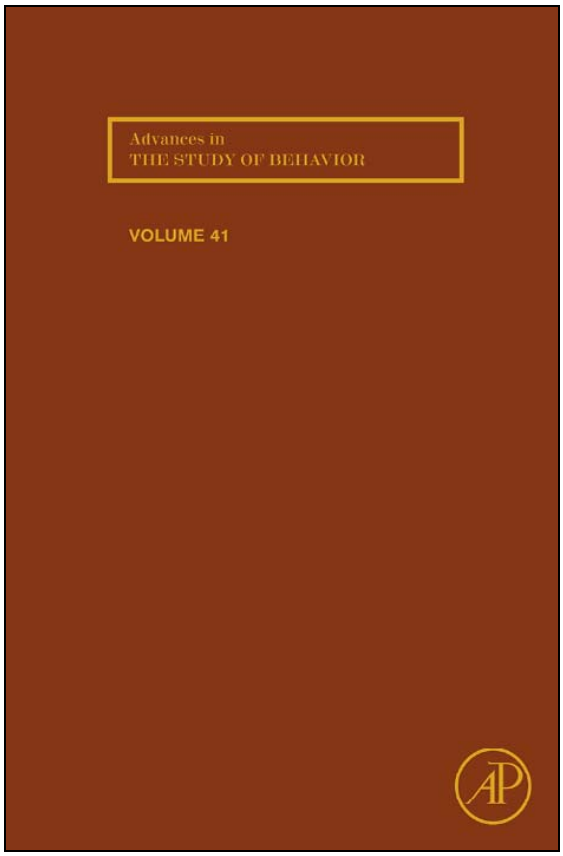

From: Robert Poulin, Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions. In H. Jane Brockmann, editor: Advances in the Study of Behavior, Vol. 41, Burlington: Academic Press, 2010, pp. 151-186.
ISBN: 978-0-12-380892-9

ADVANCES IN THE STUDY OF BEHAVIOR, VOL. 41

Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions

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I. INTRODUCTION

The idea that a parasite can modify the phenotype of its host, by either taking control of host behavior or changing the host's appearance, may have first appeared in science fiction stories, but is now a well-established concept in the study of animal behavior. From the first empirical demonstrations that amphipods harboring larval acanthocephalan parasites displayed both aberrant behavior and abnormal coloration making them more susceptible to predation by the parasite's next host (Hindsbo, 1972; Holmes and Bethel, 1972), there has been sustained interest in this phenomenon. As a result, host manipulation by parasites has now been documented in a few hundred distinct host–parasite associations spanning all major phyla of living organisms (see review in Moore, 2002).

While most of these known cases generally involve only subtle changes in one aspect of host behavior or appearance, some are truly spectacular. The two trematode species that have become classical textbook examples are among those. Both require the intermediate host, in which the parasites develop as larvae, to be ingested by a definitive host that is not normally a predator of the intermediate host. The first, *Dicrocoelium dendriticum*, must be transmitted by accidental ingestion from an ant to a sheep; it causes infected ants to climb to the tip of grass blades and stay there patiently waiting for a grazing sheep (Carney, 1969; Moore, 2002; Wickler, 1976). The second trematode, *Leucochloridium* spp., alters the size, shape, and coloration of the tentacles of its snail intermediate host and causes them to pulsate violently in response to light; these attract the attention of birds to which the parasite must next be transmitted, presumably fooling them into seeing the colorful and pulsating tentacles as potential caterpillar prey (see Moore, 2002).

0065-3454/10 $35.00
DOI: 10.1016/S0065-3454(10)41005-0

There are many more examples of host manipulation that are equally bizarre. A nematode parasite turns the abdomen of its ant intermediate host bright red, and drives the ant to go perch, with its abdomen raised, among patches of small red berries, to await the frugivorous birds that serve as definitive hosts for the nematode (Yanoviak et al., 2008). Another nematode, with a simpler life cycle, must release its own eggs in water when its mayfly host returns to a stream to oviposit; when the parasite finds itself in a male mayfly, it feminizes the host, turning it at both morphological and behavioral levels into a ''female'' that will return to water, though only the parasite will be laying eggs (Vance, 1996). A parasitic wasp larva growing inside an orb-weaving spider causes its host to start building a strange new pouch-like structure attached to its web just hours before the wasp emerges from the spider; the pouch serves to protect the wasp larva from being swept away by heavy rain as it pupates after the spider's death (Eberhard, 2000). Another parasitic wasp, using caterpillars as hosts, can even alter host behavior *after* leaving it: previously infected caterpillars, which can only survive a few days, remain right by the wasp larvae that have just exited their bodies, protecting them from approaching predators as the larvae pupate (Brodeur and Vet, 1994; Grosman et al., 2008). Finally, the widespread protozoan parasite *Toxoplasma gondii*, which must be transmitted from a rat intermediate host to a cat definitive host, reverses the innate aversion of its rat host to cat odor into an attraction toward cat odor, with obvious consequences (Berdoy et al., 2000). Humans can also become infected instead of rats. Although this is a dead-end for parasite transmission, *T. gondii* must nevertheless induce neurochemical changes in infected humans, since people with latent *T. gondii* infections show personality traits and reaction times that differ from those of uninfected controls (Flegr et al., 2000; Havlicek et al., 2001).

In a nutshell, host manipulation by parasite can be defined as any alteration in host phenotype, induced by a parasite, that has fitness benefits for the parasite. In the context of altered host behavior, this generally means that infected hosts behave in ways that facilitate the transmission or dispersal of the parasite, and therefore the completion of its life cycle. The implication here is that the phenotypic traits in the host that are modified by infection are either directly or indirectly modulated by genes in the parasite genome. Indeed, host manipulation by parasites has been proposed as one of the main concrete examples of extended phenotypes (Dawkins, 1982).

Much of the research on host manipulation by parasites has focused on the significance of the phenomenon for the parasites themselves, and on how it operates at the host–parasite interface (see reviews by Moore (2002); Poulin (1995, 2007); Thomas et al. (2005)). Recently, broader implications of host manipulation have also started to attract some attention.

For instance, the presence of manipulative parasites in an animal population can shape host evolution (Poulin and Thomas, 1999), influence the ecology of other parasite species (Lafferty et al., 2000; Poulin, 2007), alter the structure of the surrounding animal community (Lefèvre et al., 2009b; Mouritsen and Poulin, 2005; Thomas et al., 1998a), and/or have veterinary implications (Lagrue and Poulin, 2010). These issues are reviewed elsewhere, and the present synthesis focuses strictly on the original and still fundamental aspects of the phenomenon, providing an update of our current understanding of host manipulation as a parasite strategy.

The goal of this chapter is to integrate several ongoing lines of research, at both the mechanistic (proximate) and functional (ultimate) levels, into a coherent and unified overview. It does not provide an exhaustive list of examples, or even a comprehensive overview of recent ones. Instead, this chapter is structured around several frequently asked questions that still drive modern research on host manipulation by parasites. After attempting to answer these important questions, I will conclude by offering some suggestions for future research that should take us beyond our current level of understanding.

II. When Is It Adaptive Manipulation?

No one expects a sick animal to behave normally. Therefore, the simplest, most parsimonious explanation for a difference in behavior between parasitized and nonparasitized animals need only involve side-effects of pathology that may or may not be coincidentally beneficial for the parasite. In the literature from the past 30 years, three alternative explanations have been considered for changes in the behavior of an animal following its infection by a parasite. First, the change may result from something done specifically by the parasite to the host, the effect of which is to alter its behavior in ways that benefit the parasite. This is the classical interpretation of adaptive manipulation, implying the existence of genes ''for'' manipulation in the parasite genome. Second, the change may represent an adaptive response of the host to infection, serving to either eliminate the infection or mitigate its negative consequences. Third, as stated above, the change in host behavior may be merely a by-product of pathology, or of other aspects of infection that, by chance and under certain circumstances, happen to have fortuitous outcomes for parasite transmission. Thus, in its narrowest sense, adaptation may not automatically apply to all cases of parasite-induced changes in host behavior.

Nevertheless, most early studies of this phenomenon, and many recent ones, have been quick to label as ''adaptive manipulation'' any observed change in host phenotype. This lead some authors to call for greater rigor in the use of the term ''adaptive'' for any presumed case of manipulation (Moore and Gotelli, 1990; Poulin, 1995). In an attempt to provide some guidelines to limit abuse of the adaptive label, Poulin (1995) proposed four basic criteria that a parasite-induced change in host behavior had to meet in order to be seen as a case of adaptive manipulation by parasites. In retrospect, only one really matters; nevertheless, let us briefly revisit these criteria. First, changes in host behavior following infection must show some conformity to *a priori* expectations based on their purported function. Conformity between *a priori* ''design specifications'' and observed phenotypic change provides evidence of the shaping force of natural selection; in contrast, *a posteriori* attempts to find an adaptive function for an unanticipated behavioral change are unconvincing. Included in this criterion is the timing of events: the onset of behavioral changes is expected to coincide with the developmental phase of the parasite at which it is ready to benefit from these changes, if manipulation is to be considered adaptive. For instance, the larval stages of parasitic worms often only begin to induce behavioral changes in their intermediate host when they are developmentally ready to be transmitted by predation to their next host (Bethel and Holmes, 1974; Hammerschmidt et al., 2009; Poulin et al., 1992).

