**REVIEW**

Host–parasite molecular cross-talk during the manipulative process of a host by its parasite

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Summary

Many parasite taxa are able to alter a wide range of phenotypic traits of their hosts in ways that seem to improve the parasite’s chance of completing its life cycle. Host behavioural alterations are classically seen as compelling illustrations of the ‘extended phenotype’ concept, which suggests that parasite genes have phenotype effects on the host. The molecular mechanisms and the host–parasite cross-talk involved during the manipulative process of a host by its parasite are still poorly understood. In this Review, the current knowledge on proximate mechanisms related to the ‘parasite manipulation hypothesis’ is presented. Parasite genome sequences do not themselves provide a full explanation of parasite biology nor of the molecular cross-talk involved in host–parasite associations. Recently, first-generation proteomics tools have been employed to unravel some aspects of the parasite manipulation process (i.e. proximate mechanisms and evolutionary convergence) using certain model arthropod-host–parasite associations. The pioneer proteomics results obtained on the manipulative process are here highlighted, along with the many gaps in our knowledge. Candidate genes and biochemical pathways potentially involved in the parasite manipulation are presented. Finally, taking into account the environmental factors, we suggest new avenues and approaches to further explore and understand the proximate mechanisms used by parasite species to alter phenotypic traits of their hosts.

Key words: alteration of host behaviour, parasite manipulation, parasito-proteomics, proximate mechanisms.

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**Introduction**

Living organisms are constantly exposed to parasites. In any given environment, a molecular war begins when a host encounters a parasite. In many host–parasite associations, the molecular war was initiated a long time ago (Majerus et al., 1996), perhaps millions of years ago. As a result of selective pressure, most host species have acquired strategies to mislead the parasite and to win the battle during their molecular cross-talk. Conversely, many parasite species have acquired strategies, also as a consequence of intense selective pressure, to bypass the host defences and to win the struggle, thereby enhancing the chances of completion of their life cycle. Such finely tuned host–parasite molecular cross-talk is the result of the molecular arms race between a host and its parasite, where the host uses different molecules and biochemical pathways to counter parasite invasion and where the parasite deploys many molecules to counter the host defences. How a parasite or its propagules modify the behaviour of a host in ways that seem to increase the parasite’s chances of completing its life cycle is a central topic in parasite ecology (Combes, 2001; Hurd, 2003; Lefèvre and Thomas, 2008; Moore, 2002; Poulin, 1995; Thomas et al., 2005). These alterations of host behaviours enhance host-to-host transmission, ensure that the parasite or its propagules are released in an appropriate habitat or increase parasite survival. In host–parasite associations, alteration of host behaviours by the parasite can include a change in preferred substrate, temperature preferences, locomotor activity, visual cycle, circadian rhythms, geo- or phototrophism, cessation of food consumption, feeding rate or any other behaviours enhancing survival and transmission of the parasite (Adamo, 2002; Beckage, 2002; Hurd, 2003; Lefèvre and Thomas, 2008; Rogers and Bates, 2007; Schaub, 2006; Thomas et al., 2005; Webster, 2001). Many examples of parasite manipulation, including manipulation of the parasites’ life cycles, are given in this special issue; for instance, the summit disease (also called tree top disease) induced by baculovirus (Hughes, 2013), the manipulation of ants by nematodes and fungus species (Hughes, 2013), the ‘fatal feline attraction’ of *Toxoplasma gondii*-infected rodents to their predatory cat definitive host (Webster et al., 2013), the bodyguard behaviour (Maure et al., 2013a; Maure et al., 2013b), and hijacking of free will of a host induced by parasitic wasps (Libersat and Gal, 2013).

Although there are numerous examples of host manipulation by a parasite (Libersat et al., 2009; Moore, 2002; Poulin, 2010; Thomas et al., 2005), the mechanisms underlying these ethological changes are by no means well characterized nor understood (Klein, 2003; Lefèvre and Thomas, 2008; Libersat et al., 2009; Poulin, 2010; Thomas et al., 2005; Thompson and Kavaliers, 1994). Questions concerning the molecular manipulative mechanisms have received much less attention than questions of function (i.e. adaptive significance). The central nervous system (CNS) integrates the information that it receives from its sensory receptors and coordinates the activity of all parts of the bodies of bilaterian animals (i.e. all multicellular animals except radially symmetric animals such as sponges). The CNS is responsible for a variety of functions, including receiving and processing sensory information, perceiving and
deciphering environmental stimuli, controlling motor function and converting patterns of activity in sensory receptors into patterns of muscle activity that constitute appropriate behaviours to a given situation. Thus, any changes in host behaviour would be expected to have a molecular basis in the CNS. The simultaneous study of host and parasite proteomes during their interaction is a promising avenue in order to decipher and understand the manipulative tactics used by parasites and to reveal new products such as neuropeptides and neurotransmitters related to the alteration of host behaviour. In an attempt to clarify the present state of this relatively new research area, the current knowledge on proximate mechanisms and on molecular manipulative tactics is here presented. The parasito-proteomics used to decipher the cross-talk involved during the manipulative process by certain parasite species, the pioneer results and the pitfalls and lessons derived from previous studies are also described. Finally, a five-year overview is given concerning future prospects in the field of parasito-proteomics and parasite manipulation.

