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REVIEW

Giving microbes their due – animal life in a microbially dominant world

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ABSTRACT

The new technology of next-generation sequencing is changing our perceptions of the form and function of the biological world. The emerging data reveal an array of microbes that is more vast and more central to all biological processes than previously appreciated. Further, evidence is accumulating that the alliances of microbes with one another and with constituents of the macrobiological world are critical for the health of the biosphere. This contribution summarizes the basic arguments as to why, when considering the biochemical adaptations of animals, we should integrate the roles of their microbial partners.

KEY WORDS: Microbial biosphere, Symbiosis, Woese

Introduction

From the 1970s to the present, biologists have witnessed the extraordinary growth of two largely independent, major areas of research: (i) the molecular, biochemical and physiological adaptation of animals to their environments and (ii) the organismal biology of microbes. The former, pioneered by the global community of comparative biologists and brought together in comprehensive volumes by Hochachka and Somero (1973, 1984, 2002), integrated biology across the hierarchy of animal life, from molecules to ecosystems. Research efforts in this arena depended upon a deep understanding of the phylogenetic relationships of the subject organisms and their biogeography. In contrast, while basic biochemistry was characterized using microbes such as *Escherichia coli* and although microbiologists had devised many ways to distinguish microbes, before the advent of low-cost, high-throughput sequencing of nucleic acids, biologists could not know the identity and relationships among constituents of the microbial world nor their species-specific roles in the biosphere. The application of this new 'lens' of molecular biology began with the work of Carl Woese and colleagues in the late 1970s (Pace et al., 2012), was facilitated by the invention of polymerase chain reaction (PCR), and rendered accessible to nearly all biologists by the development of inexpensive, next-generation sequencing techniques. The use of these technologies has opened the doors to a world far beyond our expectations. We now know that the greatest diversity of organisms occurs within the microbes, and that the relationships with the microbial world shape animal biology, whether in intimate symbioses or as life forms that share and modify a common habitat. This contribution will explore our new-found awareness of the centrality of the microbial world and its implications for biology's future.

Biology at an inflection point

In 2008, the US National Institutes of Health (NIH), the National Science Foundation and the Department of Energy commissioned a study by the National Academy of Sciences (NAS) to examine the role of biology in addressing the challenges of the 21st century. The committee, which included members of academia, industry and private foundations that had been chosen to develop a roadmap for the 'New Biology', first examined the landscape to define a focus for their work. The group's consensus was that biology is at an inflection point, a time of significant change in trajectory brought about by societal needs and technological advances in key disciplines.

Following World War II (WWII), we entered an era in which we would witness dramatic changes in all measures of biosphere health. The 'Anthropocene', an informal geological epoch that began with the industrial revolution in the early 1700s, was so named to recognize the impact of the most fateful change during this time, i.e. the increase in human population growth. This increase has become exponential since the late 1940s, and has been concurrent with an astonishing increase in the demand for energy. The effects of uncontrolled population growth have produced a series of looming crises that not only have biology at their base but also present an arena in which practitioners of the biological sciences will be critical contributors. The analyses that emerged from the NAS study (National Research Council Committee, 2009) identified four areas in which biology is poised to make pivotal contributions: (1) the provision of food for the growing population; (2) the development of alternative energy sources; (3) the promotion of health; and (4) environmental protection.

The growing need for solutions to biological problems is driving innovation, not only in biology itself but also in the physical sciences, engineering and mathematics. Recent years have witnessed technological advances in biology that are revolutionizing both the theory and the practice of the discipline. Concomitant with the crises driven by human population growth, the post-WWII era witnessed the dawn of molecular biology. Following the discovery of DNA as the genetic material in the early 1950s, biology began a reductionistic, decades-long focus into nucleic acid chemistry. Then, with the advent of PCR, followed by less and less expensive high-throughput nucleic acid sequencing, molecular approaches became more accessible for the study of questions at all levels of the hierarchy of life. The Joint Genome Institute website (http://jgi.doe.gov/) provides data to show that sequencing on all platforms will have gone from about 33 Gb (gigabases) in 2006 to a projected 100,000 Gb by the end of 2015, with the cost plummeting from ~\$600,000 Gb^{-1} to <\$100 Gb^{-1} (Fig. 1; Boyle and Gill, 2012). In addition, physicists, chemists, engineers and mathematicians increasingly lend their expertise to biology, and biology is providing these groups with new frontiers in their fields (e.g. Cohen, 2004; Davies, 2004). With these issues in mind, the 'New Biology' committee recommended that the most fruitful directions for the near future would be all about integration, i.e. research across the hierarchy of biology itself and through collaborative efforts of biologists with practitioners of other key disciplines.

