The Journal of Experimental Biology 216, 148-160

doi:10.1242/jeb.073825

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.

Received 17 April 2012; Accepted 13 August 2012

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 hence 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 sitmuli, 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 zooparasite *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 gammarid 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 or 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 of the chart 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

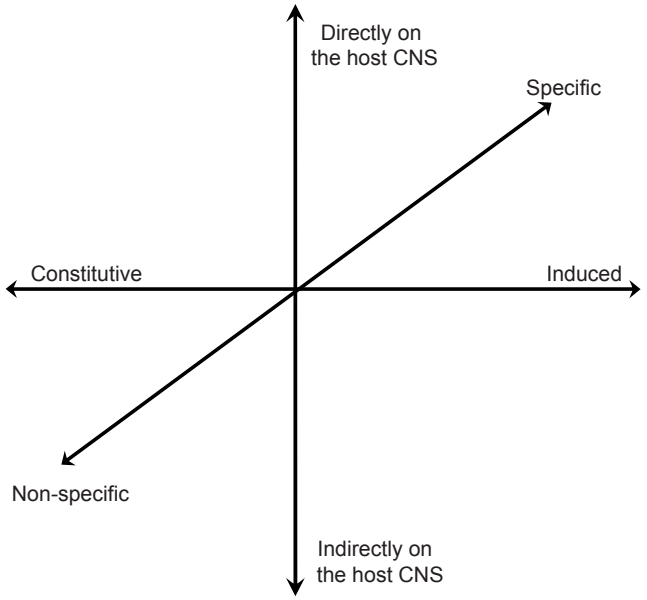

Fig. 1. Manipulative tactics used by parasites to alter phenotypic traits of their hosts.

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 parasite 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).

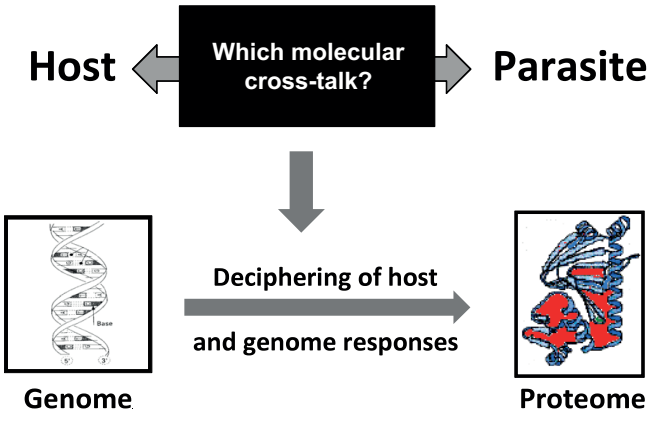

Fig. 2. Parasito-proteomics, a promising approach to decipher host–parasite cross-talks.

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.

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)! Previous field experiments have shown that water-seeking behaviour exists in two Orthoptera–hairworm associations in the same natural habitat of southern France: (1) the cricket, *Nemobius sylvestris* (Bosc) (Orthoptera, Gryllidae), parasitized by the hairworm *Paragordius tricuspidatus* (Dufour) (Nematomorpha, Gordiidae), and (2) the long-horned grasshopper, *Meconema thalassinum* (De Geer) (Orthoptera, Tettigoniidae), parasitized by the hairworm *Spinochordodes tellinii* (Camerano) (Nematomorpha, Spinochordidae) (Thomas et al., 2002). Because the size of the parasites is very large relative to their orthopteran hosts (i.e. hairworm length exceeds that of the host by 3–4 times), it is very easy to separate the host and the parasite, thereby allowing the simultaneous study of proteomes of both animals without the risk of contamination.