**The hypothesis of parasite manipulation and proximate mechanisms**

**Knowledge on proximate mechanisms**

Many surveys have demonstrated the ability of parasites to manipulate the physiology of their host through the secretion of chemical compounds. Some proteins, including peptides, are used by phytophagous or animal parasites to modify the genome expression of their host, which should be seen as an extended phenotypic effect of the parasite’s genes. For instance, phytoparasites such as the nematode *Meloidogyne* sp. secrete substances (mainly proteins such as superoxide dismutase, proteases and calreticulin) in their hosts to induce a giant cell that is used as a feeding site (Vanholme et al., 2004). A similar system has been observed for the zoo parasite *Trichinella spiralis* (Stichosomida, Trichinellidae) (Govers et al., 2000). To alter the behaviour or the phenotype of its host, a parasite must be able to disturb the functioning of the host CNS. A parasite could accomplish this by modulating the amounts of neuroactive compounds within the host CNS. Some studies have identified various neurotransmitters and hormones related to parasite manipulation (Adamo, 2002; Kavaliers et al., 1999; Klein, 2003; Klein, 2005; Thompson and Kavaliers, 1994). Hence, many acanthocephalan and trematode species alter the escape behaviour of their gastropod hosts via the host serotonergic systems (Adamo, 2002; Helluy and Thomas, 2003; Tain et al., 2006; Tain et al., 2007). An update on the current knowledge on proximate mechanisms is given by Adamo (Adamo, 2013).

Despite a widespread belief that there is little empirical proof that parasites change host behaviour by secreting substances (i.e. biogenic amines, neuromodulators, neurohormones or neurotransmitters) that act directly on the host CNS (Adamo, 2002; Thomas et al., 2005; Lefèvre and Thomas, 2008; Biron et al., 2011), little is known about the cross-talk during the manipulative process. In many host–parasite associations, it appears that the host and not the parasite produces the effective molecules (i.e. neuromodulators, neurotransmitters, biogenic amines and neurohormones) that result in an alteration in host behaviour. This arrangement may be a common one in many host–parasite associations, especially for parasites that are not physically in contact with the host CNS and are not located within the host CNS. Producing potent concentrations of neuroactive compounds may be energetically expensive for many parasites. For this reason, it is generally argued that parasites should mainly exploit indirect and less energetically expressive methods to alter host behaviour (Adamo, 2002; Libersat et al., 2009; Poulin, 2010).

**Molecular manipulative strategies**

In host–parasite associations, many molecular manipulative tactics have been selected as a result of natural selective pressure to improve the parasite’s chances of completing its life cycle. Such molecular strategies have been interpreted by many behavioural ecologists and evolutionists as the sophisticated products of natural selection favouring the parasite manipulation. From an evolutionary point of view, such alterations of host phenotypes are classically seen as compelling illustrations of the ‘extended phenotype’ concept, as originally proposed by Dawkins (Dawkins, 1989), in which genes in one organism (i.e. the parasite) have phenotypic effects on those of another organism (i.e. the host). Based on the cross-talk in host–parasite associations, Fig. 1 shows the high diversity of molecular tactics that may be used by a parasite to alter its host’s behaviour. The first (x) axis of the chart refers to molecular manipulative tactics used by a parasite with two extreme cases: (1) a constitutive molecular mechanism used by the parasite to manipulate host behaviour [i.e. the parasite continuously releases substances (e.g. mimetic and/or non-mimetic host molecules)] and (2) an induced molecular mechanism to avoid a costly permanent manipulative capability [i.e. parasites release products (e.g. mimetic and/or non-mimetic host molecules) at a specific moment of the manipulative process]. The second (y) axis refers to the level of action on the host CNS with two extreme cases: (1) a direct action by secreting mimetic and/or non-mimetic host molecules directly into the host CNS and (2) an indirect action by secreting mimetic and/or non-mimetic host molecules indirectly, for instance via the haemolymph into the host CNS or by inducing the host immune system to produce the appropriate neuromodulators. The third (z) axis relates to the degree of specificity of the molecular manipulative tactic.

Hosts and parasites have dynamically, and often sequentially, co-evolved molecular cross-talks during their interactions. Each player attempts to win the ongoing ‘arm races’. Parasites steadily evolve to optimize host exploitation while hosts evolve in order to

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**Fig. 1.** Manipulative tactics used by parasites to alter phenotypic traits of their hosts.
minimize the loss of fitness consequent upon parasite infection. To date, no study has so far demonstrated the existence of a general mechanism by which the host modulates the host CNS. Hitherto, many researchers working on proximate mechanisms of parasite manipulation have limited their experiments to trying to find and quantify known manipulative molecules previously identified in earlier studies (Adamo, 2002; Libersat et al., 2009; Poulin, 2010; Thomas et al., 2005). Molecular studies using the ‘omics’ tools are necessary to stimulate a new impetus and to improve our understanding – general as well as more specific – of the proximate mechanisms involved in the molecular manipulative strategies used in parasitic lifestyles.

**Deciphering host–parasite cross-talks involved in parasite manipulation**

**What is parasito-proteomics?**

The topic of parasito-proteomics is defined as 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; Biron et al., 2005b). Parasito-proteomics offers an excellent way to examine the host and parasite genomes in action through the revelation of the host and the parasite proteomes during the host–parasite cross-talk. The simultaneous study of host and parasite proteomes presents a promising option not only for studying the manipulative process(es) per se, to test the ‘manipulation hypothesis’, but also to reveal molecules as neuromodulators and neuropeptides related to the host manipulation by a parasite (Fig. 2).

