CHAPTER 3

Invasion of the Body Snatchers: The Diversity and Evolution of Manipulative Strategies in Host–Parasite Interactions

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Abstract   Parasite-induced alteration of host behaviour is a widespread trans-
mission strategy among pathogens. Understanding how it works is
an exciting challenge from both a mechanistic and an evolutionary
perspective. In this review, we use key examples to examine the

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proximate mechanisms by which parasites are known to control the behaviour of their hosts. Special attention is given to the recent developments of post-genomic tools, such as proteomics, for determining the genetic basis of parasitic manipulation. We then discuss two novel perspectives on host manipulation (mafia-like strategy and exploitation of host compensatory responses), arguing that parasite-manipulated behaviours could be the result of compromises between host and parasite strategies. Such compromises may occur when collaborating with the parasite is less costly for the host in terms of fitness than is resisting parasite-induced changes. Therefore, even when changes in host behaviour benefit the parasite, the host may still play some role in the switch in host behaviour. In other words, the host does not always become part of the parasite's extended phenotype. For example, parasites that alter host behaviour appear to induce widely disseminated changes in the hosts' central nervous system, as opposed to targeted attacks on specific neural circuits. In some host–parasite systems, the change in host behaviour appears to require the active participation of the host (e.g., via host immune-neural connections). Even when the change in host behaviour results in clear fitness benefits for the parasite, these behavioural changes may sometimes be produced by the host. Changes in host behaviour that decrease the fitness costs of infection could be selected for, even if these changes also benefit the parasite.

3.1. INTRODUCTION

Animal behaviour is complex. The dance of a honeybee, the dawn chorus of birds, the group hunting of wolves or the cognitive behaviour of tool-using crows and chimps are examples that illustrate this point. Organisms choose where to go, when and where to forage, how and with whom to mate, and whether or not to invest in parental care of the resultant offspring. The study of animal behaviour is a one of the oldest and most established of the natural sciences and remains one of the more accessible arenas of science for the general public through natural history programming. However, the behaviour of parasites is usually neglected. This is not to say that parasites do not behave but only that their behaviour has been considered simple and not particularly interesting. Most parasites are phage or bacteria whose behaviour consists of habitat choice. For multi-cellular parasites such as the liver fluke, *Fasciola hepatica*, a wider range of behaviours exist such as choosing an optimal location, choosing to mate or whether to self-fertilise, and when to reproduce. But it is hardly behaviour on par with the courtship dance of a bower bird.

However, in some host–parasite systems parasites induce complex behavioural changes in their host. For example, some parasitoid wasps

coerce spiders into spinning 'sleeping bags' suspended from branches, thus providing a safe pupating site for the wasp (Eberhard, 2000). Crickets, ants and other insects infected with hairworms (nematodes) dive into water, allowing the aquatic parasite to exit their body and mate (Maeyama *et al.*, 1994; Thomas *et al.*, 2002a). Rats infected with *Toxoplasma* develop a fatal attraction for cats, increasing parasitic transmission to their next host (Berdoy *et al.*, 2000). These are just some of the many examples of the dramatic behavioural impact parasites can have on their host. Parasites can also have less extreme changes on host behaviour such as shifts in foraging, location or activity (reviewed in Moore, 2002). If the change in host behaviour benefits the parasite (e.g., by increasing transmission to a new host) we suggest that the parasite has been selected to produce this behavioural change in its host. In this case, the altered behaviour of the host is a phenotype of the parasite and is controlled by the parasite's genes. Therefore, it is part of the parasite's extended phenotype.

This concept of the extended phenotype was first developed by Richard Dawkins (1982). In his book, *The Extended Phenotype*, he argues that a gene can produce a phenotype that extends beyond the body of a single individual, if such a phenotype results in increased transmission of the gene to the next generation. A phenotype is usually defined as a trait of the individual organism such as eye or flower colour. The extended phenotype perspective includes abiotic structures such as birds' nests. Birds' nests increase the fitness of bird genes; therefore, a nest is an example of an extended phenotype. Parasitic manipulation of host behaviour leading to increased transmission of the parasite's genes is another example of an extended phenotype (Dawkins, 1982, 1990, 2004).

The extended phenotype perspective is not a theory, but is merely a 'way of viewing the facts'. We know that the presence of parasites alters the behaviour of their hosts in ways that range from simple to dramatic. Behaviour is a phenotype and has a genetic basis. In infected hosts either parasite genes or host genes are responsible for its altered behaviour. The extended phenotype perspective merely postulates that in some host–parasite interactions the parasite genes are responsible for the aberrant behaviour. This parasite-centred view has been relatively ignored in evolutionary ecology (Poulin, 2007), sometimes for good reason (Thomas *et al.*, 2005, see Box 3.1).

In this review we will examine the mechanisms by which parasites are known to control the behaviour of their hosts. Despite years of study, we lack unequivocal evidence that parasite genes cause host behavioural change. Thus we advocate that future studies should use a more sophisticated approach than has been previously adopted (e.g., the incorporation of more molecular techniques for determining the genetic basis of manipulation). We review the advances that have been made in the field using proteomic tools. We also explore the degree to which host behavioural changes could be a compromise between host and parasite strategies.

BOX 3.1   Brief history of parasitic manipulation

Cram (1931) and **Van Dobben (1952)** first suspected that parasites might have the ability to modify the behaviour of their hosts in a way that increases their transmission efficiency. The pioneering works of Bethel and Holmes on acanthocephalan worms (1973, 1974, 1977) significantly advanced this hypothesis. However, it was only with the publication of Richard Dawkins' book entitled '*The Extended Phenotype*' (1982, see main text for details) that the field of parasitic manipulation acquired a conceptual framework. Henceforth, parasitologists considered that host alteration may be regarded as the expression of the genes of the parasite in the host phenotype and that some of the parasite's genes are selected for their effect on host phenotype.

Dawkins' way to view facts has led researchers to consider all behavioural changes observed in an infected organism as beneficial for the parasite. However, not all parasite-induced alterations of the host phenotype necessarily enhance parasite transmission. Some alterations can be adaptations of the hosts to defend themselves against parasites (e.g., behavioural fever, Moore, 2002). Moreover, changes might be pathological consequences of infection, adaptive to neither host nor parasite. These are termed 'boring by-products' of infection (Dawkins, 1990; Edelaar *et al.*, 2003; Webster *et al.*, 2000).

Robert Poulin (1995) wrote an important paper that helped address the issue of adaptive versus non-adaptive host behavioural changes by highlighting the need for a clear approach to interpreting potential cases of parasitic manipulation (Poulin, 1995). Four criteria were proposed in order to consider changes as adaptive for the parasite in the context of transmission: complexity, purposiveness of design (i.e., conformity between *a priori* design and the host phenotypic alterations), convergence (similar changes in several independent lineages) and fitness consequences. Poulin's (1995) paper marked the start of a new period during which many studies taking into account these recommendations appeared in the scientific literature. It also marked a departure from purely adaptationist reasoning.

This paper has nonetheless left one point obscure: should we consider the changes that are pathological consequences of infection *and* are coincidentally beneficial for the parasite as adaptations? In his paper, Poulin (1995) distinguished between 'true' parasite manipulation and 'by-products' of infection (the latter being changes coincidentally beneficial that may be a fortuitous payoff of other adaptations). This point faced criticism since it is almost impossible to distinguish between the primary focus of historical selection and concomitant effects on transmission (see Lefèvre and Thomas, 2008; Moore, 2002 and Thomas *et al.*, 2005 for details).

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Two scenarios of parasitic manipulation are presented (i.e., the exploitation of host compensatory responses and 'mafia-like manipulation') in which the parasite-manipulated behaviours are not necessarily an illustration of the extended phenotype and can benefit both partners.

As has been common in the field of parasitic manipulation of host behaviour, our discussion will span multiple fields. For parasitologists we aim to provide key information regarding parasite-mediated activities; for behavioural ecologists, whose focus is behaviour, we want to highlight the myriad forms of manipulation and how we are beginning to understand the proximate mechanisms underlying them; for evolutionary biologists, who are interested in trait evolution and co-evolutionary processes, we want to review this exciting field for them; and for applied scientists who either deal with human or veterinary diseases or use parasites as biocontrol, we want to emphasise that behavioural studies are highly relevant to applied fields.

3.2. HOW PARASITES ALTER HOST BEHAVIOUR

Most research on parasitic manipulation of behaviour has focused on the effects of parasites on host neural function. This emphasis is reasonable given that behaviour is controlled by the central nervous system (CNS). Below we review two examples in which parasites are known to alter the neural function of their host (for more examples see Adamo, 1997, 2002; Klein, 2003; Moore, 2002; Thomas *et al.*, 2005). These examples illustrate that the mechanisms mediating host behavioural change are often complex. Parasites do not manipulate the brain of their hosts the way a puppeteer controls a puppet, delicately tweaking only those neural circuits responsible for specific behaviours. Instead parasites appear to slug the host's brain with a number of diffuse and widespread effects, some of which induce changes in host behaviour. We continue by showing how post-genomic era approaches can lead to great advances in our understanding of the proximate mechanisms mediating host behavioural change. In particular we discuss the recent parasito-proteomics studies of infected host brains. We end with a discussion of the importance of this new technique, especially in light of the complex mechanisms that are typically involved in host behavioural change.