The proteome of *M. thalassinum* reacts more strongly than the proteome of *N. sylvestris* to the manipulative process by its hairworm (Fig. 3A) (Biron et al., 2005c; Biron et al., 2006a). For the hairworms, the percentage of qualitative proteome related to the manipulative process is similar (Fig. 3B) (Biron et al., 2005c; Biron et al., 2006a). In Figs 4 and 5, the altered functions during the manipulative process for the two Orthoptera–hairworm associations are shown, while the protein family of the identified proteins is given in Table 1 (Biron et al., 2005c; Biron et al., 2006a). The altered functions are similar for both Orthoptera species except for some families of proteins, particularly those involved in geotactic behaviour (Armstrong et al., 2006; Bland et al., 2009), protein biosynthesis and recovery following an infection, but are differentially expressed in *M. thalassinum* (i.e. are qualitatively and, to some extent, quantitatively different in terms of gene expression between the two hosts). Thus, in the CNS of *M. thalassinum* and *N. sylvestris*, differential expression of proteins specifically related to neurogenesis (i.e. an increase in neurogenesis

Table 1. Synopsis of parasito-proteomics studies

Host–parasite association		Proteomics tools			Pfam of proteins identified*		
Host species	Parasite species	Protein separation	Isoelectric point; molecular mass	Protein identification	In head of host	In parasite	Reference
Nemobius sylvestris (Bosc) (Orthoptera, Gryllidae)	*Paragordius tricuspidatus* (Dufour) (Nematomorpha, Gordiidae)	2-DE	pH 5–8; 19–122 kDa	MS, MS/MS, protein sequencer	6-phosphogluconate dehydrogenase, actin; 1, ATPase α/β, BIR; 2, calcineurin-like phosphoesterase, Clathrin_lg_ch; 1, Collin_ADF; 1, CRAL_TRIO_C, DUF52; 1, glycosyltransferase O-Fuc, GST_N; 1, NAD_Gly3P_dh; 1, PCI; 1, PGAM; 1, Ras; 1, SHMT; 1, transposase, trypsin, Wnt 1	DUF976; 1, ECH; 1, F-box; 1 Glycoside hydrolase, KH; 1 PCI; 1, PIR; 1, proteasome; 1, tubulin; 1, WD40; 4, Wnt;1 Zf-C_3CH_4; 1	Biron et al., 2006a
Meconema thalassinum (De Geer) (Orthoptera, Tettigoniidae)	*Spinochordodes tellinii* (Nematomorpha, Spinochordodidae)	2-DE	pH 5–8; 19–122 kDa	MS	Actin; 1, band_41; 1, DnaJ; 1, flotillin; 1, Ig; 2, Ribosomal_L10e; 1, SNAP-25; 1, SNARE; 1,Tubulin; 1, Wnt; 1, Zf-C_2H_2; 6	ATP-gua_Ptrans; 1, bestrophin; 1 biopterin_H; 1, CARD; 1, CPN60_TCP1; 1, DS; 1, filament; 1, FKBP_C; 1, G-alpha; 1, HGTP_anticodon; 1, Kunitz_BPTI; 2NOA36; 1, Sec1; 1, troponin; 1, tubulin_C; 1, Wnt; 1, Zf-C_2H_2; 8	Biron et al., 2005c
Anopheles gambiae (Giles) (Diptera, Culicidae)	*Plasmodium berghei* (Haemosporida, Plasmodiidae)	2D-DIGE	pH 3–10; 14–100 kDa	MS, MS/MS	14-3-3, ADK, annexin, BSD, COX5A, Efhand; 3, HAD-SF_hydro_IIA, HSP20, PGAM; 1, tropomyosin	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	AAA, C2, CK_II_beta, Concanvalin A- like, Enolase, G6PD_C, MAM, Pkinase, Pyridoxal_dec; 1, Znf_C_2H_2; 6	No data	Lefèvre et al., 2007b
Gammarus insensibilis (Amphipoda, Gammardiae)	*Microphallus papillorobustus* (Trematoda, Microphallidae)	2-DE	pH 3–6; 20–122 kDa	MS	Acetyltransf_1; 1, ATP-gua_Ptrans; 1, Carb_anhydrase; 1, CRAL_TRIO; 1, CUB; 1, Efhand; 3, Histone; 1, P450; 1, PBP_GOBP; 1, Pyridoxal_deC; 1, SGS; 1	No data	Ponton et al., 2006
Gammarus pulex (Amphipoda, Gammardiae)	*Polymorphus minutes* (Acanthocephala, Polymorphidae)	2-DE	pH 3–6; 20–122 kDa	MS	ATP-gua_Ptrans, EIF-5a, Haemocyanin, KOW, MAM, Ras, Sushi,TFIIE; 1, tropomyosin	No data	Ponton et al., 2006

*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.

