The Journal of Experimental Biology 216, 27-35

doi:10.1242/jeb.074005

REVIEW

Multidimensionality in parasite-induced phenotypic alterations: ultimate *versus* proximate aspects

Frank Cézilly[1,2,*], Adrien Favrat[1] and Marie-Jeanne Perrot-Minnot[1]

[1]Université de Bourgogne, Equipe Ecologie Evolutive, UMR CNRS 6282 Biogéosciences, Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France and [2]Institut Universitaire de France

*Author for correspondence (frank.cezilly@u-bourgogne.fr)

Summary

In most cases, parasites alter more than one dimension in their host phenotype. Although multidimensionality in parasite-induced phenotypic alterations (PIPAs) seems to be the rule, it has started to be addressed only recently. Here, we critically review some of the problems associated with the definition, quantification and interpretation of multidimensionality in PIPAs. In particular, we confront ultimate and proximate accounts, and evaluate their own limitations. We end up by introducing several suggestions for the development of future research, including some practical guidelines for the quantitative analysis of multidimensionality in PIPAs.

Key words: adaptationism, host manipulation, parasite.

Received 17 April 2012; Accepted 6 September 2012

Introduction

Parasites can bring about various phenotypic alterations in their hosts that appear to increase their own fitness at the expense of that of their hosts (Moore, 2002; Thomas et al., 2005). In particular, parasites with complex life cycles often modify the behaviour and/or the appearance of their intermediate hosts, in ways that appear to increase trophic transmission to final hosts (Moore, 2002; but see Shirakashi and Goater, 2005; Leung and Poulin, 2006; Fermer et al., 2011). Such phenomena are regularly interpreted in relation to the concept of extended phenotype introduced by Dawkins in his eponymous book (Dawkins, 1982). Thus, according to the 'parasite manipulation' hypothesis, the ability of a parasite species to modify its host's phenotype is the product of natural selection acting on the genes of the parasite (Thomas et al., 2005; but see Cézilly et al., 2010).

Early studies of the impact of parasites on the phenotype of their host typically focused on a single trait at a time, such as modified reaction to light (Bethel and Holmes, 1973), reduced fecundity (Skorping, 1985), respiration (Rumpus and Kennedy, 1974) or altered pigmentation (Oetinger and Nickol, 1981). Most often, however, a single parasite species alters more than one phenotypic trait in its host. Recently, Cézilly and Perrot-Minnot (Cézilly and Perrot-Minnot, 2005) coined the term 'multidimensionality' to address this phenomenon. Following Thomas et al. (Thomas et al., 2010a), we consider here that the alteration of a host by its parasite is multidimensional if more than one phenotypic trait is altered, regardless of whether the altered traits belong to different categories (e.g. behavioural *versus* life-history traits). Multidimensionality in parasite-induced phenotypic alterations (PIPAs) is actually a widespread, if not systematic, phenomenon, and has generated a growing interest among parasitologists (Cézilly and Perrot-Minnot, 2005; Cézilly and Perrot-Minnot, 2010; Benesh et al., 2008; Thomas et al., 2010a; Thomas et al., 2012). Multidimensionality in PIPAs is of interest to evolutionary biologists as well, in direct relation to the evolution of complex life cycles (Parker et al., 2009). For instance, one crucial point for the understanding of the evolution of complex life cycles is to establish whether PIPAs are the cause or the consequence of infecting several hosts in succession (Cézilly et al., 2010). Multidimensionality in PIPAs is also of interest in ecology, in connection with the complex role of parasites in ecosystems (Thomas et al., 1997). Phenotypic alterations brought about by parasites in their hosts can have several consequences for ecosystems, e.g. by releasing the prey community from predation pressure (Sato et al., 2012) or by affecting the availability of trophic resources to plant and animal communities (Hernandez and Sukhdeo, 2008; Boze et al., 2012). However, although the influence of PIPAs on ecosystem functioning is widely acknowledged (Thomas et al., 1997; Hatcher et al., 2012), we do not know to what extent the ecological impact of a parasite is related to the number of phenotypic alterations it brings about in its host.

The 'adaptationist' view regards the multiple phenotypic alterations caused by parasites as effectively discrete, with each dimension that contributes to transmission being an adaptation designed by natural selection (Thomas et al., 2010a; Thomas et al., 2012). From a proximal point of view, in contrast, the various phenotypic alterations observed in infected hosts, independently of their effect on trophic transmission, may find their origin in a single or a few physiological processes (Thomas et al., 2010a; Cézilly and Perrot-Minnot, 2010), possibly linked (but necessarily limited) to the crosstalk between the immune system and the nervous system of the host (Adamo, 2002; Perrot-Minnot and Cézilly, 2012). Although the ultimate and proximate approaches to multidimensionality in PIPAs are not necessarily contradictory (Thomas et al., 2010b; Thomas et al., 2012), their differences are likely to improve our understanding of the evolution of host–parasite interactions.

In the present review, we first consider how multidimensionality manifests itself in various host–parasite interactions, with a particular emphasis on one particular host–parasite association for which several dimensions of the altered phenotype have been quantified. Second, we present various adaptive explanations that can be put forward to account for multidimensionality in PIPAs, before discussing various kinds of limitations associated with them. Third, we introduce a proximate perspective on multidimensionality in PIPAs and argue about its relevance to the question of adaptive manipulation of hosts by parasites. Finally, we propose some directions for future research with the hope of promoting a more integrative approach to PIPAs.