Second, the complexity of parasite-induced behavioral changes can also reveal their adaptiveness. Simple traits are more likely to arise by chance, for instance as by-products of pathology, than complex ones; the latter require an organizing principle such as natural selection. The appearance of completely novel phenotypic features seems to meet this criterion. For instance, in ants whose abdomen turns from black to bright red and that go perch among red berries following infection by nematodes, the phenotypic change seems both too complex and too well-fitted to parasite transmission to be anything other than an adaptive manipulation (Yanoviak et al., 2008). Similarly, nematomorphs, or hairworms, must emerge from their terrestrial insect hosts into freshwater bodies to continue their life cycle. At the right time in the development of the parasite, infected insects suddenly display a completely novel behavior: they seek water and throw themselves into it (Thomas et al., 2002). Again, this is a complex change in behavior that seems unlikely to arise by chance as a side-effect of worm development within the host. In truth, it is the complexity of the mechanism used by the parasite to alter host behavior that should matter for this criterion, and not that of the eventual change in behavior. However, so little is known about the underlying mechanisms that we must judge complexity of manipulation by its phenotypic manifestation only.

Third, convergence between unrelated parasite lineages with respect to the type of behavioral changes they induce in their host can provide strong hints that these are cases of adaptive manipulation. Evolving under similar selective pressures, we would expect different taxa to solve similar problems with analogous traits performing similar functions. For example, mermithid nematodes and hairworms belong to different and unrelated phyla (Nematoda and Nematomorpha, respectively). However, they have independently evolved very similar life cycles: in both groups, at some point late in their development within a terrestrial arthropod host, the parasite must emerge in water or water-saturated soil to pursue its life cycle. As seen earlier, hairworms induce their hosts to find water and jump in it (Hanelt et al., 2005; Thomas et al., 2002). Remarkably, mermithids do the same to their hosts (Maeyama et al., 1994; Poulin and Latham, 2002; Vance, 1996). The independent evolution in unrelated parasite lineages of almost identical changes in host behavior following infection points toward adaptation.

Fourth, an adaptive trait must confer fitness benefits to its bearer, and so parasites capable of manipulating the behavior of their hosts must achieve greater transmission success than conspecifics not capable of altering host behavior. Although this has only been confirmed in a small proportion of documented cases of parasite-induced changes in host behavior, it remains the strongest evidence one can obtain of adaptive manipulation. For trophically transmitted parasites, this would typically consist in a predation test where equal numbers of parasitized (manipulated) and nonparasitized (not manipulated) intermediate hosts are available to a definitive host of the parasite in a seminatural setting (Lafferty and Morris, 1996; Lagrue et al., 2007; Moore, 1983); any bias toward greater ingestion rate of manipulated prey is seen as enhanced transmission achieved via host manipulation.

In hindsight, Poulin's (1995) criteria as a whole were probably much too strict, or at the very least overly conservative. It is only the fourth, concerning fitness effects for the parasite, that should really matter. Whether the manipulation is simple or complex, whether it seems a good fit to its function or not, and whatever its evolutionary origins, it will be favored and/or maintained by selection if it improves the fitness of the parasite, making it a true adaptation. As pointed out by Thomas et al. (2005), if we elevate the coincidental by-product scenario to the status of null hypothesis against which other interpretations must be evaluated, then it should be testable. In practice, there is no straightforward experimental way of distinguishing between an advantageous by-product and an advantageous direct product of selection. Historically, parasite traits evolved for other functions that happened to have concomitant effects on transmission may have been co-opted for manipulation, and the boundary between the original and the new function is rarely clear-cut. Consider what happens to coral polyps infected by the trematode

Podocotyloides stenometra. Infected polyps turn bright pink, and their increased visibility causes them to be preferentially eaten by the butterfly fish that serve as the parasite's definitive hosts (Aeby, 2002). Recently, the compound responsible for the pink coloration of infected polyps has been identified as a protein involved in the host's cytotoxic defense system (Palmer et al., 2009). Therefore, the altered pigmentation of infected corals is part of their normal immune response to infection, but as it is harmless to trematodes and even beneficial to their transmission, selection should favor parasites that induce more pronounced color changes in their host. At some point, beneficial side-effects simply become adaptations.

Therefore, in order to move forward, it is probably time to put to rest the argument over adaptiveness: if a parasite-induced change in host behavior leads to improved transmission of the parasite, then, as long as there is a genetic basis for this effect, and whether it is fortuitous or not, it is a case of adaptive manipulation.

III. What Kinds of Parasites Manipulate Their Host?

This question can be answered on two levels. From a taxonomic perspective, manipulation has been documented in representatives of most of the major lineages of parasitic organisms. We know that at least some species are capable of host manipulation in the animal phyla Platyhelminthes (classes Trematoda and Cestoda), Acanthocephala, Nematoda, Nematomorpha, and Arthropoda, as well as in the viruses, bacteria, fungi, and the mixture of single-celled eukaryotes still conveniently lumped together as ''Protozoa'' or ''Protista'' (see Moore, 2002). In some of these groups, such as the Nematomorpha or Acanthocephala (Hanelt et al., 2005; Moore, 1984), the ability to alter host behavior in ways that benefit the parasite appears to be widespread, possibly even shared by all species within the group. This suggests that the ability to manipulate host phenotype is an ancestral trait, inherited by most (or all) living parasite species within a lineage. In contrast, in other groups such as the Platyhelminthes or Nematoda, host manipulation has only been documented from certain families, often phylogenetically distant from each other, indicating that it has evolved independently more than once within each of these phyla. Overall, a conservative estimate suggests that the ability to manipulate host behavior has evolved at least 20 separate times among parasite lineages during the history of life on Earth.

The second level at which the question heading this section can be answered is an ecological one that focuses on the commonalities between the transmission routes used by manipulating parasites. Massive losses of larval infective

stages during transmission events create inevitable bottlenecks at one or more stages in the life cycle of parasites, and these in turn exert strong selective pressures on parasites. Given that different parasite lineages have often converged on similar life cycles characterized by similar transmission routes (Poulin, 2007), it is perhaps not surprising that similar obstacles to transmission have lead to similar adaptations in widely different parasite lineages. Manipulation of host behavior is one such adaptation, serving to increase transmission success during one of these bottlenecks. Clearly, manipulation of host behavior can only benefit the parasite if its transmission success is linked to what the host does. Many parasites, such as monogeneans ectoparasitic on fish (e.g., Shirakashi et al., 2008), cause alterations in the behavior of their host; since monogeneans are transmitted simply by releasing eggs into the water, no one would argue that these are parasite adaptations serving to enhance transmission success. Focusing only on parasite-induced alterations of host behavior that meet the definition of adaptive manipulation given at the end of the previous section, a survey of documented cases of host manipulation shows that the vast majority involve parasites using one of four general transmission routes (Fig. 1). Although the details of the manipulation, such as which host phenotypic trait is altered, vary widely from one situation to the next, the transmission obstacle that needed a solution is roughly the same across all examples within each of the four categories.

The first type of transmission route in which host manipulation is widely manifested is trophic transmission (scenario A in Fig. 1). In this situation, the larval or juvenile stages of a parasite living inside an intermediate host must be transmitted to the parasite's definitive host by predation. Manipulation consists in altering the appearance or behavior of the intermediate host to render it more visible or otherwise susceptible to predation by a suitable definitive host (Lafferty, 1999). Many parasitic worms with complex life cycles, including trematodes, cestodes, acanthocephalans, and nematodes discussed throughout this chapter, employ this type of manipulation, as do several parasitic protozoans (Berdoy et al., 2000; Hoogenboom and Dijkstra, 1987).