Next-generation sequencing, which came onto the scene just 2 years before the New Biology committee would embark on their work (<10 years ago), led to an explosion in the descriptions of the microbial world. For example, the extensive sampling and metagenomic analyses of the world's oceans, undertaken by the scientific crew

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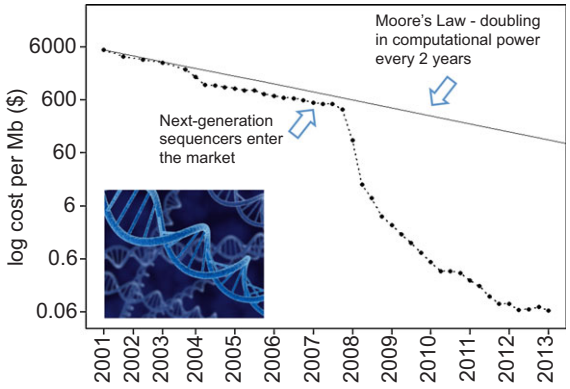

Fig. 1. The decrease in the cost of nucleic acid sequencing over recent years. Modified from Boyle and Gill (2012). Mb, megabases.

of the J. Craig Venter Institute sailboat the *Sorcerer II*, provided the first detailed view of the diversity of the planktonic microbiota (Rusch et al., 2007). Similarly, the first phase of the Human Microbiome Project was underway and had already demonstrated unprecedented diversity and stability of the microbiota of the human body (Proctor, 2011). Considering the emerging data from these efforts, the New Biology committee suggested that, among all of the subdisciplines of biology, microbiology appears to be uniquely positioned to play a major role in the efforts identified by the group. The general consensus was that no field would be affected more by the recent progress in biotechnology, and no field had the potential to contribute more broadly than microbiology to the challenges highlighted by the New Biology committee.

Microbes as the foundation of the biosphere

The ability to use molecular characters for phylogenetic analysis is revealing the vastness of the microbial world (Fig. 2), a recognition that continues to revolutionize biology. For over two millennia, biologists used visual information to classify organisms, first with the unaided eye, and later with increasingly sophisticated microscopy. Then, in the 1960s and 1970s, biologists began to use a new 'lens', i.e. the gene sequence, to study the relatedness of organisms. Comparative analyses of DNA sequences had shown that the genes of a given organism evolve at different rates, i.e. some relatively slowly and some relatively quickly. Sequence comparisons of slowly evolving genes, such as those encoding ribosomal RNA (rRNA), could be used to identify relationships among organisms of great evolutionary distance.

In the late 1970s, Carl Woese and colleagues at the University of Illinois began to apply the methods of molecular systematics to 'prokaryotes'. Prior to this work, because these microbes often have relatively similar sizes and morphological appearance, microbiologists relied on physiological and biochemical characters to classify them, using such resources as *Bergey's Manual of Determinative Bacteriology*. In addition, microbiologists widely held that prokaryotic diversity is low, with only a few thousand species, and that biologists would never see the level of insight into the evolutionary biology of prokaryotes and other microbes that had been possible with the macrobiological forms (Stanier and van Niel, 1962). However, Woese's early papers reported meaningful differences in the sequences of the rRNA among various microbial and non-microbial life forms. In a benchmark paper, Woese and his colleague George Fox described their evidence for three domains of life, or 'aboriginal lines of descent', which they called the eubacteria, archaebacteria and the eukaryotes (Woese and Fox, 1977). The development of PCR technology in the mid-1980s allowed Woese and colleagues to obtain a huge data set of rRNA sequences, which provided strong support for the domain structure they proposed earlier, although the names had been changed to the Bacteria, Archaea and Eucarya (Woese et al., 1990). This analysis placed the 'Last Universal Common Ancestor' or LUCA, with the Bacteria (Fig. 2, inset,