Multidimensionality of phenotypic alterations in infected hosts

Infection with parasites generally induces the alteration of several phenotypic traits, simultaneously or in succession. Simultaneous phenotypic alterations have been observed in a large number of host–parasite associations, involving for instance crustacean and/or insect intermediate hosts infected with acanthocephalans (Bakker et al., 1997; Fuller et al., 2003), cestodes (Franceschi et al., 2007) or trematodes (McCurdy et al., 1999; Leung and Poulin, 2006). However, the evidence for simultaneous multidimensionality in PIPAs is most often cumulative, as specific studies generally address only one or a few dimensions at a time. Still, particular host–parasite interactions have received more attention than others, eventually resulting through time in an impressive list of phenotypic alterations brought about by a single parasite in a single host species. For instance, no less than 15 different phenotypic alterations have been reported in the crustacean amphipod *Gammarus pulex* infected by the acanthocephalan parasite *Pomphorhynchus laevis* (Table 1). It is therefore likely that, in several cases, the number of PIPAs known for a given host–parasite association is more representative of the number of studies that addressed that association than of the true number of phenotypic alterations induced by the parasite. To test for such a confounding effect, we related the number of known PIPAs in 11 host–parasite associations [including crustacean–helminth, insect–nematomorph, insect–fungus, insect–nematode, insect–parasitoid and fish–cestode; table 1 in Thomas et al. (Thomas et al., 2010a)] to the natural logarithm of the number of papers published on the same associations, based on the information collected from the Web of Science, using the scientific names of both host and parasite species as key words. There was indeed a significant and positive relationship between the two variables (Spearman rank correlation coefficient, r_S=0.8809, P=0.0003; Fig. 1). Such a correlation is open to different interpretations. For instance, a specific parasite could induce changes that are dependent on the local context such that a positive correlation occurs simply because different studies are identifying different traits that function in different contexts. In this case, however, the total number of traits identified through the multiple studies would be an overestimate of the number of traits expected to function in any particular context. Hence the importance of addressing multidimensionality of PIPAs in empirical studies in order to assess to what extent multidimensionality is context-dependent. Alternatively, the correlation may arise because some host–parasite systems, being more abundant, more ubiquitous and easier to study in the field or to be maintained in the laboratory than others, attract more scrutiny. Whatever its interpretation, however, this observed correlation is problematic to some extent, as it would make comparative studies based on literature surveys relatively difficult to undertake. Still, it would be worth investigating at the interspecific level whether particular phenotypic alterations co-occur more often than by chance in infected hosts, after controlling for the effect of phylogenetic inertia, and, eventually, whether some alterations precede others historically (see Dubois et al., 1998; Pagel and Meade, 2006).

However, there is a risk that multidimensionality in PIPAs is overestimated when using cumulative evidence collected from different populations of infected hosts if the exact taxonomic identity of either the host or the parasite (or both) is not always correctly established. For instance, Perrot-Minnot (Perrot-Minnot, 2004) reported contrasting levels of altered reaction to light induced by two closely related, and previously confounded, species of acanthocephalans in their common amphipod intermediate host. Symmetrically, the existence of cryptic species in hosts (see Westram et al., 2011), eventually living in sympatry, may also affect the assessment of multidimensionality in PIPAs, as the effect of a parasite is known to vary between closely related species of intermediate hosts (Bauer et al., 2000; Bauer et al., 2005; Tain et al., 2007; Cornet et al., 2010). Ideally, then, such a study should be conducted in a single host–parasite system; a single system is advantageous in that it allows the researcher to assess whether all infected hosts express the same combination of modified traits at the same intensity. However, the potentially large number of PIPAs

Table 1. Multidimensionality in the phenotypic alteration induced by the acanthocephalan *Pomphorhynchus laevis* in its amphipod intermediate host, *Gammarus pulex*

Phenotypic trait	Alteration (relative to uninfected individuals)	Source
Response to olfactory predator cues	Reversal	Baldauf et al., 2007; Kaldonski et al., 2007
Reaction to light	Reversal	Cézilly et al., 2000
Activity	Increase	Dezfuli et al., 2003
Drift	Increase	McCahon et al., 1991; Lagrue et al., 2007
Pairing success	Decrease	Bollache et al., 2001; Bollache et al., 2002
Female fecundity	Decrease	Bollache et al., 2001; Bollache et al., 2002
Fluctuating asymmetry	Increase	Alibert et al., 2002
Oxygen consumption	Decrease	Rumpus and Kennedy, 1974
Immunocompetence	Decrease	Rigaud and Moret, 2003; Cornet et al., 2009b
Brain serotonergic activity	Increase	Tain et al., 2006; Tain et al., 2007
Appearance	Increased conspicuousness	Bakker et al., 1997; Kaldonski et al., 2009
Glycogen level	Increase	Plaistow et al., 2001
Carbohydrate titres in haemolymph	Increase	Bentley and Hurd, 1996
Hemolymph protein concentration	Increase	Bentley and Hurd, 1993
Protein and copper content of the midgut gland	Decrease	Bentley and Hurd, 1995