The second transmission situation where host manipulation is commonly observed involves parasites that must either exit the host themselves, or release their propagules, in a habitat other than the one in which the host lives (scenario B in Fig. 1). Here, manipulation by the parasite induces the host to move to a different habitat, sometimes one that is completely unsuitable for the host. The nematomorphs and mermithid nematodes discussed earlier, which cause their terrestrial arthropod hosts to seek and enter water, are perfect examples of this type of manipulation (Hanelt et al., 2005; Maeyama et al., 1994; Poulin and Latham, 2002; Thomas et al., 2002; Vance, 1996). Other examples include trematodes that induce their snail

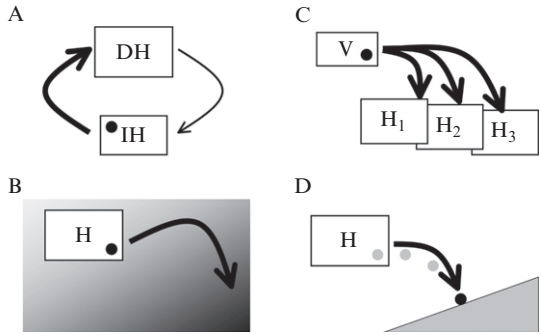

FIG. 1. The four main scenarios in which host manipulation by parasite has been observed. In each case, hosts are represented by boxes and the parasite by a black circle; the thick arrow indicates the step in the life cycle where manipulation is seen. (A) In trophically transmitted parasites, manipulation of the behavior or appearance of the intermediate host, IH, can increase the probability of transmission by predation to the definitive host, DH. (B) In parasites that must either exit the host themselves, or release their propagules, in a habitat other than the one in which the host lives, manipulation can cause the host, H, to move to a different but suitable habitat, as shown by the gradient in the figure. (C) In vector-borne parasites, manipulation of the vector, V, can induce it to visit more hosts, H_n, to which the parasites can be transmitted than it would otherwise. (D) In parasitoids that must exit the host and pupate on external substrates, manipulation can alter the behavior of the host in ways that will protect the parasite pupae from predators or other dangers.

intermediate hosts to move to different microhabitats that are ideal for the release of the parasites' infective stages (Curtis, 1987; Lowenberger and Rau, 1994), and parasitic fungi that force their insect hosts to go to the top of shrubs or trees, or to settle on the underside of leaves, where the conditions are better for wind-assisted dispersal of fungal spores (Andersen et al., 2009; Maitland, 1994; Pontoppidan et al., 2009).

The third situation where host manipulation appears regularly is in cases of vector-borne transmission (scenario C in Fig. 1). The best-known examples involve pathogens transmitted among vertebrate hosts by blood-sucking insects such as mosquitoes; they are picked up by the vector during one blood meal, and injected later in a new host during a subsequent blood meal. Since transmission opportunities for the parasite depend entirely on how many potential hosts are visited by the vector, manipulation of vector behavior can serve to shorten the duration of individual blood meals and increase the number of different hosts visited (Moore, 1993). Parasites known to induce behavioral alterations in their vectors include viruses, protozoans such as trypanosomes and *Plasmodium* spp. (the causative agents of malaria), and filarial nematodes (Hurd, 2003; Moore, 1993; Rogers and Bates, 2007).

Finally, the fourth general type of transmission mode in which host manipulation is common is that used by most insect parasitoids, both Hymenoptera and Diptera, that must exit their host after growing inside it and pupate on external substrates (scenario D in Fig. 1). In these cases, manipulation can alter the behavior of the host in ways that will provide protection to the parasite pupae from predators or other dangers. This can be achieved by the host moving to specific microhabitats prior to the emergence of the parasitoid (Brodeur and McNeil, 1989), by the host producing physical structures that will protect the parasitoids following their emergence (Eberhard, 2000), or by the host remaining next to the pupating parasitoids to defend them against predators (Brodeur and Vet, 1994; Grosman et al., 2008).

The success of parasites with other types of transmission modes is also tightly coupled with host behavior, but evidence of host manipulation is either lacking or not convincing for such parasites. For instance, parasites transmitted during physical contact between two individual hosts could, in principle, boost their transmission opportunities by increasing the frequency of such contacts. There are some reports of sexually transmitted parasites altering the sexual behavior of their host in ways that lead to further contacts with mating partners (e.g., Abbot and Dill, 2001). Similarly, the rabies virus is transmitted when an infected host bites a susceptible host, and much has been made of the fact that rabid animals display increased aggression. However, the story is more complex, with increased aggression being only one possible manifestation of rabies (see Hemachudha et al., 2002; Rupprecht et al., 2002). Therefore, the evidence that contact-transmitted parasites manipulate host behavior is on the whole not very convincing. This may be in part because these parasites have not been studied explicitly within the context of host manipulation, and possibly future evidence will change this assessment. Alternatively, it may be that selection pressures favoring host manipulation are not very strong in contact-transmitted parasites, since the normal behavior of their hosts is often sufficient to guarantee regular sexual or social contacts with conspecifics. Nevertheless, if we consider all transmission routes where host behavior plays a determinant role in parasite success, it is fair to say that the ability to manipulate the host has evolved repeatedly, in a broad taxonomic range of parasites, as an adaptive parasite strategy.

IV. What Host Traits Are Manipulated by Parasites?

Underlying any externally visible changes in host phenotype, there must be a series of parasite-induced alterations in biochemical and physiological pathways. However, the majority of studies on host manipulation by

parasites have focused exclusively on visible changes in coloration, morphology, or behavior. In some cases, manipulation by the parasite results in completely novel behavioral patterns, such as crickets jumping in water, or spiders producing a totally new type of structure within their web. Most often, though, the manipulation targets existing behaviors and is manifested by small changes in their expression. For instance, the outcome of manipulation may be only a slight shift in the proportion of time an animal spends in one particular microhabitat, or performing a particular behavior. Parasites are frequently seen to modify basic host tropisms (e.g., responses to light, gravity, humidity), reactions to threat stimuli (disturbances associated with large moving objects such as predators), or activity levels; Moore (2002) provides comprehensive lists of documented examples of each type. Changes in these simple behavior patterns are generally sufficient to cause the host to move toward a different microhabitat, become more vulnerable to predation, or do whatever it takes to enhance parasite transmission success.

This has been the predominant view for many years. Increasingly, researchers are now recognizing the fact that manipulated hosts are not merely normal hosts with one or few altered traits, but instead they are deeply modified organisms (Thomas et al., in press). Parasite manipulation occurs along several phenotypic dimensions, and it is the complex outcome of relationships among these dimensions that yields transmission benefits for the parasite. Consider the previously mentioned case of the parasitic nematode *Myrmeconema neotropicum*, found in tropical ants, which must be transmitted to frugivorous birds in order to complete its life cycle (Yanoviak et al., 2008). Turning the abdomen of infected ants from black to bright red is not sufficient to ensure transmission; the parasite must also cause its ant host to find patches of red berries on tree branches, stay within these patches, and maintain their abdomen raised at an angle close to vertical, in order for it to convincingly mimic a small fruit and deceive a bird (Yanoviak et al., 2008). This, like most other manipulations by parasites, requires a suite of traits to be altered at once, or in a particular sequence, for the probability of transmission to be increased. Manipulative parasites must channel several associated traits in particular directions, whether or not these traits are linked by common neurological pathways. From an historical or phylogenetic perspective, manipulative parasites most likely derive from nonmanipulative ones, and it is more parsimonious to assume that the original manipulation involved only a single host phenotypic dimension. Any parasite capable of modifying one dimension of its host phenotype with a resulting increase in its transmission success would have been favored over its conspecifics by natural selection. Several selective forces can explain why fitness benefits could be achieved by adding new dimensions to an

originally simple manipulation (Thomas et al., in press). For instance, the modification of additional host traits can synergistically boost the efficiency of the original manipulation, or increase its specificity, for example, by making an intermediate host even more susceptible to predation by definitive hosts but less susceptible to predation by other predators that are unsuitable as definitive hosts (see Médoc and Beisel, 2008; Médoc et al., 2009).

Quantitative analyses of multiple phenotypic traits of hosts manipulated by parasites can reveal details about both the evolution of manipulation and its underlying mechanistic basis. In particular, evaluating both the independent and combined effects of each manipulated trait on parasite transmission, and quantifying the correlations among traits, would be important steps forward (Benesh et al., 2008; Cézilly and Perrot-Minnot, 2005; Thomas et al., in press). Two different host traits, each with its own independent effect on parasite transmission success, may or may not have additive or synergistic effects. Also, their respective effects may depend on external conditions, with one having a major effect and the other no effect at all under certain conditions, and vice versa under different circumstances. Each of these patterns would be consistent with a different evolutionary scenario. For instance, if different manipulated traits have independent but nonadditive effects, they may represent either a back-up or contingency system evolved to ensure that at least one of many redundant traits succeeds given locally variable transmission conditions, or separate manipulations with different ''target'' definitive hosts (Thomas et al., in press). Similar hypotheses have been put forward to explain the evolution of multiple cues in mate choice (Candolin, 2003). Alternatively, if the efficiency of each manipulated trait is dependent on external conditions, then they may have evolved in different populations that experience different selective forces, because of regional variation in community composition. In some areas, one species of definitive host may be the dominant predator of intermediate hosts; elsewhere, another suitable definitive host species may be numerically dominant. Therefore, an altered trait that works efficiently for the parasite in one area may be ineffective elsewhere. Parasites coevolve with their hosts in a heterogeneous environment, following the general principles of the geographic mosaic theory of coevolution (Thompson, 2005). Multidimensional manipulation may have evolved, and may be maintained, in response to spatially variable external conditions affecting the probability of transmission. The panoply of traits manipulated by a parasite may increase the probability that at least one will fit the current local conditions.