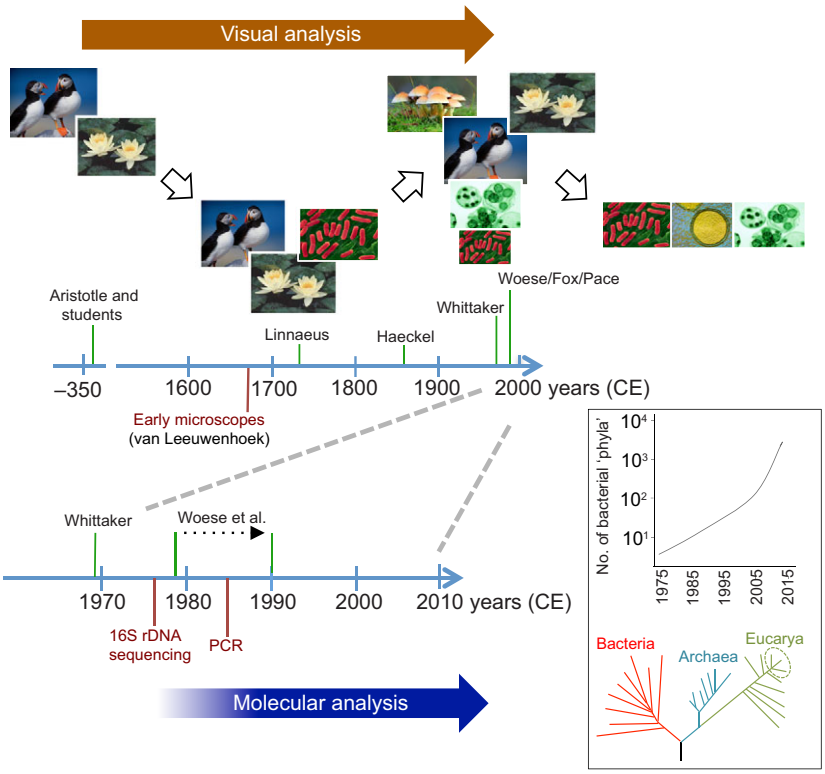

Fig. 2. The classification of organisms. The change in tools, from visual to molecular analyses, used to characterize the relationships among constituents of the biological world. Inset: top, number of bacterial phyla resolved by molecular methods (Yarza et al., 2014); bottom, the tree of three domains generated by Woese and colleagues.

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bottom). The sensitivity of PCR was also critical for defining the diversity of bacteria, as it could amplify rRNA from microbes that had not been brought into culture. These data, gathered over the last 20+ years, have revealed a microbial world more expansive than anyone had imagined. A recent analysis of the SILVA REF 114 database (reviewed in Yarza et al., 2014), which compiled 1.3 million high-quality rRNA sequences of >900 bp, estimated the number of bacterial phyla at over 1000, two orders of magnitude larger than thought to exist only 20 years ago (Fig. 2, inset, top). It should be noted here that the new sequencing capacity has also led to recent extensive comparisons and analyses of full genome sequences of microbes, with findings suggesting that the LUCA is basal in the Archaea, and that the Bacteria are more closely related to the Eucarya (Kim et al., 2014).

Microbiology as unifying the field of biology

Although Bacteria and Archaea are now recognized as the oldest and most diverse life forms, many biologists are relatively unfamiliar with these microbes as organisms, i.e. beyond their use as molecular tools and biochemistry subjects, or their membership in complex communities. This happenstance results from a variety of drivers. As mentioned above, microbiology as an evolutionary construct was previously not accessible. In addition, coincident with the molecular revolution, we have witnessed the development of deep silos in biology over the last 50 years into exclusive sub-disciplines often associated with departmental structures that have not fostered a comprehensive view of biology. The concept was that biology needed to drill down to understand mechanism. As a result, we have gained a wealth of knowledge in each sub-discipline, something that should never be taken lightly. However, this knowledge has been gained at the expense of providing students, even from their very first exposures, with a more holistic view of the field. In addition to this trajectory of biology as a whole, two strong schools of microbiology had emerged in the mid-19th century and remain today – pathogenic and environmental microbiology, a conceptual dichotomy that had largely ignored healthy relationships of microbes with macrobiological taxa. The legacy of these divergent areas is reflected in the structure of the societies of microbiologists, the journal formats of the field, and the focuses of undergraduate and graduate majors at universities. In response to this strong culture, biologists studying animals and plants largely ignored the microbial world in their considerations of forces that shape evolution, restricting their efforts to considerations of abiotic pressures, such as temperature, salinity, oxygen tension and light.