Many researchers rely heavily on parasito-proteomics to decipher host–parasite cross-talk. Parasito-proteomics studies are in their infancy but have already led to new insights concerning molecular pathogenesis and microorganism identification (Biron et al., 2005b; Levy et al., 2004; Moura et al., 200; Moura et al., 2003; Vierstraete et al., 2004). However, many parasito-proteomics studies performed so far have employed powerful tools but lack a conceptual approach to disentangle the host and parasite genome responses during their interactions. We have therefore proposed a new holistic approach based on the chart of manipulative tactics used by parasites (Fig. 1) (Biron et al., 2005a; Biron et al., 2005b). In effect, parasito-proteomics bridges the gap between our understanding of the genome sequences and cellular behaviour; it can be viewed as a biological assay or tool for determining gene function (Biron et al., 2005a; Biron et al., 2005b).

**Orthoptera–hairworm associations**

Initially, parasito-proteomics approaches were applied in order to explore the mechanisms in host CNS underlying the suicidal behaviour of orthopteran species (crickets and grasshoppers) when manipulated by their infecting hairworms (Biron et al., 2005c; Biron et al., 2006a). From an ecological point of view, hairworms have astonishing life cycles, developing in their insect hosts until ready to exit these in water, usually a stream, river or lake (Schmidt-Rhaesa, 1997; Schmidt-Rhaesa, 2001). Hairworms must thus make two critical transitions during their life cycle. The first is from the aquatic larva to the terrestrial definitive host; the second from the definitive host to water. Orthopteran species harbouring mature hairworms display, in the first part of the night, a behaviour originally not present in their repertoire: they seek water and jump into it (Thomas et al., 2002)!

Recently, pioneer studies concerning the topic of parasite manipulation were performed on six arthropod host–parasite associations: two Orthoptera–hairworm associations, two insect–vector–pathogen associations and two gammarid–parasite associations. These parasito-proteomics studies utilized the conceptual approach suggested by Biron et al. (Biron et al., 2005a; Biron et al., 2005b). Table 1 summarizes the proteomics tools used and the proteome responses of host CNS (i.e. protein families differentially expressed during the manipulative process) for each host–parasite association. Many biological treatments were applied in each study to control the potential confounding effects to exclude the proteins that are non-specific to the manipulative process and to find those proteins potentially related to host behavioural changes.

**Table 1**

<table>
<thead>
<tr>
<th>Host association</th>
<th>Proteomics tools</th>
<th>Proteome responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairworm control</td>
<td>Mass Spectrometry</td>
<td>Differentially expressed proteins</td>
</tr>
<tr>
<td>Hairworm infection</td>
<td>Mass Spectrometry</td>
<td>Differentially expressed proteins</td>
</tr>
<tr>
<td>Control</td>
<td>Mass Spectrometry</td>
<td>Differentially expressed proteins</td>
</tr>
<tr>
<td>Tarantula</td>
<td>Mass Spectrometry</td>
<td>Differentially expressed proteins</td>
</tr>
</tbody>
</table>

**Fig. 2.** Parasito-proteomics, a promising approach to decipher host–parasite cross-talks.
Proteomics and parasite manipulation in N. sylvestris and a decrease in neurogenesis in M. thalassinum), visual process, signalling and neurotransmitter activities has been observed (Fig. 4). Interestingly, these proteomics studies suggested that P. tricuspidatus induces an inhibition of apoptosis in the N. sylvestris CNS, while S. tellinii causes an induction of apoptosis in the M. thalassinum CNS (Biron et al., 2005c; Biron et al., 2006a).

Thus, these two different parasite tactics may potentially disrupt host CNS functions (Klein, 2003; James and Green, 2004). Finally, these two pioneering proteomics studies suggest that the adult hairworms produce host mimetic proteins from the Wnt family that seemingly act directly on the host CNS during the manipulative process.

### Table 1. Synopsis of parasito-proteomics studies

<table>
<thead>
<tr>
<th>Host–parasite association</th>
<th>Proteomics tools</th>
<th>Pfam of proteins identified*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Host species</strong></td>
<td><strong>Parasite species</strong></td>
<td><strong>Protein separation</strong></td>
</tr>
<tr>
<td>Nemobius sylvestris (Bosc) (Orthoptera, Gryllidae)</td>
<td>Paragordius tricuspidatus (Dufour) (Nematomorpha, Gordiidae)</td>
<td>2-DE</td>
</tr>
<tr>
<td>Meconema thalassinum (De Geer) (Orthoptera, Tetrigonidae)</td>
<td>Spirochordodes tellinii (Nematomorpha, Spirochordodidae)</td>
<td>2-DE</td>
</tr>
<tr>
<td>Anopheles gambiae (Giles) (Diptera, Culicidae)</td>
<td>Plasmodium berghei (Haemosporida, Plasmodiidae)</td>
<td>2D-DIGE</td>
</tr>
<tr>
<td>Glossina palpalis gambiensis (Diptera, Glossinidae)</td>
<td>Trypanosoma brucei brucei (Kinetoplastida, Trypanosomatidae)</td>
<td>2-DE</td>
</tr>
<tr>
<td>Gammarus insensibilis (Amphipoda, Gammaridae)</td>
<td>Microphallus papillorobustus (Trematoda, Microphallidae)</td>
<td>2-DE</td>
</tr>
<tr>
<td>Gammarus pulex (Amphipoda, Gammaridae)</td>
<td>Polymorphus minutus (Acanthocephala, Polymorphidae)</td>
<td>2-DE</td>
</tr>
</tbody>
</table>

