 2010. Targeting the cancer kinase through polypharmacology. *Nature Reviews Cancer* **10**: 130–137.

Koziol, U., T. Rauschendorfer, L. Zanon Rodríguez, G. Krohne, and K. Brehm. 2014. The unique stem cell system of the immortal larva of the human parasite *Echinococcus multilocularis*. *EvoDevo* **5**: 10.

Laurent, S. A-L., J. Boissier, F. Coslédan, H. Gornitzka, A. Robert, and B. Meunier. 2009. Synthesis of “Trioxaquartel”® derivatives as potential new antischistosomal drugs. *European Journal of Organic Chemistry* **2008**: 895–913.

Leathwick, D. M. 2013. Managing anthelmintic resistance—parasite fitness, drug use strategy and the potential for reversion towards susceptibility. *Veterinary Parasitology* **198**: 145–153.

Lee, H., A. Moody-Davis, U. Saha, B. M. Suzuki, D. Asarnow, S. Chen, M. A. Arkin, C. R. Caffrey, and R. Singh. 2012. Quantification and clustering of phenotypic screening data using time-series analysis for chemotherapy of schistosomiasis. *BMC Genomics* **13** (Suppl. 1): S4.

Lewis, F. A., M. A. Stirewalt, C. P. Souza, and G. Gazzinelli. 1986. Large-scale laboratory maintenance of *Schistosoma mansoni*, with observations on three schistosome/snail host combinations. *Journal of Parasitology* **72**: 813–829.

Lim, S., I. Sakuma, M. J. Quon, and K. K. Koh. 2014. Differential metabolic actions of specific statins: Clinical and therapeutic considerations. *Antioxidants and Redox Signaling* **20**: 1286–1299.

Liu, X., F. Zhu, X. H. Ma, Z. Shi, S. Y. Yang, Y. Q. Wei, and Y. Z. Chen. 2013. Predicting targeted polypharmacology for drug repositioning and multi-target drug discovery. *Current Medicinal Chemistry* **20**: 1646–1661.

Lockery, S. R., S. E. Hulme, W. M. Roberts, K. J. Robinson, A. Laromaine, T. H. Lindsay, G.M. Whitesides, and J. C. Weeks. 2012. A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans*. *Lab on a Chip* **12**: 2211–2220.

Luni, C., E. Serena, and N. Elvassore. 2014. Human-on-chip for therapy

development and fundamental science. *Current Opinion in Biotechnology* **25**: 45–50.

Lycke, R., A. Parashar and S. Pandey. 2013. Microfluidics-enabled method to identify modes of *Caenorhabditis elegans* paralysis in four anthelmintics. *Biomicrofluidics* **7**: 064103.

Manneck, T., O. Braissant, Y. Haggenmüller, and J. Keiser. 2011. Isothermal microcalorimetry to study drugs against *Schistosoma mansoni*. *Journal of Clinical Microbiology* **49**: 1217–1225.

Mansour, N. R., and Q. D. Bickle. 2010. Comparison of microscopy and Alamar blue reduction in a larval based assay for schistosome drug screening. *PLoS Neglected Tropical Diseases* **4**: e795.

Marcellino, C., J. Gut, K. C. Lim, R. Singh, J. H. McKerrow, and J. Sakanari. 2012. WormAssay: A novel computer application for whole-plate motion-based screening of macroscopic parasites. *PLoS- Neglected Tropical Diseases* **6**: e1494.

McCall, J. W., J. B. Malone, A. Hyong-Sun, and P. E. Thompson. 1973. Mongolian jirds (*Meriones unguiculatus*) infected with *Brugia pahangi* by the intraperitoneal route: a rich source of developing larvae, adult filariae, and microfilariae. *Journal of Parasitology* **59**: 436.

Morphy, R., C. Kay, and Z. Rankovic. 2004. From magic bullets to designed multiple ligands. *Drug Discovery Today* **9**: 641–651.

Nwaka, S., D. Besson, B. Ramirez, L. Maes, A. Matheeussen, Q. D. Bickle, N. R. Mansour, F. Yousif, S. Townson, S. Gokool, F. Cho-Ngwa, et al. 2011. Integrated dataset of screening hits against multiple neglected disease pathogens. *PLoS Neglected Tropical Diseases* **12**: e1412.

Osei-Atweneboana, M. Y., K. Awadzi, S. K. Attah, D. A. Boakye, J. O. Gyapong, and R. K. Prichard. 2011. Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. *PLoS Neglected Tropical Diseases* **5**: e998.

O'Reilly, L. P., C. J. Luke, D. H. Perlmutter, G. A. Silverman, and S. C. Pak,. 2014. *Caenorhabditis elegans* in high-throughput drug discovery. *Advanced Drug Delivery Reviews* **69–70**: 247–253.

Paolini, G. V., R. H. Shapland, W. P. van Hoorn, J. S. Mason, and A. L. Hopkins. 2006. Global mapping of pharmacological space. *Nature Biotechnology* **24**: 805–815.

Paveley, R. A., N. R. Mansour, I. Hallyburton, L. S. Bleicher, A. E. Benn, I. Mikic, A. Guidi, I.H. Gilbert, A. L. Hopkins, and Q. D. Bickle. 2012. Whole organism high-content screening by label-free, image-based Bayesian classification for parasitic diseases. *PLoS Neglected Tropical Diseases* **6**: e1762.

\_\_\_\_\_, and Q. D. Bickle. 2013. Automated imaging and other developments in whole-organism antihelminthic screening. *Parasite Immunology* **35**: 302–313.

Peak, E., I. W. Chalmers, and K. F. Hoffmann. 2010. Development and validation of a quantitative, high-throughput, fluorescent-based bioassay to detect *Schistosoma* viability. *PLoS Neglected Tropical Diseases* **4**: e759.

Pharmaceutical Research and Manufacturers of America. 2013. *Biopharmaceutical Research Industry Profile*. PhRMA, Washington, D.C. 78 p.

Pink, R., A. Hudson, M-A. Mourès, and M. Bendig. 2005. Opportunities and challenges in antiparasitic drug discovery. *Nature Reviews Drug Discovery* **4**: 727–740.

Portela, J., J. Boissier, B. Gourbal, V. Pradines, V. Collière, F. Coslédan, B. Meunier, and A. Robert. 2012. Antischistosomal activity of trioxaquines: *In vivo* efficacy and mechanism of action on *Schistosoma mansoni*. *PLoS Neglected Tropical Diseases* **6**: e1474.

Ramot, D., B. E. Johnson, T. L. Berry, L. Carnell, and M. B. Goodman. 2008. The parallel worm tracker: A platform for measuring average speed and drug-induced paralysis in nematodes. *PLoS One* **3**: e2208.

Rojo-Arreola, L., T. Long, D. Asarnow, B. M. Suzuki, R. Singh, and C. R. Caffrey. 2014. Chemical and genetic validation of the statin drug target to treat the helminth disease, schistosomiasis. *PLoS One* **9**: e87594.

Selimović, S., M. R. Dokmeci, and A. Khademhosseini. 2013. Organs-on-a-chip for drug discovery. *Current Opinion in Pharmacology* **13**: 829–833.

Singh, R., M. Pittas, I. Heskia, F. Xu, J. H. McKerrow, and C. R. Caffrey. 2009. Automated image-based phenotypic screening for high-throughput drug discovery. *IEEE Symposium on Computer-Based Medical Systems (CBMS)*, p. 1–8.

\_\_\_\_\_. 2012. Quantitative high-content screening-based drug discovery against helminthic diseases. In *Parasitic helminths: targets, screens, drugs and vaccines*, C.R. Caffrey (ed.) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 159–179.

