

Adaptive host manipulation by parasites: From phenomena to a causal framework

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ABSTRACT

Over the past decades, numerous cases of parasite-induced host phenotypic alteration have been documented across diverse taxa. However, a central challenge in this field lies not in the scarcity of examples, but in distinguishing true adaptive manipulation from host-driven responses or incidental pathological byproducts. Although the adaptive manipulation hypothesis proposes that such alterations enhance parasite transmission and reproductive success, empirical support remains fragmented and is often based on correlative evidence. Here, we critically reassess the adaptive manipulation hypothesis by synthesizing current evidence and identifying key limitations in linking host phenotypes to parasite fitness and underlying molecular mechanisms. We propose a structured evaluative framework based on four criteria: stage-specific temporal alignment, independence from host compensatory responses, net fitness benefit to the parasite, and mechanistic causality. Together, these criteria form a hierarchical approach for assessing the adaptive nature of host alterations. Applying this framework to well-studied host-parasite systems, we show that many widely cited examples remain inconclusive when evaluated against rigorous causal standards. We further synthesize current knowledge of molecular mechanisms, highlighting common themes such as neural interference, immune modulation, endocrine disruption, and gene regulatory changes, while noting that most systems lack direct functional validation. Finally, we outline an integrative research strategy that leverages emerging genomic and experimental approaches, including spatiotemporal transcriptomics, single-cell sequencing, and targeted perturbation, to bridge the gap between correlation and causation. By shifting the focus from descriptive phenomena to causal inference, this review provides a

conceptual and methodological roadmap for evaluating adaptive host manipulation and advances a more rigorous understanding of host-parasite interactions.

Keywords: Host manipulation; Adaptive significance; Parasite-host interactions; Molecular mechanisms; Causal inference

Table of Abbreviations

Abbreviation	Full Name
AcMNPV	Autographa californica multiple nucleopolyhedrovirus
5-HT	5-hydroxytryptamine (Serotonin)
20E	20-hydroxyecdysone
CcBV	<i>Cotesia congregata bracovirus</i>
CNS	Central Nervous System
CRISPR	Clustered regularly interspaced short palindromic repeats
DA	Dopamine
EGT	Ecdysteroid UDP-glucosyltransferase
GABA	Gamma-aminobutyric acid
GPCRs	G-protein-coupled receptors
JAK/STAT	Janus kinase/signal transducer and activator of transcription
miRNA	MicroRNA
NO	Nitric oxide
PTP	Protein tyrosine phosphatase
RNAi	RNA interference
scRNA-seq	Single-cell RNA sequencing
SSPs	Small secreted proteins
STT	Spatiotemporal transcriptomics

INTRODUCTION

Parasites live on or within host organisms, deriving nutrients at hosts' expense. Many also modify host physiology or behavior to facilitate their own survival and transmission (Poulin & Morand 2000). They exist across diverse taxa, including viruses, bacteria, fungi, protozoa, helminths, and arthropods (Humber 2000; Poulin & Morand 2000; Schmid-Hempel 2021). Estimates suggest that 40% of known species are parasitic (Dobson et al. 2008), and that parasitic interactions may account for up to 75% of links in ecological food webs (Lafferty et al. 2006). Given this pervasive presence, parasites are not only widespread biological entities but also major drivers of ecological and evolutionary processes (Dobson et al. 2008; Lefèvre et al. 2009). They regulate host populations, drive co-evolutionary dynamics, and contribute substantially to the global burden of infectious diseases such as malaria and schistosomiasis (Hotez & Kamath 2009; Shen & Luo 2025). Importantly, natural host populations are frequently exposed to multiple co-infecting parasites, such that host-parasite interactions typically occur within complex, multi-parasite ecological networks rather than simple pairwise associations (Poulin & Morand 2000; Dobson et al. 2008).

To maximize survival and spread, many parasites have evolved highly specialized and often adaptive transmission strategies tailored to specific ecological niches. These include direct, vector-borne, fecal-oral, sexual, and environmental routes. Notably, some lineages use trophic transmission, where parasites frequently induce phenotypic changes in intermediate hosts that increase predation risk, thereby ensuring transfer to the next host in the life cycle (e.g., trematodes in fish and birds) (Woolhouse et al. 1997; Combes 2001;

Poulin 2007). This strategy highlights a critical feature of many parasitic systems: transmission success may depend on the ability to modify host traits. Such modifications are not restricted to trophic systems, suggesting that active intervention in host biology is a widespread feature of parasitic life histories.

One of the most striking manifestations of such trait modification is host manipulation, in which parasites alter host behavior or physiology in ways that enhance their own transmission. This phenomenon has been interpreted as the extended phenotype, a concept proposed by Dawkins (1982), in which an organism's genetic influence extends beyond its body to shape its environment, including other organisms (Dawkins 1982). Parasite-induced behavioral changes can range from subtle shifts in activity levels to extreme modifications that increase host susceptibility to predation. A well-documented example is *Toxoplasma gondii*, which is associated with altered predator avoidance behavior in rodents, including reduced aversion to feline cues, which may increase the likelihood of predation by cats (Webster et al. 1994; House et al. 2011). Similarly, the parasitic hairworm *Spinochordodes tellinii* induces infected grasshoppers to enter water, a behavior that is lethal to the host but essential for parasite reproduction (Biron et al. 2005).

Despite the growing number of documented cases, a central challenge in this field lies not in identifying examples of host manipulation, but in determining whether these phenotypic changes represent true adaptive strategies of the parasite. From an evolutionary perspective, host manipulation is considered a highly adaptive strategy that enhances parasite's ability to reproduce and successfully complete its life cycle (Hernandez-Caballero et al. 2022). However, direct evidence linking these manipulations to increased

98 parasite fitness remains limited, and some behavioral changes attributed to parasites may,
99 in fact, result from the host's own immune responses rather than active manipulation
100 (Jensen et al. 2023). This ambiguity highlights a fundamental problem: correlations
101 between infection and host phenotype do not necessarily imply adaptive manipulation.

102 A related challenge concerns mechanism. Although behavioral manipulation has been
103 documented in over 100 host-parasite systems, only about 10% have been examined at the
104 mechanistic level (Moore 2002; Poulin & Maure 2015; Li & Poulin 2025). Current research
105 suggests that parasites exploit multiple molecular pathways, including neurotransmitter
106 interference, immune modulation, endocrine disruption, and epigenetic regulation, to exert
107 control over host behavior (Adamo 2013). However, these mechanisms are often examined
108 in isolation, and their causal relationships with host phenotypes are rarely established. As
109 a result, much of the current evidence remains correlative, limiting our ability to distinguish
110 mechanistic drivers from secondary consequences of infection.

111 Taken together, current research is characterized by two major limitations: the
112 absence of a unified framework for evaluating the adaptive nature of host manipulation,
113 and the lack of causal linkage between molecular processes and fitness outcomes. To
114 address these challenges, this review aims to move beyond descriptive accounts of host
115 manipulation by providing a structured framework for evaluating adaptive significance and
116 mechanistic causality. Specifically, we first synthesize representative cases to illustrate the
117 diversity of host manipulation, then critically reassess the adaptive manipulation
118 hypothesis, and subsequently propose a set of operational criteria for distinguishing
119 adaptive manipulation from alternative explanations. Building on this framework, we

integrate current knowledge of molecular mechanisms across systems and identify key gaps that hinder causal inference. Finally, we outline future research directions that combine emerging genomic tools with experimental approaches to directly link molecular mechanisms to fitness-relevant phenotypes.

THE WIDESPREAD PHENOMENON OF HOST MANIPULATION

Host manipulation is a widespread and often adaptive phenomenon (Poulin & Maure 2015), frequently observed in parasites with complex life cycles to facilitate trophic transmission. However, such strategies also occur in species with direct life cycles, such as “zombie ant” fungi, to optimize spore dispersal (Andersen et al. 2009). Parasites capable of inducing host phenotypic changes occur across all major parasite groups, from viruses to worms, and affect a wide range of hosts, including both invertebrates and vertebrates (Moore 2002). These influences extend beyond subtle behavioral adjustments in feeding or movement to encompass profound physiological, morphological, socio-biological, and reproductive alterations that enhance parasitic fitness.

Parasitic influence manifests through diverse and often extreme phenotypic shifts. For example, trematode infections (*Ribeiroia ondatrae*) induce severe limb malformations in amphibians, such as supernumerary or missing hind limbs, which significantly impair locomotion and increase susceptibility to avian predators (Johnson et al. 1999). Similar morphological shifts occur in terrestrial systems, where the “ant-slaying” nematode (*Myrmeconema neotropicum*) causes the black gaster of its ant host (*Cephalotes atratus*)

to turn bright red, mimicking a ripe berry. This visual deception increases the probability of the ant being consumed by frugivorous birds, thereby facilitating the nematode's dispersal (Yanoviak et al. 2008).

Beyond physical form, parasites subvert fundamental social and reproductive instincts. In social insects, the strepsipteran parasite *Xenos vesparum* suppresses the social instincts of its paper wasp host, driving infected individuals to abandon their colony and gather at specific mating sites for the parasite—a phenomenon termed “social zombification” (Beani 2006). Furthermore, some parasites influence host reproductive systems; for instance, *Wolbachia* bacteria can induce cytoplasmic incompatibility, parthenogenesis, or feminization in arthropods to prioritize the transmission of these maternally inherited endosymbionts (Werren et al. 2008). In extreme cases, parasites even replace host anatomy, as seen in the isopod *Cymothoa exigua*, which physiologically replaces the host's tongue to act as a functional but resource-draining mechanical substitute (Brusca & Gilligan 1983).