With evolutionary approaches becoming available to microbiology, we have a ‘new day’. This change in vantage point is one in which the microbial world is basal, wherein microbes provide the foundation for and major contribution to the genomic makeup of all organisms through crown group animals, such as humans (Domazet-Loso and Tautz, 2008). This new view promises to drive the integration of microbiology with the other sub-disciplines of the field and to change our basic frame of reference. For example, consider the hierarchy of life, the construct that structures the biological world into organizational levels, spanning from atom to biosphere. Viewing the abbreviated hierarchy of life as it occurs in the microbes (Fig. 3A), i.e. without the levels spanning organelles to organ systems, has interesting implications. Complex functions are coordinated in an animal or plant by these intervening levels of the hierarchy. In contrast, the physiology, behavior, population biology and ecology that enable a microbe to function within the environment are largely discovered and understood within the context of its molecular biology and biochemistry. As such, microbes integrate the synthetic and reductionistic approaches to biology. The study of biochemical adaptations in animals and plants, which does integrate across the hierarchy, requires focusing on a few relevant levels (Fig. 3B).

In addition to being evolutionarily basal and having a simplified hierarchy of life, microbes also have certain traits that, when considered, provide insight into the fundamental capacities and limitations of life processes. Relevant to the discussion here is the widespread occurrence of horizontal gene transfer (HGT) in the Bacteria (Darmon and Leach, 2014) and Archaea (Wolf et al., 2012), and their metabolic diversity (Kluyver and van Niel, 1956) (Fig. 4). While concepts of evolutionary selection, as developed by Darwin, apply to both microbiological and macrobiological organisms, the Mendelian vertical inheritance of traits and associated genetic patterns is largely restricted to groups within the Eucarya and, as such, is a derived feature. Further, the propensity

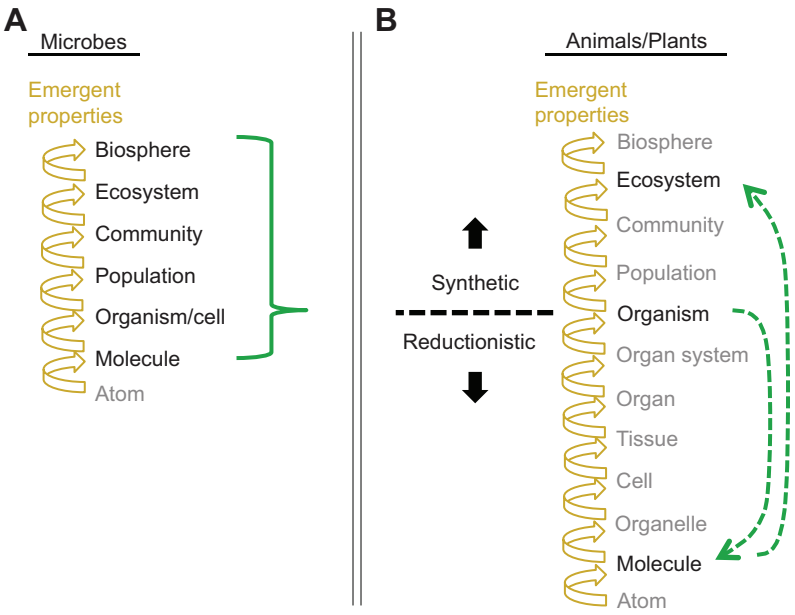

Fig. 3. The ‘hierarchy of life’ of different sets of organisms. (A) Microbes collapse the hierarchy of life, such that all levels are typically studied using biochemical and molecular methods (green bracket). (B) In animals and plants, the level of the organism has a series of defining lower levels that reflect their multicellularity. While most fields of biology focus on one level or contiguous levels, an animal or plant’s ecology can be studied by characterizing its biochemical adaptations to the environment in which it evolved (green arrows), an area of emphasis developed by Hochachka and Somero.