3.2.1. Parasitic effects on host neural function

3.2.1.1. Rabies
Rabies is often cited as a classic example of parasitic manipulation of host behaviour (Klein, 2005). As in other cases of parasitic manipulation, the parasite is thought to commandeer the neural circuits that regulate

specific host behaviours. Changing these specific host behaviours benefits the parasite. Below we examine the evidence for this scenario.

Rabies is caused by RNA viruses of the genus *Lyssavirus* (Rupprecht *et al.*, 2002). Rabies virus infects the CNS of its host and induces profound behavioural changes (Rupprecht *et al.*, 2002). Some of these behavioural changes (e.g., aggressiveness and hyper-salivation) increase viral transmission (Hemachudha *et al.*, 2002). The rabies virus docks with specific neural receptors suggesting specificity in its attack on the host's CNS (Hemachudha *et al.*, 2002). Once inside a neuron, the rabies virus alters ion homeostasis and synaptic physiology, both of which alter neural transmission (Dhingra *et al.*, 2007). This effect may explain why neural transmission is abnormal in some brain regions in rabies (Fu and Jackson, 2007). Interestingly, changes also occur in neurons that do not appear to be directly infected with the virus, suggesting that the virus can also influence neural function indirectly (Fu and Jackson, 2007). Neuronal damage is minimal during the period in which an infected animal is transmitting the virus (i.e., prior to severe motor symptoms, Scott *et al.*, 2008). Therefore, the virus has the tools to selectively alter host behaviour by manipulating specific target neurons without killing them.

Nevertheless, the rabies virus does not selectively alter either behaviour or neural function. For example, rabies virus induces more than just aggression and hyper-salivation in its host. Infected hosts also suffer from a lack of appetite and have reduced co-ordination (Ruppecht *et al.*, 2002). These behaviours are unlikely to enhance viral transmission and demonstrate that the effects of rabies are not entirely selective. Although non-specific effects might be expected from any virus that infects the brain, behaviours that are important for enhanced transmission (e.g., increased aggression) would be expected to occur in all hosts of a manipulative parasite. However, not all animals infected with rabies are aggressive (Hemachudha *et al.*, 2002). Most rabies victims can be divided into two groups based on their behavioural symptoms: encephalitic (furious) and paralytic (dumb) (Hemachudha *et al.*, 2002). Aggressive behaviour is observed only in encephalitic rabies. In paralytic rabies, the host gradually loses motor control and consciousness (Hemachuda *et al.*, 2003). Although these symptoms would increase the host's contact with predators, this is unlikely to lead to increased viral transmission because the rabies virus is fragile and non-bite transmission of rabies (e.g., via mucous membranes) is rare (Rupprecht *et al.*, 2002). In paralytic rabies, the lack of aggression coupled with the animal's decreased mobility and increased lethargy probably results in reduced viral transmission. Nonetheless, paralytic rabies is not a rare form and about 25% of infected humans have paralytic rabies (Hemachudha *et al.*, 2002). The paralytic form of rabies is also common in dogs (Laothamatas *et al.*, 2008), even though dogs are the co-evolved host for the canine variant of the virus

(Hemachudha *et al.*, 2003). Differences in the genetic code of the virus are not responsible for the differences in the behaviour of infected hosts (Hemachudha *et al.*, 2003). For example, Hemachudha *et al.* (2002) report a case in which the same rabid dog induced paralytic rabies in one victim and encephalitic rabies in the other. Therefore, the rabies virus induces aggression in only some of its hosts, despite the likely importance of this behaviour for viral transmission. Moreover, the virus also induces other behaviours in its host that probably impede viral transmission.

The rabies virus does not selectively target those brains areas responsible for regulating aggression (Laothamatas *et al.*, 2008). For example, in humans, the amygdala (a part of the limbic system) and the orbitofrontal cortex regulate aggression (Coccaro *et al.*, 2007). Although the virus reliably strikes the limbic system, the virus does not infect these structures exclusively, or even preferentially (Laothamatas *et al.*, 2003). During rabies in humans, magnetic resonance imaging (MRI) shows changes in brainstem, cerebellum, hippocampi (and other parts of the limbic system, including the amygdala), hypothalamai, deep and sub-cortical white matter, and deep and cortical gray matter (Laothamatas *et al.*, 2003). The amygdala is thought to be critical for the regulation of aggression in non-human mammals too (Kandal *et al.*, 1991). Nevertheless, the rabies virus does not target the amygdala in non-human hosts either. For example, rabid dogs have high concentrations of rabies viral messenger RNA (mRNA) in the basal ganglia, caudate nucleus, cerebellum, hippocampus (and other parts of the limbic system), medulla, mid-brain, pons, thalamus and the frontal, parietal, occipital and temporal lobes of the cerebrum (Laothamatas *et al.*, 2008). Interestingly, the distribution of virus in the brain is the same in both paralytic and encephalitic forms of rabies (Laothamatas *et al.*, 2003, 2008; Smart and Charlton, 1992). The limbic system is attacked in both forms, but only results in increased aggression in encephalitic rabies.

The evidence above demonstrates that the simplest postulated mechanism of manipulation—that is, that the rabies virus selectively infects and manipulates those brain areas that regulate aggressive behaviour in mammals—is false. How then does rabies produce enhanced aggression in a large portion of its hosts? Hemachudha *et al.* (2002) suggest that the immunological reactions provoked by the virus (see Hooper, 2005) play a role in changing host behaviour. For example, cytokines, released during the body's response to the rabies virus, could alter limbic system function (Hemachudha *et al.*, 2002) and this may increase aggression. Increases in some cytokines can increase aggressiveness, supporting this hypothesis (Kraus *et al.*, 2003). In fact, immune reactions alone can produce aggressive behaviour. For example, during auto-immune disorders such as paraneoplastic limbic encephalitis, the immune system damages the limbic system (Osborne, 1994) and this induces aggressive behaviour

in some patients (Tardiff, 1998). Therefore, the effect of the virus on host behaviour may depend on the host's immune response (Hemachudha *et al.*, 2002), and this would explain why the effects of the virus on host behaviour are variable. However, Charlton *et al.* (1984) found no significant change in the aggressiveness of rabid skunks given the immunosuppressant cyclophosphamide compared to controls (Charlton *et al.*, 1984). Rabies virus replication was increased by cyclophosphamide treatment (i.e., brains of treated animals had higher viral titres), demonstrating that cyclophosphamide did suppress host immune responses (Charlton *et al.*, 1984). Recently, Laothamatas *et al.* (2008) found that dogs with paralytic rabies had a more robust immune response to the virus and showed greater cytokine release in all brain areas (including the limbic system) than dogs with encephalitic rabies, the opposite to what would be predicted if cytokines are driving the increase in aggression. Moreover, MRI studies revealed greater brain abnormalities in the non-aggressive paralytic dogs than in encephalitic dogs, even though dogs with paralytic rabies have less viral mRNA expressed in their brains compared to dogs with encephalitic rabies (Laothamatas *et al.*, 2008). These results suggest that the robust immune response of some animals may prevent the virus from altering host behaviour, leading to the paralytic form of rabies. Further studies are required to clarify the role of the host's immune system in the production of aggressive behaviour during rabies.

The Borna disease virus (BDV) is another virus of the CNS that induces aggressive behaviour in its host (Klein, 2003). However, similar to rabies, not all infected animals show an increase in aggressive behaviour (Carbone *et al.*, 1987). As with rabies, BDV infects multiple brain areas, including the limbic system (see Klein, 2003). In BDV infections, the virus replicates first in the hippocampus (Carbone *et al.*, 1987). However, by the time behavioural symptoms such as aggressive behaviour occurs, the virus has widely disseminated throughout the brain (Carbone *et al.*, 1987). Moreover, the behavioural symptoms occur at the same time that the host's immune response produces widespread inflammation in the brain (Carbone *et al.*, 1987). Therefore, the immune system may play a role in inducing host aggression in both rabies and BDV.

3.2.1.2. Gammarids, parasites and serotonin

Unravelling the connections between parasites, neural transmission and altered host behaviour may be easier to discover when the host is an invertebrate rather than a vertebrate host (Helluy and Holmes, 2005). In this section we review the mechanisms used by different parasites to alter the behaviour of small crustaceans known as gammarids. Gammarids are attacked by parasites that often have complex life cycles in which the parasite requires transmission to a vertebrate host to complete its development (e.g., Kennedy, 2006). In some of these systems, once the parasite

reaches the infective stage, the parasitised host shows changes in escape behaviour resulting in an increased likelihood that the infected gammarid will be consumed by the parasite's appropriate vertebrate host. Some of these changes probably occur because of parasite-induced changes in the host's serotonergic neural signalling system (Table 3.1).