Among these diverse forms of host alteration, behavioral manipulation has attracted particular attention because it often provides the clearest and most striking examples of parasite-induced phenotypic change (Moore 2002; Poulin & Maure 2015). The best-known cases, including “zombie ants,” “suicidal crickets,” and “lighthouse snails,” illustrate how parasites can modify host behavior in ways that appear highly specific and closely aligned with transmission or reproductive success.

Representative behavioral manipulation cases

The phenomenon of "zombie ants" is one of the most iconic examples, primarily caused by the parasitic fungus *O. unilateralis* (Moore 2002; van Roosmalen & de Bekker 2024) (**Figure 1A**). This fungus infects ants, predominantly in tropical forest ecosystems, where it manipulates hosts' behavior to leave its colony, ascend vegetation, and bite down onto a leaf or twig in a fixed position. This biting behavior, known as the "death grip," secures the ant's body in place even after death (Hughes et al. 2011). The location at which infected ants die is highly specific, reflecting a parasite-induced behavioral bias that positions hosts in microhabitats with favorable light, temperature, and humidity. Such precise placement enhances fungal development and maximizes spore dispersal potential, ultimately increasing transmission success (Evans et al. 2011; Andriolli et al. 2019). Phylogenetic evidence suggests that such manipulative behaviors may have evolved following host shifts from beetle larvae to ants (Araujo & Hughes 2019). Other *Ophiocordyceps* species induce similar host behaviors, demonstrating a sophisticated strategy for ensuring the parasite's transmission (Hughes et al. 2016; van Roosmalen & de Bekker 2024).

Another striking example is the parasitic hairworm *Spinochordodes*, which induces crickets to engage in suicidal behavior (**Figure 1B**). The parasitic hairworm has a complex lifecycle with an aquatic reproductive stage and uses terrestrial insects, such as crickets, as intermediate hosts. It infects crickets during their larval stage, typically through ingestion of contaminated water or food. Once matured inside a host, the parasite induces behavioral changes that bias infected crickets toward seeking and entering water, a fatal act for the

host that allows the hairworm to exit the cricket's body, searching for mating partners, and transition into its adult form (Libersat et al. 2009).

The "lighthouse snails" phenomenon, caused by the parasitic flatworm *Leucochloridium paradoxum*, demonstrates another form of host manipulation (**Figure 1C**). The parasite's larval stage migrates to the ocular tentacles (eyestalks), where it forms an enlarged, pulsating, caterpillar-like structure that mimics prey to attract birds, the parasite's definitive host (Bernot 2003). Beyond these morphological changes, infected snails exhibit altered behavior by moving to exposed, elevated, and well-lit sites, which significantly increases their visibility to avian predators (Bernot 2003). This behavior is colloquially known as the "lighthouse" effect, as the infected snail's eye stalks resemble a lighthouse beacon, guiding birds to infected snails (Wesołowska & Wesołowski 2014).

Certain species take parasitism to another level. For instance, some *Glyptapanteles* wasp larvae, after emerging from their caterpillar host, seal the exit wounds using a molted exoskeleton, ensuring the caterpillar's survival (**Figure 1D**). Remarkably, the caterpillar then takes the role of a bodyguard, ceasing to feed and vigilantly guarding the wasp pupae from potential predators with aggressive head swings (Grosman et al. 2008). Eventually, the caterpillar succumbs to starvation while protecting the parasites.

Another well-documented example of host manipulation is the "fatal attraction" induced by the protozoan parasite *T. gondii*, which infects rodents and alters their behavior to enhance transmission to its definitive host—typically cats (House et al. 2011). Infected rodents lose their natural aversion to the scent of predators, particularly cat urine. Instead of avoiding areas marked with feline scent, they are attracted to them, significantly

increasing their chances of being preyed upon by cats (Berdoy et al. 2000; Hari Dass & Vyas 2014). This change in behavior is hypothesized to enhance the likelihood of the parasite's transmission to its definitive host, where it completes its reproductive cycle (Weinersmith & Faulkes 2014). Similarly, *Plasmodium* species, responsible for malaria, have been suggested to influence their mosquito hosts in ways that may contribute to optimizing transmission. Initially, affected mosquitoes exhibit reduced attraction to humans and decreased feeding activity. Because the parasite has not yet reached the transmissible sporozoite stage, such behavioral changes may reduce risky host contact and thereby increase mosquito survival, indirectly favoring parasite development (Smallegange et al. 2013). However, in the later stages of infection, when the mosquitoes carry infectious sporozoites, they are more attracted to humans and tend to bite more frequently at night, potentially aiding in the spread of the parasite (Smallegange et al. 2013).

Research biases and challenges in evaluation

The examples highlighted above, while striking, represent only a small fraction of the vast array of behavior manipulations observed in nature (Poulin & Maure 2015; Herbison 2017). Given that approximately 40% of all known species on Earth are parasitic, documented cases likely underestimate the true diversity of host-parasite interactions. However, current research exhibits a significant taxonomic and methodological bias, traditionally focusing on conspicuous behavioral shifts, such as "suicide behavior", dramatic visual mimicry, or other extreme behavioral shifts (Poulin 2010). As a result, the

literature is likely skewed toward the most visible endpoints of manipulation, rather than providing a representative view of its full biological diversity.

This emphasis on overt phenotypes creates an important limitation for evaluating adaptive host manipulation. In many understudied systems, we lack the observational and mechanistic resolution needed to determine whether altered host traits reflect active parasite manipulation, generalized pathological effects, or host-mediated responses to infection. Consequently, many frequently cited examples rely on compelling behavioral correlations, while the evolutionary significance of these changes remains unresolved. A broader understanding of host manipulation therefore requires moving beyond anecdotal and highly visible cases to include less conspicuous taxa, subtler physiological or developmental alterations, and systems in which manipulation may be harder to detect but no less evolutionarily important (Lafferty & Shaw 2013).

More importantly, phenotypic change alone is not sufficient evidence of adaptive manipulation. To infer that a trait has been shaped by selection to benefit the parasite, it is necessary to distinguish active parasitic control from non-adaptive consequences of infection, including sickness behavior, host stress responses, or pathological side effects that do not increase parasite fitness (Poulin 1995). This distinction is central to the adaptive manipulation hypothesis. Accordingly, the following sections move from descriptive examples to critical evaluation, assessing iconic case studies against explicit evolutionary criteria in order to determine when parasite-induced host alterations can be interpreted as genuinely adaptive.

A REFINED EVALUATIVE FRAMEWORK FOR ADAPTIVE HOST MANIPULATION

Adaptive host manipulation: Definition and controversies

Parasite-induced host manipulation is often regarded as an adaptive strategy, encapsulated in the “adaptive host manipulation hypothesis.” This hypothesis posits that parasites can alter their hosts' behavior, physiology, or morphology in ways that enhance the parasite's fitness, such as by facilitating transmission to subsequent hosts or improving reproductive success (Holmes & Bethel 1972). These changes are often interpreted as extensions of the parasite's genetic influence and framed as part of the parasite's “extended phenotype” (Dawkins 1982). However, this interpretation is not without controversy and remains a topic of ongoing debate (Poulin 2021). Not all phenotypic changes observed in hosts are directly controlled by parasites; some may arise as byproducts of host immune responses or represent host-driven adaptive strategies to combat infection (Poulin 1995; Will et al. 2020). These responses may, by chance, aid in parasite transmission and could therefore be mistakenly interpreted as manipulation by the parasite (Jensen et al. 2023). Distinguishing true parasite adaptation from incidental or host-mediated effects is therefore one of the major conceptual challenges in this field.

Several classic examples of manipulation have been re-evaluated in light of this concern. For instance, malaria parasites (*Plasmodium* spp.) are well-known for altering the behavior of infected mosquitoes: early-stage infections are associated with decreased attraction to human hosts, whereas later-stage infections (carrying infectious sporozoites) result in increased attraction to humans and more frequent biting behavior at night

(Smallegange et al. 2013). These behaviors were thought to be adaptive because they improve the parasite's chances of transmission to new hosts. However, similar behavioral changes were observed in mosquitoes treated with heat-killed *Escherichia coli* (Cator et al. 2013), and these changes were linked to alterations in insulin signaling within the mosquito gut (Cator et al. 2015). This suggests that the behavioral changes in mosquitoes may arise from generalized immune or physiological mechanisms rather than direct manipulation by *Plasmodium* (Cator et al. 2015). Furthermore, the phenomenon of rats infected with *T. gondii* being attracted to cat odors has often been cited as a classic example of adaptive parasite manipulation. However, some researchers have argued that these behavioral changes could simply reflect non-adaptive pathological byproducts. For instance, *T. gondii*-induced non-specific neural damage or a localized inflammatory response could non-specifically disrupt the hosts' innate fear circuitry, leading to an accidental loss of predator avoidance (Hrdá et al. 2000; Worth et al. 2013). These cases highlight the inherent complexity in distinguishing between specialized parasitic manipulations and broader phenotypic traits resulting from host responses.