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.

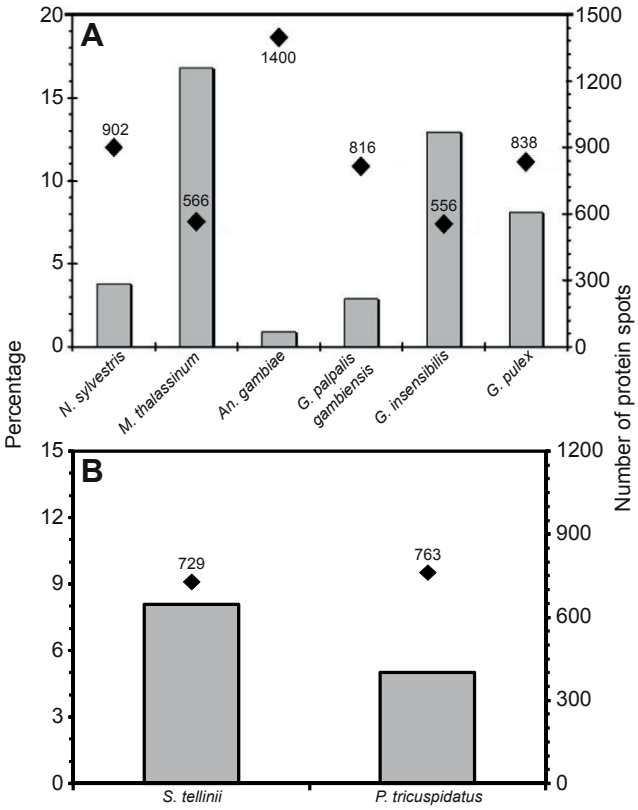

Fig. 3. Total number of protein spots detected (black diamonds) and percentage of protein spots differentially expressed (grey bars) in (A) the head proteomes of arthropod hosts and (B) the proteomes of manipulative hairworms during the expression of the water-seeking behaviour.

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 Jefferies, 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 palpalis 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 (Lefèvre et al., 2007a; Lefèvre et al., 2007b). For the two dipteran species, the qualitative proteome of *G. palpalis 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-vector–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

