Trends in Parasitology

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Opinion

The Adaptiveness of Host Behavioural Manipulation Assessed Using Tinbergen's Four Questions

Upendra R. Bhattarai,[1,*] Jean-François Doherty,[2] Eddy Dowle,[1] and Neil J. Gemmell[1]

Host organisms show altered phenotypic reactions when parasitised, some of which result from adaptive host manipulation, a phenomenon that has long been debated. Here, we provide an overview and discuss the rationale in distinguishing adaptive versus nonadaptive host behavioural manipulation. We discuss Poulin's criteria of adaptive host behavioural manipulation within the context of Tinbergen's four questions of ethology, while highlighting the importance of both the proximate and evolutionary explanations of such traits. We also provide guidelines for future studies exploring the adaptiveness of host behavioural manipulation. Through this article, we seek to encourage researchers to consider both the proximate and ultimate causes of host behavioural manipulation to infer on the adaptiveness of such traits.

Highlights

Host manipulation has evolved as a primary life history strategy in all major parasitic lineages, and its adaptive significance is well established.

Although adaptiveness is an evolutionary concept, the complexity of analysing the evolutionary parameters of a manipulative trait has given rise to an ongoing debate on the criteria of adaptive host manipulation.

The importance of both proximate and ultimate mechanisms for inferring the adaptiveness of host manipulation is discussed by reviewing Poulin's criteria considering Tinbergen's four questions of ethology.

There are important parallels between Tinbergen's four questions and Poulin's criteria of adaptive host behavioural manipulation, from an ethological perspective. Their one-to-one analysis provides deeper insights into the importance of both proximate and ultimate mechanisms of adaptive host behavioural manipulation.

Adaptive Significance of Host Manipulation

Host manipulation (see Glossary) has evolved as one of the primary life history strategies in all major parasitic lineages [1,2]. It can produce a wide range of physiological, morphological, and behavioural changes in the host [3]. Intriguing examples include: the fungi *Ophiocordyceps* that cause ants to climb up plants or trees, clamp their mandibles around a leaf stem, and die [4]; the protozoan *Toxoplasma gondii* that perhaps causes the 'fatal attraction' of rats to felines [5]; and the nematomorphs and mermithid nematodes that somehow drive their terrestrial arthropod hosts into water [6,7]. Although the **adaptive significance** of altered host behaviours for some well studied parasites is now very much established [2,8–10], the criteria used to infer adaptiveness is still debated [1,11,12].

It has been nearly 50 years since host manipulation was first treated as an adaptive trait [13] and our understanding of the phenomenon has since changed remarkably, including new theories and many more examples [14,15]. However, adaptation alone is intrinsically complex and determining the adaptiveness of a trait is not straightforward [16]. Two primary schools of thought exist on what criteria a trait requires to be adaptive (Box 1). Some consider a trait adaptive if it confers a higher fitness benefit in individuals with it than those without [17]. A contrasting view is that, in addition to bestowing a higher fitness benefit, it is also necessary to identify the specific selective pressures that may have contributed to the higher fitness value of the trait before it can be deemed adaptive [18,19]. The first school of thought is more likely to consider fortuitous outcomes as an adaptive trait, unlike the second one which provides a more rigorous analysis involving both proximate and ultimate mechanisms. These differing criteria of adaptiveness could ultimately impact our research approaches, and thus, our understanding of host behavioural manipulation by parasites. In this article we focus on the manipulative traits that supposedly benefit parasites and discuss the importance of exploring these using both proximate and ultimate criteria as laid out by Poulin [10] (Box 2) in light of Tinbergen's four questions of ethology [20] (Box 3).

[1]Department of Anatomy, University of Otago, Dunedin 9016, New Zealand
[2]Department of Zoology, University of Otago, Dunedin 9016, New Zealand

*Correspondence:
bhaup057@student.otago.ac.nz
(U.R. Bhattarai).

Box 1. The Criteria for Adaptive Traits

The first school of thought considers fitness benefits as the sole criterion of a trait's adaptiveness (orange) (Figure I). This widely used definition denotes that a trait that offers higher fitness to an organism, compared with others lacking it, is adaptive. This school of thought also assumes that a particular trait is either present or absent, which is very unlikely to occur in nature, and views adaptation as a trait [19,20].

The second school of thought (blue) (Figure I) views adaptive traits as a function of fitness benefit (orange) and evolutionary selective pressures (blue). It refers to adaptation in a population driven by specific selective pressures through evolutionary timescales and processes through which traits with better survival and reproductive success emerge as adaptive. This also refers to the idea that adaptation is an ongoing process and continually acts to fine-tune traits according to the environment [19,21].