Operational criteria for evaluating adaptive manipulation

Given the persistence of this debate, deciphering whether phenotypic shifts are adaptive for the parasite or merely incidental requires a robust evaluative framework (Worth et al. 2013; Poulin & Maure 2015; Doherty 2020; Hernandez-Caballero et al. 2022). An influential early attempt was provided by Poulin (1995), who argued that infection-induced behavioral changes should be considered adaptive only if they meet specific

criteria: exhibiting complexity, displaying evidence of purposeful design, evolving independently across multiple parasite lineages, and ultimately enhancing parasite fitness. However, these criteria are often difficult to apply empirically. For instance, convergent evolution is nearly impossible to detect in highly specialized or unique host-parasite systems where no comparable lineages exist, and the concept of “complexity” remains subjective without mechanistic evidence. To address these limitations, we propose a more refined and operational framework that move from ecological observation to mechanistic causality. Specifically, adaptive host manipulation can be evaluated based on four criteria: (1) stage-specific temporal alignment, (2) independence from host compensatory responses, (3) net fitness benefit to the parasite, and (4) mechanistic causality (**Figure 2**). Together, these criteria provide a hierarchical basis for distinguishing genuine adaptive manipulation from incidental pathological effects or host-mediated responses. Each criterion is discussed in detail below.

Stage-specific temporal alignment

A defining feature of adaptive manipulation is precise temporal coordination between parasite development and host behavioral change, such that altered phenotypes are expressed specifically when they enhance transmission rather than emerging gradually with infection progression. Several canonical systems strongly support this expectation. In malaria parasites, infected mosquitoes reduce host-seeking behavior during early, non-transmissible stages, but increase attraction to humans and biting frequency once infectious sporozoites are present, indicating that behavioral modulation is tightly aligned with

transmission potential (Smallegange et al. 2013; Cator et al. 2013, 2015). Similarly, *Dicrocoelium dendriticum* induces ants to climb vegetation and attach to grass tips specifically during cool evenings, when grazing mammals are most likely to ingest them, while allowing a reversion to normal activity during the day (Wickler 1976), providing striking evidence of reversible, stage-dependent control. The “zombie ant” system caused by *Ophiocordyceps* fungi also exhibits consistent spatial and temporal precision, with infected ants dying in microhabitats that optimize fungal growth and spore dispersal (Hughes et al. 2011; Evans et al. 2011; Andriolli et al. 2019), although the exact timing dynamics are less well resolved. In contrast, cases in which behavioral changes scale monotonically with parasite burden—such as progressively impaired locomotion or coordination in heavily infected hosts—offer weaker support, as such patterns are equally consistent with generalized physiological deterioration or sickness behavior. Thus, stage specificity is best regarded as an initial screening criterion: it provides an important first indication that a phenotypic change may be aligned with parasite interests, but it does not by itself establish adaptive manipulation.

Independence from host compensatory responses

A second critical criterion is whether the observed phenotype is independent of host compensatory or defensive responses. Whereas the first criterion, stage-specific temporal alignment, identifies phenotypes that appear at times potentially favorable to parasite transmission, it does not determine whether those changes are actually imposed by the parasite. Many infection-associated behaviors arise from the host’s own plasticity rather

than active parasitic control, making it essential to distinguish parasite-driven effects from host-mediated responses.

For example, female bush crickets infected with gut protozoans show reduced fecundity but compensate by increasing mating frequency and reducing selectivity; however, similar behavioral shifts occur under non-parasitic stressors such as starvation or senescence, indicating that these changes are better interpreted as generalized life-history responses (Simmons 1994; Jennions & Petrie 1997; Moore 2002). Likewise, behavioral fever in grasshoppers and accelerated maturation in parasitized snails are broadly understood as host-driven strategies that enhance immune performance or secure some reproductive output before death (Boorstein & Ewald 1987; Lafferty 1993), rather than parasite-imposed manipulations. More ambiguous cases, such as increased foraging in infected fish (Crowden & Broom 1980), further highlight this difficulty: although elevated foraging can increase predation risk and potentially benefit trophically transmitted parasites, it can also be parsimoniously explained as a host response to reduced energetic efficiency.

In contrast, highly specific and stereotyped behaviors that are difficult to reproduce under general stress—such as the precise climbing and biting behavior in *Ophiocordyceps*-infected ants or the water-seeking behavior of hairworm-infected crickets—provide stronger, albeit still indirect, support for parasite-driven control. Because such traits are unusually specific, context-dependent, and difficult to interpret as generic sickness or stress responses, they are less easily explained as host compensation alone. Even so, they do not conclusively establish active parasite control without additional evidence.

Accordingly, when host responses and parasite manipulation cannot be cleanly separated, the most defensible approach is not to force a binary classification. Instead, such cases should be treated as consistent with adaptive manipulation, but unresolved with respect to control. In other words, the framework should explicitly allow an intermediate category between confirmed manipulation and simple pathology. A phenotype may satisfy the first criterion of temporal alignment and may even appear to confer plausible fitness benefits, yet still remain uncertain because host compensation, sickness behavior, inflammatory damage, or generalized stress responses have not been excluded. This is particularly important in systems such as *T. gondii* in rodents, where altered responses to feline odors have often been interpreted as adaptive manipulation, but critics have argued that evidence for increased transmission to cats remains insufficient and that non-specific neural disruption or inflammation cannot be ruled out.

Taken together, this criterion clarifies the logical limits of the first standard. Stage specificity identifies candidate cases, whereas independence from host compensatory responses evaluates whether those candidates truly reflect parasite-driven control rather than host-mediated effects. As such, the second criterion does not replace the first, but deepens it by excluding many superficially compelling cases that are more likely to reflect host plasticity than adaptive manipulation.

Fitness benefits to the parasite

Adaptive manipulation must ultimately be evaluated in terms of its consequences for parasite fitness, because natural selection operates at the level of individuals and favors

traits that reliably enhance transmission or reproductive success. Several classic systems are broadly consistent with the expectation of fitness enhancement. For instance, in *L. paradoxum*, the enlarged, pulsating eyestalks of infected snails, coupled with their tendency to occupy exposed and illuminated microhabitats, increase visibility to bird definitive hosts and thereby facilitate transmission (Bernot 2003; Wesołowska & Wesołowski 2014). Similarly, *T. gondii* reduces innate predator aversion in rodents, increasing the likelihood of predation by felids, the parasite's definitive hosts (Berdoy et al. 2000; Hari Dass & Vyas 2014; Weinersmith & Faulkes 2014). In parallel, hairworms (*Spinochordodes* spp.) induce infected crickets to enter aquatic environments, a behavior that is lethal to the host but essential for parasite emergence and reproduction (Libersat et al. 2009). In trophically transmitted trematodes such as *Euhaplorchis californiensis*, infection markedly elevates predation risk by bird hosts, in some cases by orders of magnitude, further suggesting a direct transmission advantage (Lafferty & Morris 1996). Taken together, these systems provide convergent ecological evidence that parasite-induced behavioral changes can enhance individual fitness by increasing the probability of reaching the next host.

Importantly, assessing fitness benefits also requires considering the costs of manipulation. Parasite-induced behavioral control is unlikely to be free; it may require energetic investment, the production of neuroactive compounds, or the evolution and maintenance of highly specific interactions with host neural, endocrine, or immune pathways (Poulin 2010). These investments create trade-offs, because resources allocated to manipulation could otherwise support growth, reproduction, dispersal, or immune

evasion (Poulin & Thomas 1999). In addition, highly specialized manipulation strategies may reduce ecological flexibility by tying parasite success to particular host species, developmental stages, or environmental contexts, thereby increasing vulnerability to environmental instability or host community change (Thomas et al. 2012). A manipulation strategy should therefore be considered adaptive only when its transmission or reproductive benefits outweigh these physiological, ecological, and evolutionary costs.

This emphasis on net rather than gross benefit is especially important because some phenotypes may appear advantageous in a narrow context while proving costly across the full life cycle. From an evolutionary perspective, manipulation should be favored only when it consistently improves parasite fitness across relevant ecological settings and over repeated transmission opportunities. Over time, such traits may be refined by selection, becoming more efficient, stage-specific, and less costly, which may explain why some manipulative systems appear highly precise and stereotyped (Poulin 2010). Conversely, where benefits are weak, conditional, or context-dependent, manipulation-like phenotypes may remain evolutionarily unstable or may be better interpreted as byproducts rather than adaptations.

Taken together, this criterion moves the framework from control to adaptive value. The first two criteria ask whether a phenotype is plausibly parasite-associated and distinguishable from host-mediated responses; the third asks whether that phenotype actually improves parasite fitness. Only when such benefits can be shown to outweigh associated costs can a host alteration be confidently interpreted as an adaptive manipulation rather than a merely intriguing infection-associated trait.

Mechanistic causality

Finally, the most stringent criterion for adaptive manipulation is the demonstration of mechanistic causality, which moves the argument beyond ecological inference to direct evidence that parasite-derived factors actively generate the host phenotype. While temporal specificity, independence from host compensatory responses, and apparent fitness benefits can provide strong inferential support for adaptive manipulation, these criteria remain inherently indirect and, in some cases, ambiguous. Indeed, even well-known systems such as *Plasmodium* spp. in mosquitoes and *T. gondii* in rodents have been reinterpreted upon closer examination, as their associated phenotypes may arise from generalized immune activation or non-specific neural disruption rather than targeted parasitic control. These reassessments highlight a fundamental limitation of ecological inference: even when multiple criteria appear to be satisfied, they do not uniquely distinguish adaptive manipulation from incidental byproducts of infection.