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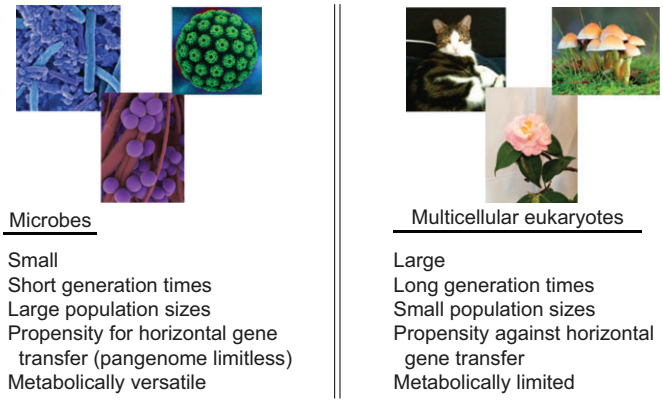

Fig. 4. Traits of the microbiological and macrobiological worlds that promote their partnering intimate association.

for HGT seems to have attenuated in the radiation of the Eucarya, particularly among the animals, although evidence is increasing that it is more prevalent than previously thought (Boto, 2014). Because we now know that the most common mode of acquisition of traits occurs by HGT in Bacteria and Archaea, we can no longer use the 'Modern Synthesis' to describe the genetic basis of evolution. With this change in perspective, Koonin has suggested that we need a 'Post-Modern Synthesis' that encompasses the genetic mechanisms of all branches of the Tree of Life (Koonin, 2012; Koonin and Wolf, 2012). Along with the relative instability of their genome, a defining theme in microbes is their metabolic diversity. The array of microbial metabolisms includes all the known ways in which organisms can obtain carbon (autotrophy or heterotrophy), reducing equivalents (lithotrophy or organotrophy), and energy (phototrophy or chemotrophy), and they combine these in nearly every way possible. These metabolic strategies use a wide variety of terminal electron donors and acceptors. By definition, the acquisition of the mitochondrion is the hallmark characteristic of the Eucarya; this innovation nevertheless has almost exclusively limited the Eucarya to oxygen as a terminal electron acceptor for respiration.

Taken together, a student who grasps the biological scope of microbes would understand the full array of gene organization and inheritance, as well as the possible metabolisms available to life forms as we know them. In addition, because it was the microbes that established the biosphere ~3.5 billion years ago, viewing the microbial world as the foundation of biology provides historical context. Turning the field 'on its head' in this way may provide a powerful mechanism for unifying biology into a set of basic principles, in much the same way that chemistry and physics identified a series of unifying themes decades ago.

Partnering with microbes

Another byproduct of advances in sequencing technology has been the recognition that intimate animal and plant interactions with microbes are now, as they have been throughout geological history, a common theme among life forms. With the seminal work of Lynn Margulis, biologists became aware that the eukaryotic cell arose through a symbiotic event (Sagan, 1967). Interestingly, this event, i.e. the acquisition of the mitochondrion, appears to have occurred once and spurred the radiation of all of the Eucarya (Lane, 2014). The presence of mitochondria augmented the efficiency of cellular bioenergetics by increasing the membrane surface area for the production of ATP through cellular respiration. In addition, over evolutionary history, much of the mitochondrial genome has been transferred to the nucleus, such that the mitochondria energetically support the much larger nuclear genome. These relationships between the nucleus and mitochondria of the cell provide eukaryotes with orders of magnitude more energy per gene than is available per gene for the Bacteria and Archaea (Lane, 2014). In addition to its well-recognized role as the 'powerhouse' of the cell, recent studies have indicated that the mitochondria control many more cellular functions (Mitra and Lippincott-Schwartz, 2010; Antico Arciuch et al., 2012; Friedman and Nunnari, 2014). For example, the mitochondrial network in the cell appears to be electrically coupled (De Giorgi et al., 2000) and coordinates such critical processes as cell death, cell proliferation, autophagy (Rambold and Lippincott-Schwartz, 2011), cell differentiation (Mitra et al., 2012) and aging (Lee and Wei, 2012). The view seems to be emerging that perhaps the mitochondrial network is actually the 'brains' of the cell, controlling much of the cell's processes, including activities of the nucleus.