Significant correlations between the degree of expression of different manipulated traits could indicate that they are end-products of the same cascades of physiological alterations induced by the parasite (Benesh et al.,

2008; Thomas et al., in press). More importantly, correlations between host traits may be the real targets of manipulations, instead of the traits themselves (Coats et al., 2010). For example, a parasite may best increase its probability of transmission from its intermediate host to its final host by strengthening, reversing, or breaking up an existing association between host traits. An effect of parasite infection on correlations between host traits can have gone undetected in many earlier studies, because the average trait values may not differ between parasitized and nonparasitized individuals if they have not been altered by the parasite (see Fig. 2). If a parasite could uncouple two antipredator traits, such as cryptic coloration and a freezing response following a threat stimulus, without changing the average magnitude of these traits, it could achieve greater transmission success by ensuring that the host fails to evade predation in one way or another. In such a case, a simple comparison of mean trait values between parasitized and nonparasitized hosts would be inadequate; one would need also to compare the way the traits are correlated within the two groups of hosts (see Fig. 2). Recently, behavioral ecologists have become interested in behavioral syndromes, that is, suites of correlated behavioral traits, as the defining characteristic of animal personalities (Sih and Bell, 2008; Sih et al., 2004). Applying this more holistic view of animal behavior to the study of host manipulation will be a promising way of determining what, exactly, gets modified by parasites.

V. Why Do Some Parasites Manipulate Their Host but Others Do Not?

Most of the early studies on host manipulation by parasites reported very clear-cut, sometimes spectacular changes in host phenotype following infection, and it took several years for reports of very small effects, or even of no manipulation at all, to appear in the literature (Poulin, 2000). This temporal trend may reflect the simple truth that to be noticed in the first place, manipulation by parasites had to be evident, such that the first studies were inevitably carried out on host–parasite systems where obvious behavioral changes were induced by parasites. There may also have been publication biases against the acceptance of statistically nonsignificant differences between the behavior of parasitized and nonparasitized hosts, since nonsignificant results are often seen as boring. In any event, it is now universally accepted that some parasite species are capable of manipulating the behavior of their hosts, whereas others are not. In addition, even within species otherwise considered to be manipulative, there is considerable variation among individual parasites in the magnitude of the host

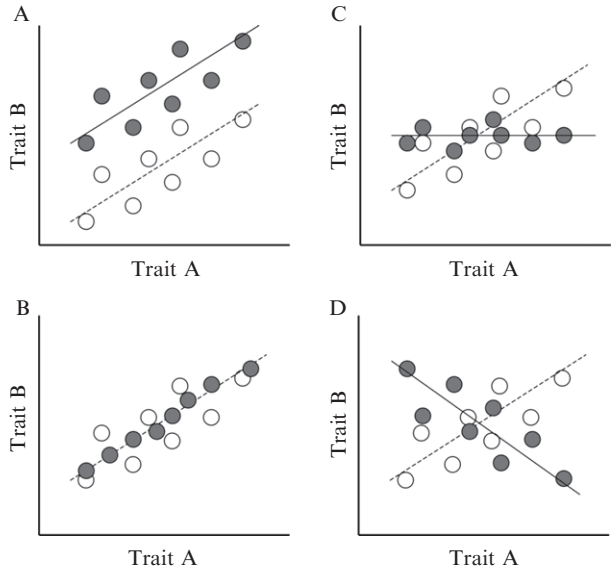

FIG. 2. Possible effects of a manipulative parasite on the correlation between two host traits. Each point represents an individual host, either parasitized (filled circles) or not (open circles); the correlation between the two traits is shown for parasitized (solid line) and nonparasitized (broken line) hosts. (A) The manipulation results in an increase of values for trait B only, but no change in the correlation between traits. (B) The manipulation results in a strengthening of the correlation between traits, that is, less scatter around the trend line. (C) The manipulation results in an uncoupling of the association between the two traits, such that their values are no longer significantly correlated. (D) The manipulation results in a reversal in the direction of the correlation between traits, from positive for nonparasitized hosts, to negative for parasitized ones. Note that in the last three scenarios (B–D), the parasite induces no change in the average trait values, only a change in how they correlate with each other; therefore, a comparison of mean trait values between parasitized and nonparasitized hosts would completely miss the effect of manipulation.

manipulation induced, with some individuals causing no detectable changes in host behavior. Therefore, the question of why certain parasites manipulate their hosts while others do not can be answered at both interspecific and intraspecific levels.

A. Interspecific Variation

Manipulation of host behavior is an adaptation serving to improve transmission and the completion of a parasite's life cycle. Like any other adaptation, the net benefits it confers on its bearer vary depending on the nature

of the host–parasite association, in particular on the specific transmission obstacles and other constraints faced by a parasite. For some parasite species, the net benefits of manipulation may be high and selection should favor this strategy, whereas for others, the benefits may be nil and manipulation would not be favored.

Underpinning all theoretical analyses of net benefits of manipulation is the notion of costs that must be outweighed, in fitness terms, by the gross benefits of manipulation for that strategy to be favored. These costs of manipulation come in two forms. First, there may be induction costs, that is, the physiological costs associated with the mechanism used by the parasite to induce a change in host behavior. Some parasites may produce changes in host behavior without incurring any costs, simply by being in the right organ and fortuitously impairing tissue function, as long as living in that organ as opposed to another does not result in lower parasite growth or survival. However, most alterations of host behavior appear to result from active interference with host neurochemistry that may involve the secretion and release of substances by the parasite (Hurd, 1990; Thomas et al., 2005; Thompson and Kavaliers, 1994). The development of specialized glands or tissues for the production of chemicals must be costly. It must be noted that such glands have never been found in any parasite, and that induction costs have never been quantified.

Second, in addition to any physiological costs associated with inducing the manipulation, parasites that manipulate their host may also pay consequential costs, measurable as a higher probability of early death (Poulin et al., 2005). These can be estimated in situations where manipulative parasites have conspecifics that benefit from manipulation without themselves inducing it. For instance, larval stages of the trematode *Microphallus papillorobustus* that encyst in the cerebral region of their amphipod intermediate host induce a strong positive phototaxis and aberrant evasive responses in the host. This manipulation of host behavior results in infected amphipods being more susceptible to predation by aquatic birds, which serve as definitive hosts for the parasite. However, not all *M. papillorobustus* encyst in the head of amphipods, some also encyst in the abdomen. Amphipods are capable of mounting an immune response against invading parasites, involving both encapsulation and melanization of the trematodes. Thomas et al. (2000) have found that 17% of cerebral parasites are killed by encapsulation, whereas less than 1% of abdominal parasites suffer this fate. Three other trematode species parasitize the same amphipod; they all encyst in the amphipod's abdomen, and none of them is attacked by the host immune system (Thomas et al., 2000). The host's defenses target specifically those parasites most likely to cause it harm. The end result is that manipulative individuals incur a much greater probability of death from immune attack than their conspecifics opting not to manipulate the host.

Another example involves the trematode *Curtuteria australis*, which infects the cockle *Austrovenus stutchburyi* as intermediate host; the parasites encyst in the foot of cockles and await predation by oystercatchers, their definitive host. As parasites accumulate in the foot of a cockle, they replace host muscle tissue and debilitate the foot, such that heavily parasitized cockles lose their ability to burrow, and are left stranded on the sediment surface of intertidal areas (Thomas and Poulin, 1998). Field experiments have shown that manipulated cockles are about 5–7 times more likely to be eaten by bird definitive hosts, than healthy, buried cockles (Mouritsen, 2002; Thomas and Poulin, 1998). The trematodes tend to encyst near the tip of a cockle's foot, where their debilitating effect on the host's burrowing ability is most intense (Mouritsen, 2002). Importantly, however, many *C. australis* are found in the middle of the foot or near its base. The benefits of host manipulation are shared by all parasites: although only those near the tip of the foot impair host burrowing ability, an oystercatcher feeding on a cockle eats all parasites along with host tissues. However, an opportunistic predatory fish also picks on surface-stranded cockles, eating exclusively the tip of the foot of those cockles that try in vain to burrow. This fish predator is not a suitable definitive host and any *C. australis* ending up in it dies. A third of all cockles show signs that part of their foot has been cropped by the fish (Mouritsen and Poulin, 2003). In manipulated cockles lying on the sediment surface, close to one-fifth of parasites are lost to fish predation (Mouritsen and Poulin, 2003). All of those were encysted near the tip of the foot of cockles, not at its base. Therefore, in this system, parasites that induce host manipulation face a greater risk of mortality than conspecifics that do not.