Against this backdrop, mechanistic causality provides the most rigorous standard for evaluating adaptive manipulation by shifting the focus from correlation to direct evidence of parasite control. In practice, this requires moving from correlation to intervention. Identifying parasite-derived genes, secreted effectors, metabolites, or neuroactive compounds provides an essential starting point, but such associations alone remain insufficient. Stronger support requires showing that these factors are expressed at the relevant developmental stage, delivered to the appropriate host tissues, and capable of altering host physiology or behavior in a manner consistent with the phenotype under study. The most convincing evidence comes from perturbation-based approaches, such as gene

knockout, gene silencing, or targeted gene editing, that directly test whether disrupting the candidate factor abolishes or modifies the phenotype.

This criterion is especially important because many of the most iconic systems remain mechanistically suggestive rather than mechanistically resolved. In *Ophiocordyceps*-infected ants, for example, the highly stereotyped “death grip” and the precise positioning of dying hosts strongly imply parasite-driven control, yet the exact molecular effectors and neural targets remain incompletely characterized. Likewise, in *T. gondii* and *Plasmodium*, plausible links to host neural, endocrine, or metabolic pathways have been proposed, but the strength of the evidence varies, and full causal chains from parasite factor to host phenotype to transmission outcome are still rarely established. In the absence of such evidence, even highly specific and repeatable behaviors remain open to alternative explanations. Mechanistic specificity therefore serves as the final step that distinguishes compelling ecological inference from direct demonstration.

Importantly, the absence of mechanistic proof should not automatically be interpreted as evidence against adaptive manipulation. In many systems, technical limitations make functional validation difficult, especially where parasites are hard to culture, manipulate genetically, or observe across complex life cycles. For this reason, mechanistic causality should be viewed not as an all-or-none prerequisite for considering a case plausible, but as the highest evidential tier in the framework. Cases that satisfy the first three criteria may still represent strong candidates for adaptive manipulation, but only mechanistic evidence can most confidently establish that the parasite is the proximate cause of the host phenotype.

Evolutionary context

From a broader evolutionary context, mechanistic causality also helps place host manipulation within a dynamic coevolutionary process between parasites and their hosts. Manipulative traits will be favored by selection only when they reliably increase parasite transmission or reproductive success and when these benefits outweigh the metabolic, developmental, and ecological costs required to produce them (Moore 2002; Adamo 2013). At the same time, hosts are expected to evolve countermeasures, including behavioral avoidance, immune defenses, resistance to parasite effectors, and compensatory life-history responses that reduce the fitness consequences of infection (Gandon et al. 2008; Papkou et al. 2016). As a result, host manipulation should not be viewed as a static trait, but as the outcome of an ongoing coevolutionary “arms race” in which parasite control and host resistance are continually refined.

This perspective also helps explain why manipulation varies so markedly across systems. In some clades, such as acanthocephalans, manipulation appears widespread and phylogenetically recurrent, suggesting that it may have originated early and diversified across lineages (Moore 2002; Poulin 2011). In other cases, however, similar host phenotypes may emerge repeatedly without necessarily reflecting the same underlying mechanism or adaptive function. Some infection-associated behaviors may instead provide indirect benefits to the host, its kin, or neither party in a consistent manner, further complicating interpretation. In addition, the expression and fitness consequences of manipulative traits are likely to be shaped by environmental context. Stressors such as pollution, habitat disturbance, or other anthropogenic changes may alter host physiology,

immune performance, and behavioral responsiveness, thereby modifying both the manifestation of parasite-associated phenotypes and the selective conditions under which manipulation evolves. Although these environmentally mediated effects remain insufficiently studied, they highlight the need to evaluate host manipulation not only in terms of parasite and host traits, but also within the broader ecological conditions in which these interactions occur. Mechanistic evidence is therefore important not only for identifying immediate causal pathways, but also for understanding how manipulative traits originate, persist, diversify, or are lost over evolutionary time.

Taken together, these considerations show that mechanistic causality is the strongest criterion in the framework because it links ecological pattern, functional process, and evolutionary interpretation into a single line of evidence. A phenotype can be regarded as robust evidence of adaptive manipulation only when it is not merely correlated with infection, but can be traced to parasite-derived factors that consistently generate host changes conferring net fitness benefits under natural conditions. In this sense, mechanistic causality does not replace the preceding criteria; rather, it integrates and validates them, providing the clearest path from descriptive pattern to a fully supported claim of adaptive host manipulation.

MECHANISTIC BASIS AND CAUSAL INFERENCE OF HOST MANIPULATION

As discussed above, ecological criteria alone provide only indirect evidence, and even widely cited systems can remain ambiguous in the absence of causal validation.

Understanding the molecular and physiological basis of host alteration is therefore essential, not only for explaining how parasites influence host biology, but also for determining whether these changes represent true adaptive manipulation. Given the vast taxonomic range of parasites, including viruses, bacteria, fungi, protozoa, worms, and arthropods, it is reasonable to assume that their mechanisms of host manipulation are equally diverse (Poulin 2010). Although significant progress has been made in identifying key components of these mechanisms, the exact processes through which parasites influence host behavior often remain complex and only partially understood (Lafferty & Shaw 2013). In this context, the framework developed in the previous section provides an important reference point. Only when molecular processes can be causally linked to specific, fitness-enhancing phenotypes can they be confidently interpreted as adaptive.

Baculoviruses: viral control of insect hosts

One of the clearest examples of mechanistic causality is provided by baculoviruses, which alter insect behavior to enhance viral transmission. Baculoviruses encode ecdysosteroid UDP-glycosyltransferase (EGT), an enzyme that inactivates host ecdysteroid hormone, 20-hydroxyecdysone (20E), which is essential for regulating molting and development (O'Reilly & Miller 1989) (**Figure 3**). Beyond its role in molting, 20E has been implicated in a broad range of physiological processes, including immunity, circadian rhythm regulation, and neurological function (Adamo 2019). Experimental manipulation of EGT expression has demonstrated that disruption of 20E signaling directly contributes to altered locomotor activity and the development of “tree-top disease,” in which infected

larvae climb to elevated positions prior to death, thereby enhancing viral dispersal (Hoover et al. 2011; Masoudi et al. 2024; Gasque et al. 2019). This system provides a clear proof-of-concept for adaptive manipulation, as it satisfies several criteria outlined earlier, including stage-specific behavioral expression, measurable fitness benefits, and, most importantly, a direct causal link between a parasite-derived gene and a host behavioral phenotype.

This system also illustrates how genomic and functional approaches can be integrated to distinguish adaptive manipulation from non-specific sickness behaviors. By experimentally linking EGT activity to a specific behavioral outcome, the baculovirus model establishes a direct connection between genotype and phenotype, thereby providing a framework for evaluating more complex host–parasite interactions. At the same time, the mechanisms underlying baculovirus-induced behavior are not limited to hormonal disruption. Because ecdysteroids are also involved in circadian regulation, baculovirus infection has been proposed to influence host circadian gene expression in the nervous system (Kumar et al. 2014; Mark et al. 2021). Consistent with this hypothesis, some studies suggest that baculoviruses affect phototactic pathways, making infected larvae more likely to climb and remain in well-lit environments (van Houte et al. 2014b).

However, further work has revealed that similar behavioral outcomes can arise through alternative molecular routes. In *Spodoptera exigua* larvae infected with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), summing behavior does not require EGT (van Houte et al. 2014a). Instead, another viral factor, protein tyrosine phosphatase (PTP), plays a critical role in inducing hyperactivity. PTPs are

neuroactive molecules that modulate kinase signaling pathways in the host nervous system, thereby directly influencing locomotor activity and behavioral arousal (Kamita et al. 2005). These findings indicate that baculoviruses can achieve similar behavioral outcomes through distinct but functionally convergent mechanisms. Through a combination of hormonal interference, circadian modulation, and direct neural signaling, baculoviruses exert sophisticated control over their hosts, ensuring conditions that favor transmission. This system exemplifies the concept of the parasite “extended phenotype” (Dawkins 1982) and represents one of the strongest and most experimentally supported cases of adaptive host manipulation.

Fungal, hairworm, and parasitic wasp models

Similarly, the parasitic fungus *Ophiocordyceps* spp., known for inducing the "zombie ant" phenomenon, provides another important system (**Figure 3**). Genomic analyses have identified an abundance of enterotoxin-encoding genes in *Ophiocordyceps* species that manipulate ants, with one enterotoxin uniquely expressed during the manipulated biting event (De Bekker et al. 2017a; Will et al. 2020; Ding et al. 2023). These enterotoxins typically target G-protein-coupled receptors (GPCRs), which are crucial for neurotransmission and circadian rhythms (Bloom 2006; Burchett & Hicks 2006; Kobmoo et al. 2018; Will et al. 2023). Transcriptomic analyses have further revealed that many fungal genes encoding small secreted proteins (SSPs) exhibit elevated expression levels during host manipulation (De Bekker et al. 2015). Machine learning approaches suggest that some of these SSPs interact with ant GPCRs, particularly those mediate light and odor

perception (Will et al. 2023). Supporting the hypothesis that the parasite hijacks the host's circadian clock, several clock genes were significantly downregulated in manipulated ants compared with healthy ants (De Bekker et al. 2015). These molecular changes are associated with the parasite's behavioral control and coincide with the precisely timed summing and biting behaviors in infected ants (De Bekker et al. 2017b; Das & de Bekker 2022; van Roosmalen & de Bekker 2024). However, direct experimental evidence linking individual fungal molecules to specific behavioral outputs remains limited. As a result, the criterion of mechanistic causality is not yet fully satisfied.

