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Opinion

Host Manipulation by Parasites: A Look Back Before Moving Forward

Robert Poulin[1,*] and Fanny Maure[1]

The ability to manipulate host behaviour is among the most fascinating and best-studied adaptations of parasites. In this opinion article, we highlight trends and biases in the study of this phenomenon that may cloud or limit our understanding of its evolution. For instance, reviews and theoretical studies have shown a disproportionately sharp increase in the past decade relative to empirical studies. We also discuss taxonomic biases in the host–parasite systems investigated, as well as recent declines in the use of experimental infections and in the proportion of these systems in which fitness benefits for the parasites have been confirmed. We finish this opinion article by offering recommendations for the continued success of research in this area.

Trends

A huge body of literature now exists demonstrating that numerous taxa of parasites are capable of modifying the behaviour of their hosts in ways that favour the completion of the parasites' life cycles.

The nature of recent research on host manipulation by parasites is changing, however. In particular, theoretical and conceptual studies account for a growing fraction of new publications, at the expense of primary empirical studies.

The taxonomic coverage of empirical studies is also narrowing down toward a focus on a few model host-parasite systems.

At the same time, some basic principles for sound empirical research are increasingly being overlooked.

These recent changes in the way research on host manipulation by parasites is carried out can have important consequences for the future growth of this fascinating area of parasitology.

Parasites as Puppeteers

Host manipulation by parasites, that is, the ability of certain parasites to modify host behaviour, appearance or physiology to their own advantage [1–4], has remained an active and trendy area of research since the 1980s, after the groundwork was laid in the 1970s. We have learned much about fungi that cause ants to die perched high above ground [5]; nematodes that induce fruit mimicry in their ant hosts [6]; parasitoid insects that induce their host to care for them after their emergence from that host [7]; hairworms that drive their hosts to jump in water [8]; and of course *Toxoplasma gondii* and how it subverts the anti-predator behaviour of its rodent host [9] and incidentally alters our personality [10]. Host manipulation by parasites is now accepted as a widespread adaptive strategy of parasites and serves as the focus of intense research efforts. Indeed, over the years, *Trends in Parasitology* has published several articles on this topic (e.g., [10–15]). To ensure ongoing progress, however, and to identify any weakness in how we accumulate knowledge, it may be time to take a critical look at past approaches used in this field and set the course for the next several years.

In this short article, we want to highlight historical trends and biases in the study of host manipulation by parasites, and use them as a basis to make recommendations for future research on this fascinating phenomenon. Our focus is not on the phenomenon itself, but rather on how it has been investigated over the years. We focus specifically on the study of host manipulation by trophically transmitted helminths, that is, parasitic worms that must be transmitted by ingestion from an intermediate (prey) host to a definitive (predator) host in order to complete their life cycle. These parasites commonly modify the behaviour or appearance of their intermediate host to make it more susceptible to predation by their next host. They have been extensively studied for decades in the context of host manipulation, providing an ideal historical overview of how researchers have tackled the phenomenon. Using a comprehensive database of all studies published on host manipulation by these parasites (see Supplementary Material online), we comment on the disparity between the rate at which new ideas are proposed versus

[1]Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

*Correspondence: robert.poulin@otago.ac.nz (R. Poulin)

that at which new data accumulate, as well as reveal generally overlooked methodological and taxonomic biases that may impede further expansion of our knowledge.

Is Theory Outpacing Empirical Research?

The publication of Holmes and Bethel's [16] landmark paper in 1972 established the field by placing behavioural alterations induced by parasites firmly within an adaptive framework. It marked the beginning of an era of empirical research during which most new studies contributed additional examples to the growing list of parasite species known to be manipulative. In the past 10–15 years, however, empirical research has concentrated on model host–parasite systems to address questions going beyond the mere use of host manipulation, ranging from how manipulative parasites avoid enhancing capture of their intermediate host by predators other than their next host [17,18] to how the presence of other parasites influences the phenotypic changes induced in the host by manipulators [19,20]. From the beginning, empirical research has been accompanied by theoretical research providing a growing conceptual framework for our understanding of host manipulation by parasites. In recent years, this has included mathematical models identifying the conditions under which manipulation by parasites can evolve [21–23], as well as verbal arguments either proposing new and more sophisticated hypotheses, or building conceptual bridges between host manipulation by parasites and other research areas to open new research directions [15,24–26]. Of course, the accumulating information has been periodically synthesised over time, either in reviews providing a critical assessment of our growing knowledge [1–4], or by various types of meta-analyses seeking to extract general quantitative patterns from the available evidence [27–29].