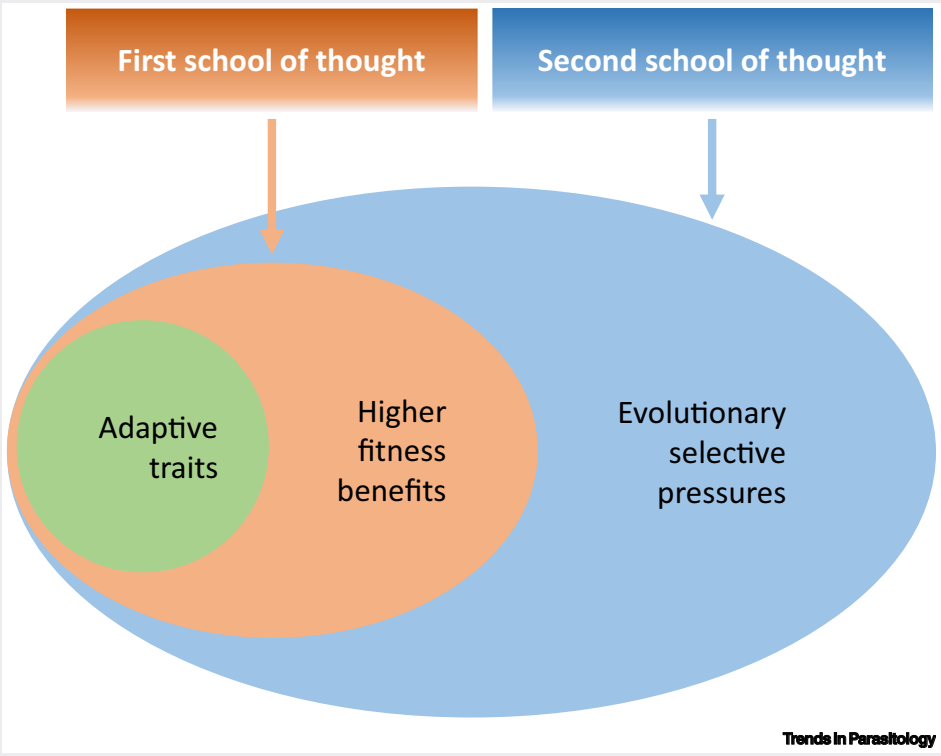

Figure I. Venn Diagram for the Two Schools of Thought on the Criteria of Adaptiveness.

Box 2. Poulin's Criteria of Adaptive Host Manipulation

In 1995 Poulin presented the following four criteria for parasite manipulative traits to be adaptive [11].

(i) Fitness effects. Fitness effects are among the most important criteria of all but are not conclusive evidence of adaptation on their own. In order to be adaptive, the behavioural change in the host must confer fitness benefits to the parasite.
(ii) Complexity. Complex traits are more likely to be adaptive as opposed to simpler ones. They require an organising principle and are less likely to be an accidental outcome. However, judging the complexity of mechanisms responsible for host behavioural change can be challenging. Such evaluations are often subjective and possibly blinded by the complexity of the mechanism. Therefore, complexity is regarded as the least useful of the criteria.
(iii) Purposiveness of design. Adaptive traits show a purposiveness of design and are well fitted to their functions and environment. For such an arrangement to arise by chance is unlikely, which could reflect a seemingly purposive developmental design.
(iv) Convergence. Species from different lineages in different environments under similar selection pressures might evolve with similar adaptation strategies. Such convergent evolution provides strong support for adaptive host manipulation.

Glossary

Adaptive significance: refers to any behaviour that evolved through generations and was shaped by natural selection and influenced the survival and/or reproduction of the organism.
Epigenators: the extracellular signals responsible for triggering an epigenetic pathway.
Epigenetic: refers to genetic control by factors other than an individual's DNA sequence. It involves mechanisms such as methylation, acetylation, phosphorylation, and ubiquitylation.
Extended phenotype: the genes or gene products of an organism that are expressed into a phenotype even beyond their physical boundaries.
Gradualism: a concept which proposes that large morphological changes in organisms occur through a series of smaller steps over a long period of time.
Host manipulation: the ability of certain parasites to alter host phenotype for their own fitness benefit, at the expense of the host.
Host manipulation hypothesis: a hypothesis which states that parasites can evolve to modify specific aspects of their host's traits to increase their own chances of survival and reproduction.
Hypomethylation: the loss of a methyl group on the 5-methylcytosine nucleotide.
Maternal effects: environmental influences provided by mothers on offspring that interact with offspring genotype and other external influences on offspring.
Teleological: relates to the explanation of phenomena in terms of the purpose they appear to serve rather than their cause or origin.
Treetop disease: *Baculovirus*-infected lepidopteran larvae typically climb toward the top of trees and die, thereby facilitating the dispersal of viral particles onto the broader canopy area.
Trophically transmitted parasites: parasites that are naturally transmitted when their current host is consumed by their next host.

Box 3. Tinbergen's Four Questions of Ethology

Niko Tinbergen, a founding father of ethology, suggested the following four important questions to better understand animal behaviour [23].

(i) Function –what is it for? Also referred to as the current utility of a behaviour, Tinbergen invested a substantial amount of his work investigating the function of animal behaviours. This explains the utility of a trait with respect to the organism's survival and reproductive success.
(ii) Causation – how does it work? Questions of causation help to determine and explain the mechanisms underlying a trait. This can help to identify the molecular, biological, morphological, or external stimuli constructing the trait.
(iii) Ontogeny – how does it develop during the lifetime of the organism? This explains the developmental changes an individual goes through during its lifespan and the traits that are developed during these phases. It can help to explain the effects of the external environment and individual learning that shape a trait.
(iv) Phylogeny – how did it evolve during the evolutionary history of the organism? This gives an evolutionary explanation about the history of the trait. How the trait was shaped through an evolutionary timescale and what selection pressures are responsible for the current form of the trait.