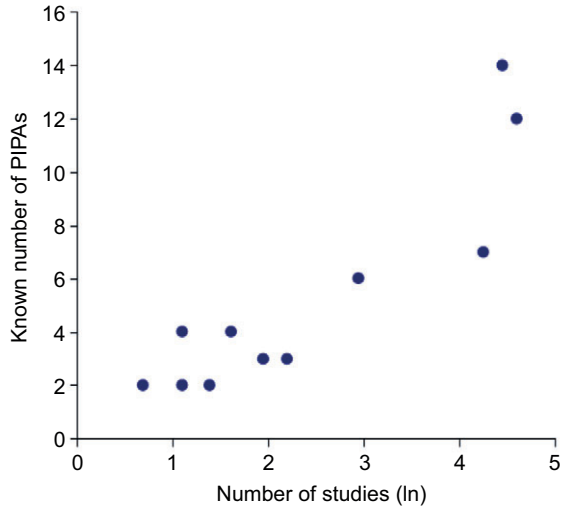

Fig. 1. Relationship between the number of known parasite-induced phenotypic alterations (PIPAs) and the number of published studies for 11 host–parasite associations (adapted from Thomas et al., 2010a).

in each host–parasite association might make such task a daunting one. Furthermore, although hosts are often infected by several parasites, visual examination of hosts for the presence of conspicuous macroparasites, such as acanthocephalans and cestodes, may overlook the presence of less detectable parasites such as protists, *Rickettsia*-like organisms and microsporidian parasites (see Messick et al., 2004). However, there is evidence that such parasitic organisms can affect the phenotype of their hosts and eventually interact with the effects of macroparasites (Haine et al., 2005).

Sequential multidimensionality, the alteration of several phenotypic traits in succession, appears to occur more rarely, but is particularly interesting. It might be useful to consider two types of sequential multidimensionality. In cumulative sequential multidimensionality, the various PIPAs add to one another through time, such that the number of altered dimensions in the host's phenotype increases with time since infection. However, the functional efficiency of one alteration does not appear to depend on the expression of other alterations. For instance, in the amphipod *Gammarus insensibilis* infected with the trematode *Microphallus papillorobustus*, increased lipid content can be observed before the parasite becomes infective for its final host, at which stage the infected intermediate host also shows altered reaction to light, negative geotaxis and aberrant escape behaviour (Ponton et al., 2005). In ordered sequential multidimensionality, the sequence of altered traits corresponds to some kind of dormant fixed action pattern, organised as a fixed, stereotyped, temporal sequence of behaviours, as suggested by Salwiczeck and Wickler (Salwiczeck and Wickler, 2009) in the case of *Formica* ants infected by the trematode *Dicrocelium dendriticum*. According to Salwiczeck and Wickler (Salwiczeck and Wickler, 2009), infected ants seek elevated places, crawling up twigs, and then use their mandibles to fasten themselves, moving upward in the evening and downward in the morning. Doing so, they actually exhibit a phylogenetically old sleeping behaviour, usually observed in non-social Hymenoptera (Wickler, 1976). Another example of ordered sequential multidimensionality is provided by the work of Eberhard (Eberhard, 2010) on the effect of the ichneumonid wasp *Polysphincta gufreundi* on the web-building behaviour of its host, the orb-weaving spider *Allocyctosa bifurca*. When infected by a larva of the parasitic wasp, the spider modifies its web-building behaviour in a gradual manner, with several distinct steps occurring in a consistent sequence. Interestingly, if the larva is experimentally removed, the spider returns progressively to a normal web-building behaviour following the reverse order (Eberhard, 2010).

Adaptive explanations for multidimensionality in PIPAs

Why should a parasite alter several dimensions in its host's phenotype? One answer is that some PIPAs evolved in consequence of their direct benefits for the parasite's fitness (at the expense of that of its host), whereas some others might simply be pathological by-products of infection (Thomas et al., 2010a; Thomas et al., 2012). If this is true, how might inducing multiple phenotypic alterations in its host benefit a parasite? Practically, as is often the case in behavioural ecology, the answer to that question is essentially limited by one's ability to build adaptive scenarios.

A first possibility is simply that two is better than one, three is better than two, etc. Intermediate hosts most often rely on various sensory modalities, such as vision, olfaction, and sound and vibration detection, to locate and avoid predators (Tikkanen et al., 1994; Wudkevich et al., 1997; Abjörnsson et al., 2000; Popper et al., 2001). Modifying more than one sensory modality may then increase the vulnerability of infected intermediate hosts to predation by final hosts. Accordingly, Bakker et al. (Bakker et al., 1997) found that both modified appearance and altered reaction to light in *G. pulex* infected with *P. laevis* act synergistically to increase trophic transmission to fish final hosts (but see below).

In addition, the efficiency of a phenotypic alteration in enhancing trophic transmission may vary according to local conditions. For instance, increasing the conspicuousness of the intermediate host by modifying its visual appearance might be of little consequence in environments with reduced light, such as turbid waters. Similarly, modifying the drift behaviour of aquatic intermediate hosts may have different consequences depending on current velocity. Parasites with complex life cycles may thus benefit from altering several dimensions in the phenotype of their hosts because multidimensionality ensures increased trophic transmission in a large range of environments.

In that respect, the number of dimensions that are altered should represent some optimal value, determined by the balance between the accrued benefits from multidimensionality and its potential costs (see Poulin et al., 2005). Alternatively, a trade-off may exist between the number of traits that are altered in the host and the efficiency with which each trait is altered. If this is true, one would expect a lower variation between infected hosts in the intensity of each alteration when only one or a few traits are altered, and a larger one when a large number of traits are modified. To date, however, no empirical study, to our knowledge, has addressed the question. One difficulty, though, might be to determine how many traits are actually modified by one parasite in its host.