Smillie, C. L., A. C. Vickery, B. H. Kwa, J. K. Nayar, and U. R. Rao. 1994. Evaluation of different medium supplements for in vitro cultivation of *Brugia malayi* third-stage larvae. *Journal of Parasitology* **80**: 380–383.

Smout, M. J., A. C. Kotze, J. S. McCarthy, and A. Loukas. 2010. A novel high throughput assay for antihelminthic drug screening and resistance diagnosis by real-time monitoring of parasite motility. *PLoS Neglected Tropical Diseases* **4**: e885.

Spiliotis, M., S. Lechner, D. Tappe, C. Scheller, G. Krohne, and K. Brehm. 2008. Transient transfection of *Echinococcus multilocularis* primary cells and complete in vitro regeneration of metacestode vesicles. *International Journal for Parasitology* **38**: 1025–1039.

Thompson, D. P., R. D. Klein, and T. G. Geary. 1996. Prospects for rational approaches to anthelmintic discovery. *Parasitology* **113**: S217–S238.

\_\_\_\_\_, N. F. H. Ho, S. M. Sims, and T. G. Geary. 1993. Mechanistic approaches to quantitate anthelmintic absorption by gastrointestinal nematodes. *Parasitology Today* **9**: 31–35.

Vercruyse, J., M. Albonico, J. M. Behnke, A. C. Kotze, R. K. Prichard, J. S. McCarthy, A. Montresor, and B. Levecke. 2011. Is anthelmintic resistance a concern for the control of human soil-transmitted helminths? *International Journal of Parasitology: Drugs and Drug Resistance* **1**: 14–27.

Wang, B., J. J. Collins III, and P. A. Newmark. 2013. Functional genomic characterization of neoblast-like stem cells in larval *Schistosoma mansoni* eLife **2**: e00768.

Whitesides, G. M. 2006. The origins and the future of microfluidics. *Nature* **442**: 368–373.

Wisnewski, N., and P. P. Weinstein. 1993. Growth and development of *Brugia pahangi* larvae under various *in vitro* conditions. *Journal of Parasitology* **79**: 390–398.

Woods, D. J., C. Lauret, and T. G. Geary. 2007. Anthelmintic discovery and development in the animal health industry. *Expert Opinion on Drug Discovery* **2 (Suppl. 1)**: S25–S33.

Woods, D. J., and C. S. Knauer. 2010. Discovery of veterinary antiparasitic agents in the 21st century: A view from industry. *International Journal for Parasitology* **40**: 1177–1181.

\_\_\_\_\_, V. A. Vaillancourt, J. A. Wendt, and P. F. Meeus. 2011. Discovery and development of veterinary antiparasitic drugs: Past, present and future. *Future Medicinal Chemistry* **3**: 887–896.

Xie, L., L. Xie, S. L. Kinnings, and P. E. Bourne. 2012. Novel computational approaches to polypharmacology as a means to define responses to individual drugs. *Annual Reviews of Pharmacology and Toxicology* **52**: 361–379.

Yates, J. A., K. A. Schmitz, F. K. Nelson, and T. V. Rajan. 1994. Infectivity and normal development of third stage *Brugia malayi* maintained *in vitro*. *Journal of Parasitology* **80**: 891–894.

Zhang, S., D. Banerjee, and J. R. Kuhn. 2011. Isolation and culture of larval cells

from *C. elegans*. *PLoS One* **6**: e19505.

Zhou, X., J. N. Deng, B. D. Hummel, D. J. Woods, W. T. Collard, S. X. Hu, M. J. Zaya, C. S. Knauer, D. P. Thompson, D. A. Merritt, J. K. Lorenz, and A. A. Marchiondo. 2014. Development of an *in vitro* screen for compound bioaccumulation in *Haemonchus contortus*. *Journal of Parasitology* **100**: 848–855.

Zimmermann, G. R., J. Lehar, and C. T. Keith. 2007. Multi-target therapeutics: When the whole is greater than the sum of the parts. *Drug Discovery Today* **12**: 34–42.



**Part IV**

**The First Figures Published in *The Journal of Parasitology***

# ***The Journal of Parasitology* as a visual experience: Ten landmark figures**

*The Journal of Parasitology* (*JP*) is certainly one of the richest, visually, of any biological journal, and a legitimate case could be made that it is indeed the most visually diverse of all scientific journals, if we exclude advertising. Why should *JP* be such a rich and diverse publication? The answer to that question is four-fold, and is one that any parasitologist could probably give, namely, that all animal phyla have parasitic members, parasites often if not typically have complex lives, many parasites are microscopic, thus can be a challenge to describe, and finally, all parasites have relationships with their hosts, relationships that routinely are represented by pictures. A study of any recent issue is likely to reveal figures that range, as a minimum, from magnificent drawings of new species, to phylogenies that tell a compelling story not only of organisms' histories but also scientists' efforts to recover those histories, photographs taken at scales differing by orders of magnitude, and ecological events that cover major blocks of time. Thus our search through the first hundred volumes of *JP* for "first figures" was actually a study of the way parasitologists have represented their organisms and host-parasite relationships over the decades, incorporating new technologies along the way.

We have made a serious effort to actually identify these first figures as first, and some figures were far easier to find with confidence than others. Stanley Fracker's proposal for quantifying the structural features of nematodes, using a pinworm as an example, the first figure ever published in the journal, was easy to find. So was Minnie Watson's camera lucida drawing of a gregarine from millipedes, the first figure of a new species to appear in *JP*. Later figures were a little more of a challenge. For example, we were very surprised that the first life cycle diagram similar to what you'd expect to see in a textbook did not appear until 1988, in Wes Shoop's review of trematode life cycles. Discovery of the first digital photomicrograph was also a challenge, in part because so many authors who published in the late 1980s and 1990s did not indicate, in the Materials and Methods sections, what kind of camera equipment they used.

Scanning and transmission electron micrographs in *JP* are particularly revealing in that they clearly demonstrate not only the evolution of technology, but also the consequent use of ultrastructural features as taxonomic characters. Similarly, graphs and line drawings demonstrate the diversification of statistical methods as applied to parasitological problems, obviously a reflection of computer evolution as well as graphing software. Few if any figures in current issues have the telltale signs of having been done using a Leroy lettering set. In picking out these firsts, we were reminded again that study of visual representation in *The Journal of Parasitology* reveals not only the history of technology, but also the manner in which parasitologists have used every tool they can think of to explore host-parasite interactions, then convey their discoveries to the broader scientific

community.

# First figure published in *The Journal of Parasitology*: Stanley Fracker's proposal for quantifying the structural features of a nematode



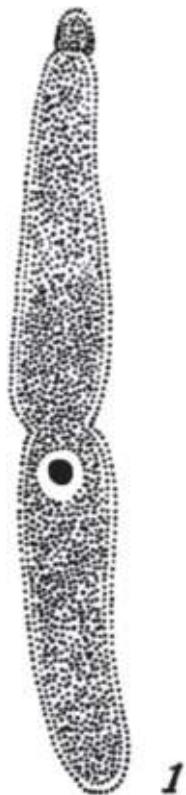
Fig. 1.—*Oxyurias vermicularis* Linn. Specimen No. 2; lateral aspect of cleared worm. 1-6, the structures and distances measured in this paper; for explanation see text, and table opposite.

This drawing of a pinworm is the first image of a parasite to be published in *The Journal of Parasitology*. The problem of how best to represent a species, and how to quantify its traits, is one that parasitologists still sometimes struggle with, even in the molecular age. The species represented above is now the familiar *Enterobius vermicularis*.