Thus, changes in host behavior that benefit the parasite are unlikely to be cost-free, even if the costs are often difficult to quantify. In the case of induction costs, trade-offs will affect the evolution of host manipulation: any energy invested by the parasite in host manipulation will not be available for growth, reproduction, or fighting the host's immune system. These trade-offs, combined with the risks of early parasite death sometimes associated with manipulation, mean that selection will not always favor parasites investing in host manipulation. Investments in manipulation, or manipulation effort (ME), should tend toward an optimal value at which parasite fitness is maximized. Under some ecological conditions, low values of ME will be favored, and the associated changes in host behavior may sometimes be very small. The first theoretical treatments of host manipulation have focused on predicting the optimal ME, or ME*, expected under different conditions (Brown, 1999; Poulin, 1994a, 2007). Even with no investment in host manipulation (ME = 0), the transmission success of a

parasite is unlikely to be nil. For instance, because definitive hosts ingest many prey over time, chances are that random prey selection will result in some infected intermediate hosts being captured by definitive hosts even without manipulation by the parasites. Investments in manipulation ($ME > 0$) will only increase the probability of transmission above the passive transmission rate, or p (Fig. 3). The gross benefits of manipulation correspond to the difference between the transmission rate achieved through manipulation and the passive transmission rate (or $m - p$ in Fig. 3). As ME gets higher, the rate of increase in the probability of transmission is likely to follow a law of diminishing returns (Fig. 3): small investments yield greater returns per investment unit than large investments. The costs associated with manipulation are also likely to increase with increases in ME. The shape of the cost function will vary, from roughly

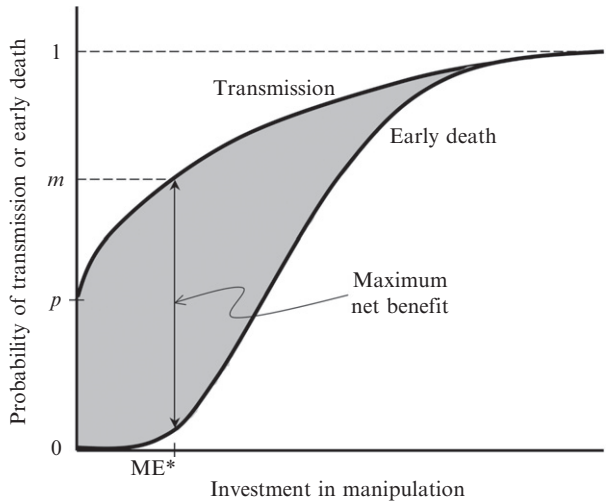

FIG. 3. Probability of parasite transmission and probability of the parasite dying early as a function of investment in the manipulation of the intermediate host (i.e., manipulative effort, ME). Without any investment in manipulation, the parasite has a passive transmission rate (p) that is greater than zero but less than one; increasing investment in manipulation yields higher transmission probabilities but with diminishing returns (top curve). At the same time, the cost of manipulation, or the probability of dying early as a consequence of investing in manipulation, increases with the level of investment, following a sigmoidal function in this hypothetical example (bottom curve). The gross benefits of manipulation equal $m - p$, or the difference between the realized transmission rate and the passive transmission rate. The optimal investment in manipulation (ME*) is the level at which the net gain (benefits minus costs) in transmission probability is maximized, that is, where the shaded area is highest. Modified from Poulin (1994a, 2007).

linear if the costs are mainly due to production of neuroactive substances inducing changes in host behavior, to an all-or-nothing step function if mortality of manipulative individuals is much higher than that of nonmanipulative individuals, as in the examples above. The investment favored by selection, ME*, will depend both on p and on the exact shape of the transmission and cost curves in Fig. 3; for some parasites, ME* could be zero or less, and manipulation would not be favored.

Several other factors will also come into play. For instance, the mean number of conspecific parasites per intermediate host, or the genetic relatedness of these parasites, can both affect the evolution of ME*, possibly leading to conditional strategies instead of a fixed ME* (Brown, 1999; Poulin, 1994a, 2007). Nevertheless, the simple theoretical framework presented in Fig. 3 still leads to predictions that are testable using an interspecific comparative analysis. For instance, we would expect fewer manipulative parasite species, or species inducing weaker manipulation, in systems where predation rates on intermediate hosts by definitive hosts are high than in host–parasite systems where these rates are relatively low.

Recently, Parker et al. (2009) have developed a modeling framework for the evolution of host manipulation in trophically transmitted parasites. They determine the conditions under which it is favorable for the parasite to manipulate host behavior in order to reduce predation on the intermediate host before the parasite completes its development within that host, and enhance it afterwards. Most parasites require some time to develop within an intermediate host before they become infective to their definitive host; from the onset of infectivity to the next host, manipulation to enhance transmission success can increase parasite fitness. However, this is only true under certain conditions. The key factors include the maximum time that the parasite can survive in its intermediate host following the onset of infectivity, and both the induction and consequential costs of manipulation (Parker et al., 2009). As explained above, the induction costs are essentially of an energetic nature, resulting via a trade-off in a reduction in growth or fecundity, whereas the consequential costs are manifested as an increased probability of dying from either host immune responses or predation by unsuitable definitive hosts. The maximum time during which the parasite survives in its intermediate host following the onset of infectivity can be limited either by the host's lifespan, or by the parasite's own mortality within the host.

Parker et al. 's (2009) model predicts the threshold value for induction costs below which a gene for manipulation can spread through a parasite population. For a given improvement in transmission rate resulting from manipulation (equivalent to $m - p$ above), genes for manipulation will be favored when postinfectivity time in the intermediate host is limited, even

at relatively high induction costs, and whatever the consequential costs (Fig. 4). As the time available in the intermediate host postinfectivity increases, however, the threshold value for induction costs below which manipulation genes are favored decreases rapidly toward an asymptote; the drop in the threshold value is even more pronounced when consequential costs of manipulation are substantial (Fig. 4). In these models, the parameter space over which genes for manipulation are favored does not vanish when consequential costs increase, but it does become rather small. Thus, if manipulation of the intermediate host routinely leads to parasites ending up in unsuitable predators where they die, manipulation may still be advantageous, but under a narrower set of conditions (see also Seppälä and Jokela, 2008). It must be remembered, however, that the probability of predation by nonhost predators is not constant in space: different localities are characterized by different communities of predators. Thus, if the consequential

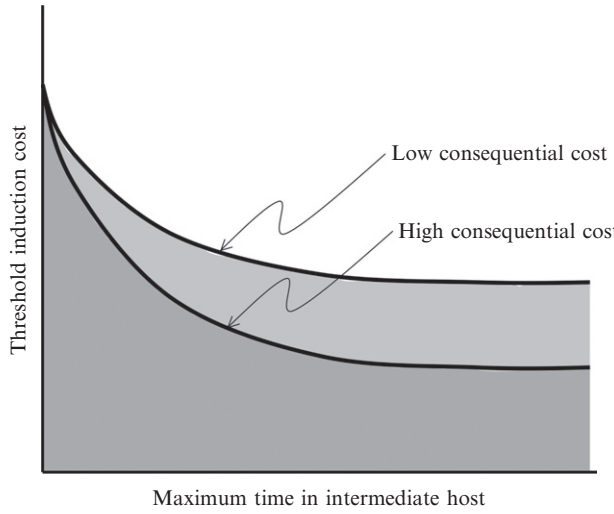

FIG. 4. Relation between the threshold fitness cost of inducing a manipulation of intermediate host behavior, and the maximum time that a parasite can survive in the intermediate host after becoming infective for its next host. Manipulation is favorable if, and only if, the induction cost lies below a given curve (i.e., in the shaded area); in that case, genes for manipulation can spread through a parasite population. The range of induction cost values for which the evolution of manipulation remains favorable decreases as the time that the parasite can survive in the intermediate host increases, in particular if the consequential cost of manipulation (such as the risk of ending up in a predator that is unsuitable as definitive host) is high. Modified from Parker et al. (2009).

cost of manipulation is low in some parasite populations, and if there is much gene flow among these populations, manipulation can exist in localities where conditions appear unlikely to favor its maintenance.

Like other models, the predictions of Parker et al. 's (2009) are amenable to empirical testing using a comparative approach. There is some evidence that different parasite species, with identical life cycles and sharing the same intermediate host species, but with different lifespan, differ in their ability to manipulate host behavior in ways that fit with theory (see Knudsen et al., 2001). It would be very instructive to perform a comparative analysis across parasite species to see how lifespan in the intermediate host and the risk of nonhost predation, possibly estimated using measures of local predator diversity or food web complexity, relate with the frequency at which manipulation is used as a transmission strategy.