Multidimensionality might also be related to the range of definitive hosts that are available to a parasite. For instance, *P. laevis* can use a very large range of fish species as intermediate hosts, including the bleak, *Alburnus baliki* (Aydogdu et al., 2011), the common barbel, *Barbus barbus* (Djikanovic et al., 2010; Dezfuli et al., 2011), the Turkish cyprinid *Capoeta antalyensis* (Aydogdu et al., 2011), the common nase, *Chondrostoma nasus* (Jirsa et al., 2011), the European chub, *Leuciscus cephalus*, the vairone, *L. souffia*, the European perch, *Perca fluviatilis* (M.-J.P.-M., unpublished data), the round goby, *Neogobius melanostomus* (Francova et al., 2011), the common minnow, *Phoxinus phoxinus*

(Dudinák and Spakulova, 2003), and the sheatfish, *Silurus glanis* (Dezfuli et al., 2011). This large diversity of final hosts includes species feeding nocturnally or diurnally, as well as ambush or cruising predators. Altering more than one dimension in host phenotypes may then expose it to a larger range of definitive hosts, and hence speed up trophic transmission. The relationship between multidimensionality and host diversity may eventually be tested by comparing the extent of multidimensionality in manipulation between parasites with trophic transmission differing in their specificity for final hosts. However, multidimensionality might be beneficial even if the parasite relies on a limited number of species as appropriate final hosts, when, for instance, the predatory behaviour of the latter shows some variation related to age (Graeb et al., 2006; Takeuchi, 2009).

A last possibility is that whereas some phenotypic alterations evolved as adaptations to enhance trophic transmission to appropriate final hosts, others actually evolved as adaptations to decrease the vulnerability of hosts to predation by non-host species (Médoc and Beisel, 2011) (but see below).

Limits to the adaptationist approach

Thomas et al. (Thomas et al., 2010a; Thomas et al., 2010b) suggested that the term 'multidimensionality' should be restricted to PIPAs that directly contribute to completion of the parasite's life cycle at the expense of its host's fitness. However, considering PIPAs as adaptations comes with three major problems that have been partly overlooked and, thus, need addressing. The first is directly linked to the limitations of the adaptationist programme as first emphasised by Stephen Jay Gould and Richard Lewontin in the famous 'spandrels' paper (Gould and Lewontin, 1979). The second, of practical concern, is linked to the very feasibility of demonstrating a causal relationship between increased probability of life-cycle completion and a single PIPA. And the third is directly related to reductionism, the definition of what constitutes a phenotypic trait and the existence of genes 'for' phenotypes (Kaplan and Pigliucci, 2001).

In its more extreme version, the adaptationist programme is 'an attempt to explain the existence and the particular forms of any phenotypic trait as the results of natural selection' (Pigliucci and Kaplan, 2000) (see also Forber, 2009). When applied to multidimensionality in PIPAs, the adaptationist view is not so extreme, but rather considers that some traits are 'true adaptations' while others should be regarded as mere pathological consequences of infection (Thomas et al., 2010a). Still, the distinction between the two types of traits is not straightforward, as the most ardent defenders of the 'host-manipulation' hypothesis tend to consider that 'if pathology is linked to transmission, then it is highly likely that natural selection has not been blind to that pathology' (Thomas et al., 2005).

The confusion here centres around the distinction between three types of traits (Thomas et al., 2005). The first type corresponds to traits that are coincidental with infection but do not seem to play any part in increased transmission (or completion of the life cycle). The second type corresponds to traits that are coincidental with infection and appear to contribute to transmission, but do not appear to have been specially designed to that end. A general decrease in stamina in infected hosts fits this category perfectly. Traits belonging to the third type are coincidental with infection, appear to contribute to transmission and are suggestive of 'purposive design' (*sensu* Poulin, 1995). Precisely, the limits of the adaptationist stance lie in the possibility to infer the selective forces that historically shaped one trait from its apparent current utility (Gould and Lewontin, 1979; Pigliucci and Kaplan, 2000).

Showing that a given PIPA contributes effectively to enhance transmission is not proof that the set of genes that presently confer to the parasite the ability to induce such an alteration has evolved at any time in relation to this advantage. A more moderate claim consists of resorting to the concept of 'exaptation' (Gould and Vrba, 1982), as suggested by Combes (Combes, 2005) and Beisel and Médoc (Beisel and Médoc, 2010). Broadly speaking, an exaptation refers to a shift in function during the evolution of a trait (Gould and Vrba, 1982). According to Beisel and Médoc (Beisel and Médoc, 2010), the tendency of amphipods infected with the bird acanthocephalan *Polymorphus minutus* to show reverse geotaxis compared with uninfected individuals is an example of exaptation as it first evolved as an adaptation to avoid predation by non-hosts species ('historical genesis'), before favouring today trophic transmission to avian definitive hosts ('current utility'). However, this is only shifting the problem, not solving it, as the avoidance of predation by non-hosts is another 'just-so story', particularly as specificity in transmission does not appear to have much influence on the evolution of PIPAs (Cézilly et al., 2010). A more parsimonious view might be that reversed geotaxis first appeared as a by-product of the infection when the ancestors of *P. minutus* had a simple life cycle, thus increasing predation by aquatic birds, and hence favouring their later inclusion as a second and definitive host in the life cycle of the parasite (Cézilly et al., 2010).