Poulin's Criteria and the Debate on Adaptiveness

Poulin, in his 1995 paper 'Adaptive changes in the behaviour of parasitized animals: A critical review' [10], highlighted the need for a clear approach in interpreting the adaptiveness of host behavioural manipulation as a trait and put forward a logical framework for the rigorous analysis of both the proximate and ultimate causes. Four criteria were presented: 'Fitness effects, Complexity, Purposiveness of design, and Convergence' (Box 2). Poulin concluded that the fitness effect is the most important criterion of all and highlighted the importance of selection history for a trait to be adaptive [10].

These benchmarks have been criticised as being too conservative [14,21,22]. Some even argued that the need for such distinction between adaptation and the coincidental side effects of infection is questionable [12,23]. Moore [24] argued strongly against altered host behaviours as being side effects of infection. According to the author, the fitness benefit of a trait is subject to natural selection and is expected to be strongly linked with the evolution between interacting species. However, it is not the fitness benefit that is under natural selection but rather the different traits; natural selection favours those with higher fitness benefits. In 2010 Poulin relaxed his original definition by including any change in host behaviour that improves parasite transmission as an adaptive manipulation, as long as it is encoded in the genes of the parasite [15]. Still, many studies continue to follow Poulin's [10] original criteria [25,26], and many more believe that studying both proximate and ultimate mechanisms is essential to understanding the adaptiveness of host behavioural manipulation [27–30].

Cézilly and colleagues [31] noted the importance of understanding the evolution of complex life cycles in parasites, arguing that manipulative traits predating the inclusion of the manipulated host in the parasite's life cycle may not be true adaptations. Therefore, investigating how such an ability came into being in the first place would provide critical information on the adaptiveness of the trait. In addition, Worth and colleagues [32] argued that accidental fitness benefits for parasites, like those mediated by the host, cannot be considered as adaptive as they are likely to be nonspecific and could occur in response to a large range of pathogens. Even though researchers in this field have persistently emphasised the investigation of the mechanistic bases of manipulation, to date we have failed to fully connect the question 'Why do parasites manipulate their host?' to 'How do they do it?'. This has left researchers unable to empirically distinguish the adaptive physiological changes induced by parasites from the pathological side effects of infection (see [33] for a review).

Host manipulation by parasites can be a multidimensional phenomenon in terms of the number of simultaneously altered behavioural traits by a single parasite [34] and/or the specific action of

each parasite in the case of multiple infections [12,35]. As mentioned before, not every change in host behaviour can be considered adaptive; they could merely be a side effect of infection or even a host reaction to the external environment, which may or may not be fortuitous to either organism [36]. For example, the reduced food consumption of *Spodoptera litura* larvae infected with a pathogenic baculovirus [37] does not have any fitness effect for either organism, whereas the hyperactivity and **treetop disease** of infected *S. litura* increase the dispersal of the virus [38]. Similarly, behavioural fever, which has long been thought to be an adaptive behaviour of the host, may not have any marked fitness effect. Behavioural fever in house flies with fungal infections does reduce parasitic growth but it also reduces egg viability when compared with infected flies not undergoing behavioural fever [39]. By contrast, host manipulation that shows a fitness effect may just be accidental, that is, not the outcome of a purposive proximate mechanistic explanation. The appearance of bright pink coloration in coral polyps infected with trematodes was considered to be the parasite's strategy to increase visibility, which would improve its chances of being eaten by its definitive host, the butterfly fish [40]. However, it was found that the pigmentation appeared as a result of the coral's cytotoxic defence system and plays a role in the immune response of the coral [41]. Even so, according to the '**host manipulation hypothesis**' [27], trematodes can evolve to control their host's coloration, to enhance their own survival and reproduction, but a thorough evolutionary investigation of this trait is lacking.

Scope of Current Opinion

The adaptiveness of host behavioural manipulation by parasites is still largely debated. However, the need for distinction between adaptive versus nonadaptive host changes has been more prominent with the rapid expansion of interest in this field [32]. This remarkable phenomenon can significantly impact ecosystems and evolutionary processes by modifying nutrient flow, food web structure, host population ecology, and species competition [22,42–44]. Moreover, some of the deadliest human diseases, like malaria and rabies, rely on host behavioural or physiological change, making it immensely important to have an in-depth understanding of the mechanisms of manipulation [45,46]. This requires a clear distinction between behavioural alterations that are adaptive for the parasite and the accidental by-products of infection.

Well designed studies of proximate and ultimate mechanisms have proven helpful in dissecting and predicting the adaptiveness of such traits [47,48]. Here, we revisit Poulin's [10] criteria, which have served as a guideline and basis for studying the adaptiveness of host behavioural manipulation for 25 years, through Tinbergen's four questions, the classical roadmap for behavioural investigations (Figure 1). Niko Tinbergen, a founding father of ethology and Nobel laureate, authored 'On aims and methods of ethology', a paper considered as one of the most important contributions to the field of animal behaviour [20]. In it, Tinbergen suggested that animal behaviour could be better understood by questioning its function, phylogeny, causation, and ontogeny. These four questions provide us with a comprehensive approach that has stood the test of time in shaping behavioural studies. We also provide guidelines for future studies exploring the adaptiveness of host behavioural manipulation, along with some examples of studies that offer answers to Tinbergen's four questions while addressing Poulin's criteria.