For example, when the acanthocephalan Polymorphus paradoxus reaches the infective stage, its gammarid host, Gammarus lacustris, changes its escape behaviour. The parasitised host swims towards the light and clings to the nearest solid material when disturbed, instead of swimming away from the light and burrowing into the mud as non-parasitised controls do. Some of the same behaviours induced by the presence of the parasites can be mimicked by injections of serotonin. Injections of other biogenic amines, such as octopamine or dopamine, do not induce these behaviours. Serotonin haemolymph concentrations need

TABLE 3.1 Relationship between presence of the parasite, increased host phototaxis and serotonin immunohistochemistry

Gammarid (Host)	Parasite	Effect of parasite on phototaxis	Effect of serotonin on phototaxis	Effect of parasite on serotonin staining of the CNS[a]
Gammarus insensibilis	*Microphallus papillorobustus* (within CNS)	Increase	Increase[b]	Decrease TGN smaller
Gammarus lacustris	*Polymorphus paradoxus* (within haemocoel)	Increase	Increase	Increase in varicosities
Gammarus pulex	*Pomphorhynchus laevis* (within haemocoel)	Increase	Increase	Increase
	Polymorphus minutus (within haemocoel)	None	Increase	None
Gammarus roeseli	*Pomphorhynchus laevis* (within haemocoel)	None	Increase	None

[a] The study on G. lacustris examined only the ventral nerve cord. All other studies examined staining in the cerebral ganglion
[b] cited in Helluy and Thomas (2003); TGN, tritocerebral giant neuron.
Notes: See text for references.

to be raised by approximately three orders of magnitude above physiological levels to produce the effect. The need for such large concentrations may suggest that serotonin is acting through the CNS and not via peripheral receptors (Helluy and Holmes, 1990).

Immunohistochemical staining for serotonin reveals that infected animals have the same number of serotonergic cells as controls. However, the fine structure of the neurons differs in infected animals. Infected individuals have an apparent increase in the serotonergic staining of structures thought to be axon terminals (Maynard *et al.*, 1996). This result could signal a change in the amount of serotonin released into the synapse. For example, reduced serotonin release would result in a build up of serotonin within the axon terminal of the neuron, creating the increase in staining. Regardless of whether serotonin release is increased or decreased, Maynard *et al.*'s (1996) results suggest that the parasite has an impact on the host's serotonergic system. How the parasite exerts this effect is unknown.

A related gammarid, *Gammarus pulex*, is infected with the acanthocephalans *Polymorphus minutus* and *Pomphorhynchus laevis*. Hosts infected with *P. minutus* show a reversed geotaxis compared to control animals and swim towards the surface. This change in behaviour probably increases the chance that the host comes in contact with its definitive host, a bird. *P. minutus* does not induce phototaxis. *P. laevis*, however, changes the photophobia of *G. pulex* into phototaxis, resulting in the host swimming towards the light. This behaviour makes the host more vulnerable to fish predation, the definitive host for this species. Injections of serotonin induce phototactic behaviour but do not change geotactic behaviour in *G. pulex*. As in *G. lacustris*, large doses of serotonin are needed to induce the effect (Tain *et al.*, 2006).

Immunohistochemical staining of the cerebral ganglion for serotonin shows no gross differences between hosts infected with either parasite and uninfected controls (e.g., in the number of serotonergic cells). Tain *et al.* (2006) also found no difference in the gross anatomy of the giant serotonergic neuron found in the brain (i.e., the tritocerebral giant neuron (TGN)) in infected animals. However, hosts infected with *P. laevis* had enhanced immunohistochemical staining for serotonin, but there was no difference in the intensity of staining when they were infected with *P. minutus*. Therefore, staining for serotonin only increased when host phototactic behaviour increased (Tain *et al.*, 2006).

A related gammarid (*Gammarus roeseli*) is also infected with the parasite *P. laevis*. However, in this gammarid, *P. laevis* does not induce phototactic behaviour, even though injections of serotonin can induce phototaxis in *G. roeseli*. *G. roeseli* shows no change in serotonergic staining when infected (Tain *et al.*, 2007), supporting the hypothesis that altered serotonin signalling is causally involved in the change in host phototactic behaviour (Table 3.1).

The gammarid *Gammarus insensibilis* is parasitised by the trematode *Microphallus papillorobustus*. In the previous examples, the parasites remain outside of the CNS (Kennedy, 2006). In this system, however, the trematode lodges within the host's protocerebrum, a part of the cerebral ganglion and CNS. The host is not debilitated, but instead shows altered responses to specific sensory stimuli such as light. Once the parasite reaches the infective stage, the host shows aberrant escape behaviours making it more likely to be consumed by the parasite's definitive host, a bird (Helluy and Thomas, 2003).

Immunohistochemical staining for serotonin reveals profound changes between infected and control animals. For example, the TGN is stunted in infected animals suggesting some degeneration of serotonergic fibres. Such a change in neural architecture is very likely to produce decreases in serotonergic signalling within the CNS because of likely decreases in the synaptic field. However, some parts of the brain showed no change in serotonergic staining, suggesting that the effect was specific to certain neurons or brain areas. The parasite does not appear to influence the TGN by mechanically squeezing it; the giant neuron and the parasite reside on opposite sides of the brain (Helluy and Thomas, 2003). How the parasite alters the morphology, and presumably the function, of this serotonergic neuron remains unknown. Unfortunately the role the TGN plays in the host's escape behaviour is also unknown.

Taken together, the results (Table 3.1) suggest that parasites can influence gammarid phototactic behaviour by altering some aspect of the serotonergic system. However, the results are puzzling because it is unclear whether an increase or decrease in serotonin release within the CNS is responsible for altering phototactic behaviour. Immunohistochemical studies do not provide a reliable estimate of neural activity or neurotransmitter release (see de Jong-Brink and Koene, 2005) especially when looking across different physiological states (e.g., parasitised vs non-parasitised, see Zitnan *et al.*, 1995).

The observation that injections of serotonin into the haemocoel induce increased phototaxis suggests that enhanced serotonergic release is responsible for the increase in phototaxis. However, all the parasites except *M. papillorobustus* remain outside the host's CNS (Kennedy, 2006). It is unlikely that the parasites residing in the haemocoel can produce enough serotonin to induce phototaxis (Holmes and Zohar, 1990; Tain *et al.*, 2006; Thomas *et al.*, 2005). Most likely the parasites induce the host's CNS to produce serotonin. Ponton *et al.* (2006a) found that one of the enzymes important for serotonin production, aromatic amino acid decarboxylase (Cooper *et al.*, 2002), was not visible on two-dimensional (2D) electrophoresis gels of the brains of *G. pulex* parasitised with *P. minutus*, but was visible on gels of uninfected brains. Ponton *et al.* (2006a) interpreted their results as indirect evidence of an increase in

aromatic acid decarboxylase activity. However, these data are equivocal, and like immunohistochemical staining, can also support the hypothesis that serotonin production has declined. Moreover, in vertebrates the decarboxylation step is not the rate-limiting step in the synthesis of serotonin; the rate-limiting step is governed by tryptophan hydroxylase and the availability of tryptophan (Cooper *et al.*, 2002). Given that the amount of *L*-tryptophan in the crayfish brain is typically more than five times greater than that of 5-hydroxytryptophan (Rodriguez-Sosa *et al.*, 1997) the situation is probably similar in crustaceans. Therefore if serotonin production is increased in parasitised brains, there should be a concomitant increase in tryptophan and tryptophan hydroxylase concentrations. More direct measurements of serotonin synthesis in parasitised brain tissue are needed (e.g., using high-performance liquid chromatography (HPLC)). Pharmacological (e.g., Tierney *et al.*, 2004) and electrophysiological studies would also be helpful in determining how altered serotonergic activity is related to the increase in phototaxis.

As with rabies, the mechanisms mediating behavioural manipulation of infected gammarids are complex. For example, host immune responses may also play a role in producing the change in host behaviour (Tain *et al.*, 2007). As in rabies, the manipulative parasites (e.g., *P. laevis*, Cézilly and Perrot-Minnot, 2005) induce other behavioural changes in their host, in addition to phototaxis. This effect may reflect the fact that serotonin signalling is involved in many behaviours in crustaceans (e.g., see Weigner, 1997). Most of the studies in Table 3.1 suggest widespread alterations in the functioning of the serotonergic system, not a selective strike on specific neural circuits.

3.2.2. Proteomics and proximate mechanisms

Post-genomic technology promises to revolutionise many fields in biology by providing enormous amounts of genetic data from non-model organisms. Proteomics is a case in point and promises to bridge the gap between our understanding of genome sequences and cellular behaviour; it can be viewed as a biological assay or tool for determining gene function (for explanations of genomic terms see Box 3.2). Parasito-proteomics is the study of the reaction of the host and parasite genomes through the expression of the host and parasite proteomes (genome-operating systems) during their complex biochemical cross-talk (Biron *et al.*, 2005a,b). Proteomics, with the ability to investigate the translation of genomic information, offers an approach to study the global changes in protein expression of the host CNS caused by parasites. Fig. 3.1 outlines the essential steps to any proteomics study of parasite manipulation of host behaviour.

BOX 3.2   Glossary for the 'omics' tools use in parasite-proteomics

Genome: The full complement of genes carried by a single (haploid) set of chromosomes. The term may be applied to the genetic information carried by an individual or to the range of genes found in a given species.

Genomics: It is the study of an organism's genome and the use of the genes. It deals with the systematic use of genome information, associated with other data, to provide answers in biology, medicine and industry.

Immunochemistry: A branch of chemistry that involves the study of the reactions and components on the immune system. Various methods in immunochemistry have been used in scientific study, from virology to molecular evolution.

Interactome: The interactome is the whole set of molecular interactions in cells. It is usually displayed as a directed graph. When spoken in terms of proteomics, it refers to protein–protein interaction network (PPI) or protein network (PN).