*Protein families or domains according to the Pfam database of the Sanger Institute (http://pfam.sanger.ac.uk) or the InterPro database of the European Bioinformatics Institute (http://www.ebi.ac.uk/interpro). Abbreviations: 2-DE, two-dimensional gel electrophoresis; 2D-DIGE, two-dimensional difference gel electrophoresis; MS, mass spectrometry.
induce alteration in the head proteome of their insect vectors (Lefèvre et al., 2007b). For the two dipteran species, the qualitative proteome of G. pallid gambiensis responded rather more strongly to its manipulative trypanosome species compared with A. gambiae manipulated by its malaria species (Fig. 3A) (Lefèvre et al., 2007a; Lefèvre et al., 2007b). Fig. 4 shows the altered functions and Table 1 shows the family of proteins differentially expressed during the manipulative process for the two insect–parasite associations, respectively. The altered functions are similar for both dipteran host species (i.e. sugar metabolism, signal transduction and heat shock response) (Fig. 4). For these two parasito-proteomics studies, an alteration in energy metabolism has also been observed (Lefèvre et al., 2007a; Lefèvre et al., 2007b). This result supports the hypothesis that a parasite can induce a nutritional stress associated with a global metabolism disorder in several tissues that lead secondarily to new feeding attempts (Lefèvre and Thomas, 2008). Finally, the studies suggest that P. berghei and T. brucei brucei can both modulate the host apoptosis pathways during the manipulative process.

**Amphipoda–parasite associations**

Several parasites, such as trematodes and acanthocephalans, alter the behaviour of their intermediate host to enhance trophic transmission to the final host, i.e. to increase the chance of it being eaten by the next and definitive host (Moore, 2002; Thomas et al., 2005). Parasito-proteomics studies were done on two Amphipoda–parasite associations: (1) the gammarid *Gammarus insensibilis* (Amphipoda, Gammaridae), parasitized by a trematode, *Microphallus papillorobustus* (Trematoda, Microphallidae), and (2) the gammarid *Gammarus pulex* (Amphipoda, Gammaridae), parasitized by an acanthocephalan, *Polymorphus minutes* (Acanthocephala, Polymorphidae) (Ponton et al., 2006). *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. minutes* displays broad ecological similarities with that of *M. papillorobustus* since it also involves a gammarid as intermediate host and an aquatic bird (mainly ducks) as definitive host. Metacercariae of *M. papillorobustus* are always encysted in the CNS of *G. insensibilis*, whereas cystacanths of *P. minutes* are located in the body cavity of *G. pulex*. Both parasites manipulate the behaviour of their gammarid host, making them much 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. minutes* induces only a negative geotaxis.

For the two gammarid species, the qualitative proteome of *G. insensibilis* responded slightly more strongly to the manipulative process by its trematode, with ~13% of the total proteins observed compared with ~8% for *G. pulex* manipulated by its acanthocephalan parasite (Fig. 3A). Fig. 4 shows the altered functions during the manipulative process for the two gammarid–parasite associations, while the protein families are given in Table 1. The altered functions are similar for both gammarid species except for some families of protein involved in visual processes, DNA binding, cell proliferation and metabolism but are differentially expressed in *G. insensibilis*. The proteomics results obtained for *G. insensibilis–M. papillorobustus* corroborates previous studies suggesting a major role of serotonin in the expression of the aberrant evasive behaviour (Helluy, 1984; Ponton et al., 2006). Furthermore, these two proteomics studies support the hypothesis that parasites can exploit host defence reactions in order to manipulate host behaviour (Adamo, 2002; Moore, 2002; Ponton et al., 2006). These studies provided evidence that the parasites can indeed induce alteration in the head proteome of their insect vectors (Lefèvre et al., 2007a; Lefèvre et al., 2007b). For the two dipteran species, the qualitative proteome of G. pallid gambiensis responded rather more strongly to its manipulative trypanosome species compared with A. gambiae manipulated by its malaria species (Fig. 3A) (Lefèvre et al., 2007a; Lefèvre et al., 2007b). Fig. 4 shows the altered functions and Table 1 shows the family of proteins differentially expressed during the manipulative process for the two insect–parasite associations, respectively. The altered functions are similar for both dipteran host species (i.e. sugar metabolism, signal transduction and heat shock response) (Fig. 4). For these two parasito-proteomics studies, an alteration in energy metabolism has also been observed (Lefèvre et al., 2007a; Lefèvre et al., 2007b). This result supports the hypothesis that a parasite can induce a nutritional stress associated with a global metabolism disorder in several tissues that lead secondarily to new feeding attempts (Lefèvre and Thomas, 2008). Finally, the studies suggest that P. berghei and T. brucei brucei can both modulate the host apoptosis pathways during the manipulative process.