What Is It for?

The most decisive criterion on whether a trait can be considered adaptive stands on whether or not it confers fitness advantages to its bearer [10,23]. However, while a critical criterion, a simple fitness advantage cannot be regarded as a sole criterion of adaptiveness. The concept of adaptation is not just **teleological**; it also refers to how a trait was produced in the past and shaped by natural selection. As natural selection acts over long periods of time, the fitness advantage could be statistically nonsignificant or undetectable [10,49]. The quantification of fitness effects is

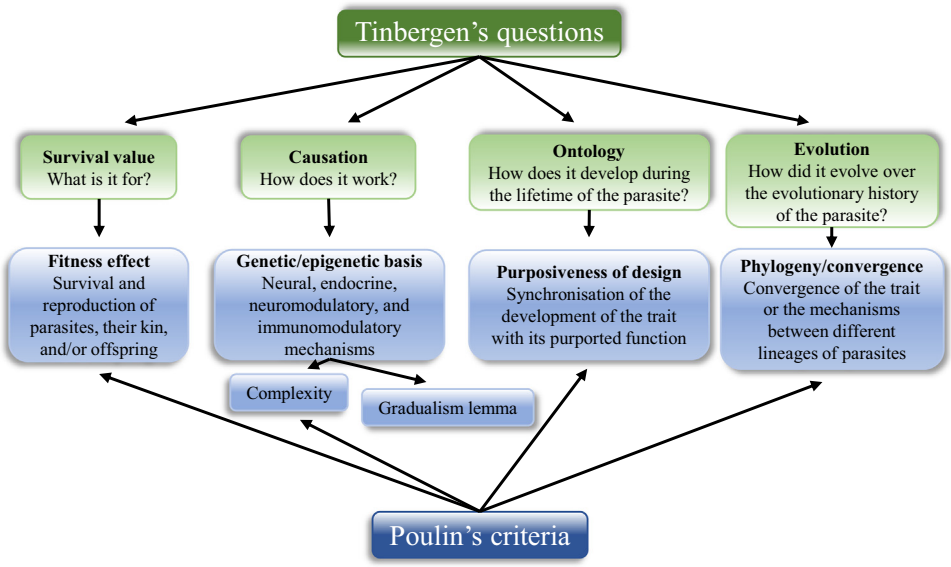

Figure 1. Tinbergen's Four Classical Questions in Ethology, Modified in the Context of Behavioural Change Induced by Parasitic Infection, Answered with Poulin's (1995) Criteria on the Adaptiveness of Host Behavioural Manipulation by Parasites [10]. Tinbergen's four questions and Poulin's four criteria are aligned in a pairwise fashion. Note that Poulin indirectly answers Tinbergen's question of causation through the concept of complexity.

another hurdle in its own way, especially when it comes to multidimensional phenotypic changes where the altered phenotypes could have occurred through multiple means such as disrupted physiologies, aberrant behaviours, changes in appearance, or a resulting combination [34,50]. On the other hand, the fitness benefit obtained through a manipulated behaviour could just be an accidental outcome of infection, and thus the altered behaviour cannot be considered an adaptive trait [32]. For example, heat-killed *Escherichia coli* induces the same behavioural and neurophysiological changes in host mosquitoes as that stimulated by malaria parasites, *Plasmodium* spp., wherein mosquitoes exhibit enhanced attraction to human hosts during the late stage of infection [51]. Such changes are found to be the result of insulin signalling-dependent changes in host resource allocation patterns and thus likely represent a general by-product of infection rather than specific host manipulation [52]. Similarly, mice, *Mus musculus,* infected with the enteric protozoan parasite *Eimeria vermiformis,* show reduced predator-induced fear or anxiety and avoidance of cat odour [53]. However, the parasite does not need to be passed on to cats to complete its life cycle, so the full adaptive significance of reduced predator-avoidance has yet to be ascertained.

Studies on the 'bodyguard' behaviour induced by braconid parasitoid (*Glyptapanteles* sp.) on their caterpillar host (*Thyrinteina leucocerae*) suggest that the fitness effect of host manipulation is highly context dependent [54]. When located on native guava trees, infected caterpillars appear to behave as bodyguards to the parasitoid's pupae; standing bent over the pupae, they violently swing their head towards approaching predators, keeping pupae safe [55]. However, when translocated to non-native eucalyptus trees, the bodyguard behaviour does not increase the parasitoid's survival [54]. Reportedly, the species composition of predators and hyperparasitoids differ between the native and non-native trees, resulting in differential fitness from the modified caterpillar behaviour. Thus, the fitness effects of such manipulation should be evaluated in a natural setting that includes all relevant components of the natural food web.