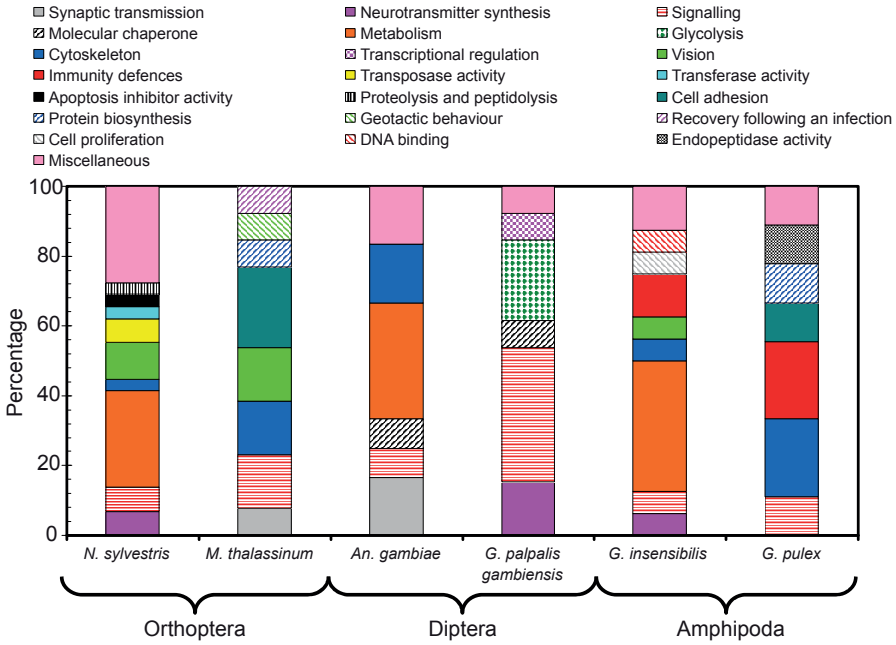

Fig. 4. Proportion of identified proteins related to a biological process and differentially expressed in the head proteomes of arthropod hosts during the manipulative process.

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.,

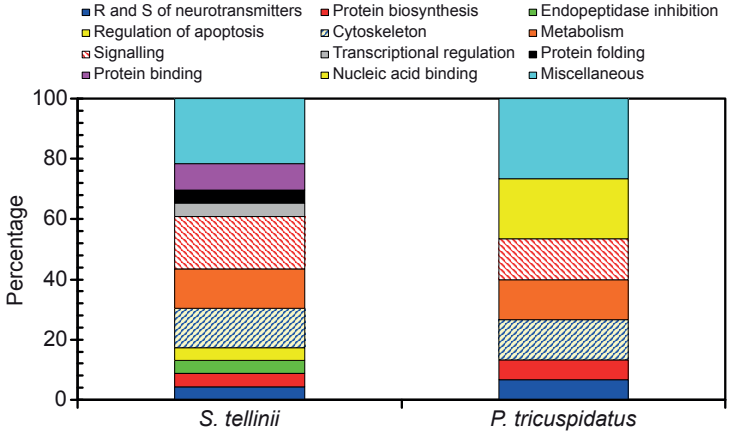

Fig. 5. Proportion of identified proteins related to a biological process and differentially expressed in the proteomes of manipulative parasites.

2005c; Biron et al., 2006a; Lefèvre et al., 2007a; Lefèvre et al., 2007b; Ponton et al., 2006). Many parasite species evolve under similar selective pressures in terms of the completion of their life cycle, exploiting either the same host species in the same sequence or different host species but in a similar context. There is, *sensu strico*, evolutionary convergence when similar behavioural changes are induced by phylogenetically unrelated parasites experiencing similar selective pressure. Thus, do phylogenetically distant parasites use the same molecular mechanisms to induce similar behavioural changes in their host? Parasito-proteomics studies performed on insect-vector–pathogen associations (Lefèvre et al., 2007a; Lefèvre et al., 2007b) and on gammarid–parasite associations (Ponton et al., 2006) have both highlighted convergence of the physiological and molecular mechanisms causing alteration of arthropod host behaviour. In the two insect-vector–pathogen associations studied so far, the manipulative process led to an aberrant host feeding behaviour (i.e. ~350 times increase of probing rate), while in the two gammarid–parasite associations, the manipulative process caused an aberrant host evasive behaviour. Interestingly, in these two cases of evolutionary convergence, the studies suggest that many of the functions in the host CNS altered during the manipulative process are similar but some are also different and thus apparently specific to the host–parasite association in question (see Table 1 and Fig. 4). For the two orthopteran–hairworm associations, it is not a true case of 'evolutionary convergence' as the two hairworms are phylogenetically related; but, interestingly, although many similar functions of the host CNS are indeed altered, to induce the water-seeking host behaviour, some biochemical changes are seen to be specific to a given orthopteran–hairworm system (see Table 1; Figs 4, 5).

