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Review

# Parasitic manipulation: where are we and where should we go?

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How a parasite (or its offspring) moves from one host to the next remains a central topic in parasitology. Understanding such strategies is at the heart of applied aspects of parasitology, but it is also important for solving more basic biological questions. One strategy of transmission that is especially intriguing is that of host manipulation, which occurs when a parasite enhances its own transmission by altering host behaviour. We begin this paper with a brief historical overview of the ‘manipulation hypothesis,’ in order to illuminate past and present research on this transmission strategy, as well as current challenges.

Scientists were beginning to suspect that parasites could manipulate their hosts early in the 20th century (e.g. Cram, 1931). In 1952, van Dobben reported that fish retrieved from cormorants (definitive hosts) were far more likely to be intermediate hosts of the cestode *Ligula intestinalis* than were fish captured by fishermen. Bethel and Holmes (1973, 1977) used laboratory experiments to show that the cystacanths of the acanthocephalan *Polymorphus paradoxus* provoke abnormal behaviours in the amphipod (*Gammarus lacustris*; intermediate host), and then verified the resulting increased predation risk from ducks (definitive hosts).

Since that time, there has been increasing enthusiasm among parasitologists for the study of phenotypic changes in parasitised animals. The idea that parasites could manipulate the phenotype of their host and thus enhance their own transmission became rapidly popular not only because it was inherently a fascinating phenomenon, but also because it offered parasitologists an opportunity to demonstrate the ubiquitous importance of parasites to a broader community of scientists. Due to an impressive number of studies performed during the last three decades on this topic, parasite-induced alterations of host phenotypes are now documented for a wide range of parasites (see Barnard and Behnke, 1990; Combes, 1991, 1998; Poulin, 1998; Moore, 2002 for reviews). These studies have demonstrated that a large range of host phenotypic traits can be altered by parasites (e.g. behaviour, morphology and/or physiology), and that the alterations can vary greatly in their magnitude, from slight shifts in the percentage of time spent in performing a given activity to the production of complex and spectacular behaviours (Poulin and Thomas, 1999; Moore, 2002).

The most popular example of parasitic manipulation in ecological textbooks seems to be the trematode “brainworm” *Dicrocoelium dendriticum*. Ants (intermediate hosts) infected with this trematode ascend blades of grass, a behaviour that probably enhances transmission to grazing sheep. However, this is not the best exemple of parasitic manipulation. As one might imagine, it is difficult to study ant predation by sheep, and the relative numbers of infected and uninfected ants that are eaten by these herbivores remain a mystery. There are however many impressive examples of apparent host manipulation that are more amenable to quantification. For instance, numerous trophically transmitted parasites have been shown to alter the behaviour of their intermediate hosts in a way that increases their vulnerability to predatory definitive hosts (Lafferty, 1999; Berdoy et al., 2000; Moore, 2002). Parasites also manipulate host habitat choice; arthropods harbouring mature nematomorphs or mermithids seek water and jump into it, thereby allowing the parasitic worm to reach the aquatic environment needed for its reproduction (Thomas et al., 2002a). Mermithid nematodes can also feminize male insect behaviour when parasite transmission is dependent on a female-specific behaviour (Vance, 1996). Parasitic wasps can make their spider host weave a special cocoon-like structure to protect the wasp pupae against heavy rain (Eberhard, 2000, see also Brodeur and Vet, 1994), or can even cause the host to seek protection within curled leaves to protect pupae from hyperparasitoids (Brodeur and McNeil, 1989). Viruses may stimulate superparasitism behaviour in solitary parasitoids and thus achieve horizontal transmission (Varaldi et al., 2003). Some digeneans drive their molluscan intermediate hosts toward ideal sites for the release of cercariae (Curtis, 1987). ‘Enslaver’ fungi make their insect hosts die perched in a position that favors the dispersal of spores by the wind (Maitland, 1994). Vector-borne parasites can render their vertebrate hosts more attractive to vectors, and/or can manipulate the feeding behaviour of vectors to enhance transmission (Hamilton and Hurd, 2002). All these spectacular phenotypic changes have been interpreted as the sophisticated products of natural selection that has favored host manipulation, thus increasing the likelihood that parasite propagules will encounter the next host or a suitable habitat. From an evolutionary point of view, these changes are classically seen as compelling illustrations of the ‘extended phenotype’ concept proposed by Dawkins (1982), in which genes in one organism (i.e. the parasite) have phenotypic effects on another organism (i.e. the host).