It is both interesting and informative to contrast the cumulative rise over time in the relative numbers of primary empirical studies, theoretical or conceptual studies, and various kinds of syntheses on host manipulation by parasites (Figure 1). The number of empirical studies has grown steadily over the years; in contrast, reviews and theoretical/conceptual studies have shown a disproportionately sharp increase in the past decade. Synergy between theory and empirical observations and experiments has fuelled major advances in many disciplines, such as ecology [30] and behavioural ecology [31]. Progress is often limited when not spurred by theory, and both verbal arguments and mathematical models play a key role in laying the bedrock of theory [32]. The observed trends indicate that theoretical research generating new and

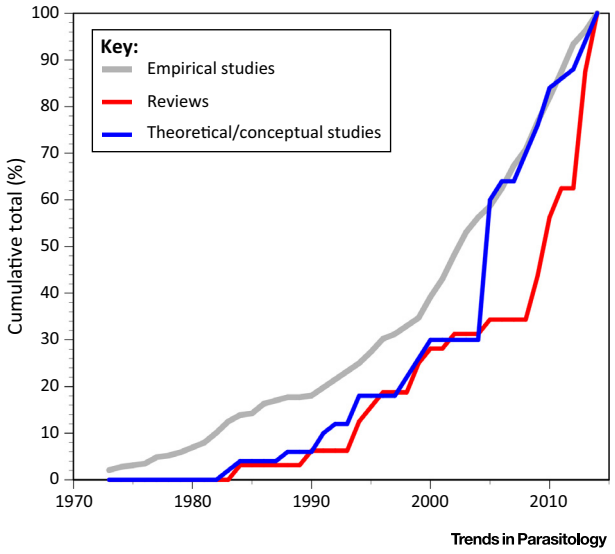

Figure 1. Cumulative Number of Published Studies on Host Manipulation by Helminth Parasites, Shown as a Percentage of the Total, in the Years 1973–2014. Empirical studies (n=288), reviews including either narrative or quantitative syntheses of the topic (n=32), and theoretical or conceptual studies (n=50) are shown separately.

increasingly sophisticated hypotheses regarding host manipulation by parasites is playing an increasingly important role in the field, relative to fundamental empirical research. One reason for the recent and rapid rise in the number of theoretical or conceptual articles may be that limited funding is pushing researchers to find cheaper ways of making novel contributions to the field or producing higher-impact papers. This is not a bad thing in itself, but it does suggest a looming imbalance between theoretical and empirical approaches. New ideas may get too far ahead of hard data, especially since our current basic understanding of the phenomenon remains full of gaps. In light of the biased and incomplete nature of the empirical foundation on which our understanding of host manipulation by parasites is based (see following sections), we encourage renewed efforts toward field and laboratory studies of manipulating parasites. It would be unwise for imaginative new ideas to get too far ahead of established facts.

Is Taxonomic Coverage Keeping up with Research Effort?

Many biological disciplines rely on a few model species from which we have extracted much of what we know about living systems. Genetics is founded on classical studies of *Drosophila* fruit flies, developmental biology on the nematode *Caenorhabditis elegans*, and so on. Because their basic biology is known and experimental techniques for their use are in place, these models allow novel questions to be explored immediately. However, focusing on a few models has its drawbacks [33]. Model systems are rarely chosen because they are truly representative of a higher taxon; instead, they are often selected out of convenience, because they occur close to research institutions, are easy to sample in the wild, and amenable to experimentation in captivity. We simply cannot assume that our in-depth knowledge of a few host–parasite associations generally applies to all other systems. We face a trade-off between deep, specific knowledge obtained by focusing on a few model systems, and broad, general knowledge gained from the more cursory study of a wider range of organisms.

The tendency for any growing research area to narrow down the number of species studied in order to achieve deeper knowledge is affecting other biological disciplines. For example, consider research on plant invasion ecology. Although the number of empirical studies on plant invasions is rising sharply, the number of alien plant species that have been the subject of at least one study is no longer keeping pace, with researchers increasingly relying on fewer preferred model plant species [34]. Plant invasion biologists appear to be focusing their efforts on species already known to have an ecological impact, thereby skewing any overall assessment of the general effect of introduced species.