Most theoretical investigations to date have considered the evolution of host manipulation in the context of single parasite species, ignoring the fact that parasite species rarely occur on their own in a host population, or even in a host individual. It is not unusual for two or more parasite species to have roughly identical life cycles, so that they share both intermediate and definitive hosts. In such a case, if one of these species is an efficient manipulator of host behavior, there would be reduced selection pressure on the other species to also evolve the ability to manipulate the host. Instead, any species co-occurring with the manipulator could be a ''hitchhiker'' that gets a free boost to its transmission chances each time it shares an individual intermediate host with the manipulator. There are indeed a few documented cases of apparent hitchhiking (Lafferty et al., 2000; Leung and Poulin, 2007; Poulin, 2007; Thomas et al., 1997). In theory, if the manipulator species is highly prevalent in the intermediate host population, then, all else being equal, there will be very little benefits for the other species to evolve the ability to manipulate the host by itself since it would often co-occur with the manipulator by chance alone (Thomas et al., 1998b). Other scenarios are also possible, in which for instance the nonmanipulator has a different destination, that is, different definitive host, from that of the manipulator, or in which both co-occurring species are manipulators but each target different host traits as they have completely different definitive hosts (Lafferty et al., 2000; Poulin, 2007). The range of circumstances under which parasites find themselves are therefore varied, and the resulting combinations of selective pressures can favor a range of transmission strategies, of which host manipulation is but one. Despite the complex nature of factors driving the evolution of host manipulation, tests of theoretical predictions remain possible, although none has been conducted to date. Some of the key determinants, such as the longevity of parasites inside their intermediate hosts, the diversity of potential predators

of the intermediate host, and the proportion of these that are suitable definitive hosts, are all quantifiable in principle and could be used in comparative analyses to test the predictions of models as to why some parasite species manipulate their hosts and others do not.

B. Intraspecific Variation

Even within species known to cause behavioral changes in their host, there is variation among individual parasites with respect to the magnitude of the changes induced. Thus, not all manipulators are equal. Some of the intraspecific variation can be explained by differences in age, size, or sex of the parasites, or their numbers per host (Benesh et al., 2009; Franceschi et al., 2008; Sparkes et al., 2004). This type of variation is expected, almost banal even. However, variation can occur on three other levels that reflect the action of complex selective forces: (i) the expression of parasite manipulation may vary with respect to the characteristics of individual hosts, (ii) there may be genetic differences among individual parasites in the tendency to manipulate that represent different transmission strategies, and (iii) the probability that a parasite opts to manipulate its host may depend on what other conspecific parasites are doing. Let's look at each of these briefly.

The expression of host manipulation cannot be considered independent of the initial phenotypic characteristics of the host, since it is measured as a change in those characteristics. It is possible that host individuals with certain trait values are more susceptible to parasite infection, or more likely to display large changes in phenotype following infection, than other hosts. We should thus expect reciprocal interactions between the intrinsic host phenotype and how parasites can alter that phenotype (see Blanchet et al., 2009a,b). This argument can also involve selective benefits for the host. In many discussions of host manipulation by parasites, there is a tacit assumption that the host is like a helpless puppet that complies with the manipulation. In reality, natural selection should favor hosts that can oppose attempts by their parasites to modify their behavior and cause them to move to unfavorable microhabitats or be captured by predators. However, the strength of the selection pressure acting on hosts to oppose manipulation may not be uniform across all host individuals. Poulin et al. (1994) have argued that intermediate hosts should oppose parasite manipulation as long as the losses in future reproduction resulting from manipulation, that is, because of an enhanced risk of predation from definitive hosts, are greater than losses associated with opposition. Assuming that the cost of opposing the parasite is age-independent, and that expected future reproductive success of the host declines following the onset of maturity, intermediate

hosts of long-lived, iteroparous species could benefit by opposing manipulation early in their adult life. Late in their reproductive life, the benefits of opposing manipulation by parasites would be much reduced. There is some empirical evidence of this effect: in freshwater fish used as intermediate hosts by manipulative trematode parasites, older fish show greater decreases in their antipredator behaviors following infection than younger fish (Poulin, 1993). In semelparous host species, opposing manipulation would benefit the infected host before reproduction, that is, for most of its life (Poulin et al., 1994). There are thus reasons to expect at least some hosts to attempt to resist manipulation by their parasites, and this is clearly one potential source of intraspecific variation in the observed magnitude of behavioral changes induced by manipulative parasites.

Of course, resistance by the host can in turn select for a more aggressive strategy on the part of the parasite (Ponton et al., 2006a). Consider the case of crickets harboring nematomorph worms. These parasites must emerge from their terrestrial hosts in water, in order to complete their life cycle, and they are widely known to induce crickets to seek water and jump in it (Hanelt et al., 2005; Thomas et al., 2002). In one particular species, if the host does not comply and remains out of water, the parasite seems to impose a greater fitness cost: compliant hosts remain physiologically capable of reproduction following the emergence of the parasite, whereas those that opposed the manipulation are invariably killed or castrated (Biron et al., 2005b). The host–parasite interaction is thus complex and can produce a range of outcomes, and it is certainly naïve to ignore the host's interests and focus research exclusively on the parasites.

The earlier discussion on the evolution of manipulation emphasized the existence of costs associated with a manipulative strategy: either inducing the change in host behavior is itself costly, or it can have consequences that are not always beneficial. Under these circumstances, there may be alternative strategies open to individuals within manipulative species. For instance, certain individuals could benefit from the efforts of others, by abstaining from manipulating the host. Such ''cheaters'' would not pay the costs of manipulation but could still enjoy the greater transmission success resulting from the action of manipulators with whom they share the same host individual. Earlier, the manipulation induced by the trematode *C. australis* in its cockle intermediate host has been described and discussed. Briefly, the parasites encyst in the foot of cockles and impair the latter's ability to burrow, causing them to be stranded on the sediment surface where they are more likely to be eaten by bird definitive hosts (Mouritsen, 2002; Thomas and Poulin, 1998). Only parasites encysting in the tip of a cockle's foot impair its burrowing ability; those in the middle or at the base of the foot have no manipulative impact (Mouritsen, 2002). Also, only parasites in

the tip of the foot are occasionally eaten by a foot-cropping fish that is not a suitable definitive host; those in the middle or base of the foot are safe from this nonhost predator (Mouritsen and Poulin, 2003). Thus, manipulation is costly, and for some reason certain parasites encyst in safe sites where they do not contribute to host manipulation. Before infecting a cockle, the trematodes multiply asexually within snails used as first intermediate hosts; there are thus clones, that is, multiple copies of the same genotype, among the parasites entering cockles. This allows tests with replicated clones to determine whether different genotypes have different propensities to take part in host manipulation. By comparing the encystment sites of different clones, Leung et al. (2010) have found up to twofold differences among genotypes in the proportion of individuals that choose the risky tip of the foot where the manipulation is induced. Some genotypes were true manipulators, whereas others appeared to act more like hitchhikers that benefit from the manipulation of others (Leung et al., 2010). Although the differences between the genotypes were only marginally significant, they hint at genetic differences among individuals within an otherwise manipulative parasite population. Certainly, genetic variation on this level needs to be examined in other species, as it may prove important in explaining background noise and data variability in many analyses.

In addition to genetic variation, individual parasites may have flexible manipulation strategies, such that the decision to commit to manipulation, when it is costly, depends on what other parasites are doing within the same host. In the above study of the trematode *C. australis* in its cockle host, the likelihood that a parasite entering a cockle would settle in the tip of the host's foot, where it could contribute to host manipulation but at a risk to itself, was significantly correlated with how many other parasites were already established in the foot tip (Leung et al., 2010). In this system, the effectiveness of the manipulation increases with the number of manipulative parasites; new arrivals apparently were more likely to incur a risk and contribute to manipulation if a threshold number of other parasites had already adopted the same strategy. In contrast, in the classical textbook example involving the trematode *D. dendriticum*, once one individual parasite commits to host manipulation, all others choose instead a cost-free nonmanipulative strategy. Ants manipulated by *D. dendriticum* display an aberrant behavior: they climb to the tip of grass blades and latch on to them with their mandibles, staying there for hours awaiting ingestion by sheep, the parasite's definitive hosts (Carney, 1969; Moore, 2002; Wickler, 1976). When one parasite reaches the ant's suboesophageal ganglion and starts to induce the altered behavior, other parasites settle in the abdomen instead; all are transmitted together when a sheep ingests the ant, though the manipulator rarely survives to adulthood. Thus, although different from

the situation in *C. australis*, the strategy adopted by *D. dendriticum* also depends on what its conspecifics have already done. These examples, along with those concerning genetic variation among parasites and life history variation among hosts, demonstrate that the magnitude of the changes in host behavior induced by parasites is far from uniform across all individuals of any particular manipulative species. At the intraspecific level, host manipulation is no doubt genetically variable, as well as displaying considerable plasticity in response to immediate conditions.

VI. How Effective Is Host Manipulation?

Some hosts manipulated by parasites display appearances or behaviors that are strikingly different from those of nonparasitized conspecifics, and even to the untrained eye there is something clearly wrong with these hosts. Examples include the appearance of completely novel behaviors or color patterns. The most extreme or bizarre cases of host manipulation seem to involve situations where the normal phenotype of the host is not compatible at all with the parasite's interests. This is when a parasite needs to get a terrestrial insect to jump in water, a male mayfly to lay eggs, a frugivorous bird to eat an ant or an insectivorous one to eat a snail. The vast majority of documented cases of manipulation, however, involve only a change in the extent to which an animal performs a particular behavior, such as a measurably longer time spent in a particular activity, or a measurably enhanced response to a given stimulus. The advantage of these more mundane manipulations is that the trait being manipulated can be measured in similar ways in both parasitized and nonparasitized animals, to allow one to quantify the extent of the manipulation. In a meta-analysis of published estimates of behavioral changes induced by parasites, Poulin (1994b) found that while most parasites induce only small changes in the behavior of their hosts, some have large effects on how the host behaves. As a rule, when host activity levels are the target of manipulation, most parasites have small effects, but when the target of manipulation is microhabitat choice, parasites can have considerable effects on host behavior, often causing a twofold or greater increase in how much time an animal spends in a particular microhabitat (Poulin, 1994b).