As with many other fields, the early exuberance of discovery and ready explanation gave way to more cautious voices. By the late 1980s and early 1990s, scientists were viewing the manipulation hypothesis with a more critical eye, demanding more rigorous evidence for the adaptive nature of a behavioural change in an infected organism. This shift was necessary to rectify a kind of unconscious bias, a uniform tendency to consider changes in parasitized hosts as beneficial for the parasite, just because intuition, not appropriate tests, suggested that they were perfectly suited to parasitic functions. Without rejecting the fact that numerous alterations in infected hosts were undoubtedly true parasitic adaptations, two important papers (Moore and Gotelli, 1990; Poulin, 1995) opened the way for a novel and more rigorous approach of the study of parasite-induced behavioural changes within an evolutionary framework. Basically, these papers underlined three main problems that persist today (see below). (1) It is difficult to ascertain whether a phenotypic change is really adaptive for the parasite or whether it is a non-adaptive and accidental pathological side effect resulting from infection. (2) There is a growing recognition that host phenotypic changes, instead of inevitably being parasitic adaptations, can often be explained as host adaptations aimed at reducing the detrimental fitness consequences of infection. (3) Phylogeny matters. Behavioural changes can be the product of natural selection in a given host–parasite interaction, but they can also be inherited from an ancestor. In that case, they may or may not still confer a selective advantage to the parasite in the present system. In an attempt to guide future investigations, Poulin (1995) suggested that several criteria should be met before one considers changes in parasitized hosts as adaptive: (1) they must be complex; (2) they must show signs of a “purposive design”; (3) it is better if they have arisen independently in several lineages of hosts or parasites and finally (4) they must be shown to increase the fitness of either the host or the parasite.

Since this transitional period, numerous papers have appeared in the literature, most of them influenced by the previous recommendations. So, where are we now, and where are we going? Is parasitic manipulation still a worthwhile research topic ? The aim of this paper is to discuss several topics related to the study of parasitic manipulation, to give an overview of the breadth offered by this research topic, and to define the main trends and prospects in this area.

## 1. Adaptive versus non-adaptive changes: is the debate clear?

It is now accepted that phenotypic changes in infected hosts are not necessarily ‘true’ parasitic (or host) adaptations, they may be ‘by-products’ of infection or ancestral legacies. Although we agree with the evolutionary relevance of these distinctions, we believe that a discussion is needed, at least to recall the limitations of the definitions and of the methods originally chosen to decide whether a change is adaptive or not.

Adaptation is a complex concept with several possible definitions (see Futuyma, 1998 for review). For instance, the definition provided by Reeve and Sherman (1993)—an adaptation is a phenotypic variant that results in the highest fitness among a specified set of variants in a given environment, refers only to the current effects of the trait on reproductive success. At the other extreme, the definition by Harvey and Pagel (1991), for a character to be regarded as an adaptation, it must be a derived character that evolved in response to a specific selective agent, explicitly requires an inference about history. Clearly, the current approach used by most researchers on manipulation is based on the second definition, that is, a historical definition of adaptation. Strict historical definitions of adaptation like that of Harvey and Pagel (1991) are reasonable, and indeed, are necessary in particular contexts such as that of comparative analyses, but their application in other contexts may be a bit confusing. For instance, what about ancestral, inherited traits that still confer a selective advantage to their bearers in derived groups? We suspect that this situation exists within several groups of parasites such as acanthocephalans or trematodes, where it seems likely that the ability to manipulate host species has been inherited from a common ancestor. These manipulations may continue to confer a selective advantage in the context of the transmission.

As for the arguments that some changes in host behaviour are not adaptive for the parasite because they are “by products” of infection, it seems that in the current literature, this term refers to at least three kinds of phenomena. A first category corresponds to what is also called 'side-effects' of parasite infection, that is, pathological consequences with no adaptive value for the parasite or the host (Minchella, 1985). A second category of 'by products' are host changes considered as 'coincidentally beneficial' for the parasite. For instance, parasitised hosts may make themselves more visible to predators (definitive hosts) because they have increased energy requirements and must forage more, not because they are manipulated to do so by the parasite (Poulin, 1995). Finally, a third situation, corresponds to changes in host phenotype that result as 'fortuitous payoffs of other adaptations'. For example, some parasites manipulate the behaviour of their hosts by being encysted in the host nervous system; such a location may have initially been favored because it affords some protection from the host's immune system (Moore and Gotelli, 1990).

We have serious reservations about these "by-products," and about the rule of parsimony that has been widely invoked to defend the by-product interpretation. In the first case, the absence of anything, be it benefit to parasite or host, is notoriously difficult to prove. As for the second and third categories, "coincidental benefits" and "fortuitous payoffs of other adaptations," it is almost impossible to distinguish between the primary focus of historical selection (depleted energy? immunological protection?) and concomitant effects on transmission, especially when enhanced transmission itself may have selective value. We are not arguing for facile adaptationist interpretations here; we believe strongly that researchers must be circumspect in their invocation of adaptation when viewing parasite-induced behavioural changes or any other trait, for that matter. However, if parsimony yields a null hypothesis of "by product" against which other interpretations be weighed, then such a hypothesis must surely be testable. To date, tests of the by-product explanation of apparent manipulation are daunting, especially when even quantitative evidence of increased transmission, rare as it is, is viewed as an accidental corollary.