From:

Fracker, S. B. 1914. Variation in Oxyurias: its bearing on the value of a nematode formula. *Journal of Parasitology* 1: 22–30.

## First new species figure: *Stenophora diplocorpa* from the intestine of *Euryurus erythropygus*



*Stenophora displocorpa* n. sp., from the millipede *Euryurus erythropygus*, collected at Urbana, Illinois, by Minnie Elizabeth Watson. This drawing was done using a camera lucida. Watson indicates it was “from the original” although doesn't tell us what that phrase actually means.

From:

Watson, M. E. 1915. Some new gregarine parasites from Arthropoda. Journal of Parasitology **2**: 27–36.

# First photomicrograph published in *The Journal of Parasitology*: A sarcocyst from an opossum

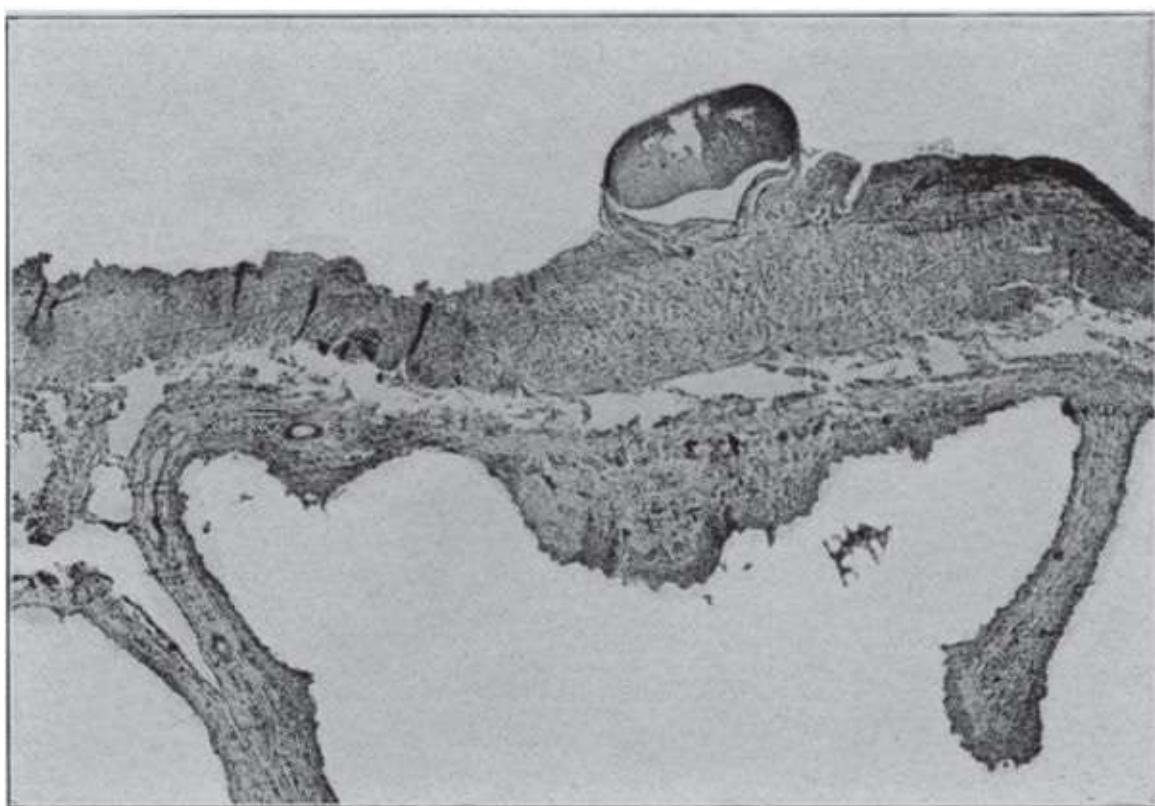


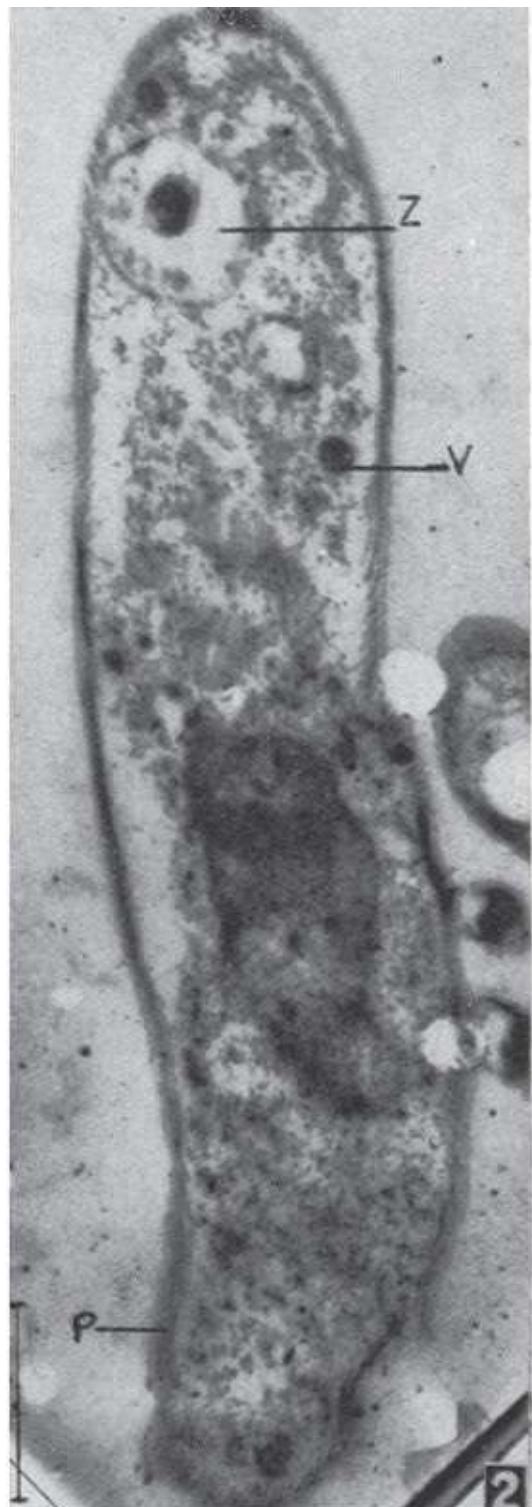
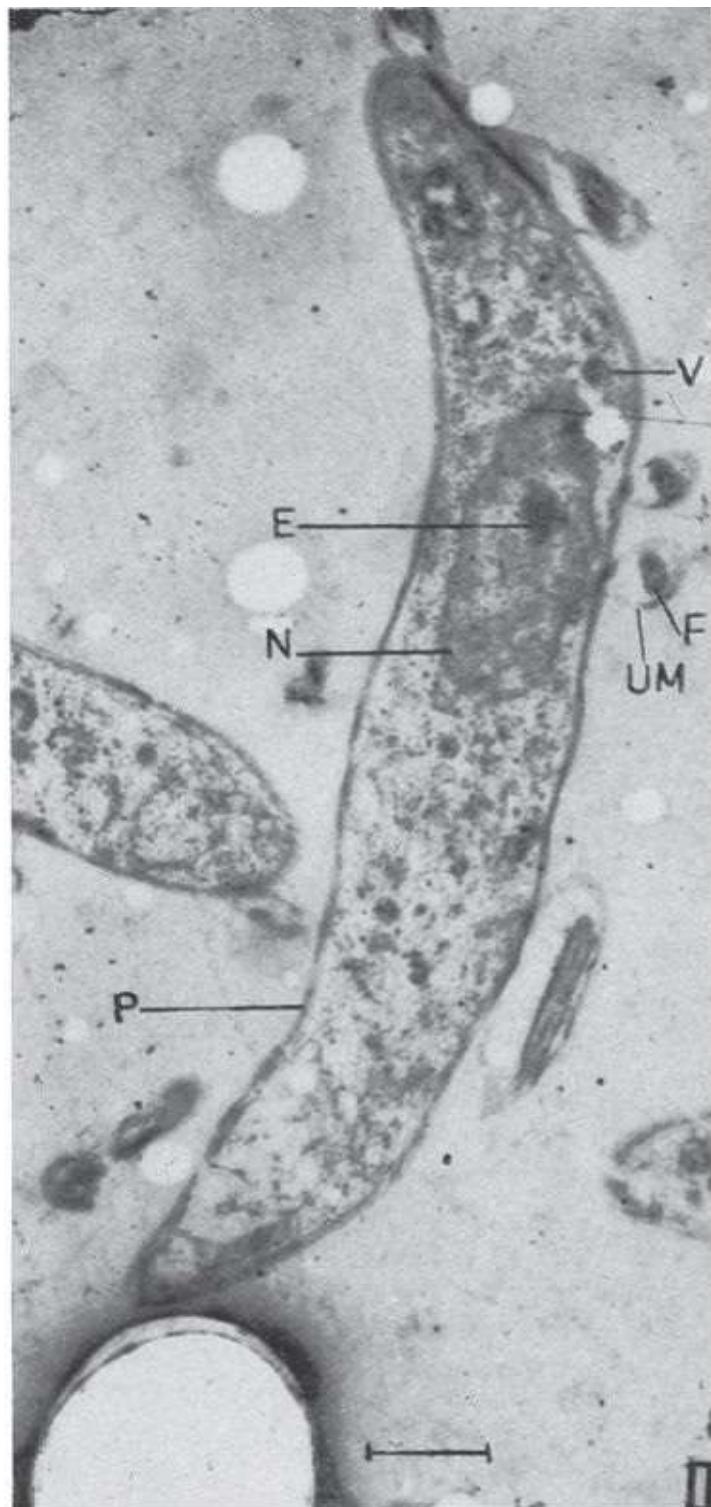
Fig. 1