Gene knock-out: This is a genetic technique in which an organism is engineered to carry genes that have been made inoperative. Gene knock-in is similar to knock out, but instead it replaces a gene with another instead of deleting it.

Neuropeptidome: In recent years, the introduction of highly sensitive mass spectrometry paved the way for rapid screening of the neuropeptide profile (neuropeptidome) even to the single cell level, in species as small as insects.

Neuropeptides: Neuropeptides are the most structurally diverse messenger molecules that influence a wide range of physiological processes. They are present in all Metazoa that have developed a nervous system.
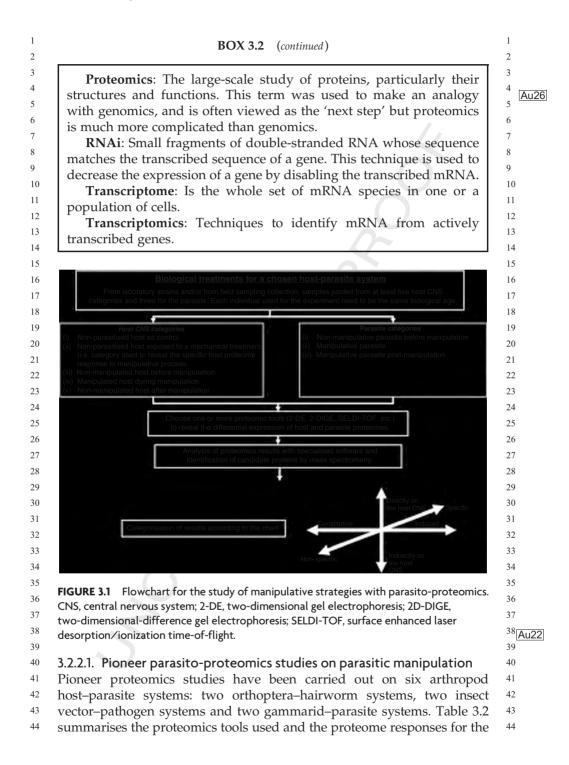
Proteome: The term proteome was first used in 1995 and has been applied to several different types of biological systems. A cellular proteome is the collection of proteins found in a particular cell type under a particular set of environmental conditions such as exposure to hormone stimulation. It can also be useful to consider an organism's complete proteome. The complete proteome for an organism can be conceptualised as the complete set of proteins from all of the various cellular proteomes. This is very roughly the protein equivalent of the genome. The term 'proteome' has also been used to refer to the collection of proteins in certain sub-cellular biological systems. For example, all of the proteins in a virus can be called a viral proteome.

BOX 3.2 (*continued*)

Proteomics: The large-scale study of proteins, particularly their structures and functions. This term was used to make an analogy with genomics, and is often viewed as the 'next step' but proteomics is much more complicated than genomics.

RNAi: Small fragments of double-stranded RNA whose sequence matches the transcribed sequence of a gene. This technique is used to decrease the expression of a gene by disabling the transcribed mRNA.

Transcriptome: Is the whole set of mRNA species in one or a population of cells.

Transcriptomics: Techniques to identify mRNA from actively transcribed genes.

FIGURE 3.1 Flowchart for the study of manipulative strategies with parasito-proteomics. CNS, central nervous system; 2-DE, two-dimensional gel electrophoresis; 2D-DIGE, two-dimensional-difference gel electrophoresis; SELDI-TOF, surface enhanced laser desorption/ionization time-of-flight.

3.2.2.1. Pioneer parasito-proteomics studies on parasitic manipulation

Pioneer proteomics studies have been carried out on six arthropod host–parasite systems: two orthoptera–hairworm systems, two insect vector–pathogen systems and two gammarid–parasite systems. Table 3.2 summarises the proteomics tools used and the proteome responses for the

TABLE 3.2 Synopsis of 'parasito-proteomics' studies on parasitic manipulation

Host–parasite association		Proteomics tools			Proteome response				References
					In head host		In parasite		
Host species	Parasite species	Separation of proteins	IP scale; Mw scale	Identification of proteins	TNSA	PPSLMP	TNSA	PPSLMP	
Nemobius sylvestris (Bosc) (Orthoptera, Gryllidae)	Paragordius tricuspidatus (Dufour) (Nematomorpha, Gordiidae)	2-DE	pH 5–8; 19–122 kDa	MS, MS/MS, Sequencer	902	3.8	729	5.0	Biron et al., 2006
Meconema thalassinum (De Geer) (Orthoptera, Tettigoniidae)	Spinochordodes tellinii (Nematomorpha, Spinochordodidae)	2-DE	pH 5–8; 19–122 kDa	MS	566	16.8	763	5.0	Biron et al., 2005
Anopheles gambiae (Giles) (Diptera, Culicidae)	Plasmodium berghei (Haemosporida, Plasmodiidae)	DIGE	pH 3–10; 14–100 kDa	MS, MS/MS	1400	0.9	No data	No data	Lefèvre et al., 2007a
Glossina palpalis gambiensis (Diptera, Glossinidae)	Trypanosoma brucei brucei (Kinetoplastida, Trypanosomatidae)	2-DE	pH 3–10; 20–122 kDa	MS	816	2.9	No data	No data	Lefèvre et al., 2007b
Gammarus insensibilis (Amphipoda, Gammaridae)	Microphallus papillorobustus (Trematoda, Microphallidae)	2-DE	pH 3–6; 20–122 kDa	MS	556	12.9	No data	No data	Ponton et al., 2006a
Gammarus pulex (Amphipoda, Gammaridae)	Polymorphus minutus (Acanthocephala, Polymorphidae)	2-DE	pH 3–6; 20–122 kDa	MS	838	8.1	No data	No data	Ponton et al., 2006a

Notes: 2-DE, two-dimensional gel electrophoresis; DIGE, difference gel electrophoresis; IP, isoelectric point; Mw, molecular weight; TNSA, total number of proteins spots analysed; PPSLMP, percentage of protein spots potentially linked to manipulative process; MS, mass spectrometry.

host CNS of each host–parasite association. In each study, multiple treatments were carried out to control for potential confounding effects and to exclude the proteins that are non-specific to the manipulative process making it easier to find the proteins potentially linked with host behavioural changes.
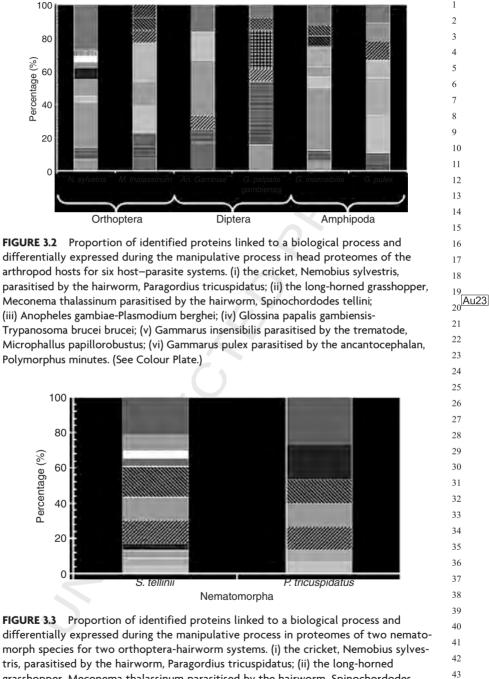
Initially, proteomics was used to explore the mechanisms in host CNS underlying the suicidal behaviour of crickets and grasshoppers when manipulated by their hairworms (Biron *et al.*, 2005c, 2006). Two orthoptera-hairworm systems have been investigated: (i) the cricket, *Nemobius sylvestris*, parasitised by the hairworm, *Paragordius tricuspidatus*; (ii) the long-horned grasshopper, *Meconema thalassinum*, parasitised by the hairworm, *Spinochordodes tellinii* (details on the background biology can be found in Thomas *et al.*, 2002a). Because hairworm parasites are very big (i.e., worm length exceeds that of the host by 3–4 times) and because they are located in the body cavity, it is very easy to separate the host CNS and the parasite, thereby allowing the simultaneous study of both proteomes without the risk of contamination.
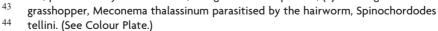
Proteomics studies suggest that adult hairworms produce host mimetic proteins and manipulate behaviour with them. These proteins are from the Wnt family suggesting a direct action of the hairworms on the host's CNS that can lead directly to an alteration of the host behaviour or indirectly via a host genome response. The analysis of the head proteomes revealed that the percentage of proteins potentially linked to the hairworm manipulative process is higher for *M. thalassinum* compared to *N. sylvestris* (see Table 3.2) (Biron *et al.*, 2006). For the hairworms, some of the proteins potentially linked to the manipulative process are the same (see Table 3.2). The altered functions are similar for both orthopteran species except for some families of proteins that are involved in geotactic behaviour, in protein biosynthesis and in recovery following an infection being only differentially expressed in *M. thalassinum* (Fig. 3.2). In the brain of manipulated orthoptera, differential expression of proteins specifically linked to neurogenesis, the visual process, the geotactic process, and neurotransmitter activities have been observed (Fig. 3.2). The altered physiological compartments are similar for both nematomorph species except for some families of proteins implicated in endopeptidase inhibition, in protein folding and in transcriptional regulation that are only expressed in *S. tellinii* (Fig. 3.3; Biron *et al.*, 2006).