As emphasized by Poulin (Poulin, 1995), one point of crucial importance to evaluate the current utility of PIPA is to show that it directly contributes to increase the probability of completion of the life cycle. However, such evidence is lacking for most PIPAs that have been qualified as 'manipulative' (Cézilly et al., 2010). Showing that the presence of a phenotypic alteration coincides with, for instance, increased predation by final hosts is not enough to infer a causal relationship between the two phenomena, as a mere correlation cannot be firm evidence for causality. We will argue that relying on an apparent logical relationship between the design of an alteration and its potential consequences (or 'purposive design') is not a reliable criterion, and can even be misleading. For instance, Lagrue et al. (Lagrue et al., 2007) observed that differences in drift between *P. laevis*-infected and uninfected *G. pulex* were in themselves sufficient to account for selective predation by bullheads, *Cottus gobio*, under field conditions. Indeed, the difference in proportions between infected and uninfected individuals in the stomach contents of bullheads did not differ significantly from what was measured in the drift. This suggests that other PIPAs known in the same host–parasite association that 'logically' appear to enhance trophic transmission, such as reversed reaction to light (Cézilly et al., 2000) and reversed reaction to olfactory cues from fish predators (Kaldonski et al., 2007; see also Perrot-Minnot et al., 2007), assessed under laboratory conditions, may not play much of a role in trophic transmission under natural conditions. Actually, recent experimental evidence (Perrot-Minnot et al., 2012) indicates that reversed reaction to light is not causally linked to increased vulnerability to fish predation in *P. laevis*-infected *G. pulex*. Clearly, evidence for a causative link between enhanced transmission and PIPAs, beyond any logical guess, is badly needed in most systems in which parasitic 'manipulation' has been advocated. The need is even more obvious in the case of multidimensionality if, as recommended by Thomas et al. (Thomas et al., 2010a), we must restrict the use of that term to PIPAs that

effectively contribute to transmission. Note, however, that the problem of partitioning the variance in increased transmission between several PIPAs and their interactions would remain (see below).

Ultimately, the adaptationist view on PIPAs crucially depends on the validity of the concept of 'extended phenotype' (Dawkins, 1982), according to which particular alleles or combinations of alleles have been selected in parasites during the course of evolution in direct consequence of their effect on the ability of 'manipulative' parasites to induce particular phenotypic alteration in their hosts. However, the existence of genes 'for' particular abilities is not always straightforward (Kaplan and Pigliucci 2001) and requires some evidence that the trait is causally linked to the gene and that the prevalence of the gene in the population results from a process of natural selection. Arguably, we know very little, if anything, about parasite genes involved in 'manipulation' in any of the major host–parasite systems studied so far. Worse, standard quantitative trait locus methods of investigation of the relationship between genes and phenotype (Lange and Whittaker, 2001; Guo and Nelson, 2008) might be of limited use in the framework of the study of PIPAs, if only for the difficulty of obtaining measures of the ability to induce PIPAs in two consecutive generations of parasites. Similarly, the use of gene knockout or knock-down methods might be of limited value if the pleiotropy of mutations (which is more relevant to evolutionary change) rather than that of the gene is involved in multidimensionality (see Wagner and Zhang, 2011).

Besides, deciding which altered traits are adaptive and which are not is contingent on the definition of 'trait'. A phenotypic trait is generally regarded as the final product of several processes taking place at the molecular and biochemical levels. But what makes a phenotypic character en entity in itself is not a trivial question (Wagner and Zhang, 2011). Imagine, for instance, that in a species of amphipod, individuals infected by a given fish acanthocephalan show both reduced photophobia and decreased use of refuges compared with uninfected individuals, with the intensities of the two behaviours being highly correlated (Fig. 2A). Imagine further that the use of refuge is shown not to be a direct consequence of altered photophobia, based on the evidence that uninfected amphipods make a more intense use of translucid refuges than infected ones (thus implying a role for thigmotactism in refuge use). Such evidence for multidimensionality could be countered on mathematical grounds by the possibility of defining a new coordinate system in which one dimension would be defined as the summation of the two behaviours. Rotating the axis (Fig. 2B) leads to an equally correct representation of the phenotypic space (see Wagner and Zhang, 2011), thus questioning the measurement of multidimensionality. Although a behavioural ecologist may see the two behaviours considered separately as more natural dimensions of the phenotype, there is no firm ground on which to prefer one representation of the phenotypic space to the other.

As emphasized by Cézilly et al. (Cézilly et al., 2010), natural selection is not supposed to act directly on host traits, but rather on the ability of parasites to alter them in a way that enhances trophic transmission. Therefore, one essential question that remains largely unanswered is: at which stage in its development is a trait altered by a parasite? Two main possibilities exist and are relevant to the question of adaptive multidimensionality. First, a parasite may directly regulate the expression of some genes in its host, eventually regulating the production of various neuromodulators that influence behaviour (Hoek et al., 1997). Such a mode of action may eventually interact with the pleiotropic effects of such genes (but