This image is the first photograph of a parasite published in *The Journal of Parasitology*. There is no legend, but the text of the Darling (1915) paper indicates it was a species of *Sarcocystis* from an opossum (scientific name not given) in Panama. Cysts occurred in a variety of tissues.

From:

Darling, S. T. 1915. Sarcosporidia encountered in Panama. *Journal of Parasitology* **1**: 113–120.

**First transmission electron micrograph published in  
*The Journal of Parasitology*: sections of *Trypanosoma*  
*equiperdum***



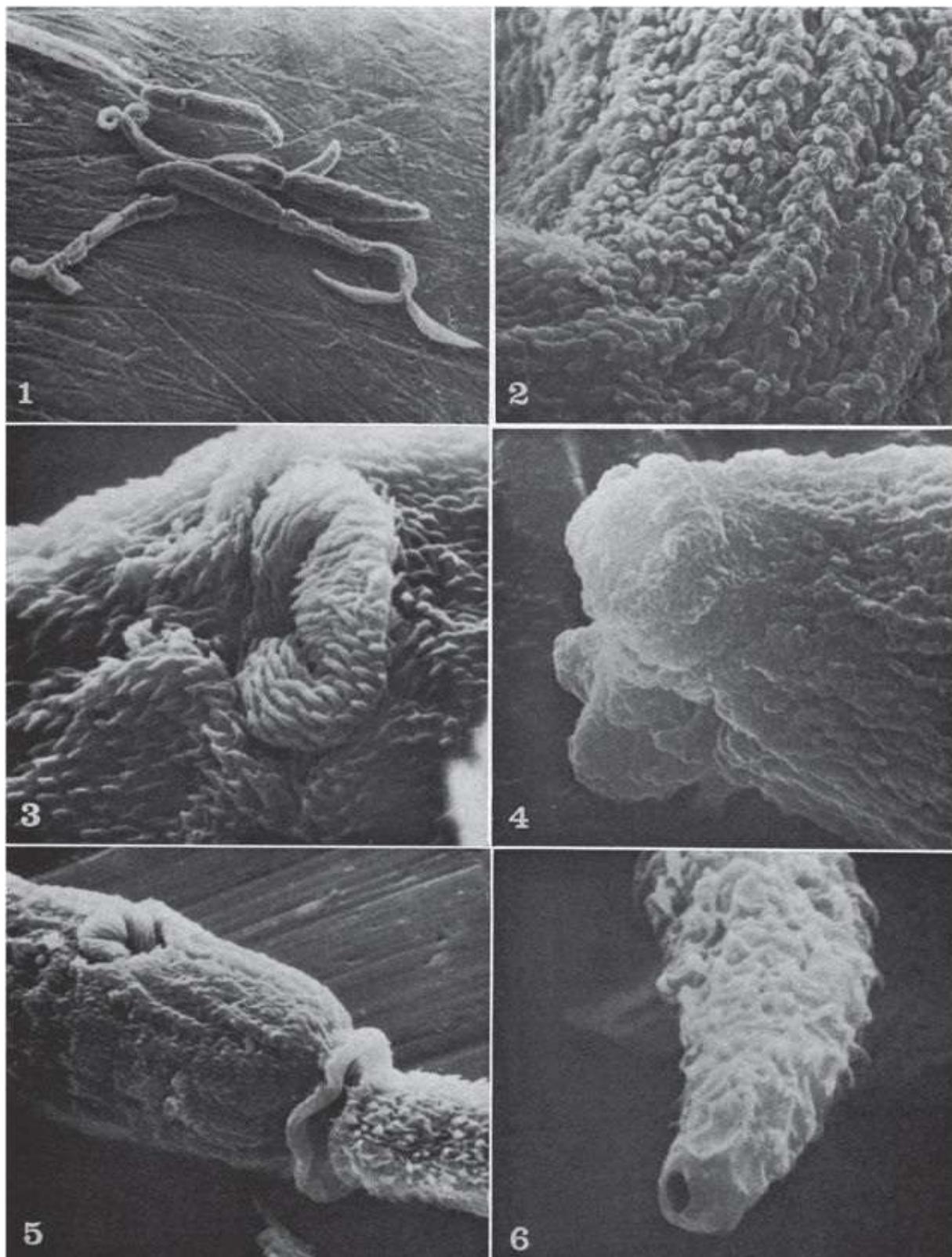
FIGURES 1 and 2. Nearly longitudinal sections showing nucleus N, endosome E, volutin granules V, striated periplast P, undulating membrane UM, and flagellum F.

The first transmission electron micrographs to be published in *The Journal of Parasitology* were these of *Trypanosoma equiperdum* in the blood of experimentally infected rats.

From:

Anderson, E., L. H. Saxe, and H. W. Beams. 1956. Electron microscope observations of *Trypanosoma equiperdum*. Journal of Parasitology **42**: 11–16.

# First scanning electron micrograph published in *The Journal of Parasitology*: Surface architecture of schistosome cercariae



FIGURES 1-6. Scanning electron micrographs of *Schistosoma mansoni* cercariae. 1. Four cercariae on the specimen holder,  $\times 200$ . 2. Spines on the body,  $\times 4,500$ . 3. Ventral sucker,  $\times 3,700$ . 4. Anterior end,  $\times 5,000$ . 5. Collar between the body and tail,  $\times 1,600$ . 6. Excretory pore at tip of caudal furca,  $\times 4,500$ .