**Insect-vector–parasite associations**

Many studies on arthropod-vector–parasite associations have been performed to test the hypothesis that parasites alter the behaviour (e.g. feeding behaviour) of their vectors in a way that increases the contact with their vertebrate host(s), thereby enhancing parasite transmission (Hurd, 2003; Lefèvre et al., 2006; Lefèvre and Thomas, 2008; Rogers and Bates, 2007; Moore, 1993; Schaub, 2006). In mosquito–malaria associations, several studies have revealed that *Plasmodium* spp. at the sporozoite stage (i.e. the infective stage for the final vertebrate host) alter the behaviour of their mosquito vectors, *Anopheles* spp. (Anderson et al., 1999; Koella and Packer, 1996; Koella et al., 1998; Koella et al., 2002; Rossignol et al., 1984; Rossignol et al., 1986; Wekesa et al., 1992). In tsetse-fly–trypanosome associations, many studies have now demonstrated that infected flies increase the probing rate and feed more voraciously than uninfected flies (e.g. Hurd, 2003; Jenni et al., 1980; Lefèvre and Thomas, 2008; Molyneux and Jeffries, 1986; Roberts, 1981). Two parasito-proteomics studies were done on two insect–vector–parasite associations to improve understanding of the proximate cause(s) leading to the alteration of the insect vector behaviour: (1) the mosquito *Anopheles gambiae* Giles (Diptera, Culicidae) parasitized by a malaria parasite, *Plasmodium berghei* (Haemosporida, Plasmodiidae) (Lefèvre et al., 2007a), and (2) the tsetse fly *Glossina pallid gambiensis* (Vanderplank) (Diptera, Glossinidae), parasitized by the sleeping sickness parasite, *Trypanosoma brucei brucei* (Kinetoplastida, Trypanosomatidae) (Lefèvre et al., 2007b).

These studies provided evidence that the parasites can indeed induce alteration in the head proteome of their insect vectors

![Fig. 3. Total number of protein spots detected (black diamonds) and percentage of protein spots differentially expressed (grey bars) in (A) the head proteomes of arthropod hosts and (B) the proteomes of manipulative hairworms during the expression of the water-seeking behaviour.](image-url)
et al., 2006; Thomas et al., 2005). It has been argued that immune responses may secondarily affect host nervous system functions and hence behaviour and it is increasingly suggested that parasites exploit host defence reactions in order to manipulate host behaviour (Adamo, 2002; Thomas et al., 2005). The proteomics results described here reveal that arginine kinase is differentially expressed in the brain of infected *G. insensibilis* and *G. pulex* compared with 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 unequivocally show that parasites do indeed exploit host defence reactions in order to manipulate host behaviour.

**Artemia–cestode associations**

As with trematodes and acanthocephalans, many cestode species alter the behaviour of their intermediate hosts to enhance trophic transmission to final definitive hosts (Amat et al., 1991; Gabrion et al., 1982; Robert and Gabrion, 1991; Sánchez et al., 2006; Sánchez et al., 2007). Recently, a parasito-proteomics study using SELDI-TOF MS was performed in order to compare the head proteome of uninfected individuals of *Artemia parthenogenetica* (Bowen and Stirling) (Branchiopoda: Anostraca) with that of infected individuals manipulated by one of the three following cestode species: (1) *Flamingolepis liguloides* (Gervais) (Cestoda, Hymenolepididea) infecting flamingos, (2) *Confluaria podicipina* (Szymanski) (Cestoda, Hymenolepididea) infecting grebes and (3) *Anomotaenia tringae* (Burt) (Cestoda, Diphyllidea) infecting shore birds (Sánchez et al., 2009). Two downregulated peptides were found in *A. parthenogenetica*-infected individuals: (1) a peptide of 4.5 kDa for the dilepidid species, *A. tringae*, and (2) a peptide of 3.9 kDa for the two hymenolepidids, *F. liguloides* and *C. podicipina*. Because parasitized individuals of *A. parthenogenetica* also typically display altered behaviour, these peptides are potential candidates for involvement in the manipulation process. However, to confirm this, further work is required, especially in terms of identification of the molecules involved and their biological functions.

**Benefits and outcomes of the early parasito-proteomics studies**

**Molecular convergence of proximate mechanisms in parasite manipulation**

Parasito-proteomics studies on the parasite manipulation concept show that proteomic tools are sensitive enough to disentangle host proteome alterations and also parasite proteome alterations related to factors including circadian rhythm, parasite status and emergence, the quality of the habitat, and the manipulative process (Biron et al.,...
Among the many tactics employed by parasites during immune evasion and host manipulation, one of the most fascinating is molecular mimicry. Parasites are confronted with host defences at multiple levels: physical barriers, innate immunity, and adaptive immune responses that need to be overcome in order to successfully establish an infection and proliferate inside a host. Molecular mimicry is well known as a tactic for immune evasion and host manipulation in viruses (Lambiri et al., 2008; Srinivasa et al., 1986). However, until now, there was little evidence that zooparasites can change host behaviour by secreting molecules that act directly on the host’s CNS.

Lately, parasito-proteomics studies on orthopteran–hairworm associations studied during the nocturnal manipulation phase (see Table 1) have shown that two proteins belonging to the Wnt family were over-expressed in the N. sylvestris and M. thalassinum CNS. This differential expression of Wnt proteins in the orthopteran CNS may well be related to a contribution of mimetic Wnt proteins synthesized by the hairworms (Table 1, Fig. 5). The mimetic Wnt proteins of the hairworms act directly on the host’s CNS, which in turn leads to an alteration of the host behaviour, or indirectly via a host genome response. It will be necessary to confirm that these hairworm proteins are the manipulating agents by isolating and injecting them into the CNS of crickets and grasshoppers.