We believe that Anderson and May (1992), and reference therein, illuminated this general problem in their seminal work, beginning in the 1970s. They addressed the evolution of virulence among other things, and they clearly pointed out that if virulence is linked to transmission, then it becomes a prime target of natural selection. This principle can be applied to parasite-induced behavioural changes (Moore, 2002). If an altered behaviour occurs sporadically in a host–parasite association, then it is not highly relevant to this discussion, unless the pattern of appearance/absence itself is somehow informative. On the other hand, if an altered behaviour occurs routinely in a host–parasite association, then it has a place among the suite of traits on which natural selection acts in that association; it is unlikely to be an accident. This does not guarantee that it is adaptive; there is a vast literature on adaptation and development that assures us of the existence of traits that are not purely adaptive. However, if pathology is linked to transmission, then it is highly likely that natural selection has not been blind to that pathology.

In a related vein, there has been some discussion of fitness costs associated with manipulation (or resistance thereto; Poulin, 1993, 1994). Despite the existence of suitable systems with which hypotheses about cost might be tested, at this stage, speculation has proven more attractive than data collection, and many workers seem to assume that costs should be inevitably associated with manipulation. We argue that the existence and amount of such costs must be closely linked to the mechanism that underlies manipulation. How expensive are the neurotransmitter molecules that probably alter gammarid behaviour (Helluy and Holmes, 1990)? Although attaining the size of a *Schistocephalus solidus* plerocercoid or an *Echinococcus* spp. cyst may be expensive, what are the benefits associated with enhanced transmission and escaping host immune response (Milinski, 1985; Moore, 1981)? What is the cost of encysting in the eye of a fish (Szidat, 1969)? The extent of manipulation may not reflect the cost in all cases [see Rigby et al. (2002) for a discussion of cost of resistance to parasites, another assumed cost that may not always be what it seems]. The issues of adaptation versus by product and of cost, as well as other questions about parasite-induced behavioural changes, may have much to gain from attention to mechanism.

## 2. How does the presence of a parasite alter host behaviour ?

Parasites can use both direct and indirect mechanisms to alter host behaviour. Parasites can alter host behaviour directly by interacting with the host's nervous system or muscle. For example, a parasite may secrete/excrete a neuroactive substance resulting in changes in host behaviour. Parasites can have indirect effects on host behaviour by affecting host tissues other than neurons and muscles, resulting in host-mediated changes in behaviour. For example, the presence of a parasite can influence host development, intermediate metabolism and/or immunity, leading secondarily to an alteration in host behaviour.

Elucidating the mechanisms mediating changes in host behaviour are unlikely to demonstrate whether the change in host behaviour is adaptive for the parasite. Both indirect and direct mechanisms can lead to increased transmission of parasites (Adamo, 2002). Mechanistic studies are essential, however, in determining the potential costs to the parasite of inducing a change in host behaviour. When researchers discuss 'parasitic manipulation' of host behaviour, they are usually referring to an active process in which the parasite expends energy to produce a direct effect on the behaviour of their host. However, because changes in host behaviour can also be induced indirectly, parasites could alter host behaviour with no additional energetic costs beyond those required to survive in the host (e.g. a suppression of host immunity that leads to a fortuitous change in host behaviour). Moreover, parasitic waste products may also influence host behaviour, and therefore even direct parasitic effects may be cost-free for the parasite. The ecological ramifications of costly parasitic manipulation versus cost-free fortuitous changes in host behaviour will be different, even if both are adaptive for the parasite (e.g. Lafferty et al., 2000). Unfortunately, differentiating between these two possibilities is problematic, as we discuss below.

### 2.1. *Direct effects*

Demonstrating that secretions/excretions from a parasite act directly on host neurons has proven difficult. Part of the problem lies with the complex interactions between immunity and the nervous system. When parasites invade any tissue, including the central nervous system (CNS), they typically invoke complex, but poorly understood, immune cascades (Roitt et al., 2001; Kristensson et al., 2002; Tomonaga, 2004). Activating these immune cascades also results in the release of neuroactive compounds (Dantzer, 1999) and these can cause a variety of changes to brain and behaviour (Bechter, 2001). These immune–neural interactions make it difficult to determine whether a change in host behaviour is a direct effect of the parasite or whether it is a result of the host's immune response. Moreover, some parasites secrete chemicals identical to those secreted by the host's immune system (Kavaliers et al., 1999; Kristensson et al., 2002). For example, the trematode *Schistosoma mansoni* secretes β endorphin and other opioid peptides (Kavaliers et al., 1999). These substances affect both immune (Duvaux-Miret et al., 1992) and neural function (Kavaliers et al., 1999). In infected hosts both opiate and opioid levels increase in the serum and CNS, but it is unclear whether the source is the host or the parasite (Kavaliers et al., 1999; Pryor and Elizee, 2000). Pharmacological tests show that these compounds play a causal role in the changes in host behaviour (Kavaliers et al., 1999). Nevertheless, *S. mansoni* has probably been selected to secrete these compounds in order to suppress local immune responses (Pryor and Elizee, 2000), not to induce changes in host behaviour. Regardless of whether *S. mansoni* affects host behaviour directly or indirectly, the change in behaviour may be an unavoidable consequence of parasite-induced immunosuppression.