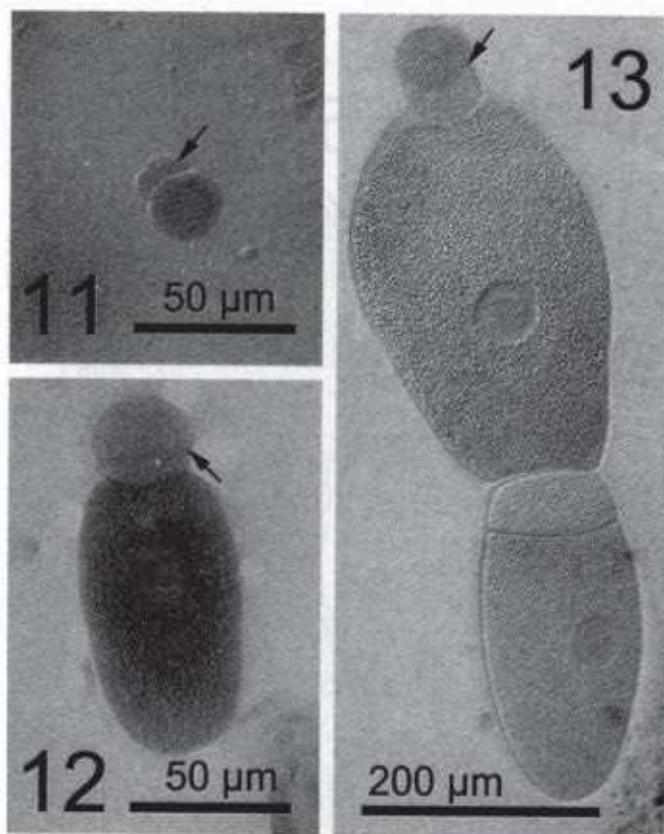
The first scanning electron micrographs to be published in *The Journal of*

*Parasitology* were of *Schistosoma mansoni* cercariae, in a research note that essentially established platyhelminth surface architecture as a suite of structural characters useful in systematics.

From:

Hockley, D. J. 1968. Scanning electron microscopy of *Schistosoma mansoni* cercariae. *Journal of Parasitology* **54**: 1241–1243.

# First digital light micrograph published in *The Journal of Parasitology*: Trophic stages of *Torogregarina sphinx*



FIGURES 11–13. *Torogregarina sphinx*, n. sp. as revealed by differential interference microscopy. Arrows indicate epimerite–protomerite junctions. 11. Young, solitary trophozoite. 12. Young, solitary trophozoite. 13. Mature gamonts in association.

These figures are the first digital light micrographs published in *The Journal of Parasitology*; the parasite is *Torogregarina sphinx*, a new gregarine species from a carabid beetle, *Bembidion laevigatum*, collected along the Missouri River in Nebraska. There are quite a few prior light micrographs that might have been taken with a digital camera, but this paper is the first one to actually state that fact in the Materials and Methods section.

From:

Clopton, R. E. 1998. Revision of the genus *Torogregarina* and description of *Torogregarina sphinx* n. sp. (Apicomplexa: Eugregarinida) from a Missouri River bank beetle, *Bembidion laevigatum* (Coleoptera: Carabidae) in southeastern Nebraska. *Journal of Parasitology* **84**: 823–827.

# First figure of a pathological condition: *Endameba gingivalis* in a pyorrheal pocket



Fig. 4

The first image of a pathological condition, published in *The Journal of Parasitology*, was this photomicrograph of amebas in a pyorrheal pocket. The Smith and Barrett (1915) paper is an unusual one in that it contains long sections, quotes, from previously published work by other authors, including Gros' description. Evidently the specimen was obtained from a patient examined by the authors. The section was stained in iron hematoxylin.

From:

Smith, A. J., and M. T. Barrett. 1915. The parasite of oral endamebiasis, *Endameba gingivalis* (Gros). *Journal of Parasitology* 1: 159–174.

# First figure of a seasonal fluctuation in parasite abundance: Cercariae from a planorbid snail

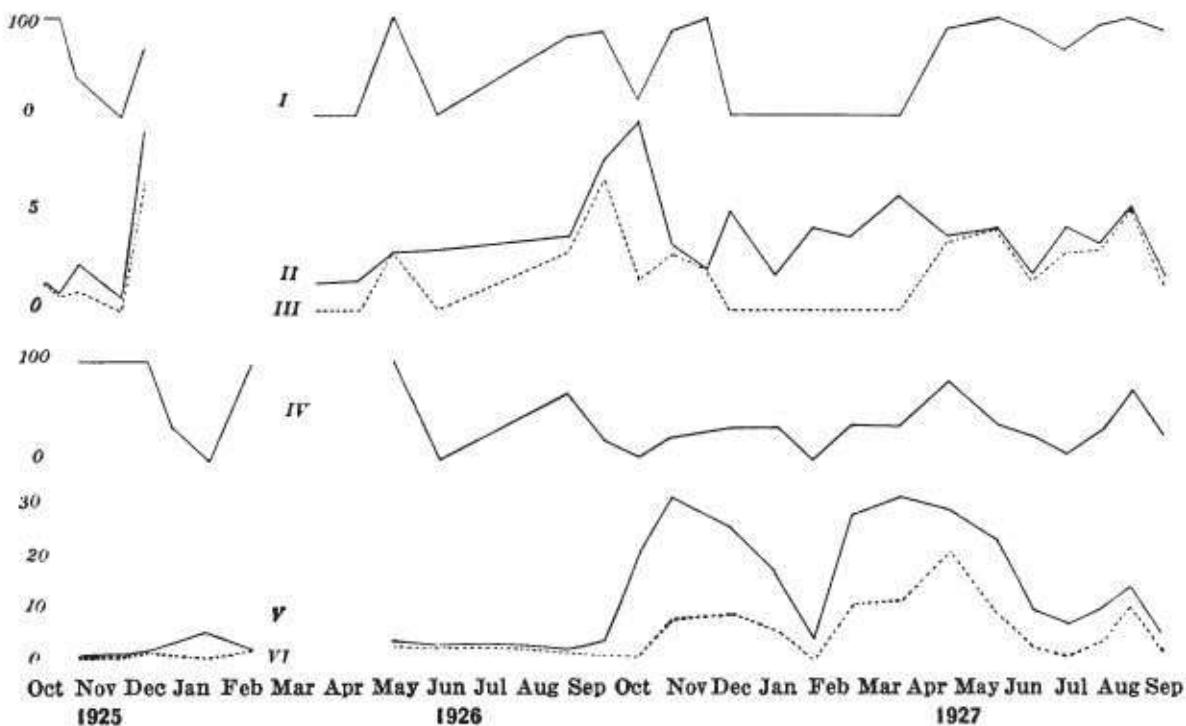


Figure 1.—Graphs showing the infestation with larval trematodes of *Planorbis trivolvis* from October, 1925, to October, 1927.

- I. *Cercaria hamata*, percentage of the infested snails which gave emerged cercariae.
- II. Percentage of snails infested with *C. hamata*.
- III. Percentage of the snails harboring mature infestations of *C. hamata*.
- IV. The cercaria of *Plagiorchis ameiurensis*, percentage of the infested snails which gave emerged cercariae.
- V. Percentage of snails infested with the cercaria of *P. ameiurensis*.
- VI. Percentage of the snails harboring mature infestations of the cercaria of *P. ameiurensis*.

McCoy's (1915) figure of cercarial production in freshwater snails over a two-year period is the first one published in *The Journal of Parasitology* that portrays seasonal fluctuation in parasite burdens. Furthermore, the figure shows data from two different trematode species.