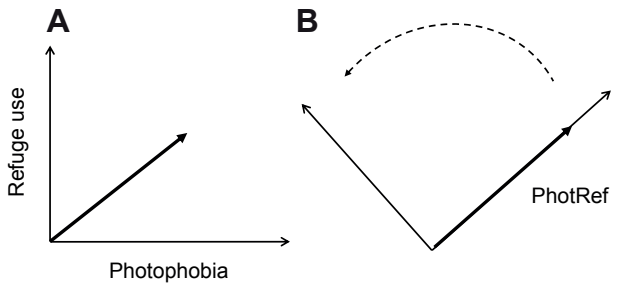

Fig. 2. Multidimensionality depends on the definition of phenotypic traits. (A) Bi-dimensional effect of a parasitic infection affecting both photophobia and refuge use in a correlated way (with both variables being standardized). (B) This estimate of multidimensionality is arbitrary because we can switch to another coordinate system in which one dimension is defined as 'PhotRef', which corresponds to the sum of the effect of infection on each of the two behaviours. A rotation of the coordinate axis leads to an equally valid representation of the phenotypic space. The dimensionality of the manipulation depends on the coordinate system (adapted from Wagner and Zhang, 2011).

see Wagner and Zhang, 2011) and contribute to the observed multidimensionality in PIPAs. However, several traits that are known to be modified by parasites in their hosts are likely to be polygenic. Controlling such polygenic traits in a way that ensures fine-tuned manipulation of the host phenotype may require an ability to control the regulation of several genes simultaneously. Second, the parasite may secrete compounds that disrupt or interfere with the physiology of its host. Such compounds may be then specific enough to alter a single trait in the host. Identifying the mechanisms behind PIPAs is therefore of paramount importance to the understanding of multidimensionality.

A proximate view on multidimensionality in PIPAs

Very little is known at present about the mechanisms underlying PIPAs, and even less so about the mechanisms underlying multidimensionality. However, identifying such mechanisms is essential to evaluate whether the various dimensions that are altered are independent of each other or are actually related at the proximate level. For instance, in proposing to include exclusively PIPAs with obvious consequences for transmission under the umbrella of multidimensionality, Thomas et al. (Thomas et al., 2010a) exclude one conspicuous alteration that is commonly observed in hosts infected with parasites with complex life cycles, i.e. partial or total castration (Bollache et al., 2001; Bollache et al., 2002). The problem here is not to decide whether castration, through forcing the host to reallocate resources to growth and maintenance, and thus contributing directly to the development of the parasite, should be considered as enhancing completion of the life cycle or not. It consists of estimating the chances that the physiological mechanisms that lie behind castration are in one way or another functionally linked to other PIPAs, such as behavioural alterations that appear to enhance transmission.

Cézilly and Perrot-Minnot (Cézilly and Perrot-Minnot, 2010) proposed that infection with parasites could result in a series of symptoms organised as an 'infection syndrome' that are all the consequences of some major dysregulation in the host's physiology. The latter phenomenon could arise as a consequence of the subversion of the host's immune system by the parasite, given the existence of crosstalk between the immune system and the nervous system in both vertebrates and invertebrates (see Adamo, 2002;

Scharsack et al., 2007). Indeed, it has been shown in various host–parasite systems (see Perrot-Minnot and Cézilly, 2012) that infection can affect various neuromodulators, such as, serotonin (5-HT). For instance, an increase in brain 5-HT immunoreactivity has been observed in amphipods infected with fish acanthocephalans (Tain et al., 2006; Tain et al., 2007), and injection with 5-HT mimics the reversed photophobia observed in amphipods naturally infected with fish acanthocephalans. Interestingly, infection with fish acanthocephalans induces partial castration in female amphipods, whereas 5-HT is known to affect ovarian development in crustaceans (Makkapan et al., 2011). Furthermore, 5-HT also controls the release of the crustacean hyperglycemic hormone (Escamilla-Chimal et al., 2002; Sathyanandam et al., 2008), and, correspondingly, altered levels of glycogen have been observed in amphipods infected with acanthocephalans (Plaistow et al., 2001). Obviously, such observations may just reflect the polyvalence of biogenic amines in the regulation of the physiology of host species. Still, they suggest that the investigation of multiple consequences of the alteration of the regulation of some key neuromodulator may shed some light on multidimensionality in PIPAs (see Perrot-Minnot and Cézilly, 2012).

A further possibility is that multidimensionality in PIPAs is related, in one way or another, to the existence of behavioural syndromes. Behavioural syndromes correspond to a suite of correlated behaviours expressed either within a given behavioural context or across different contexts and are supposed to reflect differences in so-called personality between individuals (Sih et al., 2004; David et al., 2011). The existence of behavioural syndromes has now been demonstrated in a large range of species including both vertebrates and invertebrates, and taxonomic groups that serve as intermediate hosts to ‘manipulative’ parasites (Briffa et al., 2008; Lihoreau et al., 2009; Dzieweczynski and Crovo, 2011; Hojesjo et al., 2011). According to Barber and Dingemanse (Barber and Dingemanse, 2010), behaviours that are altered following infection with ‘manipulative’ parasites often correspond to major personality axes in behavioural studies. Indeed, considerations about animal personality have often revolved around the shy–bold continuum, which is of particular relevance to inter-individual variation in anti-predator behaviour (Pellegrini et al., 2010; Jones and Godin, 2010). In parallel, several studies have shown that hosts infected with parasites with complex life cycles often show marked alterations of their anti-predatory behaviour (Libersat and Moore, 2000; Perrot-Minnot et al., 2007; Kaldonski et al., 2007). Barber and Dingemanse (Barber and Dingemanse, 2010; see also Kortet et al., 2010) further suggest that infection with parasites with complex life cycles could ‘decouple’ normally correlated behaviours that evolved in hosts as adaptations to local environments, and that such a decoupling effect would be positively selected through its effect on the vulnerability of infected hosts to predation. Similarly, Poulin (Poulin, 2010) considered that behavioural syndromes could be the targets of manipulation by parasites. Still, the precise mechanisms by which such a decoupling effect can work remain elusive. From a mechanistic point of view, however, it has been suggested that multidimensionality in personality syndromes could reflect differential patterns of activation of some key neurochemical signalling pathways (Coppens et al., 2010), such that a disruption of such pathways caused by parasitic infection may simultaneously affect several personality traits in infected hosts in a consistent way. Alternatively, individuals with different coping styles and, hence, different behavioural syndromes may show different susceptibilities to infection by parasites (see Blanchet et al., 2009), resulting in apparent multidimensionality in PIPAs, although this latter hypothesis is less likely.