The effectiveness of host manipulation should not be measured by the extent to which host traits are altered, however, but by the net increase in the transmission success of the manipulating parasite. Lafferty and Morris' (1996) study provides a good example of how important it is to distinguish between changes in host behavior and their consequences for transmission success. They showed that a trematode parasite caused a fourfold difference in the frequency of conspicuous swimming behaviors (jerking,

contorting, surfacing, etc.) between parasitized and nonparasitized hosts; however, the effect of this manipulation was a 30-fold increase in rates of predation on infected fish by avian definitive hosts of the parasite. Small effects on host behavior can therefore cause disproportionate increases in parasite transmission success. The impact of manipulation on transmission is not always this large, but is generally significant. A compilation of results from studies where predatory definitive hosts were allowed access to equal numbers of manipulated and nonmanipulated intermediate hosts under seminatural conditions indicates that, typically, the percentage of manipulated prey taken by the predator is 25–35% higher than that for nonmanipulated prey (Thomas et al., 1998b).

Despite these impressive numbers, host manipulation can go horribly wrong. The above estimates of the effectiveness of manipulation were all obtained in situations where the only predator was a suitable definitive host. In nature, an intermediate host is faced with a range of predators, many of which are not compatible hosts for its parasites. Indeed, there are numerous reports of parasites being ingested by completely unsuitable definitive hosts as a direct result of the behavioral changes they induced in their intermediate hosts (Kaldonski et al., 2008; Mouritsen and Poulin, 2003; Seppälä et al., 2008). In theory, manipulation can still evolve under those circumstances (Parker et al., 2009; Seppälä and Jokela, 2008), and parasites can even manipulate a suite of traits in their intermediate hosts in order to enhance predation by definitive hosts while decreasing predation by nonhosts (Levri, 1998; Médoc and Beisel, 2008; Médoc et al., 2009). Nevertheless, the fact remains that often manipulation fails, and manipulative parasites die after having reached the wrong host.

Once again, the trematode *C. australis* in its cockle intermediate host represents a good case study. Manipulated cockles left stranded at the surface of intertidal sediments are about 5–7 times more likely to be eaten by oystercatchers or other bird definitive hosts than healthy, buried cockles (Mouritsen, 2002; Thomas and Poulin, 1998). Despite this enhanced probability of transmission, manipulative parasites are also more likely to end up dying in nonhost predators than if they induced no manipulation (Fig. 5). Fish crop the foot of manipulated cockles, and whelks also gang up on surface cockles and ingest many encysted *C. australis*; the parasite cannot survive in either of these alternative predators of surfaced cockles (Mouritsen and Poulin, 2003). Following host manipulation, a greater proportion of parasites end up in dead-end hosts than in suitable definitive hosts (Fig. 5), because the actual predation rates on cockles by oystercatchers are very low. From the point of view of the parasite, dying inside a cockle because transmission failed and dying in a dead-end predator are equivalent in terms of fitness, and thus manipulation should remain

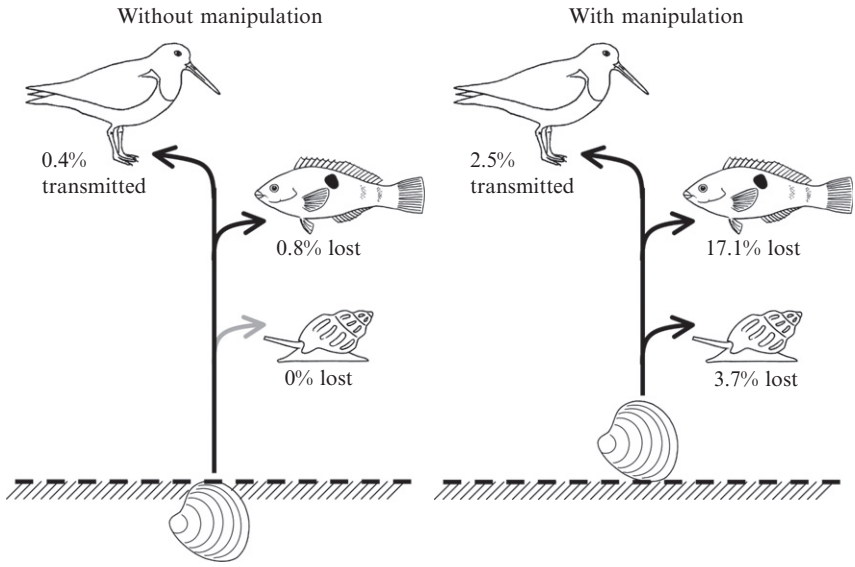

FIG. 5. Fate of the parasitic trematode *Curtuteria australis* in its second intermediate host, the New Zealand cockle *Austrovenus stutchburyi*. The data show what percentage of the total parasites are successfully transmitted to bird definitive hosts either with or without host manipulation by the parasites; manipulation results in parasitized cockles lying at the surface of intertidal mudflats, incapable of burrowing as healthy cockles do. The percentages of parasites ending up in unsuitable hosts, that is, fish or scavenging snails, where they die, are also shown for both scenarios; the remaining parasites stay within cockles until their death. Data are from Mouritsen and Poulin (2003).

advantageous. However, in certain localities where the predator community is different, that is, fewer foraging birds and more fish or whelks, the net benefits of manipulation become negligible (Tompkins et al., 2004). In this particular system, the two dead-end predators only started feeding on cockles stranded on the sediments *after* the parasites evolved the ability to manipulate cockle burrowing ability, and thus the initial conditions under which manipulation evolved differed from the present conditions. Nevertheless, in some localities within the geographic range of a manipulative parasite, manipulation may consistently fail, and in localities where it is generally advantageous, it may still cause parasites to end up in the wrong predator more frequently than they would if they did not manipulate their intermediate host. The importance of quantifying the effectiveness of host manipulation in the field should remain a central guiding principle of research in this area, as a means of ground-truthing any hypothesis about transmission benefits.

VII. How Do Parasites Do It?

The mechanisms used by parasites to alter host behavior following infection remain perhaps the least understood aspect of host manipulation by parasites. Once an animal acquires a parasite, existing behavior patterns are changed, or novel behaviors are manifested, via either direct or indirect mechanisms (Lefèvre et al., 2009a; Thomas et al., 2005). Parasites may directly secrete neuroactive substances causing a change in host behavior, or the presence of the parasite may indirectly influence or interfere with host biochemical pathways, leading coincidentally to a change in host behavior. Distinguishing between these two alternatives is rarely straightforward. Even in the very few cases where secretions from a parasite with neurological effects on the host have been identified, it is unclear whether these were secreted for manipulation or for other purposes, such as immune suppression (Thomas et al., 2005). For example, the trematode *Schistosoma mansoni* secretes opioid peptides into its host, thus influencing both host immunity and neural function (Kavaliers et al., 1999). The original function of these secretions may have been immune suppression, and have nothing to do with direct host manipulation.

From the parasite's perspective, making use of biochemical cascades and physiological processes already operating within host cells and tissues might be the ideal strategic approach to minimize the induction costs of manipulation. In particular, parasites that specifically target host compensatory responses could thus make use of existing host mechanisms to meet their own transmission needs without overly compromising host fitness (Lefèvre et al., 2008, 2009a). Vector-borne parasites, for instance, are known to change the feeding behavior of their vector, for example, increasing its probing rate, in ways that make parasite transmission more likely (Hurd, 2003; Moore, 1993; Rogers and Bates, 2007). In addition, vectors also usually incur a reduction in fecundity when they harbor parasites (Hurd et al., 1995). However, when malaria-infected mosquitos are allowed to bite more hosts per unit time (the consequence of manipulation by the parasite), they recover their normal fecundity (Rossignol et al., 1986). In this situation, the manipulation not only benefits parasite transmission, but it also allows the host to mitigate the fitness cost of infection, therefore making it less likely to be opposed by the host (Lefèvre et al., 2008, 2009a). Exploiting the existing physiological basis of host compensatory responses might be the cheapest way for a parasite to manipulate its host.