To date, parasito-proteomics studies have only been performed on arthropod host CNS. The arthropods in question have many similarities in terms of structure and function of their CNS. Thus, in light of this, a major question is whether or not phylogenetically related parasites use the same biochemical pathways to alter a function process (i.e. compartment in the host CNS) causing a similar aberrant behaviour? For instance, as seen in manipulated *G. insensibilis*, a differential expression of proteins for the CRAL-TRIO domain involved in the detection of light was observed in the CNS of the cricket *N. sylvestris* manipulated by the nematomorph *P. tricuspidatus*. Here, two phylogenetically distant parasites rely on the same molecular mechanism to alter vision in their arthropod hosts, in turn inducing different alteration of host behaviour and thereby enhancing their (the parasite's) developmental success. Also, the modulation of host apoptosis pathways seems to involve a common molecular mechanism related to host behavioural modifications. Many key genes associated with behavioural traits appear conserved during evolution across a range of host–parasite life histories, not only in terms of their sequences but also in their functions (Fitzpatrick and Sokolowski, 2004; Fitzpatrick et al., 2005; Pennisi, 2005). These pioneer parasito-proteomics studies offer new candidate genes and new biochemical pathways potentially involved in parasite manipulation of host feeding behaviour, both for host-evasive as well as water-seeking behaviours. Based on these results, PCR approaches using degenerate primers can be used to study parasite manipulation in other host–parasite associations showing similar alterations in host behaviour. Such studies open the way to understanding the evolutionary convergence of proximate mechanisms and of the molecular cross-talk involved in manipulation of a host by its parasite, including in phylogenetically unrelated species.

Molecular mimicry

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 (Lambris et al., 2008; Srinivasappa 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 orthoperan–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-protemics 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

Blueprint to make a parasito-proteomics study

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

parasite. 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 ≤4 and ≥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. Fig. 6 outlines the main steps to any parasito-proteomics study of parasite manipulation.