Another striking example is the hairworm (*Paragordius* spp.), which manipulates crickets and grasshoppers into suicidal water-seeking behavior. This behavioral manipulation is associated with significant molecular and neurological changes in the host, particularly within the central nervous system (CNS) (**Figure 3**). Proteomic analyses have revealed altered expression of neurotransmitter-related proteins, with serotonin pathways playing a key role in modulating locomotion and water-seeking behavior (Biron et al. 2005). Additionally, nitric oxide regulation is disrupted, potentially influencing neural signaling and decision-making (Biron et al. 2006; Ponton et al. 2011). The presence of small secreted proteins (SSPs) from the Wnt family, which are known to regulate CNS development, suggests a potential mechanism by which hairworms may interfere with host neural regulation (Biron et al. 2006). Visual system-related genes are also upregulated in infected crickets, which has been proposed to contribute to increased attraction to water surfaces (Biron et al. 2006; Ponton et al. 2011), whereas disruptions in circadian rhythm gene expression indicate that the parasite may synchronize host behavior with optimal

transmission conditions (Biron et al. 2006). Interestingly, hairworms employ distinct strategies depending on the host species—while they induce apoptosis in the CNS of grasshoppers, they inhibit apoptosis in crickets, suggesting a species-specific modulation of neural function to achieve the same behavioral outcome (Biron & Loxdale 2013). Beyond immediate proteomic changes, trematodes can also manipulate host behavior through epigenetic modifications. In some cases, such as infections by the trematode *Microphallus papillorobustus*, epigenetic alterations enhance the expression of enzymes involved in biogenic amine neurotransmitter synthesis, leading to persistent behavioral modifications that increase host risk-taking and parasite transmission (Ponton et al. 2006; Cossart 2011). These findings are consistent with the observed behavioral shift and suggest interference with host neural function. However, most evidence remains correlative. Although the phenotype clearly enhances parasite fitness and is difficult to explain as a host compensatory response, the lack of direct functional validation limits the strength of inference regarding adaptive manipulation.

Unlike hairworms and fungal parasites that rely solely on their own genes to induce behavioral changes, the parasitic wasp *Cotesia congregata* manipulates its caterpillar host, *Manduca sexta*, through a symbiotic bracovirus (*CcBV*) (**Figure 3**). During parasitization, the wasp injects both eggs and *CcBV* into the host, where viral genes play a crucial role in suppressing the immune response, modulating neural activity, and disrupting endocrine function to benefit wasp larval development (Espagne et al. 2005). As the wasp larvae mature and prepare to emerge, the caterpillar undergoes dramatic behavioral modifications, acting as a "bodyguard" to protect the wasp brood, a response linked to *CcBV*-encoded

protein tyrosine phosphatases (McMillan et al. 2024). These viral factors likely modulate neural function by altering synaptic transmission and motor control (Ibrahim et al. 2007; Bézier et al. 2013; Herniou et al. 2013). The “bodyguard” behavior may persist for a short period even after the larvae exit the host and begin pupation, as observed in some parasitoid-host systems (Grosman et al. 2008). In addition to neural control, *CcBV* influences the host endocrine system by disrupting ecdysteroid hormone regulation, which is essential for molting and metamorphosis. Parasitized larvae exhibit reduced hemolymph ecdysteroid levels, leading to developmental arrest at the final larval stage, thereby preventing pupation and extending the host’s suitability for parasitoid development (Gelman et al. 1998). These changes align with parasite fitness benefits and involve identifiable molecular agents, yet the causal links between specific genes and behavioral outcomes remain incompletely resolved.

***Toxoplasma gondii* and immune-neural modulation**

Early studies showed that *T. gondii* infection abolishes rodents’ aversion to predator cues and may even induce attraction to feline odors, a phenomenon known as “fatal feline attraction” (Berdy et al. 2000; Vyas et al. 2007; Hari Dass & Vyas 2014). While these findings suggested a potential increase in susceptibility to predation by felids, the specificity, consistency, and adaptive significance of these behavioral changes remain actively debated (Boillat et al. 2020; Cézilly 2010; Perrot-Minnot et al. 2012). Infection effects can vary with host strain, parasite genotype, and experimental conditions, and some studies report changes in general fear or exploratory behavior that are not specific to felid

cues (Webster 2007; Ingram et al. 2013). For example, infected mice lose aversion to cat urine even after parasite clearance, indicating that persistent behavioral alteration can occur independently of ongoing brain inflammation (Ingram et al. 2013). Recent work has highlighted that neuroinflammatory processes may play a key role in modulating host behavior and that demonstrating causal links requires experimental manipulation of candidate mechanisms (Boillat et al. 2020). Overall, current evidence suggests that *T. gondii*-associated behavioral alterations likely involve complex interactions among immune, neural, and endocrine pathways rather than a single mechanistic route (**Figure 3**).

T. gondii infection induces neuroinflammatory responses in the rodent brain, including the expression of pro-inflammatory cytokines, which have been proposed to influence neural function and behavior (Henriquez et al. 2009). Pro-inflammatory mediators such as TNF- α and IL-6 are known to modulate monoaminergic neurotransmitter systems, including dopamine and serotonin, which regulate fear responses and locomotion (Mangold & Hughes 2021). These observations support the hypothesis that immune–neural crosstalk may contribute to parasite-associated behavioral changes, although direct causal links between specific cytokines and altered behavior in *T. gondii*-infected hosts remain to be experimentally demonstrated (Henriquez et al. 2009; Zhang et al. 2024). Similar immune-associated behavioral modulation has also been reported in other host-parasite systems. For example, Helluy and Thomas (2010) observed increased nitric oxide synthase immunoreactivity in the brains of *Gammarus* hosts infected with *Microphallus papillorobustus*, suggesting altered neuroimmune signaling, although the functional significance of this response and its causal relationship to increased predation risk remain

unresolved. In contrast, in the intermediate snail host of the trematode *Trichobilharzia ocellata*, reproductive suppression is mediated by schistosomin, a parasite-derived neuroendocrine factor that reallocates host resources toward parasite development, rather than directly altering hosts' behavior (de Jong-Brink et al. 2001).

Beyond immune-mediated effects, *T. gondii* has been proposed to influence host behavior by altering host neurochemical and endocrine signaling. Early studies suggested that infection may affect dopamine synthesis and signaling through modulation of dopamine-related pathways, leading to the hypothesis that dopaminergic alterations contribute to infection-associated behavioral changes (Webster et al. 2006; Gonzalez et al. 2007). However, subsequent studies have demonstrated that such dopaminergic effects are not universally observed and may vary substantially depending on host genetic background, parasite strain, and experimental conditions (Wang et al. 2015; McConkey 2015; Tedford & McConkey 2017). Consistent with this view, neurophysiological analyses indicate that *T. gondii* infection can alter neuronal function and synaptic activity without necessarily producing uniform changes in dopamine levels (Martin et al. 2015). In addition to dopaminergic pathways, serotonergic signaling may also be affected during infection and has been proposed to contribute to altered anxiety-like and locomotor behaviors, although evidence remains largely correlative (Henriquez et al. 2009; Montazeri et al. 2022). Endocrine modulation has likewise been implicated, as infected male rodents have been reported to exhibit elevated testosterone levels, which may contribute to increased risk-taking and reduced fear responses and thereby potentially increase encounters with feline predators (Lim et al. 2013; Vyas 2013). At the molecular level, parasite-derived

microRNAs have been proposed as potential regulators of host immune and neural signaling pathways that may facilitate parasite persistence within the host. However, direct evidence linking specific parasite microRNAs to sustained behavioral manipulation in *T. gondii*-infected rodents remains limited, and this mechanism currently remains largely hypothetical (Manzano-Román & Siles-Lucas 2012). From the perspective of the framework, this system remains ambiguous with respect to adaptive manipulation.

Mechanistic convergence and multi-pathway strategies

The diverse strategies used by parasites to manipulate host behavior reveal a general pattern: despite major phylogenetic differences among parasites and hosts, many systems converge on a limited set of host regulatory hubs, particularly the nervous and immune systems (**Figure 3**). This convergence does not imply identical molecular mechanisms across taxa, but rather repeated targeting of host pathways that play central roles in behavioral regulation.

At the same time, host manipulation is rarely mediated by a single pathway. Many parasites appear to employ multi-pathway strategies, combining several molecular and physiological routes in parallel to increase the precision and robustness of host control. For example, *T. gondii* has been linked to immune–neural interactions, neurotransmitter modulation, and broader genomic or epigenetic effects (Webster et al. 2006; Henriquez et al. 2009; Manzano-Román & Siles-Lucas 2012; Zhang et al. 2024). Similarly, parasitoid wasps such as *C. congregata* manipulate hosts through a combination of viral symbiosis, immune interference, and neuromodulation (**Figure 3**). These cases suggest that

699 conspicuous behavioral phenotypes often emerge from coordinated effects across multiple
700 pathways rather than from a single molecular trigger.

701 This combination of convergence and mechanistic diversity raises an important
702 evolutionary question: are these strategies adaptive refinements shaped by long-term host–
703 parasite coevolution, or do they reflect pre-existing parasite capacities that incidentally
704 affect host behavior? Comparative genomic and functional studies indicate that at least
705 some manipulation-related traits show signatures of selection and convergence across
706 parasite lineages (De Bekker et al. 2017a; Kobmoo et al. 2018), supporting the view that
707 certain mechanisms have been shaped by adaptive evolution. Even so, distinguishing
708 adaptation from exaptation remains difficult and requires closer integration of molecular,
709 functional, and comparative evidence.