Insect vectors (e.g., mosquitoes carrying malaria) are often manipulated to increase encounter rates with vertebrate hosts in ways that enhance the pathogen's transmission (Hurd, 2003; Lefèvre and Thomas, 2008; Lefèvre *et al.*, 2006; Moore, 1993; Rogers and Bates, 2007). Two parasito-proteomics studies have been performed on such systems: (i) *Anopheles gambiae-Plasmodium berghei* (Lefèvre *et al.*, 2007a); (ii) *Glossina papalis gambiensis-Trypanosoma brucei brucei* (Lefèvre *et al.*, 2007b).

FIGURE 3.2   Proportion of identified proteins linked to a biological process and differentially expressed during the manipulative process in head proteomes of the arthropod hosts for six host–parasite systems. (i) the cricket, Nemobius sylvestris, parasitised by the hairworm, Paragordius tricuspidatus; (ii) the long-horned grasshopper, Meconema thalassinum parasitised by the hairworm, Spinochordodes tellini; (iii) Anopheles gambiae-Plasmodium berghei; (iv) Glossina papalis gambiensis-Trypanosoma brucei brucei; (v) Gammarus insensibilis parasitised by the trematode, Microphallus papillorobustus; (vi) Gammarus pulex parasitised by the ancantocephalan, Polymorphus minutes. (See Colour Plate.)

FIGURE 3.3   Proportion of identified proteins linked to a biological process and differentially expressed during the manipulative process in proteomes of two nemato-morph species for two orthoptera-hairworm systems. (i) the cricket, Nemobius sylves-tris, parasitised by the hairworm, Paragordius tricuspidatus; (ii) the long-horned grasshopper, Meconema thalassinum parasitised by the hairworm, Spinochordodes tellini. (See Colour Plate.)

These studies provide evidence that the pathogens can alter the head proteome of their insect vectors (see Table 3.2; Fig. 3.2). Some of the altered protein families are similar between dipterans (i.e., sugar metabolism, signal transduction and heat shock response) (see Fig. 3.2). An alteration in energy metabolism has been observed in the CNS of both parasitised hosts (Lefèvre *et al.*, 2007a,b). Finally, these parasito-proteomics studies suggest that *P. berghei* and *T. b. brucei* can alter host apoptosis pathways and sugar metabolisms.

Several parasites such as trematodes, cestodes and acanthocephalans alter the behaviour of their intermediate host to enhance trophic transmission (Moore, 2002; Thomas *et al.*, 2005). To date we have proteomes of two Amphipoda-parasite systems that were also discussed in Section 3.2.1.2: (i) *Gammarus insensibilis* parasitised by the trematode, *Microphallus papillorobustus*; (ii) *Gammarus pulex* parasitised by the ancanthocephalan, *Polymorphus minutes* (Table 3.2). *M. papillorobustus* has a complex life cycle, including snails as first intermediate hosts, gammarids as second intermediate hosts and various sea- and shorebirds as definitive hosts. The life cycle of *P. minutus* displays broad ecological similarities with *M. papillorobustus* since it also involves a gammarid as intermediate host and aquatic birds (mainly ducks) as definitive hosts. Metacercariae of *M. papillorobustus* are always encysted in the brain of *G. insensibilis*, while cystacanths of *P. minutus* are located in the body cavity of *G. pulex*. Both parasites manipulate the behaviour of their gammarid intermediate host, making them more likely to be eaten by predatory definitive hosts at the water surface. *M. papillorobustus* induces a positive phototaxis and a negative geotaxis to alter the behaviour of its intermediate hosts while *P. minutus* induces only a negative geotaxis (Cézilly *et al.*, 2000; Helluy, 1984).

For the two gammarid species, the proteome of *G. insensibilis* displayed a slightly stronger response to the manipulative process caused by its trematode compared to *G. pulex* manipulated by its acanthocephalan (see Table 3.2, Fig. 3.2). The altered functions are similar for both gammarid species except for some families of proteins only expressed in *G. insensibilis*: those involved in visual process, DNA binding, cell proliferation and metabolism. The proteomic results (Ponton *et al.*, 2006a) obtained for *G. insensibilis*–*M. papillorobustus* corroborated previous studies suggesting a major role of serotonin in the expression of the aberrant evasive behaviour (see Section 3.2.1.2).

It has been suggested that immune responses may secondarily affect host nervous system functions and hence behaviour and it is increasingly suggested that parasites could exploit host defence reactions in order to manipulate host behaviour (see above; Adamo, 2002; Thomas *et al.*, 2005). The proteomics results have shown that arginine kinase is differentially expressed in the brain of infected *G. insensibilis* and *G. pulex* compared to uninfected individuals. This phosphotransferase is known to be one of the

regulating factors in nitric oxide (NO) synthesis (Mori and Gotoh, 2000). NO is liberated during immunological reactions, but it also acts as a neuromodulator. Thus, these proteomic results provide supportive evidence for the hypothesis suggesting that parasites could exploit host defence reactions in order to manipulate host behaviour.

3.2.2.2. Parasito-proteomics and parasite manipulation: A bright future?

Parasito-proteomics studies have contributed to the discovery of candidate genes and new biochemical pathways potentially involved in parasitic manipulation. Future work should build upon this promising start. We suggest some additional considerations to move this work forwards. For instance, existing parasito-proteomics studies are missing: (i) the insoluble proteome linked to the manipulative process; (ii) the neuropeptidome response; and (iii) the host proteome response in a molecular weight (Mw) range of 20 kDa or less and a pH range 4 or less and 7 or greater. In addition, functional analysis in association with behavioural assays and interactome bioassays (see Box 3.2) will be necessary to confirm the involvement of the candidate proteins. Thus, a new integrative approach is necessary to bridge the gaps in our knowledge of how parasites manipulate their hosts (see Fig. 3.4).

Several new proteomics tools have been developed and can be used in the understanding and the deciphering of the manipulative process. For instance, SELDI-TOF can provide a complementary visualisation technique to two-dimensional (2D) electrophoresis. SELDI-TOF is more sensitive and requires smaller amounts of proteins than 2D electrophoresis (Bischoff and Luider, 2004; Issaq et al., 2002; Seibert et al., 2004). SELDI-TOF is most effective at profiling low Mw proteins (i.e., <20 kDa) and permits a rapid comparison of the host CNS proteome for many treatments by taking into consideration many physiochemical characteristics of proteins using SELDI protein chips with various chemical surfaces (hydrophobic, cationic, anionic, hydrophilic and metal ion preventing) (Bischoff and Luider, 2004; Issaq et al., 2002; Sanchez et al., 2008; Seibert et al., 2004). In the previous parasito-proteomics studies, no data were obtained about these key molecules (i.e., peptides and neuropeptides) influencing the physiological processes involved in the expression of host behaviour. For the proteins with a Mw greater than 20 kDa, the multi-dimensional liquid chromatography/mass spectrometry (LC/MS) offers a promising alternative and complementary approach to 2D electrophoresis for the analysis of complex protein mixtures. Multidimensional LC/MS has increased in popularity because this technique is relatively straightforward, the available software is convenient to use and once protein fractions are 'spotted' on matrix-assisted laser desorption/ionisation (MALDI) targets there are no time constraints on carrying

Biological treatments for a host-parasite system

From laboratory strains and/or from field sampling collection, recuperation of at least four host
categories of individuals and three parasite categories obtained following behavioural assays.

Non-parasitized hosts as control	Before manipulation -Non-manipulated hosts -Non-manipulative parasite	During manipulation -Manipulated hosts -Manipulative parasite	After manipulation -Manipulated hosts -Manipulative parasite

Proteomics tools used to reveal the differential expression
of host and parasite proteomes in one or more tissues.

Soluble and non-soluble proteins > 20 kDa   Soluble and non-soluble proteins ≤ 20 kDa

2D-DIGE and/or 2D-LC/MS   SELDI-TOF

Identification of candidate proteins with mass spectrometry tools (MS, MS/MS) and/or with a protein sequencer.

Functional analysis in synergy with behavioural assays to
determine the key role of the candidate proteins linked to the
manipulative process:
I. Microinjections
II. Immunochemistry
III. Suppression of the expression of the candidate proteins by
using the knock-out and/or the RNAi techniques.

Interactome bioassays:
I. Visualizing gene expression and protein
interactions with fluorescent proteins as GFPs
and new fluorescent protein variants.
II. By using bioinformatic tools, biochemical
classification of the protein-protein interactions
identified.

Some fundamental and applied perspectives
I. Measurement of the intensity of the host manipulation by a parasite within and between host populations.
II. Test the molecular convergence hypothesis for host-parasite systems showing similar host behavioural modifications.
II. Identification of biochemical signature linked to a particular habitat and/or environmental conditions.
IV. Test the hypothesis of local adaptations of the manipulative strategies.

FIGURE 3.4   New integrative approach to study the proximate mechanisms in any host–parasite system. 2D-DIGE, two-dimensional-difference gel electrophoresis; 2D-LC/MS, 2 dimensional liquid chromatography/mass spectroscopy; GFP,; MS, mass spectroscopy; RNAi, small fragments of double-stranded RNA; SELDI-TOF, surface enhanced laser desorption/ionization time-of-flight.

out further analysis for the protein identification (Brand *et al.*, 2005; Greibrokk *et al.*, 2005). However, the 2D-difference gel electrophoresis (2D-DIGE) remains a very efficient option for the analysis of the differential expression of common proteins between different treatments.