Some suggestions for future research

So far, to our knowledge, only a few studies (Cornet et al., 2009a; Benesh et al., 2008; Coats et al., 2010) have examined multidimensionality in PIPAs. Such studies have essentially investigated co-variation between a few PIPAs, under the assumption that positive correlations would provide evidence for the existence of a mechanistic link between various dimensions. For example, Benesh et al. (Benesh et al., 2008) quantified five different traits in isopods infected with acanthocephalans: hiding, activity, substrate colour preference, body coloration and abdominal colour. Although infected individuals were darker and hid less than uninfected individuals, no relationship was found between the two traits among infected individuals. However, as emphasised by Cézilly and Perrot-Minnot (Cézilly and Perrot-Minnot, 2010), the absence of correlation between two PIPAs does not necessarily demonstrate their functional independence. Indeed, the two PIPAs might be regulated by the same neuromodulator, but with different dose-dependent effects.

Future studies of multidimensionality in PIPAs would certainly benefit from adopting a common methodology. This would be particularly useful when attempting to make comparisons between studies. We therefore propose here some simple recommendations for the measurement and analysis of PIPAs, with the hope that they will help researchers to obtain reliable and comparable quantitative measures of the phenomenon.

As indicated before, assessing the total number of phenotypic dimensions altered by one parasite in its host, whether at the individual, population or species level, might be out of reach. Therefore, providing a number of phenotypic dimensions that are altered by a given parasite in its hosts makes little sense because it clearly depends on the number of traits that have been investigated in a study. Assessing multidimensionality from the percentage of phenotypic characters that is altered by infection among all characters that have been studied might be preferable, as it would be more comparable between studies. Still, in most cases, a researcher will consider that a dimension is not altered if the difference between uninfected and infected individuals is not significant at some arbitrarily chosen alpha level. Thus, with only a moderate sample size, traits that are only slightly altered by infection would not be included in the calculation of multidimensionality. However, when a large number of dimensions can be measured, even on a limited number of individuals, it might be more relevant to consider multidimensionality as a measure of the distribution of effect sizes across traits. Effect sizes obtained from population samples estimate the strength of an apparent relationship, but, unlike standard test statistics used in hypothesis testing (see Nakagawa and Cuthill, 2007), do not assign a significance level reflecting whether the relationship could be due to chance. Note, however, that there is not a single definition of effect size (Kirk, 1996). Some types of measure are adequate to estimate relationship strength whereas others provide estimates of practical mean differences. Most often, researchers interested in the quantitative assessment of PIPAs want to appreciate to what extent one alteration in one dimension might be more intense than in another dimension, or compare the intensity of the same alteration between two different host–parasite associations, or two different populations of the same host–parasite association. However, the metric used to quantify phenotypic alterations may largely vary between traits. For instance, developmental stability of infected hosts might be measured from fluctuating asymmetry (Alibert et al., 2002), whereas photophobia might be assessed from an index of time spent in a dark area *versus* a lit one (Cézilly et al., 2000). In addition, the baseline behaviour of

uninfected individuals may differ between populations. We therefore propose to use the following index, I_a, as a standardized value of alteration in a given dimension d, with:

$$I_a(d) = [\tilde{x}(d)_i - \tilde{x}(d)_u] / \text{IQR}, \quad (1)$$

where $\tilde{x}(d)_i$ denotes the median value of dimension d in infected individuals, $\tilde{x}(d)_u$ denotes the median value of dimension d in uninfected individuals, and IQR is the interquartile range of the distribution of d in uninfected individuals, equal to the difference between the upper and lower quartiles (IQR=Q_3−Q_1). From here, it is then possible to estimate the shape of the distribution of effect sizes of infection on all measured traits. If this distribution is close to normal, then the standard deviation of the effect size distribution could provide a rigorous measure of the level of multidimensionality.

We recommend the use of non-parametric estimators as most often the frequency distributions of PIPAs do not conform to a normal distribution. However, in the case where measurements conform to the normal distribution, the same index can be computed as the difference between the mean value for infected individuals and that for uninfected individuals, divided by the standard deviation for uninfected individuals (see Glass, 1976; Poulin, 1994).