Whereas biologists have known for decades about the endosymbiotic origin of the eukaryotic cell, intimate interactions of microbes with animals and plants had been thought to be rare and restricted to only a few groups. The recent, technology-enabled ability to identify and characterize uncultured microbial cells occurring in large communities has demonstrated that most animals and plants rely on interactions with microbes (Hadfield, 2011; Gilbert et al., 2012; McFall-Ngai et al., 2013). This dependence is not surprising, given that the differences in characters between

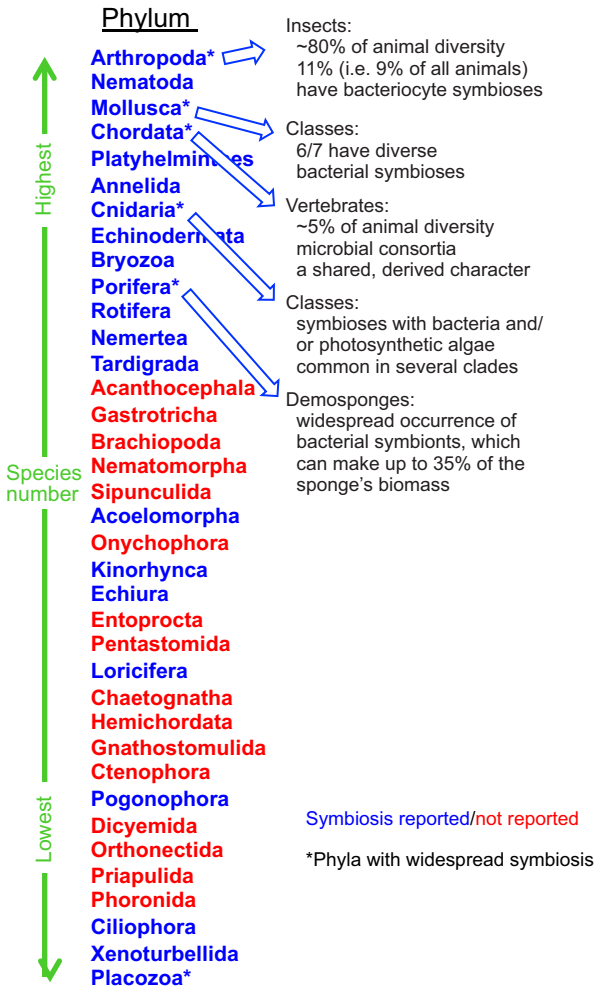

Fig. 5. The prevalence of symbiosis among the animal phyla.

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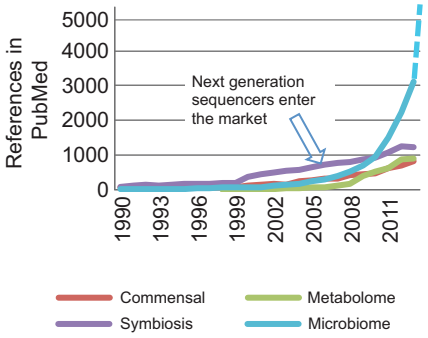

Fig. 6. The growth of the symbiosis field as reflected in references cited in National Center for Biotechnology Infrastucture (NCBI) PubMed. The commonly used key words ('commensal', 'symbiosis', 'metabolome' and 'microbiome') were searched for hits in the database.

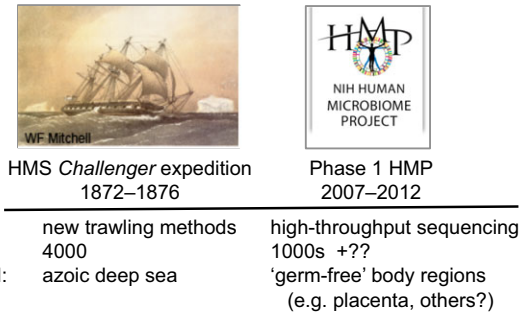

Fig. 7. Technology-enabled discoveries of organismal diversity in the 19th and 21st centuries, animals of the deep sea and microbes of the human body, respectively.