In the context of host manipulation by parasites, a comparison of the cumulative rise over time in the number of published empirical studies with the corresponding increase in the number of host and parasite species (or genera) that have been the subject of such studies suggests a similar problem may be on the horizon (Figure 2). The numbers of host and parasite taxa that have been the subject of at least one study are not keeping up with the rapidly growing body of empirical research, and the gap has become more pronounced in the past decade. The discrepancy observed indicates that researchers are gathering increasingly detailed information about proportionately fewer host and parasite taxa. In the decade from 2005 to 2010, out of 126 empirical studies of host manipulation by parasites, 34% focused on just two parasite genera, the acanthocephalans *Polymorphus* and *Pomphorhynchus*, with the remaining studies covering 36 parasite genera. Similarly, 39% of those studies investigated a single host genus, the amphipod *Gammarus*, with the rest examining a total of 34 other host genera. To this imbalance among published studies we could add the general publication bias against negative results [35], through which studies showing the absence of host manipulation are probably less likely to appear in scientific journals than those demonstrating host manipulation. This may exacerbate the focus on a few 'safe' model taxa.

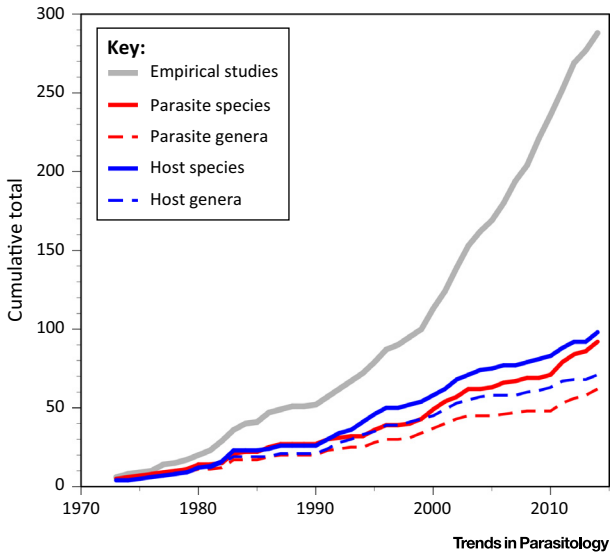

Figure 2. Cumulative Number of Empirical Studies on Host Manipulation by Helminth Parasites, and Cumulative Number of Host and Parasite Taxa (Species or Genera) that have been the Subject of these Studies, in the Years 1973–2014. Any given taxon is only counted once, in the year when it was first studied.

We are clearly entering a phase in which model systems have emerged as the main sources of our in-depth knowledge of the mechanisms and consequences of host manipulation by parasites. We are not criticising this necessary development. On the contrary, just as other fields have benefited from a focus on model systems, so will the study of host manipulation. However, each particular host–parasite combination is likely to possess unique and idiosyncratic features. We need to add new, carefully chosen model systems to our research agenda; we already have a long list of parasite species known to be manipulative from which to choose (see Supplementary Material online). These new models would serve to either confirm the universality of observed patterns, or quantify the variability of host manipulation outcomes. Here, we mean specifically new parasite species, as opposed to new host species studied in combination with a previously well-researched parasite species. Young researchers in the process of establishing a research program should be encouraged to select new model parasites, picked in light of the goals of their research but also to broaden the current taxonomic coverage. We are presently facing the consequences of the trade-off between breadth and depth of knowledge because of the increasing research focus on a few model systems. Right now, we are headed for one end of the spectrum, that is, in-depth knowledge of few systems. We need to find an acceptable compromise in order for more holistic progress in our exploration of the phenomenon of host manipulation.

Is the Nature of Empirical Studies Changing over Time?

From the early days, three components of empirical studies of host manipulation by parasites have been emphasised repeatedly as crucial for an improved understanding of the phenomenon [36,37]. These are: (i) the use of experimentally infected hosts instead of naturally infected ones; (ii) the experimental confirmation that altered behaviours in infected hosts do indeed lead to improved parasite transmission success; and (iii) the elucidation of the proximate mechanisms underpinning parasite-induced alterations of host phenotype. Experimental infections are necessary to demonstrate that infection by a parasite is the cause of any unusual behaviour displayed by an animal, and not the consequence of that behaviour. Indeed, unusual behaviour might precede infection and predispose an animal to infection. Demonstrating that, under realistic conditions, altered host behaviour leads to greater transmission by significantly raising the probability that the host is eaten by a suitable definitive host serves as the acid test of adaptive manipulation. Either the altered host behaviour improves the fitness of the parasite or it

does not, in which case it may be considered as an inconsequential side effect or a host compensatory adaptation. Finally, uncovering the physiological or neurobiological basis of altered host behaviour is akin to finding the smoking gun in a murder case: it can prove a direct link between infection and the expression of modified behaviours in the host. So how has research on host manipulation fared with respect to the highly recommended inclusion of these study components?