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

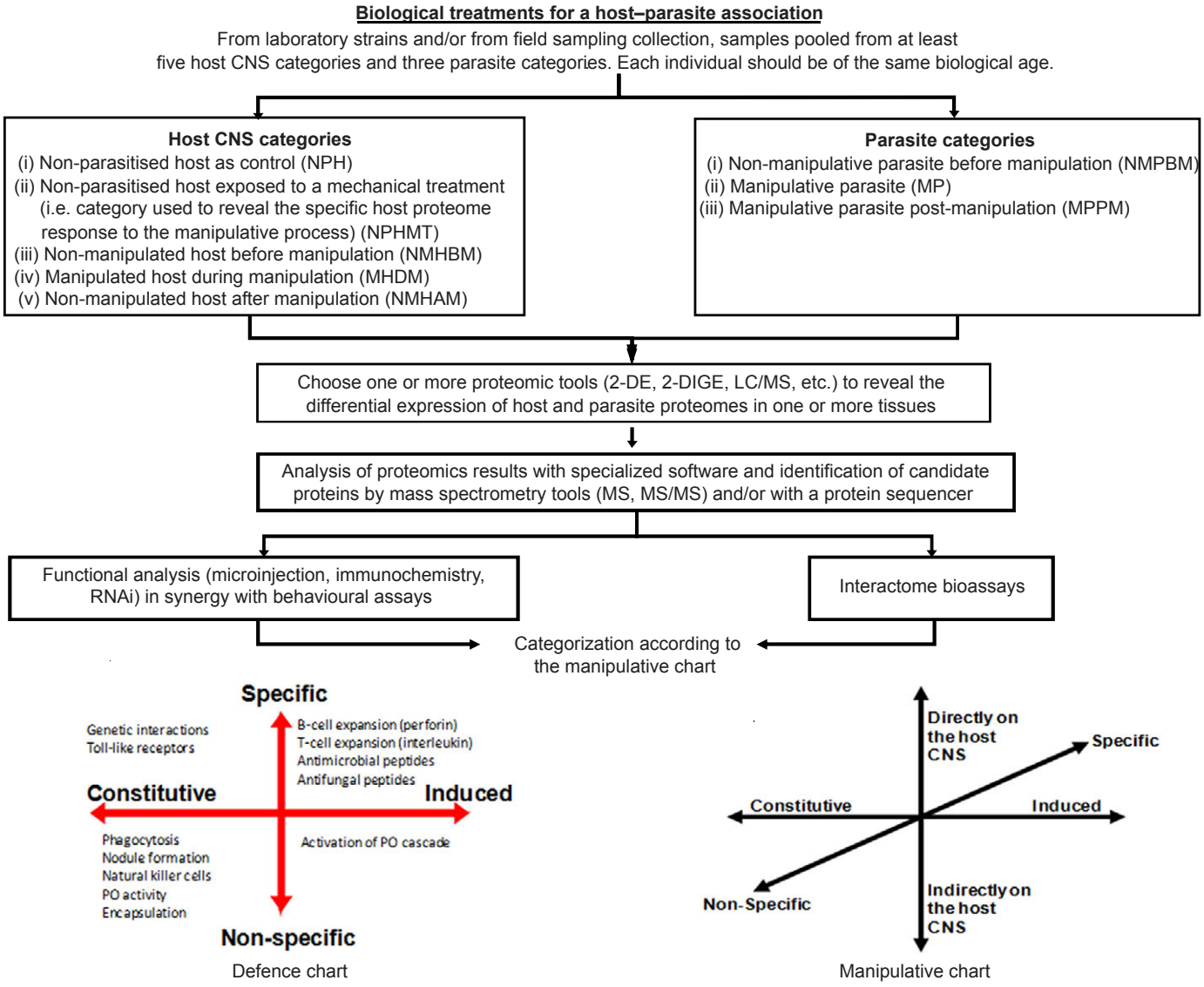

Fig. 6. Integrative approach to decipher manipulative strategies with 'parasito-proteomics'. See List of abbreviations for definitions.

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 neuropeptidome 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 host behaviour. For the proteins with a molecular mass of >20 kDa, multi-dimensional LC/MS offers a promising alternative and complementary approach to 2-DE for the analysis of complex protein mixtures for different physiochemical properties. Multidimensional LC/MS has increased in popularity because this technique is relatively straightforward, and effective analysis software is available because, once protein fractions are ‘spotted’ on MALDI targets, there are no time constraints in terms of performing further analyses for identification of the proteins involved (Brand et al., 2005; Greibrokk et al., 2005). However, to analyse the differential expression of common proteins between different treatments, 2D-DIGE remains a very useful option. The integrative approach presented in Fig. 6 permits exploration with LC/MS, SELDI-TOF, MALDI-TOF and ESI-Q-T of the host and/or parasite peptidomes expressed as a consequence of the manipulative process.

Functional analysis, in synergy with behavioural and/or interactome bioassays, provides two essential steps suggested in this integrative approach (Fig. 6). This allows confirmation of the key role of candidate proteins identified in previous pioneer studies and for any future parasito-proteomics study on the parasite manipulation hypothesis. Furthermore, these two key steps permit confirmation and/or determination of the many biochemical pathways involved in the alteration of host behaviour by a parasite. Finally, such integrative approaches allow much new data to be collected that are helpful in the study and understanding at a larger scale of the molecular cross-talk taking place in host–parasite associations – and with greater efficiency too – concerning many of 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

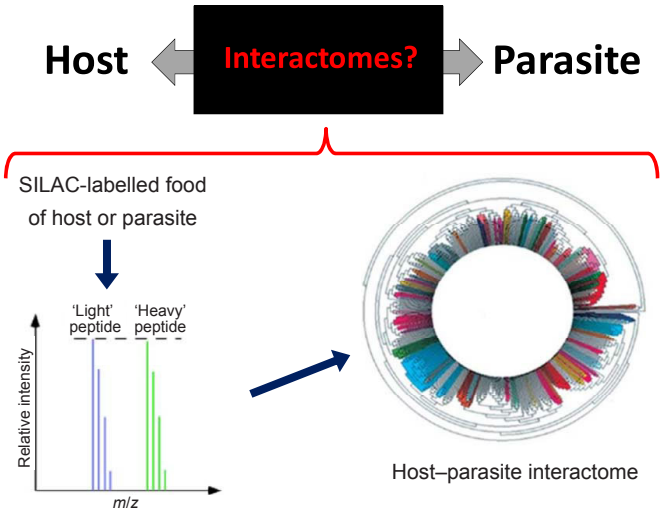

Fig. 7. Determination of the host–parasite interactome using SILAC-labelled food.