3.2.2.3. Summary
The proximate mechanisms mediating changes in host behaviour are complex. This complexity probably exists because these mechanisms evolved from the mechanisms required for the survival of the parasite within the host (see also Combes, 2005). Given the fortuitous nature of evolution, it is not surprising that parasites influence host behaviour using multiple methods (see Fujiyuki *et al.*, 2005; Tomonaga, 2004). We will need a greater understanding of parasito-proteomics, immune-neural interactions (see Adamo, 2008; Dantzer, 2008) and a more neuroethological approach to understand how parasites manipulate their host's behaviour fully. This necessitates an increase in our own 'crosstalk' with researchers investigating the proximate mechanisms of behaviour, such as neuroimmunologists and neurobiologists.

3.3. A CO-EVOLUTIONARY PERSPECTIVE

Parasitic manipulation often dramatically reduces host fitness. For this reason, the hypothesis of 'manipulation *sensu stricto*' is commonly seen as a game with evident winners (i.e., parasites) and losers (hosts) (Wellnitz, 2005) (Box 3.3(A)). The ability of parasites to manipulate host behaviour results from a long-term co-evolutionary interaction that probably leads to the mechanisms being complex (Section 3.3). Co-evolutionary dynamics implies that the host behavioural changes should thus be considered as an equilibrium state, a compromise resulting from an on-going arms race rather than a total parasite takeover (Poulin *et al.*, 1994; Wellnitz, 2005).

BOX 3.3   The three main types of manipulation

A. Manipulation *sensu stricto*
Host behavioural alteration may be regarded as a compelling illustration of the extended phenotype (Dawkins, 1982), that is, the expression of the parasite's genes in the host phenotype. The extended phenotype perspective thus postulates that in some host–parasite interactions the parasite genes are responsible for the aberrant behaviour. In this view, genes of the parasite are selected for their effect on host behaviour.

B. Exploitation of host compensatory responses
Host behavioural alteration may be regarded as a host response to parasite-induced fitness costs. Parasites may affect fitness-related traits in their hosts such as fecundity and survival in order to stimulate host compensatory responses because these responses can increase parasitic transmission. In this view, genes of the parasite are selected for their pathological effects that induce a host compensatory response. Since behavioural changes both mitigate the costs of infection for the host and meet the objectives of the parasite in terms of transmission, natural selection is likely to favour all the genes involved in this interaction.

C. Mafia like manipulation
Host behavioural alteration may be regarded as a forced collaboration. Parasites may select for collaborative behaviour in their hosts by imposing extra fitness costs in the absence of compliance. The parasite would be able to adopt a plastic strategy (i.e., facultative virulence) depending on the level of collaboration displayed by the host. In this view, genes of the parasite are selected for their ability to detect non-collaborative behaviours and their ability to produce retaliatory behaviour.

From an evolutionary point of view, these considerations are relevant as they suggest that behavioural changes in infected hosts, even when they result in clear fitness benefits for the parasite, are not necessarily pure illustrations of the extended phenotype of the parasite. In a host–parasite system, natural selection is acting on the host genome as well. At present, very few studies on manipulative changes have explored the degree to which parasite-manipulated behaviours could be a compromise between the strategies of host and parasite. We present here two scenarios in which parasitic '*manipulation*' can enhance host fitness as well.

3.3.1. Exploiting host-compensatory responses

In this section, we propose that certain parasites could affect fitness-related traits in their hosts (e.g., fecundity, survival, growth, competitiveness, etc.) in order to stimulate host compensatory responses because these host responses enhance parasitic transmission (Lefèvre *et al.*, 2008) (Box 3.3B).

3.3.1.1. Compensatory responses in the living world

The phenotype of an organism results from both its genotype and the environment in which the genes are expressed. Phenotypic plasticity is the capacity of a genotype to express different phenotypes under different environmental conditions (Pigliucci and Preston, 2004). When faced with adverse environmental conditions, many organisms are able to alter some life history traits resulting in reduced fitness loss (Metcalfe and Monaghan, 2001). For instance, when facing potential resource limitations, plants possess a remarkable degree of developmental plasticity that enables them to balance their resource acquisition and maximise their fitness (Wise and Abrahamson, 2005). Similarly, it can be adaptive for parents in many animal species to avoid producing poor-quality offspring when food is rare (e.g., by re-absorbing embryos or by reducing the production of offspring of the more vulnerable sex, see Uller *et al.*, 2007). Animals can also respond to adverse environmental conditions by using fecundity compensation (i.e., reproducing earlier in life and producing more offspring). For instance, in the presence of predatory fish, the cladoceran crustacean *Daphnia galeata* reproduce early and produce larger clutches of smaller offspring (Sakwinska, 2002).

Parasites influence the optimal strategies of their free-living hosts. Like other environmental factors, parasites have the potential to play an important role in the evolution of plastic compensatory responses. Selection will favour hosts that will react to parasite-induced fitness cost by adjusting their life history traits when they cannot resist infection by other means (e.g., immunity). Several theoretical and empirical studies back up this assumption by showing that infected hosts can adjust their

reproductive effort or growth in such a way as to increase their fitness. For example, parasitised hosts react to a fitness loss due to infection via mechanisms such as an increased rate of egg laying (Adamo, 1999; Minchella and Loverde, 1981), enhanced courtship behaviour (McCurdy *et al.*, 2000; Polak and Starmer, 1998), higher offspring number and/or size (e.g., Kristan *et al.*, 2004; Sorci and Clobert, 1995) and/or stronger parental effort (Christe *et al.*, 1996, Hurthrez-Boussès *et al.*, 1998; Tripet and Richner, 1997). High risk of infection can also select for early-onset sexual maturity in the entire population (Agnew *et al.*, 1999; Fredensborg and Poulin, 2006; Lafferty, 1993). In other cases, hosts compensate by diminishing their reproductive effort, presumably to enhance survival, which could in return increase the probability of outliving or sequestering the parasite (Forbes, 1993; Hurd, 2001; Sorensen and Minchella, 2001). Therefore, compensatory changes in behaviour may be a widespread strategy among organisms facing adverse conditions such as parasitism.

In some cases, parasites can exploit host compensatory responses that have been selected in other ecological contexts by mimicking the causes that induce them. In other cases, parasites themselves can be the triggers of the compensatory response because of their significant effects on host fitness.

3.3.1.2. Empirical support

To our knowledge, theoretical and/or experimental studies specifically designed to test this scenario have never been carried out. However, in the literature there are several examples of parasite-induced phenotypic changes that have been interpreted either as cases of adaptive host responses or manipulation *sensu stricto* but these same cases could, in fact, illustrate the exploitation of host compensatory responses. Below, we present some of these examples.

3.3.1.2.1. Foraging activity

(a) *Predation risk*. An increased predation risk is a change that can potentially be of interest for trophically transmitted parasites since, by definition, this type of parasite requires a predation event to complete its life cycle. Parasitised hosts often have increased energy requirements and forage more to compensate for the negative effect of infection. However, until now the subsequent increased predation risk has been traditionally viewed as a by-product of the infection that is coincidentally beneficial for the parasite (but see Thomas *et al.*, 2005). Parasites live at the expense of their hosts, and consequently there are many reasons, other than transmission, for parasites to divert energy away from the host (growth, maturation of gonads). We agree with this parsimonious way of thinking (Box 3.1) but we feel that in the present evolutionary context, parsimony can be viewed

differently. Host exploitation by parasites can potentially affect a broad range of fitness-related traits in hosts such as survival, fecundity or sexual attractiveness. These phenomena are expected to favour the evolution of compensatory responses in the host, such as an increased foraging or reproductive activity. For instance, three-spined sticklebacks (*Gasterosteus aculeatus*) infected by the cestode *Schistocephalus solidus* exhibit marked differences in their anti-predator, foraging and shoaling behaviour compared with uninfected conspecifics (Barber and Huntingford, 1995; Godin and Sproul, 1988; Ness and Foster, 1999). The increased nutritional demand of parasitised fish (Pascoe and Mattey, 1977) may stimulate foraging behaviour that exposes them to greater predation risk than uninfected counterparts (Godin and Sproul, 1988; Milinski, 1985). This example has been interpreted as an illustration of the 'side-effect' hypothesis according to which these changes result from pathological effects of infection that are coincidentally beneficial for the parasite (Box 3.1; Poulin, 1995). However, the behavioural changes observed in sticklebacks infected by *S. solidus* are consistent with the view that the fish benefits by obtaining more food to compensate for the resources taken by the cestodes. Thus the host will gain, at least until predated, and by that time it could have reproduced. The parasite clearly gains by making the host more vulnerable to predation. However, one cannot exclude active manipulation *sensu stricto* of host neuroendocrine systems by the parasite, for instance by the release of a neuroactive substance (Overli *et al.*, 2001). What we wish to emphasise here is that competing ideas need consideration when searching for proximate mechanisms of manipulation.