This important criterion for the adaptiveness of host behavioural manipulation answers Tinbergen's question of function or survival value. Among his questions, survival value holds a significant place in his synthesis, in which the 'adaptive significance' or the 'current utility' of a trait is emphasised [56]. For Tinbergen, fitness is largely related to survival, and behaviour ultimately contributes to the fitness of the organism. Hughes and colleagues [28] discussed the fitness effects in connection with Tinbergen's question of survival value through their experiments on the 'death grip' behaviour in ants infected with *Ophiocordyceps unilateralis* (Box 4). This behaviour causes infected ants to leave their colony and bite the underside of leaves at approximately 25 cm above ground, where they die, and the fungi can thus mature in a suitable microhabitat. When these ants were relocated either to the high canopy, the forest floor, or inside the ant colony, no fungal development occurred [57,58]. Conclusively, the apparent functionality of this behavioural alteration provides circumstantial evidence for the 'current utility' of the parasitic manipulation.

Therefore, the fitness effect or the survival value of a trait is an important criterion in determining its adaptiveness, and the difficulty in measuring this may be overcome by relating the trait to the relative performance of each candidate function. However, this criterion alone should not be regarded as the sole conclusive demonstration of adaptiveness.

How Does It Work?

Parasites, as host manipulators, target four main physiological systems in the host organism: neural, endocrine, neuromodulatory, and immunomodulatory [59]. The communications to and from these systems are accomplished with various types of neurotransmitters, neuromodulatory chemicals, hormones, proteins, enzymes, and other chemicals. Such communications can be directed through genetic or **epigenetic** mechanisms [23,60,61]. These could be the result of parasite gene expression or the result of parasite and host-secreted chemicals acting in response to one another. Investigating and understanding such mechanisms have been a challenge because pathogens affect their hosts in many ways that may or may not be beneficial to either organism. However, in recent years, technological advancements have transformed our way of studying and understanding host–parasite interactions. Increasingly, the field has transitioned from the largely qualitative and descriptive science of ethology to evolutionary, ecological, and molecular approaches utilising cutting-edge technologies and sophisticated algorithms that provide higher resolutions and detailed insights [62].

The concept of the **extended phenotype** proposed by Dawkins [63] is primarily based on the question of causation. Evidence for the extended phenotypic expression of a trait certainly adds to the adaptive significance and purposive function of said trait in host–parasite interactions. For example, the ecdysteroid UDP glucosyltransferase gene (*egt*) of *Lymantria dispar* multinucleopolyhedrovirus induces tree-top disease in *L. dispar* larvae. This causes the larvae to die higher up in the tree and increases the dispersal of viral particles [64]. The *egt* gene encodes an enzyme responsible for inactivating the moulting hormone 20-hydroxyecdysone (20E). By knocking down the downstream receptors of 20E and juvenile hormone (JH) in the host, Zhang and colleagues [65] confirmed the impacts of the viral gene on viral fitness and host climbing behaviour. They concluded that the viral infection activates JH production and inhibits the production of 20E, thereby downregulating the *BrZ2* gene and inducing climbing behaviour in the host. This shows a clear fitness benefit linked with the proximate mechanism of the virus that impacts host behaviour. Similarly, alteration of serotonergic activity is responsible for behaviour alteration in the amphipod *Gammarus pulex* infected with acanthocephalan parasites. Tain and colleagues [66] confirmed that serotonin (5-hydroxytryptamine, 5-HT) is specifically

Box 4. Parasitic Manipulation Assessed Using Tinbergen's Four Questions for the 'Death Grip' Behaviour in Ants Infected with *Ophiocordyceps*

(i) Function – the ant 'death grip' (Figure IA). Ant positioning during the 'death grip' induced by fungal infection appears to be adapted for optimal humidity and light conditions that maximise fungal spore production and dispersal [58,59].

(ii) Causation – fungal spore production (Figure IB). Infected ants appear to locate areas that favour fungal growth. The ant 'death grip' results from precise fungal penetration into the mandibular muscles, causing hypercontraction. Fungal infection also alters gene expression related to the neurobiology, odour response, circadian rhythms, and foraging behaviours of infected ants, highlighting the molecular mechanisms [68].

(iii) Ontogeny – dispersal of fungal spores (Figure IC). The fungus grows to form an extensive network of hyphae that invades the muscle fibres throughout the body but leaves the brain intact. The stage at which the fungus actively induces host behavioural change is unknown [68].

(iv) Evolution – infection (Figure ID). The 'death grip' behaviour induced by fungal infection evolved independently in two distinct lineages of parasitic fungi, *Ophiocordyceps* and *Pandora*, separated by 500 MY [82,83].