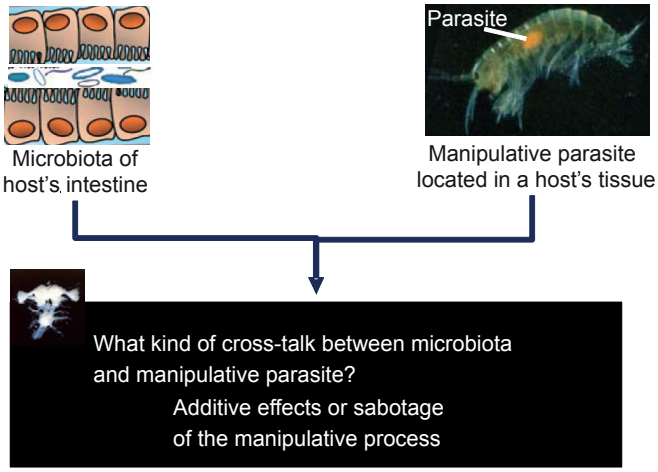

Fig. 8. Impact of host microbiota on the manipulative strategy used by a parasite to alter the phenotype trait(s) of its host.

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 broadening 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

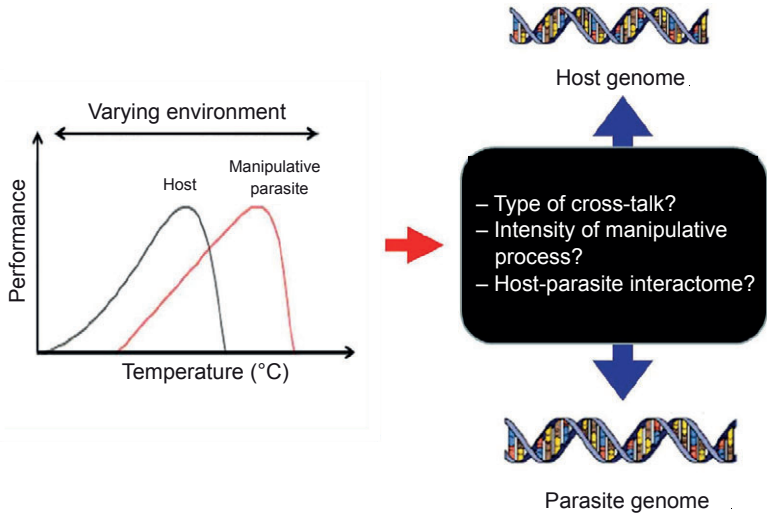

Fig. 9. Parasite manipulation in a varying environment.

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, 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

CNS	central nervous system
2-DE	two-dimensional electrophoresis
2-DIGE	two-dimensional difference in gel electrophoresis

2D-LC/MS	two-dimensional liquid chromatographic separation of peptides with tandem mass spectrometry detection
ESI-Q-TOF	electrospray mass spectrometry
LC/MS	liquid chromatography–mass spectrometry
MALDI	matrix-assisted laser desorption/ionization
MALDI-TOF	matrix-assisted laser desorption/ionization–time of flight
MS	mass spectrometry
MSIA	mass spectrometric immunoassay
MS/MS	tandem mass spectrometry
PO	phenoloxidase
RNAi	RNA interference is a process within living cells that moderates the activity of their genes
SELDI-TOF-MS	spectrum-enhanced laser desorption ionization time of flight mass spectrometry
SILAC	stable isotope labelling by amino acids in cell culture

Acknowledgements

D.G.B. thanks the members of the IHP team for many helpful discussions during the writing of this manuscript and sincerely thanks Joanne Webster, Shelly Adamo, the editors of JEB and The Company of Biologists for inviting him to attend the special meeting on neuroparasitology that directly led to this Review.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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