The proportion of empirical studies using experimentally infected hosts to test various aspects of host manipulation rose from zero in the 1970s to about half in the 1990s (Figure 3A). However, since then, it has plummeted to <10% in the present decade. To a large extent, this decrease can be attributed to the much larger annual number of studies published in recent years that have focused on new host–parasite species combinations. For many of these, no experimental infection protocol is yet available, and none is likely to be developed for a range of logistical issues. Nevertheless, the drop in the proportion of studies using experimentally infected hosts indicates a shift away from the rigorous approach to infer causality that was promoted and established earlier in the history of research on host manipulation by parasites.

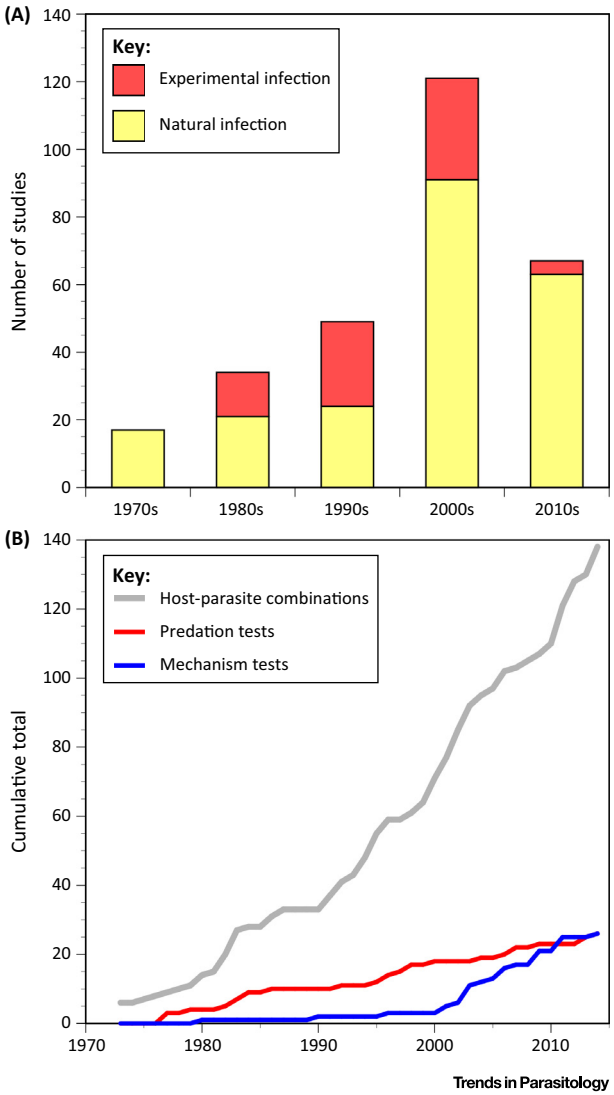

Figure 3. Trends in Empirical Studies on Host Manipulation by Helminth Parasites. (A) Numbers of empirical studies on host manipulation by helminth parasites in which hosts were infected either naturally or experimentally in each of the past few decades. (B) Cumulative number of host–parasite species combinations that have been studied empirically in the context of host manipulation by parasites. Any given combination is only counted once, in the year when it was first studied. Also shown are the cumulative numbers of host–parasite combinations for which a predation test was performed to confirm possible enhanced transmission to the definitive host, and those for which researchers have sought to elucidate the proximate mechanisms linking infection with behaviour modification.

Predation tests used to demonstrate the effectiveness of host manipulation by trophically transmitted parasites range from trials conducted under controlled laboratory conditions [38,39] to tests performed in natural or semi-natural habitats [18,40,41]. These tests remain relatively few, however, especially those conducted in natural conditions. The cumulative number of host–parasite species combinations that have been studied in the context of host manipulation by parasites has been and still is rising faster than the number of these associations for which a predation test has been carried out to confirm enhanced transmission to the next host (Figure 3B). Of the 138 host–parasite species combinations studied to date, only 26 have been the subject of a predation test. This is no doubt due in part to the logistical and, increasingly, ethical difficulties associated with the use of vertebrate intermediate hosts in predation tests. Regardless, for the vast majority of parasite taxa capable of altering host phenotype, the adaptive nature of the manipulation has not been demonstrated, and is instead tacitly assumed.

Unravelling the mechanisms by which parasites manipulate the behaviour of their hosts opens a window into the intimate biochemical interactions between the two antagonists [42,43]. Recent methodological advances have allowed increasingly sophisticated explorations of these mechanisms [44–47]. Yet, although the number of studies seeking to elucidate the proximate mechanisms linking infection with behaviour modification has risen steadily, beginning around the year 2000, they have investigated <20% of the 138 host–parasite species combinations studied so far in the context of host manipulation (Figure 3B). Apart from few acanthocephalan (*Pomphorhynchus* and *Polymorphus*) and trematode (*Microphallus*) taxa whose manipulative mechanisms have been investigated in greater depth, the majority of other parasites have been the subject of single exploratory studies. Thus, despite strong and repeated calls for greater focus on mechanisms, we generally remain in the dark as to how trophically transmitted helminth parasites manipulate their hosts.