710 A further challenge is that, although correlations between infection and host molecular
711 change are increasingly well documented, causal resolution remains limited. Many studies
712 identify shifts in host gene expression, proteomic profiles, cytokine signaling, or neural
713 activity associated with altered behavior, but such patterns do not by themselves
714 demonstrate that parasite-derived factors directly generate the phenotype. This gap remains
715 especially pronounced outside a few experimentally tractable systems, such as
716 baculoviruses. For instance, studies of hairworm-infected crickets and *Artemia*-cestode
717 interactions have detected proteomic changes associated with behavioral modification
718 (Sánchez et al. 2009), yet functional validation of specific molecular drivers is still lacking.
719 Likewise, parasite-associated changes in host cytokine expression or neurochemical

pathways may reflect adaptive manipulation, generalized pathology, or host defensive responses (Adamo 2013; Herbison 2017).

Even so, some broader patterns are emerging. One notable example is the repeated targeting of monoaminergic pathways, especially dopamine and serotonin systems, across distantly related parasites. Yet the parasite “toolkits” used to reach these common targets are often lineage-specific. Viruses may rely on genomic integration or specific genes such as *egt*, whereas fungi such as *Ophiocordyceps* appear to deploy complex secretomes of small proteins and secondary metabolites, in some cases without direct invasion of the brain. This suggests a useful distinction between conserved host targets and diverse parasite effectors.

Finally, host manipulation does not occur in isolation. In natural systems, hosts are frequently infected by multiple parasite species simultaneously, and interactions among co-infecting parasites can modify host responses and behavioral outcomes (Sanford 2022; Cézilly et al. 2014; Vaumourin et al. 2015). Co-infection is now recognized as common in wild populations and can alter the within-host environment, host immunity, and infection dynamics, all of which may influence parasite-driven phenotypes (Venter et al. 2022; Vaumourin et al. 2015). For example, studies of gastrointestinal nematodes in rabbits show that co-infection can change host immune responses, potentially affecting susceptibility and the expression of infection symptoms that correlate with host behavior (Murphy et al. 2013). In amphipods, co-occurring intracellular parasites such as Microsporidia and *Rickettsia* exhibit distinct temporal and spatial prevalence patterns within the same host

population, indicating that multi-species infections can generate complex infection dynamics that could secondarily influence host performance and behavior (Park & Poulin 2021). Such multi-parasite interactions may be synergistic or antagonistic, potentially amplifying or attenuating host manipulation outcomes. This complexity further complicates interpretation and underscores the importance of integrating molecular, functional, and ecological perspectives when evaluating adaptive manipulation, particularly within our proposed operational framework.

CHALLENGES AND FUTURE DIRECTIONS

Building on the mechanistic framework outlined in the previous section, it becomes clear that although significant progress has been made in identifying candidate molecular pathways underlying host manipulation, definitive evidence for adaptive manipulation remains limited in most systems. As discussed above, only a few cases currently satisfy the criterion of mechanistic causality, whereas many others are supported primarily by correlative or indirect evidence. Therefore, a central challenge for the field is not simply to describe molecular changes associated with infection, but to establish causal links between parasite-derived factors and fitness-enhancing host phenotypes.

A major reason for this difficulty is that host manipulation is rarely mediated by a single linear pathway. Instead, parasites often appear to target multiple interacting systems, including immune signaling, endocrine regulation, metabolism, and neural function. *T. gondii*, for example, has been proposed to influence host behavior through a combination

of immune–neural interactions, hormonal effects, and broader changes in host homeostasis (Moore 2002; Eberhard 2010; Herbison 2017). Likewise, baculoviruses and malaria parasites have provided important insights into how parasites can alter host locomotion or host-seeking behavior, respectively (van Houte et al. 2013; Emami et al. 2017). This multi-layered architecture makes manipulation difficult to dissect, because the observed phenotype may emerge from the combined action of several partially overlapping pathways rather than from a single identifiable effector.

A related challenge is that the molecular basis of manipulation may extend well beyond canonical neural pathways. Although behavioral change is often interpreted through the lens of neurobiology, parasites may also alter host behavior indirectly through metabolic shifts, immune activation, developmental pathways, or other non-neural processes. Studies in model systems have shown that genes not traditionally associated with neuronal signaling can nonetheless shape behavior by affecting pheromone perception, physiological condition, or tissue-level signaling (Bousquet et al. 2012). Similarly, parasite-induced changes in host development or homeostasis may cascade into altered behavior without requiring direct neural invasion. This broader perspective is important because it suggests that focusing exclusively on neurons or neurotransmitters may overlook key upstream mechanisms of control.

Another unresolved issue concerns timing. In many systems, it remains unclear when manipulative processes begin, whether behavioral changes arise rapidly after infection or emerge only after prolonged host-parasite interaction, and how these molecular dynamics map onto parasite developmental stage. This problem is especially important in systems

784 where adaptive interpretation depends on stage-specific expression of host phenotypes. For
785 example, developmental transitions in parasites such as *Schistocephalus solidus* may
786 provide opportunities to identify genes and pathways associated with manipulation, but
787 resolving these transitions requires fine-scale temporal sampling rather than single end-
788 point comparisons (Hébert et al. 2017).

789 Recent technological advances now make it increasingly feasible to address these
790 challenges. Whole-genome sequencing, comparative genomics, and transcriptomics have
791 already identified candidate manipulation-related genes in systems such as
792 *Ophiocordyceps* fungi and have helped clarify the evolutionary diversification of
793 manipulative traits (De Bekker et al. 2017a; Kobmoo et al. 2018). However, future progress
794 will depend on integrating these approaches with tools that provide higher spatial, temporal,
795 and functional resolution. In particular, single-cell RNA sequencing, spatiotemporal
796 transcriptomics, in situ hybridization methods such as RNAscope, and functional
797 perturbation approaches including CRISPR-Cas9 offer a powerful framework for tracing
798 how parasite and host molecules interact across tissues and infection stages. Multi-omics
799 approaches, including proteomics, metabolomics, lipidomics, and chromatin-level assays,
800 can further reveal how transcriptional changes are translated into altered cellular function,
801 signaling, and behavior.

802 A useful way to conceptualize this next phase of research is through an integrative
803 experimental design. The “zombie ant” system provides a particularly valuable model
804 because it exhibits a highly stereotyped behavioral phenotype and clear progression across
805 infection stages. Under a controlled laboratory design, infected ants could be sampled

across early infection, intermediate behavioral alteration, terminal “death grip,” and post-mortem fungal development, with uninfected ants as controls (**Figure 4**). Key tissues such as brain, muscle, and fungal structures could then be analyzed using single-cell and spatial transcriptomic approaches to identify candidate host and parasite pathways associated with each stage (**Figure 4**). RNAscope or related spatial validation methods could confirm the localization of candidate genes, while functional perturbation of fungal genes through CRISPR-Cas9 or comparable approaches could test whether predicted effectors are necessary for inducing specific host phenotypes (**Figure 4**). Although even such experiments would need to account for redundancy and compensatory mechanisms, they would move the field much closer to true mechanistic causality.

Importantly, this framework is not limited to *Ophiocordyceps*. The same logic can be extended to other systems to determine when manipulation begins, which tissues and pathways are involved, and whether parasite-derived factors are functionally necessary for the altered phenotype. More broadly, future research should aim to connect four levels of evidence that are too often studied separately: ecological phenotype, parasite fitness consequences, molecular mechanism, and evolutionary history. Only by integrating these levels can the field move from compelling descriptive examples to a more rigorous and general understanding of adaptive host manipulation.

In this sense, the most important future direction is not simply the accumulation of more molecular data, but the development of comparative, stage-resolved, and experimentally testable frameworks that can distinguish true parasite-driven control from host responses, pathology, or incidental side effects of infection. Such progress will not

only deepen our understanding of host-parasite coevolution, but also provide broader insight into how complex behaviors can be modified through multi-level biological interactions.

CONCLUSIONS

The manipulation of host phenotypes by parasites is a fascinating and complex phenomenon with important evolutionary and ecological implications. Yet, as emphasized throughout this review, not all infection-associated phenotypic changes should be interpreted as adaptive manipulation. A central challenge remains distinguishing true parasite-driven control from host compensatory responses, generalized pathology, or other incidental consequences of infection. To address this problem, we propose a structured evaluative framework based on four criteria: stage-specific temporal alignment, independence from host compensatory responses, fitness consequences for the parasite, and mechanistic causality. Together, these criteria provide a more rigorous basis for assessing when host phenotypic alterations are likely to represent adaptive manipulation rather than merely correlating with infection. At present, however, relatively few systems satisfy the strongest standard of evidence, particularly with respect to direct mechanistic validation.

Recent advances in spatial, single-cell, and functional genomic approaches provide unprecedented opportunities to dissect the molecular basis of host manipulation. Even so, major challenges remain, including resolving the complexity of host-parasite interactions, identifying key genes, and integrating multiple pathways that contribute to behavioral

change, particularly in establishing causal links between molecular processes and fitness-relevant outcomes. Addressing these challenges will require integrative approaches that combine genomic tools with neurophysiological, hormonal, and behavioral analyses, allowing the field to move beyond correlative evidence and determine when parasite-induced phenotypic changes represent true adaptive manipulation. Such efforts will allow the field to move beyond descriptive and correlative evidence toward a more robust understanding of when and how parasite-induced host phenotypes constitute true adaptive manipulation.