To further demonstrate the difficulty in determining the roles of parasite and host in mediating host behavioural change, we will examine the induction of aggressive behaviour by the rabies virus. The rabies virus lives in the brain (Rupprecht et al., 2002), affording the virus ample opportunity to directly affect host behaviour. Rabid animals do show changes in behaviour, including increased aggression and biting (e.g. dogs, Tierkel, 1975). Biting is the most effective means of transmitting rabies (Rupprecht et al., 2002). Therefore, the rabies virus could increase its transmission by infecting and then manipulating areas of the brain important for regulating aggression. This hypothesis is supported by studies showing that rabies virus can alter the neuronal function of infected cells (Ladogana et al., 1994; Iwata et al., 1999). However, closer examination of the evidence reveals complexities in the rabies-host interaction that suggests that a direct parasitic effect on the host cannot entirely explain the increase in host aggression. Virus distribution alone cannot explain the clinical features of rabies (Hemachudha et al., 2002). The rabies virus preferentially localizes in the brainstem, thalamus, basal ganglia and spinal cord (Hemachudha et al., 2002), areas that are not directly involved in regulating aggression (Pinel, 1993). Therefore, it is unlikely that the virus increases aggression by directly manipulating infected neurons.

Moreover, aggression is frequently absent in infected hosts (Rupprecht et al., 2002). Rabies has two classic forms, the "furious" (encephalitic) and the "dumb" (paralytic) (Hemachudha et al., 2002). Both forms exhibit increased salivation, but only victims of encephalitic rabies exhibit increased aggression. Virus distribution in the brain is the same in both forms (Hemachudha et al., 2002). One difference that has been found between the two forms is that patients with encephalitic rabies tend to have intact T-cell immunity and mount a robust cellular immune response against the virus (Hemachudha et al., 2002). Hemachudha et al. (2002) argue that it is the immune responses generated by the host that are responsible for the increased aggression seen in some rabies victims. Hemachudha et al. (2002) postulate that infection of the brainstem induces production of cytokines by the host's immune system and that these compounds then modify the functioning of limbic system structures (brain structures involved in the control of aggression, Pinel, 1993). Further evidence that the increased aggression observed in some rabies victims is due to a host-generated immunopathology is that the increased aggressive behaviour that occurs in humans during the final phase of rabies is also seen in other neurological disorders (both infectious and non-infectious) and is not specific to rabies (Hemachudha et al., 2002). This uncommon change in behaviour in neurological patients is probably caused by immune-generated destruction of the CNS (e.g. inflammation, Bechter, 2001). In rabies, the physiological details of an individual host's immune response may play a critical role in determining whether the virus can 'manipulate' its host.

In systems in which the host is an invertebrate, the mechanisms mediating host behavioural change may be easier to determine. Nevertheless, even in these systems, demonstrating that a parasite secretes a neuroactive substance, showing that the substance alters neuronal function and finding that the altered neuronal function is causally linked to the change in host behaviour is difficult. Recently, Helluy and Thomas (2003) suggested that the degeneration of discrete sets of serotonergic neurons in *Gammarus insensibilis* infected by the cerebral trematode *Microphallus papillorobustus* might underlie the serotonergic imbalance and thus contribute to host manipulation, but the biochemical studies remain to be done. In the *Gammarus lacustris–Polymorphus paradoxus* system, rightly famous as one of the few host–parasite systems for which we also have some evidence of the mechanism, we do not know how the host's behaviour is changed. Exogenously supplied serotonin can mimic the effect of parasitism on some host behaviours (Helluy and Holmes, 1990) and hosts show an increase in the number of varicosities exhibiting serotonin-like immunoreactivity (Maynard et al., 1996). Maynard et al. (1996) included the important control of examining the CNS of gammarids infected with a different acanthocephalan, *Polymorphus marilis*, that does not induce a change in host behaviour. *P. marilis* does not alter serotonergic staining in the host, demonstrating a correlation between the change in host serotonin-like immunoreactivity and host behavioural change. Holmes and Zohar (1990) do not believe that *P. paradoxus* is capable of raising host serotonergic levels sufficiently to alter host behaviour. They favour the hypothesis that the parasite induces the host to increase its release of serotonin (Holmes and Zohar, 1990). However, this hypothesis is difficult to reconcile with the immunohistochemical results. If the host is increasing its release of serotonin, immunohistochemical staining would be expected to decline. Without knowing whether serotonin levels are elevated in parasitized individuals, and if they are, who is responsible for its secretion, we cannot determine whether it is the parasite or host who is paying the cost of altering host behaviour.