**Reversibility of parasite manipulation**

The mechanisms used by hairworms to increase the water-seeking behaviour of their orthopteran hosts remain a poorly understood aspect of the manipulative process (Ponton et al., 2011). Results of two earlier parasito-proteomics studies suggest that phototaxis alterations (i.e. changes in the responses to light stimuli) could be a part of a wider strategy of hairworms for completion of their life cycles (Biron et al., 2005c; Biron et al., 2006a). Specifically, parasite-induced positive phototaxis could improve the encounter rate with water (Biron et al., 2006a). This assumption was derived from two arguments. Firstly, in the native forest of southern France, water areas such as ponds and rivers are, at night, luminous openings contrasting with the dense surrounding forest. Thus, light could then be a sensory cue that leads infected arthropods to an aquatic environment (Henze and Labhart, 2007). Secondly, besides this ecological reasoning, proteomics data reveal a differential expression of protein families (i.e. CRAL_TRIO) that may be functional components of the visual cycle in the CNS of Nemobius sylvestris harbouring Paragordius tricuspidatus (Biron et al., 2006a). Interestingly, the altered expressions of these proteins were only observed at a key period of the manipulative process, which is when crickets harbour a mature hairworm and when they attempt to enter water, but not in ex-infected insects (Biron et al., 2006a). Thus, this particular parasito-proteomics study suggests possible reversibility of the manipulative process by a parasite. Hosts can recover from the modified phototaxis after parasite emergence owing to the fact that the host cricket and parasitic worm no longer physiologically interact. This assumption was recently confirmed during a pioneering behavioural study (Ponton et al., 2011) which has, in turn, led to further such studies on the possible reversibility of parasite manipulation (Eberhard, 2010).

**Pitfalls and lessons of previous studies**

Early parasito-proteomics studies on parasitic manipulation contributed to finding candidate genes and biochemical pathways potentially involved in the manipulation process of a host by its
Proteomics and parasite manipulation

However, there is no doubt that more research is needed to fully disentangle the mechanisms involved in such host behavioural changes induced by parasites. For example, in the above-mentioned previous studies, there are at least three major missing data sets on the host CNS response during the manipulative process by a parasite, namely: (1) the insoluble proteome; (2) the neuropeptidome; and (3) the host proteome response in a pH range of \( \leq 4 \) and \( \geq 7 \). Furthermore, functional analysis in synergy with behavioural and interactome bioassays is necessary to determine the key role (or not) of the candidate proteins and/or peptides. Thus, an integrative parasito-proteomics approach is necessary to bridge the gap in our knowledge on the molecular cross-talk expressed between a host and its parasite during the manipulative process.

There are two important questions to ask before doing a parasito-proteomics study on parasite manipulation: (1) which host–parasite association(s) should one study and (2) how many such associations should one study? It is preferable to study a host–parasite association yielding behavioural data and scientific literature on the host aberrant behaviour and complete deciphering of genome sequences for the two partners (i.e. the host and the parasite) to avoid the pitfalls and the problems for protein identification. Otherwise it will be obligatory to use a laborious cross-species identification method (Barrett et al., 2005; Biron et al., 2006a). The minimum number of host–parasite associations to use in a parasito-proteomics study depends on the topic that a researcher wishes to explore. As far as the topic of evolutionary convergence is concerned, at least two host–parasite associations showing similar host behavioural changes induced by phylogenetically unrelated parasites experiencing similar selective pressure are needed. To study intraspecific variation of manipulative process in a host–parasite association, at least two phenotypes of a given host are required (i.e. a high ability, as well as a no ability to oppose parasite manipulation) and also two phenotypes for the parasite (i.e. efficient and inefficient manipulator). By making all the combinations of phenotypes for the host–parasite association, and by analysing the cross-talk for each combination, it is possible to determine the ‘key’ genes and proteins involved in the intraspecific variation involved in the manipulative process. Thus, to study any topic of parasite manipulation using proteomics tools (i.e. evolutionary convergence, intraspecific variation in manipulative

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**Fig. 6.** Integrative approach to decipher manipulative strategies with ‘parasito-proteomics’. See List of abbreviations for definitions.
process, sabotage of parasitic manipulation, etc.), serious planning is needed to choose the association(s) and to determine the minimal number of host–parasite associations required.

Choice of compartments to study manipulative strategies of parasites
An important question is which tissues (i.e. compartments) are preferable for a parasito-proteomics study on parasite manipulation. This depends on the localization of the parasite in the host. But whichever host–parasite association is studied, it is crucial to analyse the host CNS proteome and the neuuropeptide since the CNS functions to convert patterns of activity in sensory receptors into patterns of muscle activity that constitute appropriate behaviour. Hence, any changes in host behaviour must have a molecular basis in the host CNS (Adamo, 1997; Adamo, 2002; Hamilton and Hurd, 2002; Thomas et al., 2005). For a manipulator parasite located, for instance, in the cavity of an arthropod host abdomen, it is also very important to study the host proteome and host peptidome for the haemolymph compartment in order to find molecules potentially involved in the host–parasite cross-talk during the manipulative process.

New tools to decipher the proximate mechanisms
Using the first generation of proteomics tools (i.e. 2-DE and MS), the early parasito-proteomics studies on parasite manipulation provided many new insights into the manipulative process. However, several new proteomics tools have since been developed that can be used to collect novel data sets to understand, as well as decipher, the manipulative process. As an example of such new approaches, in order to study the peptidome response of the host CNS during a biological event or during host–parasite cross-talk, it is possible to use mass spectrometry (i.e. MALDI-TOF and ESI-Q-TOF) in positive ion mode (Predel et al., 2004). In previous studies, no data had been obtained concerning these key molecules (i.e. immune peptides and neuropeptides), which influence all the physiological processes involved in the expression of any given (i.e. immune peptides and neuropeptides), which influence all the topics associated with parasite manipulation: i.e. (1) measurement of the intensity of host manipulation within and between host populations; (2) testing the molecular hypothesis; (3) discovering biomarkers linked to a particular habitat and/or to environmental conditions; and (4) testing the hypothesis of local specialisation during manipulative strategies.