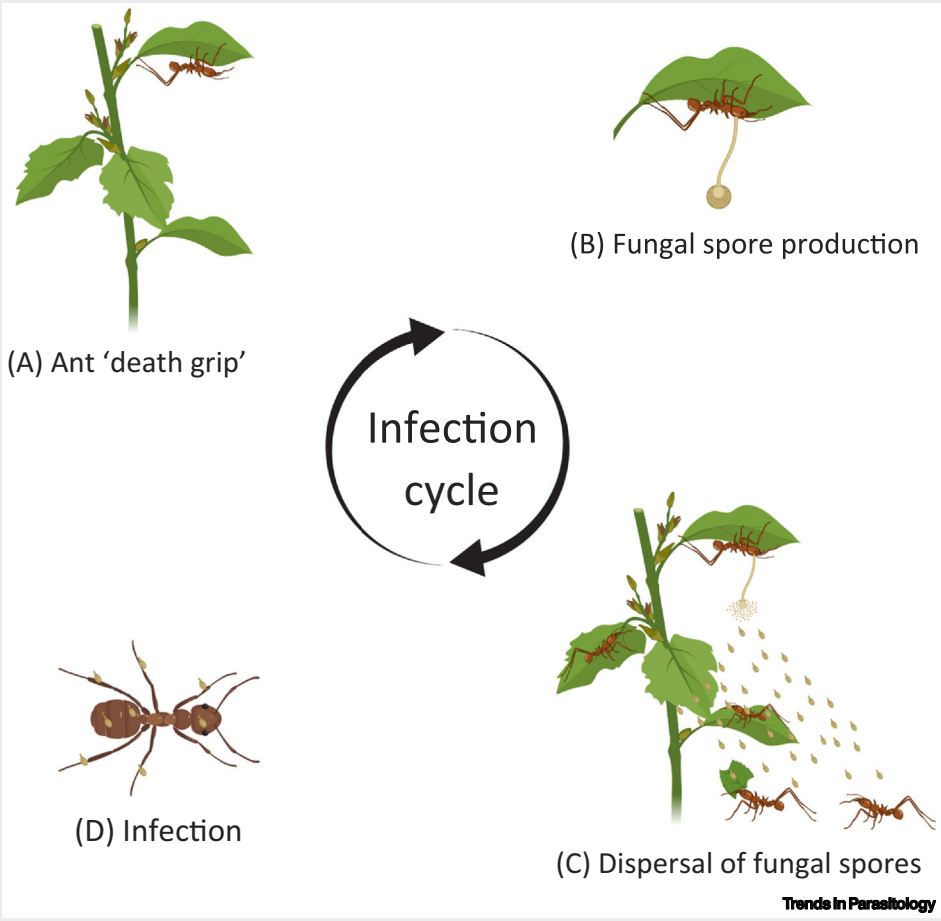

Figure I. Infection Cycle of the 'Death Grip' Behaviour in Ants Infected with *Ophiocordyceps*. Created with BioRender.com.

linked to the disruption of host photophobic behaviour by comparing the 5-HT immunoreactivity in *G. pulex* infected with different acanthocephalan parasites with the ability to induce different level of photophobic responses. Such examples of extended phenotype help us to explain how an organism can manipulate the phenotype of another [63]. Extended phenotypes produce altered traits in the host that are functionally correlated with certain alleles of the parasite and are thus subjected to reproductive bottlenecks as the benefits are passed on solely through

the genes of the organism upon which natural selection can act [67]. This type of evidence, therefore, suggests the strong adaptive significance of behavioural change for parasites.

The chances that a simple trait occurs as a by-product of other selective changes are higher than for those of a complex trait requiring an organising principle. For example, the precise penetration of fungal structures inducing mandibular hypercontraction and bypassing the brain to maintain motor neurons and neuromuscular junctions for the death grip behaviour in ants shows the level of sophistication in behaviour-manipulating parasites [68] (Box 4). Poulin [10] argued that even simple traits could be adaptive, but an increase in their complexity provides stronger evidence for adaptiveness. The author further clarified that complexity results not just from visible behavioural change but also from causal mechanisms. Seemingly simple changes in host behaviour could demand a great deal of physiological and/or morphological adjustments of the host induced by the parasite. Understanding complexity requires a detailed explanation of the mechanisms involved, which can be addressed through Tinbergen's question of causation. Dissecting causation through the complexity and **gradualism** of the underlying mechanisms, as defined by Poulin [10] and Conrad [69], respectively, may provide deeper insight into the adaptiveness of a manipulative trait.

How Does It Develop during the Lifetime of the Parasite?

Poulin [10] stated that behaviours resulting from an adaptive manipulative trait should be well fitted within their function in the environment to achieve higher parasite fitness. Therein lies the conformity between *a priori* assumptions about a manipulative trait and its purported function in host behavioural change. Importantly, the timing of these events should coincide with the developmental phase of the parasite that would benefit from these changes, which also presents a purposiveness in the induced behaviour.

Animals express particular behaviours at different stages of development, and the timing of these behaviours can be critical, for example, mate calling in insects [70]. Proper timing is essential for any behaviour to contribute to an organism's fitness, which could profit from positive selection pressure. For this reason, Tinbergen added ontogeny as a key ethological question [20]. This addition has been crucial and, as parasites induce altered behaviour(s) in their hosts, the timing and development with regard to the host and parasite's development are very important. For example, the positive phototaxis in baculovirus-infected lepidopteran larvae occurs just before the death of the host, causing it to climb high up into the tree canopy for better dispersal of the viral particles [71]. Similarly, hairworm-infected arthropods entering water coincides with the maturation of the parasite, which must emerge and mate in water [72].