### 2.2. *Indirect methods*

When parasitic alteration of behaviour has been examined in detail, the change in host behaviour is usually an indirect effect of the parasite (Adamo, 2002). There are two possible reasons for this. First, most parasites are small and it may be prohibitively expensive for them to secrete behaviourally effective amounts of a neuroactive compound, unless the parasite resides within the CNS. It might be more efficient to induce the host to make them. Second, in order to survive, parasites must evolve mechanisms to allow them to interact with host physiology, especially immunity. It may be a small evolutionary step to co-opt the chemical connections between these systems and the host's nervous system to affect adaptive behavioural change in the host. Immune–neural connections may be especially prone to this type of disruption because of the intimate contact between the parasite and the host's immune system (Adamo, 1997, 2002). If this is a common mechanism of parasitic manipulation, then most changes in host behaviour will resemble host responses to stress or infection, making it difficult to determine whether the parasite is exerting any active effect (i.e. secreting compounds that alter host behaviour). For example, the trematode *Trichobilharzia ocellata* suppresses the egg laying of its intermediate host, the snail, *Lymnaea stagnalis* using both direct and indirect methods (de Jong-Brink et al., 2001). Parasitic secretory/excretory products induce the snail's immune system to release schistosomin (de Jong-Brink, 1995). Schistosomin decreases the excitability of neuroendocrine cells responsible for releasing the peptide caudodorsal cell hormone (CDCH) that induces egg-laying behaviour (Hordijk et al., 1992). Schistosomin probably mediates a stress response in non-infected snails (de Jong-Brink et al., 1997). The parasite also exerts direct effects on gene expression for Neuropeptide Y in the snail's CNS (de Jong-Brink et al., 1999). The up-regulation of expression for Neuropeptide Y also depresses egg-laying in the snail (de Jong-Brink et al., 1999). Many parasites may be like this trematode and use multiple mechanisms to alter host behaviour.

### *2.3. Importance of understanding the physiological basis of host behavioural change*

Understanding how parasites alter host behaviour is important for practical as well as theoretical reasons. Because many parasitic effects on behaviour exploit immune–neural connections, studying these systems will increase our insight into the molecular mechanisms underlying sickness behaviour (e.g. Kristensson et al., 2002). Moreover, some parasites appear to be able to induce different behaviours in different hosts by using immune–neural connections (e.g. Hemachudha et al., 2002). Further study of this phenomenon will demonstrate how different types of immune responses induce different types of behaviour. Such information could lead to improved therapies for life-threatening host responses such as cachexia. Furthermore, infectious diseases of the CNS are thought to underlie some common forms of mental illness (Bechter, 2001; Tomonaga, 2004). Studying how parasites alter brain function may aid our understanding of these disorders. Examining how parasites alter social behaviour may also tell us something about the evolution of the brain in vertebrates. In a recent review paper, Klein (2003) reported several examples of pathogens affecting the proximate mechanisms that mediate the expression of social behaviours in vertebrates (aggressive, reproductive and parental behaviours), in ways that may increase parasitic transmission. Interestingly, the effects of parasites on social behaviour may be retained across several classes of vertebrates because parasites affect the phylogenetically primitive structures of the limbic system and related neurochemical systems (Klein, 2003). Further research in this area may increase communication and cooperation among neuroscientists, parasitologists and evolutionary biologists.

### *2.4. Implications about parasitic manipulation from recent mechanistic studies*

As demonstrated in the preceding sections, changes in host behaviour are often a mix of direct and indirect effects of parasites on their hosts' CNS. For example, the host's immunological response to infection can be involved in changing the host's behaviour into a behaviour that favours parasitic transmission (e.g. rabies). Studies attempting to differentiate between host responses (e.g. sickness behaviour) and parasitic effects on behaviour should keep this observation in mind. Even if a change in host behaviour can be mimicked by activating the immune system, this change could still be adaptive for the parasite, and it could still be a direct effect of the parasite (e.g. by the parasitic secretion of cytokines). Furthermore, finding the correct immune challenge to test whether a change in host behaviour could be a host response will not be easy. Immune responses can vary depending on the parasite (Roitt et al., 2001), and different immune responses can elicit different types of behaviour (Adamo, 1998). For example, parasites that infect the brain may induce specific changes in behaviour due to local release of cytokines, a pattern of release that would not be reproduced by a systemic challenge.

In host–parasite systems in which the host exhibits a completely novel behaviour (e.g. Thomas et al., 2002a), the causal connection between a parasitic effect and host behavioural change may be easier to establish. Activation of a unique behaviour, rather than the augmentation of a host response or a decrease/increase in a normal behaviour may also be less likely to rely on exploiting a host response to infection. Unfortunately, these types of host–parasite systems are rare and have been largely ignored by physiologists.

### *2.5. New methods in the study of how parasites manipulate their hosts*

Proteomics is the study of all the proteins produced by a cell (i.e. the proteome). Instrumental to the study of functional genomics, it incorporates protein separation methods, mass spectroscopy and bioinformatics on a massive scale. Until now, the studies in 'Parasito-proteomics' have been done using the expression of the parasite proteome during infection by a given parasite (Langley et al., 1987; Moura and Visvesvara, 2001; Cohen et al., 2002), the reaction of the host proteome following an invasion by a parasite species (Wattam and Christensen, 1992; Moskalyk et al., 1996; Thiel and Bruchhaus, 2001; Cohen et al., 2002), or the injection of immune elicitors (Han et al., 1999; Vierstraete et al., 2004). Because proteomics can rapidly provide a comprehensive view of the expression of entire genomes, Biron et al. (in press) recently indicated that proteomics would offer an excellent tool to study the host (and sometimes the parasite) genomes in action during manipulative processes. Although at the moment all the studies using proteomics to identify the mechanisms of parasitic manipulation are still in progress, preliminary results reveal a bright future for such an approach.