(b) *Qualitative change*. It has been frequently reported that parasitised organisms change their foraging behaviour (Moore, 2002). If foraging leads to increased uptake of resources that can help fight the infection it is often seen as a case of self-medication (Hart, 1994). When infected with the tachinid parasitoid *Thelaira americana*, the caterpillar host *Platyprepia virginalis*, changes its feeding preference from lupine to hemlock (Karban and English-Loeb, 1997). This change apparently reduces the costs of the infection for the host because infected caterpillars feeding on hemlock survived the emergence of the parasite and even metamorphosed into sexually mature adults without losing fecundity (English-Loeb *et al.*, 1990, 1993). This response also seems to be beneficial to the parasite. The pupal mass of flies (a good correlate of fecundity) emerging from caterpillars reared on hemlock was indeed greater than that emerging from lupine-fed caterpillars (Karban and English-Loeb, 1997). In this example both host and parasite interests are aligned (Dawkins, 1990).

(c) *Biting behaviour in haematophagous insects.* When haematophageous insects feed on their hosts, they are liable to transmit many pathogens. Vector-borne parasites manipulate several phenotypic traits of their vertebrate hosts and vectors in ways that favour parasite transmission (Hurd, 2003; Lefèvre and Thomas, 2008; Molyneux and Jefferies, 1986; Moore, 2002; Section 3.3). For instance, infected-insect vectors seem to develop an increased probing and feeding rate (e.g., tsetse flies infected with African trypanosomes, Jenni *et al.*, 1980; Roberts, 1981; bugs infected with *Trypanosoma* spp., Anez and East, 1984; Botto-Mahan *et al.*, 2006; Garcia *et al.*, 1994; sandflies infected with *Leishmania* spp., Beach *et al.*, 1985; Killick-Kendrick, 1977; Rogers and Bates, 2007; fleas infected with plague bacterium, Bacot and Martin, 1914; Gage and Kosoy, 2005; mosquitoes infected with *Plasmodium* spp., Koella *et al.*, 1998, 2002; Rossignol *et al.*, 1986; Wekesa *et al.*, 1992; and viruses, Grimstad *et al.*, 1980; Platt *et al.*, 1997). Increased biting is usually associated with mechanical interference, that is, the vector's ability to engorge fully is impaired and therefore this induces them to bite vertebrate hosts several times (Hinnebusch *et al.*, 1998; Molyneux and Jenni, 1981; Rogers and Bates, 2007). This would appear to be manipulation *sensu stricto*. However, Rossignol *et al.* (1986) demonstrated reduced fertility in *Aedes aegypti* parasitised with *Plasmodium gallinaceum.* When infected mosquitoes were free to bite more, they recovered a normal level of fecundity (i.e., equal to uninfected conspecifics). In this view, the increased biting rate of *A. aegypti* may represent a host compensatory response to parasite-induced fecundity reduction.

3.3.1.2.2. Sexual behaviour Longevity and reproduction are crucial fitness determinants of most organisms (Clutton-Brock, 1988). A trade-off between these two key life-history traits is expected so that reductions in longevity leads to increased reproductive effort (Polak and Starmer, 1998). Parasites often reduce the survival of their host, and infected hosts are expected to respond by increasing their reproductive effort. Parasites with direct transmission could benefit from decreasing the reproductive output of their host. Decreased offspring production should promote a compensatory increase in sexual behaviour, and hence parasite transmission. The sexually transmitted ectoparasite, *Chrysomelobia labidomera*, reduces the survival of its leaf beetle host (*Labidomera clivicollis).* In response, infected males exhibit increased sexual behaviour before dying (Abbot and Dill, 2001). The host compensation hypothesis predicts a positive relationship between parasite load and reproductive effort (Forbes, 1993; Polak and Starmer, 1998). As expected, the study by Abbot and Dill (2001) showed a positive relationship between male parasite load, the frequency of sexual contact and duration between males in

presence of females of copulation. This behavioural modification clearly benefits the sexually transmitted parasite since enhanced inter- and intra-sexual contact (i.e., copulation and competition) provide more opportunities for transmission (Abott and Dill, 2001; Drummand *et al.*, 1989).

In the same vein, it has been reported that females of the amphipod *Corophium volutator* compensate for the negative effect of the trophically transmitted trematode *Gynaecotyla adunca* on survival by increasing their reproductive activity (McCurdy *et al.*, 2000, 2001). Males appeared to compensate for parasitism by being more likely to mate, and perhaps by increasing ejaculate size. In amphipods mating occurs only during a narrow part of the female's moult cycle. Since, moulting is asynchronous, the operational sex ratio is strongly male biased, and males compete for access to larger, more fecund females. In response, pre-copulatory mate guarding has evolved in amphipods. Interestingly, such behaviour is known to increase the predation risk because pairs are more conspicuous, less manoeuvrable and more profitable as prey than single individuals (Cothran, 2004; Ward, 1986). Thus one can hypothesise that parasites trigger host fecundity compensation because host mating increases the chance of being preyed upon by the definitive hosts. This example, however, must be considered carefully because the increased sexual activity of parasitised gammarids may occur before the trematode is infective to vertebrate predators.

3.3.1.2.3. Inclusive fitness In the Hawaiian Islands, corals from the genus *Porites* are susceptible to infection by the digenetic trematode *Podocotyloides stenometra* (Aeby, 1991, 1992). This parasite has a complex life cycle involving a molluscan as first intermediate host, *Porites* as the second intermediate host, and coral-feeding fish as the final host. *Porites* infected with this trematode display pink swollen nodules. Given that these parasitised polyps represent a burden for the coral (reduced growth), the coral would benefit from eliminating and replacing them, for example, by offering them to predators. As a matter of fact, the Butterfly fish (definitive host) do prefer the parasitised polyps and hence contribute to the regeneration of a healthy polyp (Aeby, 1992). Whereas the higher susceptibility to fish of infected polyps seems to be a case of host manipulation *sensu stricto* by a parasite, it also agrees with the idea that the parasite relies on host compensatory responses for its transmission.

3.3.1.2.4. Gigantism Many parasite species can reduce host fecundity, either partially or via full castration, by channelling energy away from host reproduction toward their own growth (Poulin, 2007). This fecundity reduction often results in host gigantism, especially in molluscs serving as first intermediate hosts of larval trematodes (Minchella, 1985). This phenomenon is consistent with the idea that phenotypic changes following

infection can be considered as co-evolved traits. As size and fecundity are positively correlated in snails, the parasitised hosts can benefit from investing energy in growth, with fecundity compensation occurring later, after the death of the parasite. However, the parasite remains the first beneficiary of such a compensatory strategy since the larger size of the host allows the parasite to increase the biomass of the sporocyst and thus produce thousands of infective larvae.

3.3.1.3. Future directions

Most studies on parasitic manipulation assume that host phenotypic changes that benefit the parasites are compelling illustrations of the extended phenotype (*sensu* Dawkins, 1982; but see Ponton *et al.*, 2006b), that is, the expression of the parasite's genes in the host phenotype. The perspective presented above attempts to balance this view. We suggest that changes in host behaviour, even those that benefit the parasite, can be due to compromises between host and parasite strategies (i.e., a shared phenotype).

To our knowledge, it is novel to consider that parasites could achieve transmission by triggering host compensatory responses, when the latter fit (totally or in part) with the transmission route. Is this strategy common? Further studies are clearly needed at the moment to answer this question, but it may be a widespread strategy. This type of host manipulation seems parsimonious for several reasons when compared with the hypothesis of manipulation *sensu stricto*, in which the parasite must maintain a certain degree of manipulative effort with putative fitness costs. Indeed, if among the arsenal of compensatory responses displayed by the host, some are beneficial for transmission, selection is likely to favour parasites that exploit these responses, not only because this meets their objectives, but also because this requires no manipulative effort: the host is doing the job. Another good reason to believe that exploiting host compensatory responses is a likely scenario from an evolutionary perspective comes from the fact that it is also advantageous for the host: once infected, it is better for the host to behave in a way that alleviates the costs of infection, even when this also ultimately benefits the parasite (aligned desiderata, Dawkins, 1990). Under these conditions, resistance is less likely to evolve than when there is no compensation for the host.

Based on these considerations, we could predict that manipulation *sensu stricto* will exist most often in systems in which there are no host compensatory mechanisms that would result in increased parasite transmission. As a possible example of such a situation, we suggest the case of the well-known example involving the small liver fluke (*Dicrocoelium dendriticum*). It is indeed difficult to imagine what kind of compensatory responses could make the ant climb to the tip of a grass blade.

Besides the relevance of considering host compensatory responses in the context of transmission strategies, we believe that it could also be a promising approach for the study of many other aspects of host–parasite relationships.

Natural selection should favour parasites that impose specific costs on their host (with a precise schedule adjusted by selection) each time there is a host compensatory response that is beneficial for them. In our opinion, these ideas are very promising for the understanding of the ultimate basis of parasite pathogenicity and virulence (Lefèvre *et al.*, 2008).

3.2.2. Facultative virulence

The mafia-like strategy of manipulation is probably the most extreme scenario demonstrating the interactive nature of the relationship between parasites and hosts (Zahavi, 1979). This strategy suggests that parasites may select for collaborative behaviour in their hosts by imposing extra fitness costs in the absence of compliance. In this scenario the parasite would be also able to adopt a plastic strategy (i.e., facultative virulence) commensurate to the rate of collaboration displayed by the host. In response to a host's opposition to manipulation, a parasite could increase virulence because the host does not behave as expected. There-fore, non-collaborative behaviours are a more expensive option for the host than collaborative ones. This 'mafia-like strategy' can, in theory, force the host to accept behaving in ways that benefit the parasite (Box 3.3(C)). Here, we discuss and review possible evidence around this idea.