From:

McCloy, O. R. 1928. Seasonal fluctuation in the infestation of *Planorbis trivolvis* with larval trematodes. *Journal of Parasitology* **15**: 121–126.

# First modern figure of a phylogeny published in *The Journal of Parasitology*

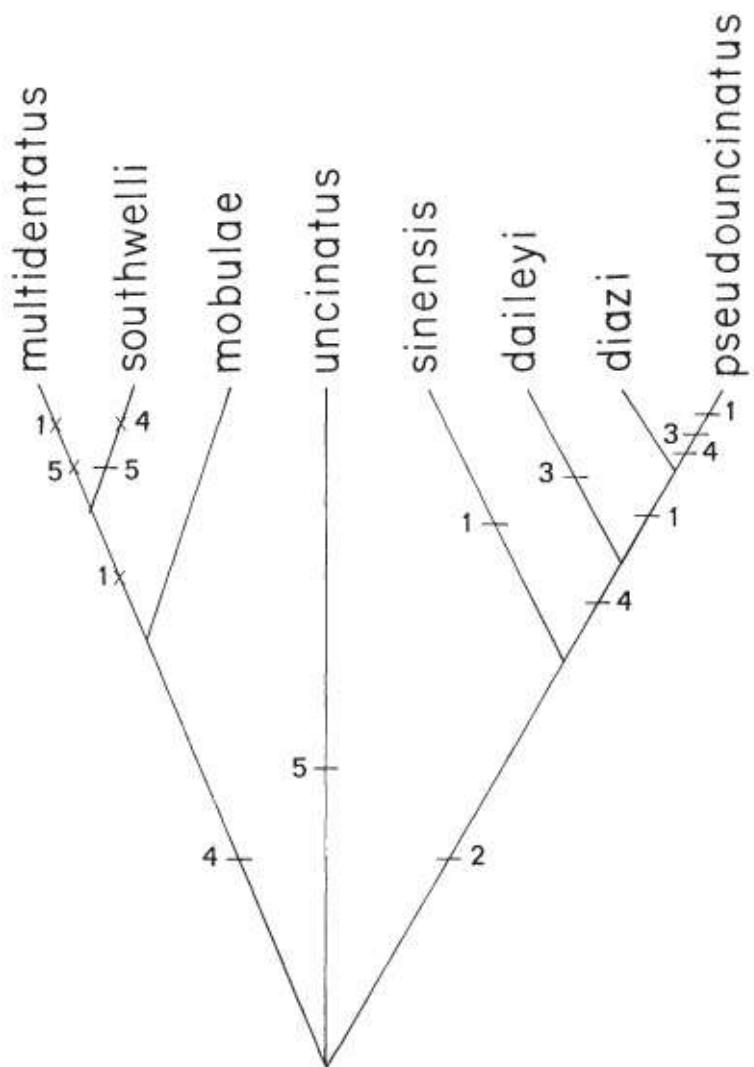


FIGURE 13. Cladogram depicting genealogical relationships of eight species of *Echinocephalus*. Slash marks indicate derived states for characters indicated by accompanying number. Single slash refers to character state with a positive notation, x-slash marks refer to character states with a negative notation. For identities of characters, see text.

The first modern phylogeny figure published in *The Journal of Parasitology*. This one is included in a paper describing *Echinocephalus daileyi*, a gnathostomid nematode named in honor of parasitologist Murray Dailey.

From:

Deardorff, T. L., D. R. Brooks, and T. B. Thorson. 1981. A new species of *Echinocephalus* (Nematoda: Gnathostomidae) from Neotropical stingrays with comments on *E. diazi*. *Journal of Parasitology* **67**: 433–439.

# First life cycle diagram, similar to what would be in a textbook, published in *The Journal of Parasitology*

*Heronimus* (Turtle Lung Fluke)

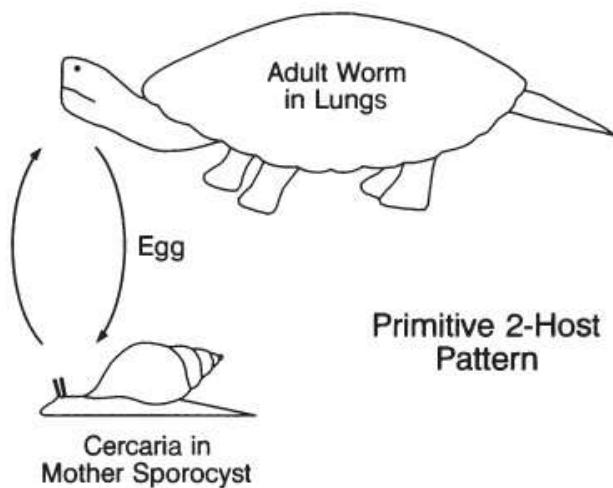


FIGURE 2. The primitive 2-host pattern of the genus *Heronimus*.

*Fasciola hepatica*

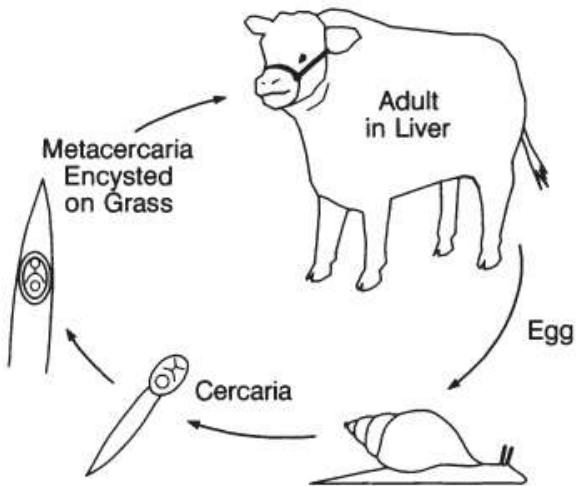


FIGURE 3. The 2-host pattern of *Fasciola hepatica* with a free-swimming cercaria encysting upon vegetation.

The first two life cycle figures, typical of those found in a textbook, to be published in *The Journal of Parasitology* appeared in a review paper by W. Shoop in 1988. The subject of this review was variation in trematode life cycles, one of the more challenging areas of parasitology, especially for those focused on evolutionary events. Although there are plenty of life cycle papers published in *JP* prior to 1988, these figures are evidently the first that are designed like a typical life cycle diagram in a textbook.

From:

Shoop, W. L. 1988. Trematode transmission patterns. *Journal of Parasitology* 74: 46–59.



## **Part V**

# **Overview and Conclusions**



# **Chapter 16**

## **Reflections: Closing comments for the centennial celebration of *The Journal of Parasitology***

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In closing this Centennial book, I want to add just a few summary notes regarding our Journal and parasitology in general. The official transfer of *The Journal of Parasitology* to the American Society of Parasitologists in 1932 marked an end to what we called the establishment era for our Journal (Esch et al., 2014). From 1932 to 1961, the Journal's middle years, there was enormous turmoil in the world. The Great Depression, World War II, the Korean War, etc., were troublesome times to be sure. Nonetheless, parasitology was becoming sophisticated during this period. Indeed, so were all of the biological sciences. Toward the end of these middle years, in 1953 to be exact, Watson, Crick, and Wilkins discovered the structure of DNA with, I must add, a significant contribution by Rosalind Franklin.