The adaptive significance of purposiveness of design for host manipulation is even more pronounced in some **trophically transmitted parasites** with complex life cycles. These parasites infect intermediate hosts during their immature stages and eventually cause a sophisticated synchronised host manipulation to get to their final host. For example, *Gammarus pulex* (intermediate host), parasitised by immature stages of *Pomphorhynchus laevis* (parasite), tends to hide under refuges and is less predated upon by trout (final host) than uninfected individuals. As the parasite reaches the developmental stage for host transmission, the behavioural response of *G. pulex* changes to increased vulnerability and decreased antipredatory behaviour [73]. Similarly, shifting of the host's antipredatory responses during the optimal developmental stage of the parasite is also found in *Engraulicypris sardella* (fish) infected with *Ligula intestinalis* (tapeworm). Infected fish behave in the same way as uninfected fish when the tapeworm is in its early developmental stages, but as the parasite matures, the infected fish change behaviour, increasing their chances of being eaten by predatory birds (final host) [26]. Thus, parasites with

complex life cycles can produce sophisticated host manipulation with precise timing of the critical events correlating with the purposiveness of design.

Tinbergen's question of ontogeny can answer the purposiveness of design by investigating how suitable the trait is in terms of the timing of its expression with regard to the developmental stage of the parasite and how well fitted the trait is with respect to the changing environment of the parasite. Furthermore, it can help reflect on the mechanisms employed during different developmental phases of the parasite, thus highlighting the purposiveness of design during the process. During critical moments in their life cycles, parasites may not only impact the phenome of the host but the genome itself through epigenetic alteration, which in a broad sense refers to a variety of processes that result in changes in gene expression without modifying the DNA sequence [74]. Epigenetic markers are established during early development by external abiotic and biotic stimuli, but later on these can have a significant impact on an organism's phenotype [75]. Intracellular parasites can act as '**epigenators**', altering the environmental state from within the cell and triggering changes to the cell's epigenetic state [76]. *T. gondii*-infected *Rattus norvegicus*, that have a decreased fear of cat odours [77], provide a strong example of such an epigenetic mechanism. Infected male rats increase testosterone production, causing epigenetic modifications in the medial amygdala of the rat's brain. These DNA **hypomethylation** modifications increase the expression of the hormone arginine vasopressin which is involved in the loss of predator aversion [25,78].

Epigenetic effects on host phenotype can extend beyond their lifetime and pass down to their offspring [79]. For example, the chicks of flea-infested great tits disperse shorter distances from their nest than the chicks from uninfected mothers, which may be due to lower concentrations of yolk androgens in the eggs of infested mothers [80]. In this context, Poulin and Thomas [81] discussed the transgenerational epigenetic consequences of parasitism. They proposed that, since **maternal effects** are so common in many organisms, could parasites also do it? They further emphasized that this field of research carries a great potential for exciting future investigations. The study of the development of interacting species and the possible epigenetic causes, as well as connecting parasitic infection with the physiological changes in the host, could help to empirically distinguish between adaptive physiological changes and incidental changes resulting from stochastic processes [33].

How Did It Evolve During the Evolutionary History of the Parasite?

Convergent evolution between distinct lineages under similar selective pressures and in the absence of constraints are compelling cases of adaptive manipulation. Poulin's [10] criterion of convergence directly addresses Tinbergen's question of evolution (Figure 1). Such convergent evolution provides considerable evidence that the trait is function-specific and adaptive rather than an accidental outcome. The integration of high-resolution molecular analyses across phylogenies could help us understand the evolutionary pathways of manipulation. For example, phylogenetic analyses show that the ant death grip behaviour (Box 4) has evolved independently in two distinct lineages of parasitic fungi, *Ophiocordyceps* and *Pandora*, that are separated by 500 million years (MY) [82,83]. The convergence of such a manipulative trait across highly divergent genetic lineages, in contrast to being shared by common descent, provides a convincing case that the death grip behaviour is adaptive. In addition, another phylogenetic host association reconstruction of the genus *Ophiocordyceps* showed that these fungi evolved from fungal parasites of beetle larvae buried in logs or soil and that niche overlap played a crucial role in the transition from beetles to ants [84]. Such strong phylogenetic reconstructions could shed light on the ancestral roots of host–parasite systems and the resulting manipulative traits, which could inform us about the kinds of selective pressures they evolved under. Similarly, the behavioural

changes induced by mermithid nematodes and hairworms in their arthropod hosts, which causes them to enter water, strongly suggest that this manipulative trait has evolved independently twice [7]. A convergence in the general function of certain regulated proteins was found across mermithid- and hairworm-infected host brains, suggesting convergence in the mechanism of manipulation across the species [85]. These parasites are not closely related (they are in separate sister phyla), but both have evolved similar lifestyles; they develop within terrestrial arthropod hosts and emerge in water to mate and/or lay eggs. The ability to somehow drive their terrestrial hosts into water greatly enhances their chances of survival and reproduction. Whether they use similar mechanisms to induce such remarkable behaviours remains open for question, but the convergence of such a trait under similar selection pressures strongly suggests that the host manipulation in both parasitic groups is adaptive [7].