3.2.2.1. Host–parasite interactions and state-dependent models

Both the host and the parasite must be able to adjust their life history decisions in a state-dependent manner for the mafia strategy of manipu-lation to evolve. Numerous lines of evidence suggest that free-living organisms are able to recognise environmental cues, including parasitic infection, and to adjust their life history traits accordingly (Section 3.3.1.1).

There are recent suggestions that parasites are also able to perceive a large set of environmental variables and respond to these in a state-dependent manner (thereby maximising their lifetime reproductive suc-cess) (Lewis *et al.*, 2002; Thomas *et al.*, 2002b). Parasites are, for instance, expected to recognise many physiological and biochemical conditions of their internal host environments that are of selective importance (age and sex of the host, presence/absence of other parasites). There are also good reasons to believe that parasites are able to perceive cues concerning the external environment of their hosts. For example, parasites can respond to host population density, the presence of predators, or the presence of sexual partners or competitors (see Thomas *et al.*, 2002b). Poulin (2003) provided empirical evidence that the environmental perception of

parasites can be much more sophisticated than traditionally thought. The trematode *Coitocaecum parvum* from New Zealand is able to accelerate its development and reach precocious maturity in its crustacean intermediate host in the absence of chemical cues emanating from its fish definitive host. Juvenile trematodes can also mature precociously when the mortality rate of their intermediate hosts is increased (Poulin, 2003). These results show that growth decisions and developmental strategies in this parasite are plastic, and conditional upon the opportunities for transmission. More generally, these results suggest that parasites can exploit several sources of information both internal and external to the host.

3.3.2.2. Mafia strategy of manipulation

By imposing extra fitness costs in absence of compliance, parasites have the potential to select for collaborative behaviour in their hosts. Of course, these collaborative behaviours do not result from conscious choices. Over time, selection is expected to produce shifts in the behaviour of infected individuals if such a shift increases their chance of survival and reproduction. In some systems, hosts that alter their behaviour in such a way that benefits the parasite may have better survival and more offspring than infected hosts that do not.

3.3.2.3. Empirical support

The cuckoo is the best exemplar of the mafia hypothesis. Zahavi (1979) hypothesised that cuckoos force their hosts to tolerate non-self eggs by making the consequences of rejection more damaging than acceptance. Soler *et al.* (1995) studied the relationship between the great spotted cuckoo (*Clamator glandarius*) and its magpie host (*Pica pica*). In this host–parasite system, the host can raise at least part of its own young along with those of the cuckoo. Soler *et al.* (1995) showed that ejector magpies suffered from considerably higher nest predation levels by cuckoos than did accepters. The interpretation being that the cuckoo retaliates and punishes non-compliant hosts. As a result, the frequency of 'accepting genes' is more likely to increase in the host population than 'rejecting genes' (Soler *et al.*, 1999). In an area with a high density of cuckoos, Soler *et al.* (1998) showed that magpies that rejected cuckoo eggs from their first clutch were more likely to be parasitised by cuckoos during their second clutch than magpies that accepted the cuckoo eggs during the first clutch.

Pagel *et al.* (1998) modelled the evolution of retaliation by brood parasites. Retaliation evolves even when hosts rear only the parasite's young (its own offspring having been ejected by the parasite, which is the case when nests are parasitised by *Cuculus canorus*). This is possible if, during the breeding season, non-ejectors enjoy lower rates of parasitism in later clutches compared to ejectors, making non-ejectors able to rear a clutch of their own following the rearing of a cuckoo nestling, while

ejectors are likely to be re-parasitised. Pagel *et al.* (1998) stressed that, for this scenario to function, it implies that brood parasites have a good memory for the location and status of nests in their territory.

Recently, Hoover and Robinson (2007) provided experimental evidence for the mafia strategy in the brood parasite, the brown-headed cowbird (*Molothrus ater*). In manipulating ejection of cowbird eggs and cowbird access to nests of their warbler host, they showed that 56 % of ejector-nests compared with only 6% of accepter-nests were destroyed by cowbirds (Hoover and Robinson, 2007). This mafia behaviour selects for collaborative hosts not only in evolutionary time by decreasing the proportion of hosts that bear rejector genes, but also within the lifetime of an individual host through a learning process. Learning probably occurs in parasitic systems in which individual host females are likely to be parasitised repeatedly within or across breeding seasons. In addition, the authors also showed that collaborative behaviours benefit the hosts as well, since warblers produced significantly more offspring by complying with the parasite.

3.3.2.4. Future directions

Examples of mafia strategy of manipulation remain scarce at the moment, but this is likely to reflect a lack of appropriate studies. We encourage researchers to imagine experiments that place infected hosts in a situation of 'disobedience' as regard to what they should do to benefit the parasite, and to study the fitness consequences of such non-compliance. It would also be necessary to determine whether pre-adaptations (physical location of the parasite with respect to the host, number and kind of hosts involved in the life cycle and phylogenetic constraints) exist for behavioural changes. In addition, do these factors matter more in cases of manipulation *sensu stricto*, than in one of the 'interactive' strategies presented above? Knowing that manipulative costs should, in theory, be lower for parasites when the host has some fitness compensation in performing the altered behaviour, we might even expect that the transition from 'pure' manipulation to 'interactive' strategies of manipulation is likely to be a scenario favoured by selection. Finally, this co-evolutionary perspective suggests that host behavioural changes can benefit the host even if they also benefit the parasite.

3.4. THE (RIVER) BLIND WATCHMAKER

In what is the most well-known argument from design, the Reverend William Paley (1802) said that just as we conclude that a watch we find lying on the ground must have had a creator, then so too must other complex things such as animals have had a creator. In Paley's case, the

creator was divine but since the publication of the *Origin of Species* (Darwin, 1859), we now accept the theory of natural selection as a more satisfying explanation. In defending Darwin's theory Richard Dawkins (1986) said of natural selection that ''*It has no mind and no mind's eye. It does not plan for the future. It has no vision, no foresight, no sight at all. If it can be said to play the role of watchmaker in nature, it is the blind watchmaker*''.

How well do we understand the interactions between parasites and their hosts? In many cases we have an enviable level of understanding of these interactions (e.g., our understanding of the antigenic variation of the protein coat of malaria, Schmid-Hempel, 2008). However our understanding is far from complete, even though parasites have the reputation of being simple organisms. (Admittedly it is non-parasitologists putting forwards this view.) Parasites are generally reduced in morphology. Also, their genomes are often reduced compared to free-living relatives (Keeling and Slamovits, 2005). The effects that parasites have on their host are likewise viewed as crude. River blindness, caused by the nematode, *Onchocerca volvulus*, is a case in point. Adult worms produce thousands of microfilaria each day and these cause a range of symptoms that often occur after these immature stages die in the human host without ever being transferred to the fly vector. The most infamous effect of these larvae is the scarring of the eye leading to blindness. River blindness causes great morbidity and mortality in hosts and reinforces the view that parasites appear to take a sledgehammer approach to the host (Section 3.3.1).

By contrast the specific changes in host behaviour that are observed in some systems can be viewed as the parasite extending its phenotype and taking control of the actions of the host. In our review we have sought to temper that view by saying that in many cases parasites appear to induce multiple and widely disseminated changes in their hosts' CNS as opposed to targeted attacks on specific neural circuits. Moreover, the behavioural change is not always the sole property of the parasite: the reaction of the host may also be important.

3.5. CONCLUDING REMARKS

When presented with a parasite causing an elaborate and often times bizarre behavioural change in its host, an obvious question that arises is how? Yet, by any admission, the field has been overly focused on why (i.e., explaining the behaviour in an adaptationist framework where the fitness benefit is ascribed to the parasite, host or neither (Box 3.1)). In this review we considered the evidence of how parasites induce changes. Overall, the evidence is slight and even the best-studied examples require further data before behavioural changes can be considered parasite

manipulation in its most strict sense. Recognising this short fall we have further tempered the manipulation *sensu stricto* view by pointing to other factors such as host immune responses, compensatory responses and facultative virulence. Our goal has been to present the current evidence for parasitic manipulation of host behaviour. To move forwards we require less debate and more evidence. How might this be achieved?

Researchers interested in behavioural manipulation need a fuller discourse with colleagues who understand how physiology, neuroanatomy and omics contribute to behavioural trait expression. This is requisite to avoid situations where the evidence of some aberration (e.g., hormone titres, smaller brain regions or distinctive proteomes) is taken as evidence of adaptive manipulation. It is possible and probable that other scenarios in uninfected hosts (e.g., stress, senescence) lead to similar signatures. In addition to greater collaboration, the field might benefit from focusing on some systems that could be developed into models of host–parasite manipulation events. Clearly, some of those reviewed above would be good contenders. In a related vein, genomic approaches will herald a new era in understanding how parasites control behaviour. A goal of the field should be a full understanding of the proximate mechanisms of how a parasite affects host behaviour. It is our hope that one day a collaborative and multi-disciplinary research approach will be able to peel back a particularly compelling example of an extended phenotype to shows its physiological, neurological and ultimately its genetic basis. Then we will know how parasites manipulate a host.

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