At a proximate level, numerous studies have shown that parasites achieve manipulation by directly or indirectly altering concentrations of hormones or neurotransmitters in their hosts. For instance, the larval stages of many parasitic worms can somehow induce changes in the concentrations

or activity of serotonin, dopamine, and/or other neurotransmitters in the brain of their intermediate hosts. This has been documented for acanthocephalans within crustacean hosts (Maynard et al., 1996; Poulin et al., 2003; Tain et al., 2007), cestodes within fish hosts (Øverli et al., 2001), and trematodes within crustacean and fish hosts (Helluy and Thomas, 2003; Shaw et al., 2009). There is good evidence also that many other types of parasites induce changes in the neurochemistry of their host's brain as part of the alterations they cause in host phenotype; these include insect parasitoids, nematomorphs, and the protozoan *T. gondii* (Adamo, 2002; Beckage, 1985; Libersat et al., 2009; Thomas et al., 2003; Webster et al., 2006). Of course, manipulation must not necessarily pass through neurological routes. For instance, although nematomorphs and mermithid nematodes have converged on very similar life cycles and host manipulations, the latter appear to rely on simple changes in host haemolymph osmolality and ionic concentration to induce water-seeking in their host (Williams et al., 2004). Whether or not brain chemistry is altered in the course of host manipulation, there is one thing common to most host–parasite systems where a partial understanding of the mechanisms has been achieved: concentrations of substances that can be synthesized by the host and that have downstream effects on behavior are altered following infection. These substances could be produced by the parasite and released into the host, or actively taken up from the host; although this would achieve the desired results, there is very little evidence that parasites induce manipulation this way. Instead, they probably take advantage of existing biochemical pathways in host cells, either up- or downregulating them to modulate the concentrations of active neurochemicals or other products. Finding altered levels of serotonin in parasitized hosts is probably just one step down a long biochemical cascade, and it may be necessary to look further up the biochemical chain to find exactly how parasites induce changes in host behavior.

Recently, a move in that direction has been made with the application of proteomic analyses to the study of host manipulation by parasites. The approach consists in comparing levels of a broad range of proteins in specific tissues between manipulated and normal hosts, and seeking the functional roles of those proteins that differ between the two types of hosts. Infection by manipulative parasites has been shown to lead to an altered profile of protein synthesis in parasitized hosts relative to nonparasitized conspecifics, with several candidate proteins identified as potentially linked with behavioral changes in parasitized hosts (Biron et al., 2005a; Lefèvre et al., 2009a; Sánchez et al., 2009). This approach may be particularly informative within a comparative framework, as it can pinpoint mechanistic differences and similarities between superficially identical host

manipulations (Ponton et al., 2006b). In some cases, proteomic studies suggest that parasites produce host mimetic proteins that may be the mechanistic origin of the manipulation, though this requires confirmation (Lefèvre et al., 2009a). Studies of the proteomes of manipulated hosts are still in their infancy, and several gaps remain. For instance, in the few host–parasite systems investigated to date, only a portion of the host proteome has been studied, with no information available on host responses involving insoluble proteins or those of low molecular weight (Lefèvre et al., 2009a). The latest proteomic tools available are now opening up these unexplored regions of the proteome, and will allow researchers to delve deeper into parasite-induced host responses at this level.

However, we may need to go further up the mechanistic chain. Surely, if protein synthesis differs between manipulated and normal hosts, it is as a result of altered gene expression within the host genome. Perhaps the least costly way for a parasite to trigger a biochemical cascade of events leading to altered behavior would be to ''reach'' into the host genome and partially suppress the expression of one or more host genes. Several environmental factors, including diet components, can cause certain genes to be turned ''off,'' with immediate phenotypic consequences and/or transgenerational epigenetic effects (Jaenisch and Bird, 2003; Richards, 2006). The process of genetic suppression is generally the outcome of DNA methylation or histone acetylation, both molecular mechanisms that mediate these phenomena (Jaenisch and Bird, 2003; Jones and Takai, 2001; Richards, 2006). For instance, a methyl group binding to a gene can silence its expression; genes can be partially methylated, and the degree of methylation correlates roughly with how active the gene remains. Many environmental factors can cause DNA methylation (Jaenisch and Bird, 2003), so why not parasites? Several indirect lines of evidence indeed suggest that parasites may alter the expression of host genes in ways that could form the proximate basis of host manipulation (see Poulin and Thomas, 2008). Solid evidence is lacking, however, in large part because no one has yet looked at parasite-induced changes in host methylation profiles and gene silencing in the context of host manipulation. And this is not the end of the causation chain: if parasites suppress the expression of certain host genes, we would then need to figure out by what mechanism they achieve this. This brings us again to the search for specific secretory products or modulators of some kind released by the parasite into the host. The complexity of the chain of events from infection to manipulation is daunting and poses real challenges for investigations at all levels, from that of the gene to the whole organism. The real source of the manipulation must lie within the parasite, and not among the existing biochemical and physiological processes of the host, even if it is the latter that are altered to produce the manipulated host phenotype. Finding that source

has proven impossible to date, for a range of reasons. Nevertheless, these obstacles must be overcome if we are to understand how, exactly, parasites manipulate the behavior of their hosts.

VIII. Looking Ahead

This review of host manipulation by parasites has centered on the questions that have driven much of the research into the phenomenon. While some of these questions now have satisfactory answers, others remain as puzzling today as they were 20 years ago. As a conclusion to this update of research in the field, I offer a list of promising directions for future investigations, that I hope could form the basis of a general research agenda for the next decade.

i. The focus of study has to shift from single traits altered in parasitized hosts to entire suites of host traits and the correlations among them. Modification of several behaviors instead of just one is likely to achieve greater transmission success in many cases, especially given synergistic effects among traits and temporal or spatial variability in transmission conditions. Investigating a narrow subset of behaviors can thus lead to an underestimation of the effectiveness of manipulation. Also, the target of manipulation may not be specific host behaviors themselves, but rather the relationships among them; strengthening or dissolving associations between host traits may in itself be what is needed to achieve increased transmission. Incorporating the ideas and approaches used in the study of behavioral syndromes (Sih and Bell, 2008; Sih et al., 2004) to the study of host manipulation seems like a very promising avenue to follow. Only then will we be in a position to appreciate the full scope of manipulative abilities possessed by parasites.

ii. We need to perform empirical tests of the predictions derived from theoretical analyses of the evolution of host manipulation by parasites (e.g., Parker et al., 2009). Some of the models make clear predictions that can be tested within a comparative framework. For instance, the models predict how lifespan in the intermediate host and the risk of nonhost predation should both determine whether or not manipulation is beneficial as a transmission strategy. Quantitative assessments of such predictions would go a long way toward validating the models and explaining interspecific variation in the use of host manipulation by parasites.

iii. The use of manipulation by parasites varies within manipulative species as well, and the determinants of this interindividual variation remain one of the great unknowns in the field. First, we need to start considering the host as an active partner in the interaction, one that may either oppose or modulate the manipulative efforts of the parasite rather then merely complying; although simplistic, experimental infections of hosts that vary in specific ways (age, reproductive status, geographical origin) followed by behavioral tests would go a long way in that direction. Second, we need to determine why individual parasites sometimes opt not to manipulate their host (see Leung et al., 2010), and we must start looking at host manipulation as a flexible strategy within the parasite's toolkit that may only be expressed under certain conditions.

iv. We need further field measurements of the effectiveness of host manipulation by parasites. In other words, we need data on what proportion of manipulative parasites actually reach a suitable definitive host or achieve successful transmission in some other way, compared to nonmanipulative parasites. At present, such data are available for a single parasite species (Mouritsen and Poulin, 2003), and the benefits of manipulation, based on that single field estimate, appear very small. Further data are needed from nature to provide better parameter estimates for mathematical models, as well as to evaluate the actual contribution of manipulation to parasite transmission and population dynamics.

v. Mechanistic studies need to push further up the chain of causation to elucidate not only the proximate mechanisms of host manipulation, but also their trigger. In particular, we need to expand the application of proteomics to host manipulation and focus on parasite modulation of host gene expression. Further, we need to identify the signal(s) originating from the parasite that initiate the biochemical cascades apparently underlying many, if not most, host manipulations.

These are the five most promising research directions I can see following my distillation of the recent literature. Of course, other researchers would no doubt come up with a slightly or totally different list. In addition, there are other important implications of host manipulation by parasites that require immediate attention, such as how it can impact the structure of entire animal communities (Lefèvre et al., 2009b; Mouritsen and Poulin, 2005; Thomas et al., 1998a). Nevertheless, the five research directions proposed here provide a strong basis for a new program of research into host manipulation, operating at both mechanistic and whole-organism levels, and designed to push our understanding well beyond its current limits.

Acknowledgments

I am grateful to Tommy Leung, Otto Seppälä, Tim Roper and an anonymous reviewer for helpful comments on this review. Over the years, many collaborators have made important contributions to my research on this topic, and to the development of my thinking on host manipulation by parasites; in particular, I would like to acknowledge Frédéric Thomas, Kim Mouritsen, Tommy Leung, and Janice Moore. Finally, I thank Tim Roper for inviting me to prepare this review, and also Frank Cézilly for inviting me to give a plenary at the 2008 European Conference on Behavioural Biology in Dijon, France, since that talk lead to the invitation to write this review!

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