microbiological and macrobiological forms (Fig. 4) render them complementary, i.e. by forming alliances they increase one another's scope. To date, over half of the nearly 40 animal phyla have members in which symbiotic associations have been described, with the most speciose third all having representatives with such alliances (Fig. 5). In several groups within these phyla, symbiosis is a defining feature, such as in the bacteriocyte symbioses that occur in ~11% of the insect species. This figure ignores the carriage of the widespread intracellular symbiont of insects, *Wolbachia* spp., which by some estimates is harbored by up to 60% of insect species (Hilgenboecker et al., 2008). Recent analyses from fish (Milligan-Myhre et al., 2011) to mammals (Ley et al., 2008) suggest that the carriage of complex consortia is a shared derived character of vertebrates. As more information becomes available, it is likely biologists will find that symbioses occur in most, if not all, major clades. Finally, it is not only all about symbiosis. Data are accumulating that implicate bacteria in the larval settlement of a large number of marine invertebrates. As such, animal–microbe interactions may play a critical role in shaping the ecology of aquatic habitats (Hadfield, 2011; Shikuma et al., 2014), such as the intertidal and shallow subtidal zone, as well as the structure of marine biofouling communities (Hadfield et al., 2014).

Among all of the animal–microbe interactions studied over the past 20 years, the human microbiota continues to receive the most attention. Researchers have identified stable, predictable communities associated with several sites on the body, including the skin and the digestive, respiratory, excretory and reproductive tracts. These communities have been studied in health and disease over the trajectory of ontogeny. Because the data have determined that the microbiota is a central player in the biology of humans, and grant support from the NIH is plentiful, the field is growing at a phenomenal rate (Fig. 6). Most notable is the inclusion of microbiome research as one of the two NIH roadmap initiatives from 2007–2012, called the Human Microbiome Project (HMP) (the other was epigenetics). The activities of this period, Phase I-HMP, represented a kind of molecular natural history that has been enabled by high-throughput sequencing to define the diversity and 'biogeography' of the human body. As such, it is not unlike the HMS *Challenger* expedition of the late 1800s (Fig. 7), the first true oceanographic expedition that set out to 'map' the geological, chemical and biological attributes of the ocean. The biological efforts of the HMS *Challenger* expedition were technology-enabled by new trawling methods to explore the deep sea and define the life forms there. With the foundation provided by Phase I-HMP, Phase II-HMP is working to define the mechanisms underlying the patterns of the microbiota that have been determined. Our understanding of the role of the microbiota in human health is still rudimentary, but biologists in this field have already linked the diversity and composition of the microbiota, and its development within a single individual, to several prevalent diseases, such as obesity (Ley, 2010), diabetes (Karlsson et al., 2013), behavioral abnormalities (Borre et al., 2014) and kwashiorkor (Smith et al., 2013). Although not much support has been forthcoming from the government to study the diversity of symbioses in other animals, the data to date suggest that the discoveries will be no less rich and compelling. Comparative studies, i.e. taking advantage of the 'experiments' that nature has done, will be valuable in defining what features of host–microbe interactions are shared throughout evolution and what features result in the diversity of symbiotic associations (Ruby, 2008; Chaston and Goodrich-Blair, 2010).

Biochemical adaptations of the holobiont – stretching the field into new dimensions

Hochachka and Somero taught students to negotiate freely across the levels of life's hierarchy in thinking about how animals adapt to their environments. This community of biologists, as well as others, has largely focused thus far on abiotic forces that influence biochemical adaptations. They have, however, a rare perspective and are uniquely poised to incorporate biotic influences into their conceptual framework, notably the partnerships with bacteria, fungi and viruses. The expansion of the field into this frontier would usher in the study of how the integrated activities of the resident two or three domains of life, i.e. the holobiont (Gilbert, 2014), are coordinated in the metaorganism in responses to various environmental pressures. Thus, as we go forward in the study of the mechanisms underlying the biochemical adaptations of animals, let's not forget their microbes!

Acknowledgements

I thank George Somero for the opportunity to work with him early in my career, for his support, and for the chance to participate with his academic family in celebration of his many contributions to the field of biology. I thank Ned Ruby for comments on the manuscript and for his constant companionship in adventures in symbiosis research. I am grateful for the opportunity to work with a phenomenal group of graduate students and postdocs over the years, as well as for the continuing collaborations with microbiologists, biochemists and biophysicists.

Competing interests

The author declares no competing or financial interests.

Funding

M.J.M.-N. has been supported by the John Simon Guggenheim Foundation, National Institutes of Health, the National Science Foundation, the Office of Naval Research, the W. M. Keck Foundation, and Gordon and Betty Moore Foundation. Deposited in PMC for release after 12 months.

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