## 3. Mafia-like strategy of manipulation: an understudied hypothesis?

The complexity of the interactions between host and parasite suggest that we may not yet know all the ways in which parasites and hosts interact. For example, it has been recently suggested that parasites may select for collaborative behaviour in their host by imposing extra fitness costs in the absence of compliance. This interaction has been called a mafia-like strategy. This process was initially proposed as a possible explanation for why several bird species accept cuckoo eggs and nestlings in their nest despite the dramatic cost to their own fitness. Cuckoos may force the bird host to tolerate nonself eggs by making the consequences of rejection more damaging than acceptance (Zahavi, 1979). Soler et al. (1995) provided empirical evidence of this phenomenon within the association between the great spotted cuckoo (*Clamator glandarius*) and its magpie host (*Pica pica*): ejector magpies suffered from considerably higher levels of nest predation by cuckoos than accepters, suggesting that the cuckoo retaliates, 'punishing' the ejector host by destroying its clutch. As a result, the frequency of 'acceptor genes' is more likely to increase in the host population than is the frequency of 'rejector genes'. Such interactions could be far more common among host–parasite systems than the few existing studies might indicate (Ponton et al., submitted).

Host species from all taxa are under selective pressure not only to eliminate parasites, but also to compensate for the effects of parasites when elimination is impossible. When parasites are able to increase their virulence in the absence of host compliance, cooperating with the parasite rather than resisting it might mitigate fitness costs associated with parasitism. This scenario implies the ability of both host and parasite to perceive a large set of fitness-related environmental cues and adjust their life history decisions (*sensu lato*) in a state-dependent manner. Increasing evidence suggests that this faculty is indeed present (see Lewis et al., 2002; Thomas et al., 2002b; Poulin, 2004 for parasite examples and Minchella and Loverde, 1981; Polak and Starmer, 1998; Adamo, 1999; Agnew et al., 1999 for host examples). Theoretically, mafia-like strategies could be observed even when manipulative parasites strongly reduce the survival of infected hosts. After all, from an evolutionary point of view, a reduction in survival is not synonymous with a reduction in fitness. Net fitness, not survival, is the primary consideration. Thus, a host that cooperates with the parasite, even to the point of "suicide" (manipulated behaviour), could be favored if its fecundity was merely reduced compared to total castration inflicted on an uncooperative host by a retaliatory parasite.

The range of effects that parasites have on fecundity—from slight reduction to total, irreversible castration—offers promising models for further investigations of mafia-like strategies of manipulation. For instance, an investigator could force non-compliance, and compare the fitness of non-compliant hosts to that of hosts allowed to express parasite-induced behaviour. In this way, we can distinguish between a collaborative host and a truly manipulated one, and measure the relative costs. From an evolutionary point of view, these considerations are relevant as they suggest that phenotypic changes in infected hosts, even when they result in clear fitness benefit for the parasite, are not necessarily an illustration of the extended phenotype of the parasite alone (sensu Dawkins, 1982). They can also be the direct product of natural selection acting on the host genome as well.

## 4. Considering manipulated hosts within ecosystems

Many studies of parasitic manipulation have been performed without considering the ecological context in which they occur. This is unfortunate, for it compromises both our understanding of the evolution of parasitic manipulation and our understanding of the ecological consequences of manipulation within ecosystems.

A full understanding of the evolution of parasitic manipulation requires knowledge of the selective pressures experienced by both the host and the parasite. Conditions used in laboratory studies as well as in semi-natural experiments may be poor approximations of processes that occur in the field. For instance, the proportion of manipulated hosts/uninfected hosts classically used in predation experiments is huge compared to that observed in natural conditions. How this affects the behaviour of predators, and hence the conclusions derived from these studies, is not known. Most experiments do not take into account the fact that, in natural conditions, other predators unsuitable as hosts may also take advantage of the manipulation (see for instance Mouritsen and Poulin, 2003), or that several suitable host species may vary in their predation efficiency. This phenomenon is nonetheless critical to our understanding of the costs and the benefits of parasitic manipulation. In some cases, certain features of parasite-induced behavioural changes seem more relevant to limiting the risk of predation by the wrong (non-host) predator than to increasing transmission to appropriate hosts (e.g. Levri, 1998). Such altered behaviour cannot be understood without considering manipulated hosts within their ecological context.