Since that discovery, the science we accomplished has been enhanced, even made possible, by a number of huge technological innovations. A great many of us have lived through at least a part of this period. Think about it. Electron microscopy opened up the cell so that we could actually see inside. Physiology and biochemistry were both greatly advanced by the invention of microchips. Many of us can also remember a time when an average-sized dataset saved on punch cards required several days to be analyzed by computers so large that they occupied an average sized living room. Today, a computer so small that it can be held in one hand can easily examine the same dataset in a few seconds. I believe most of us would agree that some of the greatest breakthroughs have come with the huge proficiency associated with molecular biology. Not only have molecular and cell biology been well served, the same technologies have added incredible applications to other disciplines such as systematics. For example, who could have predicted that rotifers and acanthocephalans are closely related? Or, how about current knowledge that fungi are more closely related to animals than they are to plants, or that malaria cells, like all plant cells, and unlike the cells of any animal, can synthesize aromatic amino acids via the so-called Shikimate pathway?

What about our present parasites, drug therapies, etc.? Where are we now compared to 1914? Regrettably, we still have species of *Necator*, *Ancylostoma*, *Ascaris*, *Trichuris*, *Entamoeba*, *Giardia*, *Schistosoma*, *Fasciola*, etc. And we still have quinine, although it does not work as well as it did 300 years ago. There are several other drugs discovered in the 1930s that are still used for the treatment of malaria, but these are also showing signs of wear and tear. Ivermectin and praziquantel are still effective, but resistance is beginning to show up in both

cases.

We also have cryptosporidiosis and several other emerging diseases of which we either were not aware, or did not know much about in the early years of our Journal. The only helminth parasite that has been effectively managed in recent years is *Dracunculus medinensis*. It is almost gone now and ironically, not because of a huge technological discovery, but because of a small piece of cloth. And, perhaps we are now ready to accept the idea that viruses and some bacteria are also parasites, in the same way as schistosomes and trypanosomes. At long last, we are on the threshold of de-balkanizing these disciplines and recognizing their true parallels to basic parasitology.

It can be concluded that parasitology has changed significantly, but that parasitism has not. This way of life is with us still. An effort to predict where we will be 100 years from now will not be made here, except to remind us that parasitism has been described by some as the most successful life strategy on Earth.

In closing, I am reminded of a statement made by Sir Isaac Newton in a letter to Robert Hooke, the noted British microscopist of the seventeenth century, and a contemporary of Antonie von Leeuwenhoek. Newton wrote, "If I have seen further, it is only by standing on the shoulders of giants." The quote stimulated John and me to identify some of our leaders during the first 100 years of the Journal. Out of curiosity, he and I separately created a list of 10 parasitologists we considered as our giants. Our results matched on six names, i.e., Henry Baldwin Ward, Clark Read, William Taliaferro, John Holmes, William Trager, and Horace Stunkard. If we had extended the list into South America, we are certain Carlos Chagas would have been included by both of us. All of us are naturally biased by our own experiences, and we are certain that many in our Society would have included other names such as Joseph Leidy, Harold Manter, William Cort, Norman Stoll, Janine Caira, William Campbell, J. P. Dubey, Robert Rausch, and about a dozen others. Whatever our choice, it is agreed that during the history of *The Journal of Parasitology*, we have been blessed in having a remarkable group of scientists who significantly contributed to our Journal.

We are confident that the second 100 years will produce even more talented and productive investigators who will publish excellent work in our Journal, and thereby add to our very gifted assembly of giants. Parasitology is a fascinating science and our Journal has served it well. We believe that the present Centennial book is a wonderful reflection on this service!

## Literature cited

Esch, G., S. Desser, and B. Nickol. 2014. A history of *The Journal of Parasitology*. *Journal of Parasitology* **100**: 1–10.



# Taxonomic index

**Note:** In the index, terms in the original Journal of Parasitology papers are identified by an abbreviation consisting of senior author initials and the original JP page numbers as follows: BE61=Bemrick, 1961; CR34=Cross, 1934; HA26=Hall, 1926; JA60=Jacobs et al., 1960; LI15=Linton, 1915; LI16=Linton, 1916; MC28=McCoy, 1928; NA95=Nadler, 1995; RA15=Ransom and Hall, 1915; SA32=Sarles, 1932; TO14=Todd, 1914; VC14=Van Cleave, 1914; WA15=Watson, 1915