Theoretical significance

This review makes several theoretical contributions to evolutionary biology and behavioral ecology. First, it proposes a more operational evaluative framework for studying adaptive host manipulation. By integrating stage-specific temporal alignment, independence from host compensatory responses, parasite fitness consequences, and mechanistic causality, this framework organizes a previously fragmented debate into a hierarchical set of criteria that can guide both comparative analysis and empirical testing. In this way, the review helps shift the study of host manipulation from descriptive interpretation toward a more rigorous and testable framework. Second, this review contributes to a broader evolutionary perspective by highlighting both convergence and diversification in parasite strategies across taxa. Although parasites from different lineages often target similar host regulatory systems, particularly neural, endocrine, and immune pathways, they do so through diverse lineage-specific effectors and multi-pathway strategies. Third, this review extends beyond

conceptual synthesis by proposing an integrative experimental framework for future research. Using systems such as the “zombie ant” model as an illustration, we outline how stage-resolved sampling, spatial and single-cell transcriptomics, in situ validation, and functional perturbation approaches can be combined to identify candidate parasite effectors and test their causal roles in generating host phenotypes. Taken together, these contributions provide a conceptual and methodological foundation for future work aimed at refining the adaptive host manipulation hypothesis, identifying causal mechanisms, and understanding the ecological and evolutionary conditions under which host manipulation evolves and persists.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 32471695), the Fundamental Research Funds for the Central Universities (Grant No. CCNU25JC033), and the Open Funds of the State Key Laboratory of Genetic Evolution & Animal Models (Grant No. GEAM2025-03), all awarded to Prof. Zhi-Qiang Ye (School of Life Sciences, Central China Normal University, Wuhan 430079, China). Additional support was provided by the National Institutes of Health (Grant No. 2R35GM122566), the National Science Foundation (Grant No. DBI-2119963), and the Gordon and Betty Moore Foundation (Grant No. 12186), all awarded to Prof. Michael Lynch (Biodesign Center for Mechanisms of Evolution, Arizona State University, Tempe, AZ 85287, USA).

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893 **Author contributions**

894

895 Z. Y conceived and designed the research; S. Z, M. L, R. D, and F. G analyzed data and
896 performed research; Z. Ye, M. Liu, and S. Zhou wrote the paper. All authors read and
897 approved the final version of the manuscript.

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900 **Disclosure Declaration**

901 The authors confirm that there are no known conflicts of interest associated with this
902 publication.

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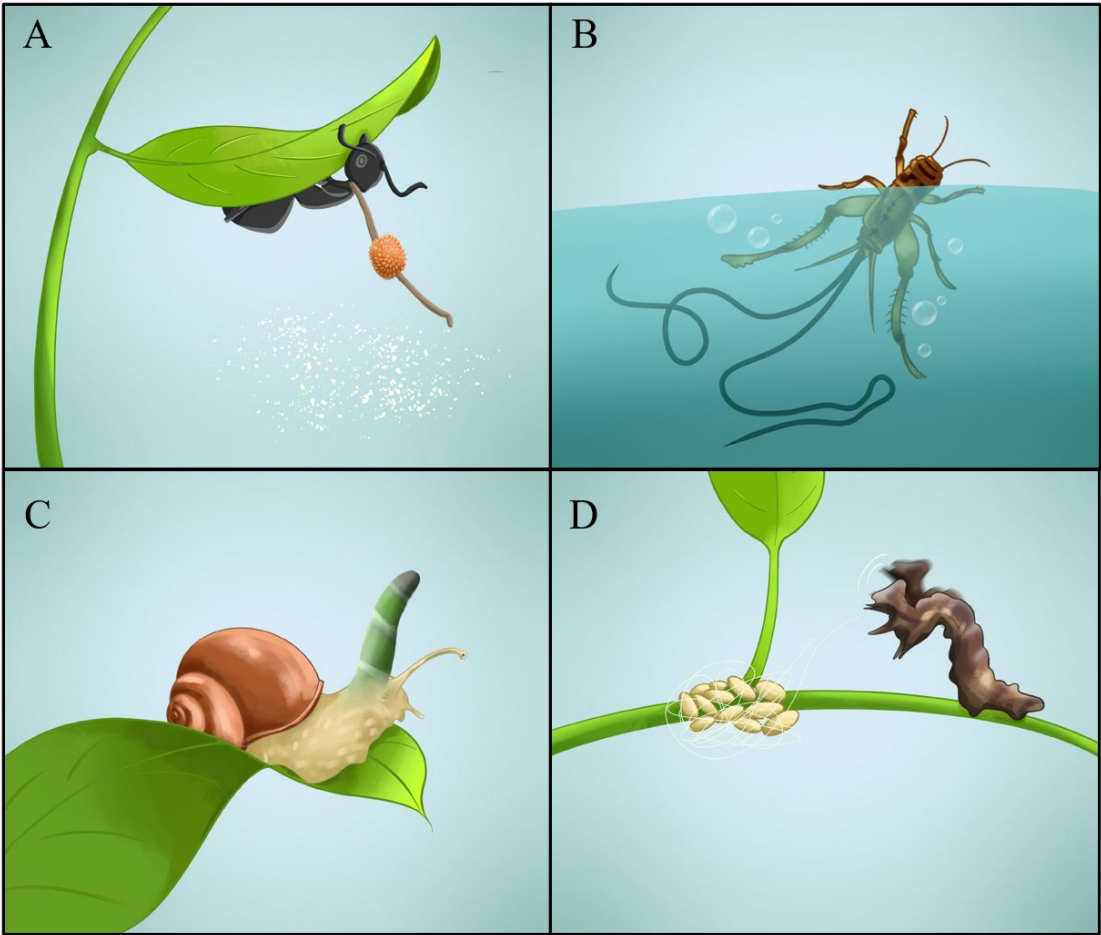
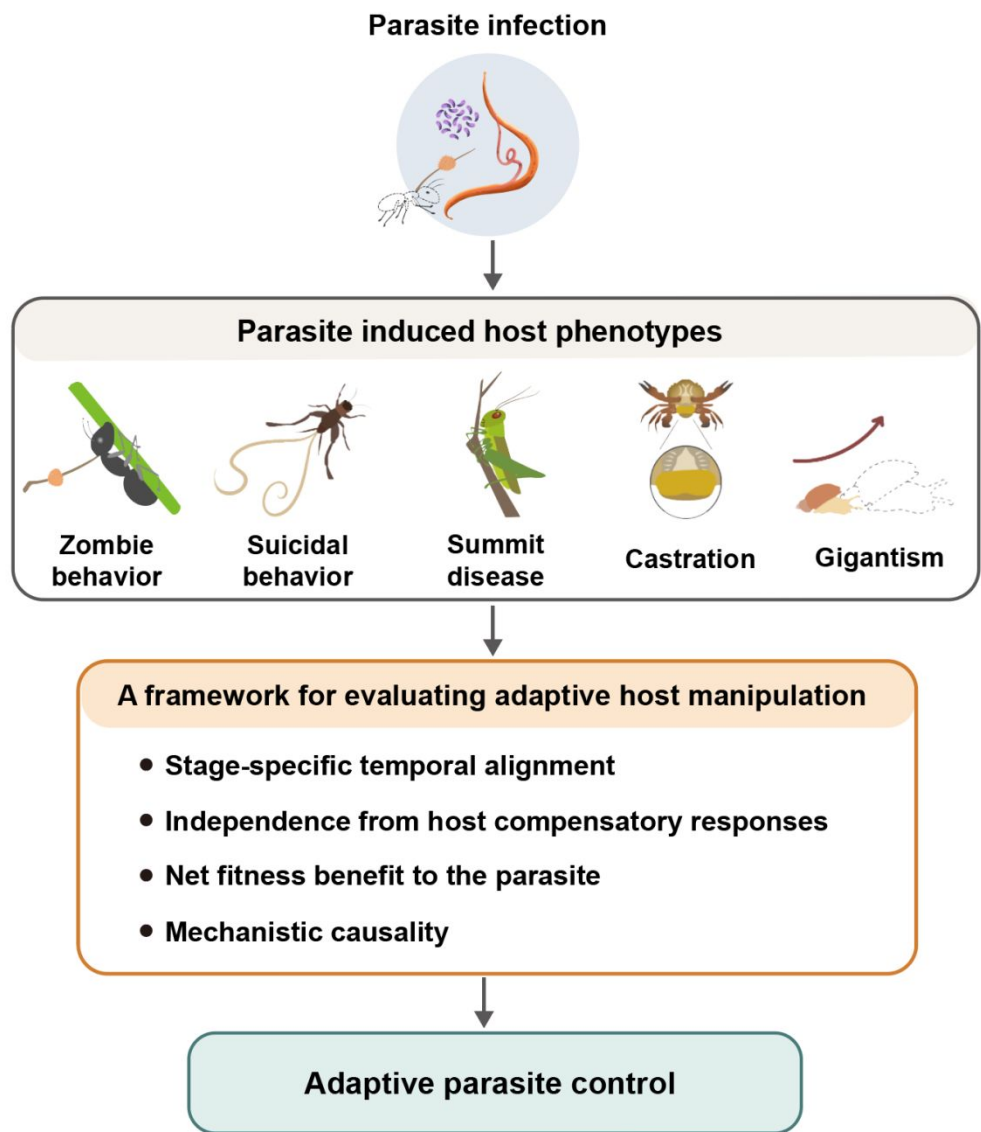


Figure 1. Classical examples of host phenotype manipulation by parasites: A. "Zombie ant" – An ant infected by *Ophiocordyceps unilateralis*, with the fungus emerging from its neck. B. "Suicidal cricket" – Hairworms *Spinochordodes* emerge from a cricket after it drowns in water. C. "Lighthouse snail" – A snail infected by the trematode *Leucochloridium paradoxum*, with the parasite occupying its left tentacle. D. "Zombie caterpillar" – A caterpillar with *Glyptapanteles* parasitoid wasp pupae. After larvae emerge and form cocoons nearby, the caterpillar spins protective silk over the pupae and guards them with head swings before dying.