Finally, an important limitation of virtually all recent theoretical and experimental studies of the evolution of parasitic manipulation is the fact that these phenomena occur in a metapopulational context (e.g. Tompkins et al., 2004). Like most animal species, host and parasite species are likely to exhibit a classical metapopulation structure over their entire geographic range, occupying habitats that are fragmented and heterogeneous in space and/or time. In heterogeneous environments, local populations might be permanently maladapted because of migration from other habitats with contrasting selection pressures (e.g. sink populations, see Hanski, 2002). Such concepts have not to our knowledge been tested in the context of manipulative changes, but research in this direction could well provide examples of adaptive changes that are locally maladapted. For instance, given that predator communities frequently vary in space and/or time, the fitness benefits for trophically transmitted parasites that result from manipulation differ from one place to another, with some sink populations being net importers of individuals and genes. In similar fashion, adaptive baseline behaviours of uninfected animals may vary across host ranges, thus changing the behavioural substrate on which the manipulative parasite may work (Moore, 2002). General conclusions about the possible adaptive value of host changes induced by parasites must therefore be considered with caution when derived from local and/or short term field studies. Consideration of the spatial structure of both host and parasite populations as well as the heterogeneity of environmental conditions is as desirable as it is daunting.

In the case of parasites in ecosystems, considerable progress has been made in understanding the functional importance of parasites, but much less is known about the more specific role(s) of manipulative parasites. Investigating how manipulative parasites interfere with ecological and evolutionary processes that generate, maintain or reduce biological diversity in ecosystems is a research area of interest to both parasitologists and ecologists. For instance, manipulative parasites have been shown to interfere with apparent competition phenomena, playing the role of mediator by manipulating some host species more than others (Thomas et al., 1995; Bauer et al., 2000, see also Milinski, 1984, 1985). A second process through which manipulative parasites could influence community structure in ecosystems is by their effect on the predator community.

Predators risk infection when feeding on manipulated prey but they also often benefit from enhanced prey capture (Moore, 1983; Lafferty, 1992; Norris, 1999; Hutchings et al., 2000). By increasing the accessibility of prey that is normally difficult to capture, we could expect that manipulative trophically transmitted parasites in ecosystems will enhance the trophic potential of these habitats. To our knowledge, no study has yet investigated whether there is a positive relationship between the local abundance of manipulative parasites, the prey availability for predators and the local richness/diversity of predator communities.

A third mechanism through which manipulative parasites can influence community ecology is through interference with engineering processes. [Ecosystem engineers are organisms, plants or animals that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials, see Jones et al. (1994, 1997).] Manipulative parasites, by altering host phenotype, can either have impacts on existing ecosystem engineers, or act as engineers themselves (Thomas et al., 1999). For instance, by manipulating the cockle *Austrovenus stutchburyi* so that it changes from one physical state (buried) to another (surface), the trematode *Curtuteria australis* is an "engineer," modifying both the availability and the quality of habitats for benthic invertebrates living on the cockle's shell (Thomas et al., 1998a). The net effect of this manipulation on local biodiversity seems positive; by reducing competition for space, local coexistence of limpets and anemones is likely to be facilitated. Many trematodes have been shown to impair the burrowing ability of their molluscan hosts (see Lauckner, 1987). It is not known, however, whether similar behavioural changes induced by parasites in similar ecological contexts yield the same ecological consequences. Answers to such questions are needed before ecological generalizations about the role of manipulative parasites can be made.

Thus, manipulated hosts must be considered as complete participants in the function of ecosystems, both in terms of their own activities and in terms of the altered activities of their hosts. Manipulated hosts keep some of the properties and attributes of uninfected conspecifics but they also display novel properties, so much so that some parasitologists view the host–parasite amalgam as a distinct entity (e.g. Curtis, 1990, 1993).

## 5. How complex are 'parasitically modified organisms'?

A full understanding of the manipulation processes requires the study of other phenotypic traits in hosts in addition to the most obviously altered behaviours. Indeed, there are several reasons to think that we have until now only studied the visible part of the iceberg, manipulated hosts being probably more complex than traditionally viewed.

Studies on phenotypic plasticity and evolution have shown how a single phenotypic change (for instance induced by a minor genetic mutation) can result secondarily in more important phenotypic changes, owing to a series of compensatory responses via a shift in the expression of related traits (see West-Eberhard, 1989 and Nijhout and Emlen, 1998). Poulin and Thomas (1999) argued that the ability of infected hosts to undergo large phenotypic alterations, such as a change of microhabitat, may depend on the capacity for some other traits to accommodate this novelty. However, to our knowledge, no one has explored the idea that manipulative parasites could act as a developmental switch channelling several associated traits in particular directions.