## A

*Acanthobothrium tortum*

*Acanthocephala*,

*Acanthocephalooides plagiuseae*

*Acanthocephalus*

*A. dirus*

*A. lucii*

*A. parksidei*

*A. tumescens*

*Adelobothrium*

*A. aetiobatidis*

*Aetobatis narinari*

*Alaria*

*A. marcianae*

*Alveolata*,

*Apicomplexa*,

*Amblyomma*

*A. americanum*

*A. hebraeum*

*A. maculatum*

*A. ovale*

*A. americanum*

*Amphoroides calverti*

*Anaplasma phagocytophilum*

*Ancylostoma caninum*

*Andracantha*

Anenteraeti

*Anisakis*

*Apeltes quadracus*

*Aphodius*

*A. coloradensis*

*A. congregatus*

*A. femoralis*

*A. fimetarius*

*A. granarius*

*A. inquinatus*

*A. vittatus*

*Aporohynchus hemignathi*

Apororhynchidea, 41

Arachnida

Archiacanthocephala

*Argas*

*A. persicus*

*A. walkerae*

*Ascaridia lineata*

*Ascaris*

*A. lumbricoides*

*A. suum*

Aschelminthes

*Asellus intermedius*

*Aythya valisineria*

**B**

*Babesia microti*

*Bartonella*

Bdelloidea

*Bembidion laevigatum*

*Bordetella bronchiseptica*

*Bothriocephalus acheilognathi*

*Brugia*

*B. malayi*

*B. pahangi*

*Bufo americanus*

**C**

*Caenorhabditis elegans*

*Callipus lactarius*

*Caryophylleus laticeps*

*Cavia cobaya*

*Centrorhynchus*

*C. milvus*

*Cercaria hamata*

*Cestoda*

*Ceuthophilus*

*C. utahensis*

*C. stygicus*

*Coccinella*

*Coleoptera,*

*Contracaecum*

*Corynebacterium diphtheriae*

*Corynosoma*

*C. constrictum*

*C. hamanni*

*Cotylurus flabelliformis*

*Cryptocotyle lingua*

*Ctenodactylus gundi*

*Cyclops*

*C. serrulatus*

*C. vernalis*

*Cyclopsetta chittendeni*

*Cyprinella lutrensis*

**D**

*Daphnia*

*Dermacentor*

*D. alibictus*

*D. andersoni*

*D. auratus*

*D. occidentalis*

*D. variabilis*

*D. venustus*

*Dermocystis ctenolabri*

*Dicrocoelium dendriticum*

*Diplopoda*,

*Diploptera punctata*

*Diplostomum*

*Dirofilaria*

*D. immitis*

*Distomum lingua*

**E**

*Echinocephalus*

*E. daileyi*

*Echinococcus multilocularis*

*Echinogammarus stammeri*

*Echinopardalis atrata*

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*Echinorhynchus*

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*E. cinctulus*

*E. coregoni*

*E. gadi*

*E. lageniformis*

*E. rutilli*

*Echinostoma*

*Ectobia germanica*

Elasmobranch

Elateridae,

*Encyptolophus sordidus*

*Endameba gingivalis*

*Enterobius*

Eoacanthocephala

Eorhynchidae,

*Eorhynchus*

*E. cylindratus*

*E. emydis*

*E. gracilisentis*

*E. tenellus*

*Euryurus erythropygus*

**F**

*Fasciola hepatica*

*Fascioloides magna*

*Fessisentis*

*Floridosentis*

*Fundulus*

**G**

*Gadus morhua*

*Gambusia affinis*

*Gammarus*

*G. lacustris*

*G. pseudolimnaeus*

*G. pulex*

Gastrotricha

*Gavia immer*

*Geomysdoecus actuosi*

*Giardia*

*G. duodenalis*

*G. intestinalis*

*G. lamblia*

*G. muris,*

Gigantorhynchidea

Gnathifera

Gnathostomulida

*Gongylonema*

*G. neoplasticum*

*G. pulchrum*

*G. scutatum*

Gorgoderidae

*Gorgoderina attenuata*

*Gracilisentis gracilisentis*

*Graphidium strigosum*

*Gregarina*

*G. abbreviatus*

*G. achetae-abbreviatae*

*G. barbarara*

*G. calverti*

*G. fragilis*

*G. galliveri*

*G. globosa*

*G. gracilis*

*G. illinensis*

*G. intestinalis*

*G. katherina*

*G. melanopli*

*G. monarchia*

*G. nigra*

*G. rigida*

*G. stygia*

*G. tenebrionella*

Gregarinida

*Gryllus*

*G. abbreviatus*

*G. pennsylvanicus*

Gyracanthocephala

*Gyraulus*

*G. armigera*

*G. parvus*

## H

*Habronema muscae*

*Haematoloechus*

*H. coloradensis*

*H. complexus*

*H. longiplexus*

*H. medioplexus*

*H. parviplexus*

*Haemonchus contortus*

*Halipegus*

*H. eccentricus*

*H. occidualis*

*Hebesoma*

*Heligmosomoides*

*H. bakeri*

*Helisoma*

*H. anceps*

*H. campanulatum*

Heteracanthocephalidae

Heteramorphida

*Histomonas meleagridis*

*Hyalella azteca*

*Hyalomma truncatum*

*Hyla versicolor*

*Hymenolepis*

*H. citelli*

*H. diminuta*

*H. nana*

## I

*Ixodes*

*I. hexagonus*

*I. holocyclus*

*I. pacificus*

*I. pilosus*

*I. redikorzevi*

*I. ricinus*

*I. rubicundus*

## K

*Katsuwonus pelamis*

Kinorhyncha

## L

*Lagopus scoticus*

Lecanicephalidea

*Lecithaster confusus*

*Leishmania*

*Lepeophtheirus salmonis*

Lepocreadiidae

*Lepomis macrochirus*

*Leptorhynchoides*

*L. polycristatus*

*L. thecatus*

*Lepus cuniculus*

*Lithobates*

*L. catesbeianus*

*L. pipiens*

*L. sylvaticus*

*Litosomoides carinii*

**M**

*Macaca rhesus*

*Macracanthorhynchus*

*M. hirudinaceus*

*M. ingens*

*Margaropus annulatus*

*Mediorhynchus*

*M. africanus*

*M. centurorum*

*M. gallinarum*

*M. grandis*

*Melanoides tuberculatus*

*Melanoplus*

*M. bivittatus*

*M. coloradensis*

*M. differentialis*

*M. femur-rubrum*

*Meriones unguiculatus*

*Microphallus opacus*

*Microtus agrestis*

*Moniliformis moniliformis*

*Mus*

*M. decumanus*

*M. musculus*

*M. rattus*

*Mustela putorius furo*

Myxosporidia

**N**

*Nassa obsoleta*

Nematoda

Nematomorpha

Neoacanthocephala

*Neoacanthorinchus*

*Neobenedenia girellae*

Neoechinorhynchidae, 41

*Neoechinorhynchus*

*N. brentnickoli*

*N. carinatus*

*N. cristatus*

*N. cylindratus*

*N. emydis*

*N. golvani*

*N. gracilisentis*

*N. longirostris*

*N. rutili*

*N. saginatus*

*Neorhynchus*

*Nippostrongylus muris*

*Notemigonus crysoleucas*

**O**

*Obeliscoides cuniculi*

*Octospinifer macilentis*

*Octospiniferooides chandleri*

*Odostomia trifida*

*Oligacanthorhynchus*

*O. atratus*

*O. tortuosa*

*Onchobothrium tortum*

Onchoproteocephalidea

*Oncomegas wageneri*

*Onthophagus*

*O. hecate*

*O. pennsylvanicus*

Opeocoliidae

*Ornithodiplostomum*

*Ornithodoros*

*O. coriaceus*

*O. lahorensi*

Orthoptera,

*Oryctolagus cuniculus*

*Ostertagia ostertagi*

*Oxyurias vermicularis*

**P**

Palaeacanthocephala

*Paralichthys olivaceus*

*Paratenuisentis ambiguus*

*Passalurus ambiguus*

*Paulisentis fractus*

*Perca flavescens*

*Periplaneta americana*

*Peromyscus maniculatus*

Phyllobothriidea

*Physa integra*

*Pimephales promelas*

*Pinguiculum*

*Plagiorchis ameiurensis*

*Plagiorhynchus cylindraceus*

*Planorbis*

*P. exustis*

*P. trivolvis*

*Plasmodium*

*P. cathemerium*

*P. falciparum*

*P. lophurae*

*P. malariae*

*P. vivax*

Platyhelminthes

Polyacanthocephala

Polymorphidae

*Polymorphus*

*P. contortus*

*P. kenti*

*P. marilis*

*P. paradoxus*

*Pomphorhynchus*

*P. bulbocolli*

*P. laevis*

*P. patagonicus*

*P. tereticollis*

Porrorchinae

*Posthodiplostomum*

Priapuloidea

*Profilicollis*

*P. altmani,*

*P. formosus,*

*Prosthenorchis elegans*

*Prosthorhynchus formosus*

*Proteocephalus exigus*

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*Pseudacris regilla*

*Pseudoacanthocephalus nickoli*

*Pseudocorynosoma*

*Pseudodiplorchis americanus*

*Pseudoterranova*

Pyrirhynchidae

## R

*Rhadinorhynchus ornatus*

Rhinebothriidea

*Rhinoptera*

*R. bonasus*

*R. quadriloba*

*Rhipicephalus*

*R. evertsi*

*R. praetextatus*

*R. simus*

*Ribeiroia ondatrae*

Rotifera

## S

*Sarcocystis tenella*

*Scaphiopus couchii*

*Schistocephalus solidus*

*Schistocerca americana*

*Schistosoma*

*S. haematobium*

*S. mansoni*

*S. spindale*

*Semotilus*

*S. atromaculatus*

*S. corporalis*

*Spiroptera obtusa*

*Stagnicola*

*S. emarginata*

*Stenophora*

*S. diplocorpa*

*S. erratica*

*S. impressa*

*S. lactaria*

Stenophoridae,

Strongyloidea

*Strongyloides*

*S. ovocinctus*

*S. ratti*

*S. stercoralis*

Stunkard, Horace

*Syacium gunteri*

*Symphurus plagiUSA*

Syndermata

*Syngamus trachea*

**T**

*Taenia ovis*

*Takifugu rubripes*

*Tanaorhampus longirostris*

*Tautoga onitis*

*Tautogolabrus adspersus*

*Tenebrio molitor*

*Tetraphyllidea*

*Tocotrema lingua*

*Torogregarina sphinx*

*Toxoplasma*

*T. gondii*

ME49 strain,

M7741 strain,

RH strain,

S-2 strain,

S-6 strain,

*Trichinella spiralis*

*Trichomonas foetus*

*Trichostrongylus*

*T. calcaratus*

*T. retortaeformis*

*T. tenuis*

*Trichuris*

*T. muris*

*T. trichiura*

*Trypanorhyncha*

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*T. equiperdum*

*T. lewisi*

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*T. pingue*

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*Uncinaria stenocephala*

*Uvulifer ambloplitis*

**X**

*Xenopus laevis*



# Index

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    antihelmintics

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John Hopkins University

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ZooBank



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