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Figure 2. A hierarchical framework for assessing adaptive parasite control.

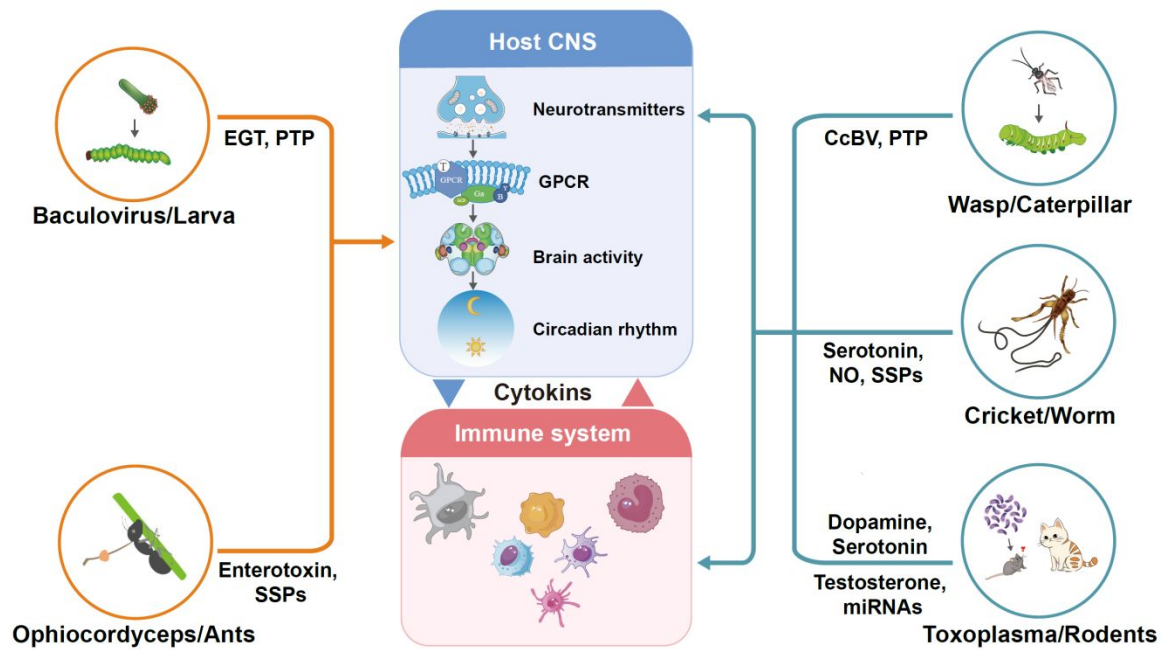


Figure 3. Mechanistic convergence and multi-pathway strategies in parasite-induced host behavioral manipulation. This figure illustrates the diverse strategies parasites employ to manipulate host behavior. Each blue circle represents a specific parasite-host system: *baculoviruses* manipulate insect larvae, *Ophiocordyceps* spp. control ants, the wasp *Cotesia congregata* influences its caterpillar host, *Paragordius* spp. manipulate crickets, and *T. gondii* encysts in the brains of rodents. The text beneath each parasite-host system highlights key molecular factors implicated in host behavioral regulation. Abbreviations: *EGT*, ecdysosteroid UDP-glycosyltransferase; *PTP*, protein tyrosine phosphatase; *SSPs*, small secreted proteins; *NO*, nitric oxide; *CcBV*, *Cotesia congregata* bracovirus; *miRNA*, microRNA.

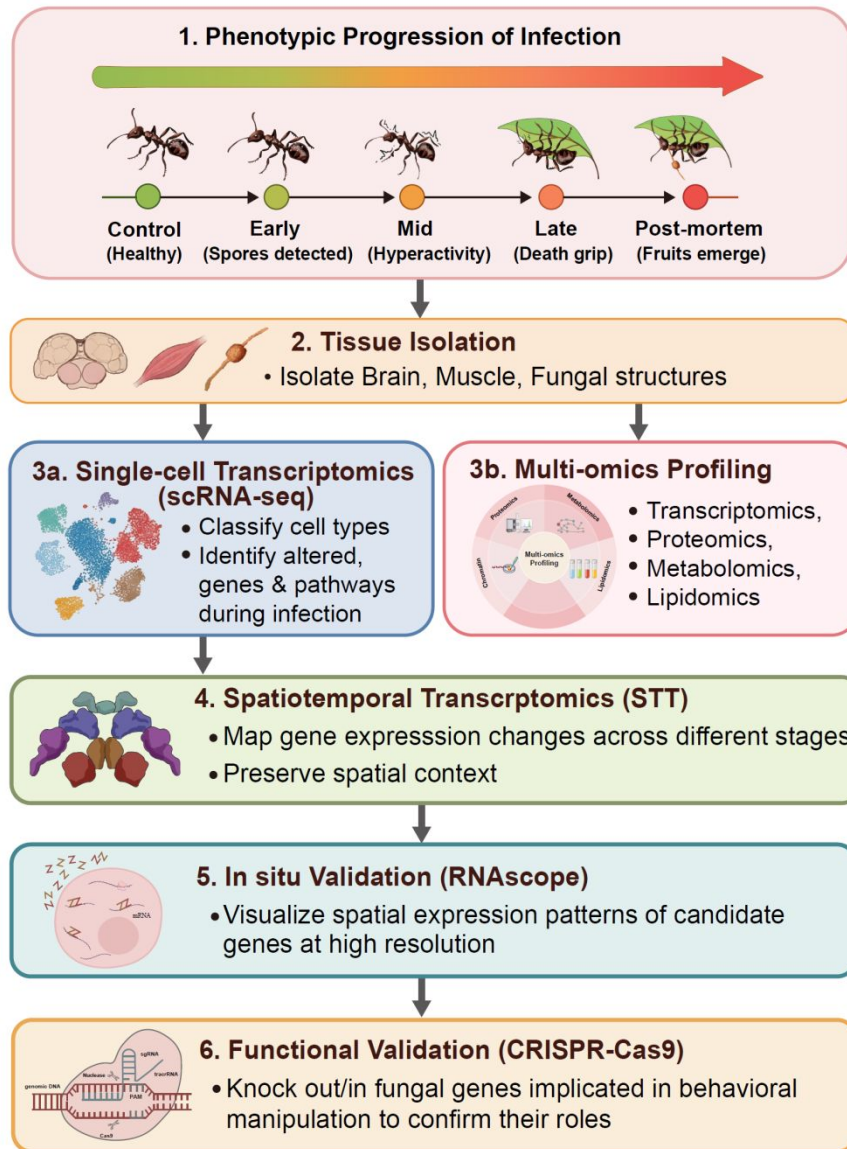


Figure 4. Integrative experimental framework for investigating *Ophiocordyceps*-induced behavioral manipulation in carpenter ants.

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寄生虫对宿主的适应性操纵：从现象到因果框架

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1308 寄生虫诱导宿主表型改变的现象已在多种类群中广泛报道, 然而, 准确区分这些改
1309 变是由寄生虫适应性操纵还是宿主自身应答所致, 仍然是该领域面临的核心挑战。
1310 根据适应性操纵假说, 寄生虫可通过诱导宿主表型发生改变, 提高自身的传播效率
1311 和繁殖成功。然而, 现有证据仍较为零散, 且多停留在相关性层面, 缺乏严格的因
1312 果验证。基于此, 本文梳理了现有研究, 对适应性操纵假说进行批判性审视, 并揭
1313 示将宿主表型与寄生虫适合度直接联系的局限。在此基础上, 本文提出了一个用于
1314 评估寄生虫适应性操纵的结构化框架, 包括四项核心标准: 其一, 操纵发生的时间
1315 应与宿主行为反应在阶段上相匹配; 其二, 相关表型变化应独立于宿主的补偿性反
1316 应; 其三, 这种改变应能够为寄生虫带来净适合度收益; 其四, 在分子机制水平应
1317 具备明确的因果性。将该框架应用于若干研究较为深入的宿主—寄生虫系统后可以
1318 发现, 许多被广泛引用的经典案例在严格的因果判定标准下仍缺乏充分证据。在机
1319 制层面, 本文系统整合了当前关于寄生虫操纵宿主的分子机制研究进展, 同时指
1320 出, 多数研究仍缺乏直接的功能验证, 因此尚难完整揭示寄生虫诱导宿主表型改变
1321 的因果链条。基于此, 本文进一步提出了一种整合性的研究策略, 主张结合时空转
1322 录组学、单细胞测序和靶向扰动等新兴基因组学与实验方法, 以推动该领域研究从
1323 相关性描述走向因果性解释。

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1325 **关键词:** 宿主操纵; 适应性意义; 寄生虫—宿主相互作用; 分子机制; 因果推断