Such cases of convergence inform our ideas about the process of adaptation [86], ecological and phenotypic bases of convergence [18], and the molecular basis of evolution [87]. Lee and Coop [88] suggested a conceptual framework to analyse the degree to which evolutionary change represents an independent case of adaptation owing to natural selection. They also highlighted the ways that quantitative genetics and genomics can address questions of convergent adaptation. According to the authors, the first step to identify an adaptation is to establish that the set of observed convergent characters are unexpected by chance. Some phylogenetic methods that address this issue at macroevolutionary scales [89,90], as well as measures for quantifying and assessing the significance of convergence, have been developed [91]. Several studies have highlighted the importance of fossil evidence as a calibration point to complement molecular phylogenies [28,47,92]. For example, the fossil record suggests the existence of entomopathogenic fungi from the Cretaceous period [93]. The record also suggests that the death grip behaviour induced by fungi in its ant host dates back to at least 47 MY [94].

Furthermore, like any other evolutionary adaptation, host behavioural manipulation by parasites must be costly [63,95]. So, a trade-off between manipulation and other vital functions of the parasite, such as reproduction or growth, is expected [15]. Analysing the process of natural selection or determining the cost of a trait may not be straightforward. However, their combined analysis could provide a greater insight into the evolutionary adaptiveness of the trait. Despite an abundance of theoretical discussions [15,61,96], there are not many experimental studies on this topic. However, an experimental selection study conducted by Hafer-Hahmann [8] on the parasitic cestode *Schistocephalus solidus* and its first intermediate host, the copepod *Macrocyclops albidus*, is a very good illustration of this approach. *S. solidus* is a trophically transmitted parasite that is known to manipulate the activity of its first intermediate host (*M. albidus*) by reducing its risk of predation by fish (final host) before the parasite is ready for transmission. The author selected for parasite lineages that were more or less capable of manipulation over three generations and analysed the evolutionary adaptability of the manipulative trait while identifying the potential trade-offs. They showed that host manipulation responded to the selection pressure, confirming that the trait is heritable. Another study on parasitic wasps (*Dinocampus coccinellae*), which are known to induce a bodyguard behaviour in their host, the spotted lady beetle (*Coleomegilla maculata*), showed that parasitoid pupae protected by manipulated lady beetles were predated upon less than pupae found under dead lady beetles. They also demonstrated that the duration of the bodyguard behaviour is negatively correlated with the fecundity, not the longevity, of the parasitoid. Hence, this suggests that the cost of manipulation decreases fecundity of the parasitic wasp larvae [97].

Concluding Remarks

Overall, the definition of adaptiveness in host manipulation remains an important question and the dilemma still lies in whether the evolutionary and developmental history of a trait needs to be

Outstanding Questions

How do manipulative traits contribute to the fitness effects of the parasite, its kin, or offspring?

What are the parasite's mechanisms (genetic or epigenetic) for the manipulation, and what is its target system in the host (neural, endocrine, neuromodulatory, immunomodulatory systems)?

How is the trait developed during the parasite's life cycle to synchronise with its developmental need?

How did the trait evolve during the evolutionary history of the parasite?

considered separately from the apparent fitness benefits in order to infer its adaptiveness. This confusion has significantly impacted research strategies in this field of ethology. Discussing Poulin's [10] criteria through the lens of Tinbergen's four questions provides new insight on the importance and integration of both the proximate and ultimate mechanisms, as well as including the genetic and epigenetic causes for a holistic understanding of host behavioural manipulation. From an ethological perspective, we can draw important parallels between the four classical behavioural questions proposed by Tinbergen and Poulin's (1995) benchmark criteria, which reiterate the importance of understanding animal behaviour as a whole and not only in part. From a parasitological point of view, Poulin's (1995) criteria highlight the impacts of host–parasite evolution and resulting interactions, which in turn provide key answers to the four behavioural questions while addressing the true adaptiveness of manipulative parasite traits.

Determining the fitness effects of a supposed manipulative trait is very important yet difficult to achieve in many cases. Relating the relative performance of the trait to a candidate function or to the survival and/or reproductive value of the parasite, its kin or offspring, can provide a quantitative measure of fitness effects, but such tasks are complicated and have thus far been achieved for very few systems. Similarly, understanding the genetic or epigenetic bases of these traits is critical to identifying their causal mechanisms. By isolating the gene products of the parasite and their actions on target systems in the host, for example, neural, endocrine, neuromodulatory, and immunomodulatory systems, we may increasingly unravel the various ways in which parasites influence their hosts [98]. The expression of a supposed manipulative trait within the host environment should be synchronised with the developmental needs of the parasite, which can be subsequently used to help shed light on its purported function. The evolutionary history of a trait may not be easy to determine at times. However, this can be achieved by studying the molecular phylogenies of manipulated behaviours. If one can identify any other parasite lineage(s) displaying a similar trait and calibrate the phylogeny, it may be possible to study the trade-off costs and adaptability of a supposed manipulative trait over evolutionary time, perhaps providing a deeper insight into the evolutionary history of that trait. In this light, we offer four directing guidelines for future studies exploring the adaptiveness of host behavioural manipulation (see Outstanding Questions). We hope that researchers consider both the proximate and ultimate causes of host behavioural manipulation to infer the adaptiveness of such traits.

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Declaration of Interests

The authors declare no competing interests.

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