Five year view in deciphering and understanding proximate mechanisms
Host–parasite interactome
The past few years have witnessed the birth of new biological entities named interactomes. In an ‘ideal world’, they correspond to the complete set of protein–protein interactions existing between all the proteins of an organism (Biron et al., 2006b). Although the deciphering of the interactomes of main model organisms (i.e. yeast, nematode, Drosophila and human) so far investigated is not yet complete, studies of the interactomes of parasites are increasing (Biron et al., 2011). It is thus likely that in the near future, as initiated by Uetz et al. (Uetz et al., 2006), the ‘docking’ (i.e. physical protein and molecular genetic interactions) of the interactomes of parasites onto those of their hosts during the manipulative process will be possible. The analysis of the host–parasite interactome (i.e. ‘docked interactomes’) during the manipulative process of the host phenotype to ensure the completion of a parasite’s life cycle is certainly a very promising and exciting aspect of ‘interactomics’ because of its obvious potential impacts on human and animal health. The deciphering of host–parasite interactomes will allow identification of host and parasite protein networks related to specific functions during their interaction.

Moreover, a recent labelling method, SILAC (stable isotope labelling by amino acids in cell culture), opens up new avenues to decipher host–parasite cross-talk involved in parasite manipulation. SILAC is now widely used to quantify protein abundance in tissue culture cells (e.g. Ong et al., 2002; Mann, 2006). Hayter et al. demonstrated that chickens can be partially labelled at the amino acid level by feeding them with a diet containing stable isotope-labelled valine (Hayter et al., 2005). More recently, Krüger et al. achieved essentially complete labelling of laboratory mice by feeding them a diet containing a stable isotope (Krüger et al., 2008), while Sury et al. did the same with laboratory fruit flies (Drosophila) (Sury et al., 2010). Up until now, so-called ‘SILAC mouse’ and ‘SILAC fly’ represent the very few multicellular organisms completely labelled via this approach. But fascinatingly, in relation to the present article, SILAC can also be used to label host and/or parasite in order to follow, and thereby decipher, the host–parasite cross-talk taking place during parasite–host manipulation, as well as when a host counters parasitic manipulation itself. Hence, SILAC provides a promising opportunity to help in the identification of the host–parasite interactomes related to manipulative strategies used by parasites to ensure completion of their life cycle (Fig. 7).

Microbiome and parasite manipulation
Each and every metazoan species is a residence for a multitude of commensal and mutualistic microbial species. These species represent the normal host microbiota, which have coevolved with their hosts over millions of years (Margulis, 2003). The metazoan host represents several distinct niches for microbial species: for example, skin and the intestinal, respiratory and urogenital tracts. Recent studies suggest that the microbiota are intimately involved...
in modulation of the maturation and function of both the central and peripheral nervous systems, ultimately leading to modulation of host behaviour (Collins and Bercik, 2009; Cryan and O’Mahony, 2011). Thus, when the intestinal tract of a host is infected by a manipulative parasite species, the microbiota–manipulative-parasite undoubtedly influences molecular cross-talk between the host microbiota and the invading organism. The microbiota will have additive effects on the host’s CNS, altering its behaviour, or, alternatively, the effective molecules of the manipulative parasite will be sabotaged by the microbiome (Fig. 8).

No data are yet available on the cross-talks between the host and its microbiota and the manipulative parasite. Understanding and deciphering such cross-talks from cell to ecosystem in interactions involved in temporal sharing of host niches by microbiota and manipulative parasites is an interesting and promising avenue. It will unquestionably lead to the collection of new data on the influence of manipulative parasite species on the temporal dynamics of sympatric parasite species, including microbial communities inside an individual, within a host population, as well as on the biodiversity dynamics in a given ecosystem. In light of this, it is therefore important for parasitologists to examine the entire community of parasites in manipulated hosts, to study and decipher the proximate factors that mediate cooperative and conflicting relationships between parasites and the microbial community sharing a manipulated host.

**Population proteomics**

What exactly the host–parasite cross-talk produces at both the individual and population scales within a habitat is a fascinating question, one that is slowly being answered using a plethora of modern proteomics approaches. Indeed, it might be asked whether it is actually possible to detect and to decipher the variability of the host and parasite proteome responses within a habitat during the manipulative process. One limiting factor of the first generation of proteomics tools, such as 2-DE, was the amount of proteins required to study the host and/or parasite proteome expression(s) during their interactions. Most surveys were done by pooling many individuals for each treatment required to answer a particular query. With this kind of experimental protocol, no data could be acquired on the inter-individual variation relating to the expression of host and parasite proteomes during the parasite manipulation process.

Newer proteomics tools and methods, such as 2D-LC/MS and MSIA, can, by contrast, allow study of the inter-individual variation of molecular cross-talk in host–parasite associations (Brand et al., 2005; Nedelkov, 2005; Nedelkov, 2006; Predel et al., 2004).