In addition to changes resulting from plastic adjustments of the hosts to novel conditions, complex alterations of the host phenotype could result from parasites being able to manipulate several traits in their hosts. Because studies on manipulation have usually focused on the most spectacular change displayed by infected hosts, this idea has rarely been explored (but see Haye and Ojeda, 1998; Latham and Poulin, 2001). When possible, the simultaneous and/or the successive manipulation of several host traits by parasites should however be greatly favoured by selection. For instance, a physiological manipulation could in many cases enhance the efficiency of a behavioural manipulation. Indeed displaying an aberrant behaviour is not only likely to be an energetically costly task for host species, it is also a period during which they usually have a reduced foraging activity. Because hosts with high levels of energy reserves could be manipulated for longer periods than those with poor reserves, parasites that cause hosts to increase energy reserves should be favored by natural selection. Indeed, this is alleged to be one advantage that accrues to parasitic castrators—they are thought to shift resource allocation from reproductive to somatic (and hence, parasite) uses. If intermediate hosts are ‘vehicles’ taking the parasites to their definitive hosts, then parasites should make sure that they contain enough ‘motor-fuel’.

We thus encourage researchers to analyse a larger number of phenotypic traits in manipulated hosts. We believe that such research will eventually reveal that parasitically modified hosts are not simply normal hosts with one aberrant trait (e.g. behaviour); instead they are deeply modified organisms with a range of modifications, some of which may favour parasites, and some of which may favour hosts. Such integrative study requires collaboration among parasitologists and researchers from other disciplines, especially physiology, morphology and developmental biology.

## 6. Multiple parasites within manipulated hosts

Recently there has been a growing interest in studying the influence of manipulative parasitic species on the evolution of sympatric parasite species. Lafferty et al. (2000) have proposed a series of predictions about transmission strategies that should be favored by natural selection depending on the ecology of the co-occurring parasite species in the manipulated host. For instance, when manipulation is costly to achieve and when non-manipulative and manipulative parasites have shared interests (i.e. they have the same intermediate and definitive hosts), non-manipulator parasites should preferentially infect hosts that are already manipulated so as to increase their chance of transmission without having to invest in manipulation (hitchhiking strategy, Thomas et al., 1997, 1998b). When two manipulative parasites have shared interests and affect different aspects of host phenotype (e.g. colour, behaviour) in ways that increase transmission additively, natural selection could favour mechanisms allowing these ‘co-pilots’ to co-occur more often than expected by chance in intermediate hosts (Lafferty et al., 2000). Finally, when one or more parasite species share an intermediate host with a manipulative species but do require different definitive hosts, conflicts of interests will emerge. At least three evolutionary solutions to such a conflict have been proposed: (1) avoiding intermediate hosts containing a manipulator, (2) killing the manipulator and (3) overpowering the manipulation of the manipulator. The last instance is called “hijacking,” in the case of a second trophically transmitted manipulator (Lafferty et al., 2000) and ‘sabotage’ when the second parasite is not a manipulator, is not trophically transmitted and benefits most from having the host with a normal phenotype (Thomas et al., 2002c).

The number of empirical studies focusing on multiple parasites within manipulated hosts is low, but increasing (but see Lafferty and Morris, 1996; Thomas et al., 1997, 1998a, 1998b, 1998c; Cézilly et al., 2000; Dezfuli et al., 2000, 2001; Fauchier and Thomas, 2001; Poulin and Valtonen, 2001; Outreman et al., 2002; Poulin et al., 2000, 2003). We therefore encourage parasitologists to examine the entire community of parasites in manipulated hosts. In addition, once the proximate causes of parasitic manipulation are better understood, multiple parasite systems will invite the study of proximate factors that mediate cooperative and conflicting relationships between parasites sharing a manipulated host (see for instance Poulin et al., 2003 for a unique example). Do different parasite species that have evolved under similar cooperative/competitive scenarios rely on similar mechanisms?

## 7. Determining the causes of intraspecific variation in manipulative processes

It is common to find substantial variation in the intensity of the phenotypic changes displayed by infected hosts, even when they are collected in the same environment and at the same time. As pointed out by Perrot-Minnot (2004), the analysis of the intraspecific variability in these patterns is nonetheless essential to an understanding of their evolution. When a character is variable for both genetic and environmental reasons, two individuals may differ because they differ in genotype, because the have had different environmental experiences, or both. Unfortunately, the extent to which different individual parasites display different manipulative abilities and the variability in the ability of individuals hosts to oppose manipulation is poorly documented. Despite the difficulty of maintaining parasites with complex life cycle in the laboratory, we need more studies aimed at identifying not only the relationship between phenotype and fitness, but also on the phenotypic variance and the degree to which manipulation is heritable. Such efforts will undoubtedly provide a much better basis for understanding the evolution of traits involved in the manipulative process.

## 8. Concluding remarks

In the year 2000, Poulin published a paper entitled "Manipulation of host behaviour by parasites: a weakening paradigm?" (Poulin, 2000). Although some of Poulin's concerns are well justified, we find that on the whole, the study of manipulation is far from being weak. If anything, it is moving into a new era, characterized by challenging questions that demand interdisciplinary approaches. These little worms, tiny protozoa and viruses are on the brink of accomplishing what has eluded large scientific societies—promoting communication among groups as disparate as conservation biologists, epidemiologists, neuroethologists and evolutionary ecologists. If this is weakness, then it is greatly desired.

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