The evidence suggests that the research community in general has not made these three key elements (experimental infections, predation tests, and the search for mechanisms) standard practice in the field. With some notable exceptions, the guiding principles devised to steer research into rigorous and fruitful directions have not been, and still are not, widely applied.

Concluding Remarks

We are still in the early learning phase about several aspects of host manipulation by parasites. In fact, basic information on species used as textbook examples, such as the trematode *Leucochloridium* in its snail host [48], is only now coming to light. Yet, it is not too early to look back at our past trajectory and make course adjustments for future research. Here, we offer general recommendations stemming from the patterns observed in the literature and summarised above (see Outstanding Questions). These recommendations are aimed at the scientific community as a whole and not at individual studies, where it may be impossible to follow them all at the same time. Also, they do not apply strictly to host manipulation by trophically transmitted helminths, but may to a certain extent be relevant to other parasite taxa and/or other modes of transmission.

First, theory should continue to develop but without getting too far ahead of itself; it should instead keep pace with the rate at which sound empirical foundations are being laid. In the 2012–2014 period, ~35% (19 out of 55) of published articles on host manipulation have been either reviews or theoretical/conceptual studies. That is a 2:1 ratio of empirical work versus theory/synthesis; in contrast, this ratio was about 4:1 in 2007–2009, and 15:1 in 2000–2004. Reviews allow general rules to be uncovered, and research without a theoretical framework can be likened to a ship without a rudder. Nevertheless, existing results can only be synthesised so many times, and recent empirical studies rarely address the key predictions of recent theoretical

Outstanding Questions

What is the ideal balance between empirical research that generates new data, and theoretical research that seeks to establish a broad conceptual framework for host manipulation by parasites?

How many new model systems do we need for a detailed study of host manipulation by parasites, and what taxa should they include if we are to achieve broad generalisations?

How important is it to use experimentally infected hosts to establish the causal link between infection and behavioural changes in the hosts?

What level of empirical proof is acceptable to conclude that an observed alteration in host behaviour does in fact improve parasite transmission?

papers. We recommend that the scientific community aims at substantially increasing this 2:1 ratio to redress the imbalance between facts and ideas. Currently, new data are just not accumulating fast enough to justify further synthesis or to support the proliferation of new hypotheses being proposed.

Second, we need to develop new model systems for the in-depth study of host manipulation by parasites. In particular, we need to investigate new parasite species that are phylogenetically distinct from the small group of taxa (mainly *Polymorphus*, *Pomphorhynchus*, *Diplostomum*, *Microphallus*, and *Schistocephalus*) currently receiving intense scrutiny. From a career perspective, there is little incentive to do this. Establishing a new model system requires time and effort, whereas adopting a well-established system can guarantee rapid publications. Institutions and funding agencies need to support researchers willing to take some risks by bringing new manipulative parasites into the laboratory. We need to encourage this broader view, to avoid the pitfalls associated with a small and unrepresentative set of model species from which practically all data are obtained.

Third, we need to return to the sound principles laid down years ago for the rigorous study of host manipulation as an adaptive strategy of parasites. In spite of all the logistical difficulties this entails, researchers must strive to use experimentally infected hosts to validate the causal link between infection and behavioural changes, and confirm that altered behaviours in infected hosts improve parasite transmission by conducting predation tests under conditions as natural as possible. The decreasing proportion of studies using experimental infections, and the decreasing proportion of host–parasite systems in which predation tests have been performed seen over the past 10–15 years are somewhat alarming. We cannot take for granted that parasites cause host behavioural changes that benefit them without empirical proof.

Finally, we could reiterate the need to elucidate the proximate mechanisms underpinning parasite-induced alterations of host phenotype. This plea has been made many times in the past decades. It has not fallen on deaf ears, and several excellent studies have already uncovered physiological and neurobiological pathways linking infection with changes in host behaviour. These investigations have been limited to model host–parasite systems, however, and as stated earlier, new systems have to be explored to allow generalisation on this front. Overall, the above recommendations are only meant as small adjustments to what has been, globally, a successful research effort into one of the most fascinating adaptations displayed by parasites.

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Supplemental Information

Supplemental Information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pt.2015.07.002.

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