At the beginning of the present century, Dobrin Nedelkov proposed a new scientific field in proteomics: population proteomics (Nedelkov, 2005). He defined population proteomics as the study of protein diversity in human populations, or, more specifically, targeted investigation of human proteins within and across populations to define and understand protein diversity, the main aim being to discover disease-specific protein modulations (Nedelkov, 2008). Biron et al. have proposed to broaden the ‘population proteomics’ concept to all living organisms with the aim of complementing population genetics (Biron et al., 2006c). Such a holistic approach offers a new avenue to decipher the cross-talk diversity involved in trophic interactions within a habitat. In the present context, this includes the manipulative strategies used by parasites to ensure the completion of their life cycle, since the execution of the genetic plan is carried out by the activities of proteins, and natural selection acts initially at the protein (phenotypic) level (Karr, 2008; Cieslak and Ribera, 2009). The apparent separation between genomics and proteomics that leads to different perspectives on the same ecological reality is a fundamental limitation that needs to be overcome if complex processes such as adaptation, parasite virulence and parasite manipulation are to be understood.

**Parasite manipulation and environment**

In relation to the parasite manipulation concept, the main assumption is that, over ecological timescales, host ability to counter parasite manipulation and, in turn, parasite ability to manipulate host behaviour are fixed at the onset of the initiation of cross-talk (Bull, 1994; Dieckmann et al., 2002; Poulin, 2010). Furthermore, environmental factors are traditionally viewed as ‘setting the scene’ for the cross-talk rather than having any explicit role once it is underway. As a result, the effect of extrinsic factors on host opposition to manipulation and a parasite’s ability to manipulate during the cross-talk has received little attention. Even so, it is common to find a substantial variation in host manipulative...
ability in populations of a parasite species, even when parasites are collected in the same environment and at the same time (Thomas et al., 2005). When a character such as manipulative ability is variable, both in terms of genetic and environmental factors, two individuals may differ because they differ in genotype, because they have had different environmental experiences, or both. Unfortunately, the extent to which different individual parasites and parasite ecotypes display different manipulative abilities is as yet poorly documented and deciphered.

The biological phenotype of an organism is not directly related to the genotype because of epigenetic information (Wolffe and Matzke, 1999). Epigenetic pathways control and modify gene expression. Almost all the elements of epigenetic control pathways are proteins (Anderson and Anderson, 1996). What happens with the epigenetic pathways of the host and of its manipulative parasite during their cross-talk? We stand on the threshold of a new research area, an important area that can yield profound insights into the understanding of parasite manipulation. There is a growing body of evidence indicating that parasites can also have trans-generational consequences; with infection of a host leading to changes in the phenotype of its offspring, though the latter are not parasitized (Hakkarainen et al., 2007; Poulin and Thomas, 2008). Poulin and Thomas assume that epigenetic pathways involving the turning ‘on’ or ‘off’ of genes may represent a general proximate mechanism for trans-generational modulation of host phenotype (Poulin and Thomas, 2008).

Life-history traits of hosts and parasites are shaped by co-evolutionary processes (Wolinska and King, 2009). Parasite manipulation measured under laboratory conditions has shown that the environment in which hosts and parasites interact may affect the range of host genotypes that can be manipulated with a given parasite genotype in host–parasite associations (Thomas et al., 2005; Poulin and Thomas, 2010). Despite this important fact, environmental fluctuations and epigenetics are often excluded in surveys on parasite manipulation. Since most host–parasite interactions occur in heterogeneous environments, there is a pressing need to take into account environmental conditions in parasito-proteomics surveys in relation to the parasite manipulation concept (Fig. 9). Population proteomics is a promising approach to resolve interesting issues specific to host–parasite cross-talk during the manipulation of a host by its parasite in a varying environment. This kind of survey would involve the gathering of pioneer molecular data in order to understand exactly why parasites sometimes evolve in a given environment toward acquiring a high ability to manipulate and hosts toward the opposite extreme, namely to counter such manipulation. Also, these surveys would allow assessment of the stability of host–parasite interactomes involved in a host–parasite association during the parasite manipulative process in an otherwise varying environment.

Concluding remarks

In recent years, the first generation of proteomics tools has been successfully used to study parasite manipulation in several model arthropod host–parasite associations. These pioneer studies have shown that proteomics tools are sensitive enough to disentangle the host proteome response during the host–parasite cross-talk, along with the parasite proteome response related to various factors including the circadian cycle, parasite status, infective stage and manipulative process. One critical result of these studies suggests that hairworms, for example, can produce host mimetic molecules that act directly on the host CNS. In addition, such studies have, for the first time, allowed questions of molecular convergence to be tackled in relation to the proximate mechanisms used by parasites to alter the behaviour of arthropod host species. Many candidate genes and biochemical pathways potentially involved in alteration of host behaviour by phylogenetically unrelated parasite species have been discovered in this way. Yet there is no doubt that more research is needed to fully disentangle the molecular mechanisms involved in the alteration of host behaviour by a parasite. In particular, these include the host and parasite peptidome responses during the manipulative process, which are very important gaps in the previous studies, because peptides are important messenger molecules that influence nearly all physiological processes. Thus, whatever the new technological advances, it is clear that both parasitologists and molecular biologists should attempt to improve their experimental design by taking into account the environmental factors involved in such interactions. This new approach will surely improve the reliability of the data derived from proteomics studies and will open the way for an enhanced comprehension of the intricacies of parasite manipulation.

List of abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CNS</td>
<td>central nervous system</td>
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<td>2-DE</td>
<td>two-dimensional electrophoresis</td>
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<tr>
<td>2-DIGE</td>
<td>two-dimensional difference in gel electrophoresis</td>
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Hughes, D. P. (2013). Pathways to understanding the extended phenotype of parasites in their hosts. *J. Exp. Biol.* 216, 142-147.