One important line of investigation for the future is to provide direct rather than correlational evidence that one phenotypic alteration effectively contributes to enhanced transmission. This can be achieved through combining refined experimental design with phenotypic engineering (Kaldonski et al., 2009; Perrot-Minnot et al., 2012). Furthermore, such experiments will allow one to estimate whether the effects of altered traits on enhanced transmission are additive or interactive. If traits are highly redundant, then their summation should explain better the increased vulnerability to predation. If they are not, their summation should not improve the prediction of vulnerability for a given predator but would eventually for a series of predators, under the assumption that multidimensionality is adaptive (see above). In contrast, multiplication would perform better if one poorly manipulated dimension is sufficient to decrease the overall effect on trophic transmission. Analysing the results of experiments combining predation trials with phenotypic engineering of both infected and uninfected hosts (Bakker et al., 1997; Kaldonski et al., 2009; Perrot-Minnot et al., 2012) using multiple regression techniques might provide interesting insights in this respect.

Information is also missing on the genetic and physiological determinism of traits that are altered by infection. Future research may then benefit from addressing the mechanistic basis of traits in uninfected individuals, and attempting to establish whether traits which are altered by infection are polygenic.

We would like to end this review with a friendly note of caution. Too much (naive) adaptationism may kill adaptationism. Although we do not deny that the study of host–parasite interactions benefits a great deal from an evolutionary approach, it might be time for evolutionary parasitologists to reconsider the extended phenotype framework and include in their reflection modern views on the relationship between genes and phenotype (Pigliucci, 2003; Dalziel et al., 2009; Wagner and Zhang, 2011). In particular, a better integration of proximate mechanisms with ecological aspects might be, in our opinion, a much more valuable advance in our understanding of the evolution of PIPAs than the endless formulation of *ad hoc* adaptive scenarios for which, most often, no critical test is available.

Acknowledgements

We wish to thank two anonymous referees for useful comments on an earlier version of the manuscript.

Funding

This work was supported by the Agence Nationale de la Recherche (programme blanc PARADIV).

References

Åbjörnsson, K., Dahl, J., Nyström, P. and Brönmark, C. (2000). Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquat. Ecol.* **34**, 379-387.

Adamo, S. A. (2002). Modulating the modulators: parasites, neuromodulators and host behavioral change. *Brain Behav. Evol.* **60**, 370-377.

Alibert, P., Bollache, L., Corberant, D., Guesdon, V. and Cézilly, F. (2002). Parasitic infection and developmental stability: fluctuating asymmetry in *Gammarus pulex* infected with two acanthocephalan species. *J. Parasitol.* **88**, 47-54.

Aydogdu, A., Emre, Y., Emre, N. and Kucuk, F. (2011). Two new host records for *Pomphorhynchus laevis* (Muller, 1776) recorded from Antalya, Turkey: small bleack (*Alburnus baliki* Bogutskaya, Kucuk and Unlu, 2000) and Antalya barb (*Capoeta antalyensis* Battalgil, 1944). *Turk. J. Zool.* **35**, 897-900.

Bakker, T. C. M., Mazzi, D. and Zala, S. (1997). Parasite-induced changes in behaviour and color make *Gammarus pulex* more prone to fish predation. *Ecology* **78**, 1098-1104.

Baldauf, S. A., Thünken, T., Frommen, J. G., Bakker, T. C. M., Heupel, O. and Kullmann, H. (2007). Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *Int. J. Parasitol.* **37**, 61-65.

Barber, I. and Dingemanse, N. J. (2010). Parasistim and the evolutionary ecology of animal personality. *Philos. Trans. R. Soc. B* **365**, 4077-4088.

Bauer, A., Trouvé, S., Grégoire, A., Bollache, L. and Cézilly, F. (2000). Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. *Int. J. Parasitol.* **30**, 1453-1457.

Bauer, A., Haine, E. R., Perrot-Minnot, M. J. and Rigaud, T. (2005). The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *J. Zool.* **267**, 39-43.

Beisel, J. N. and Médoc, V. (2010). Bird and amphipod parasites illustrate a gradient from adaptation to exaptation in complex life cycle. *Ethol. Ecol. Evol.* **22**, 265-270.

Benesh, D. P., Valtonen, E. T. and Seppälä, O. (2008). Multidimensionality and intra-individual variation in host manipulation by an acanthocephalan. *Parasitology* **135**, 617-626.

Bentley, C. R. and Hurd, H. (1993). *Pomphorhynchus laevis* (Acanthocephala) – elevation of hemolymph protein concentrations in the intermediate host, *Gammarus pulex* (Crustacea, Amphipoda). *Parasitology* **107**, 193-198.

Bentley, C. R. and Hurd, H. (1995). Depressed protein and copper content of the midgut gland in an intermediate host, *Gammarus pulex* (Crustacea), infected with cystacanths of *Pomphorhynchus laevis* (Acanthocephala). *J. Inv. Pathol.* **66**, 1-5.

Bentley, C. R. and Hurd, H. (1996). Carbohydrate titres in the haemolymph and midgut glands of *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis*. *J. Helminthol.* **70**, 103-107.

Bethel, W. M. and Holmes, J. C. (1973). Altered evasive behaviour and responses to light in amphipods harbouring acanthocephalan cystacanths. *J. Parasitol.* **59**, 945-956.

Blanchet, S., Méjean, L., Bourque, J.-F., Lek, S., Thomas, F., Marcogliese, D. J., Dodson, J. J. and Loot, G. (2009). Why do parasitized hosts look different? Resolving the "chicken-egg" dilemma. *Oecologia* **160**, 37-47.

Bollache, L., Gambade, G. and Cézilly, F. (2001). The effects of two acanthocephalan parasites, *Pomphorhynchus laevis* and *Polymorphus minutus* on pairing success in male *Gammarus pulex* (Crustacea: Amphipoda). *Behav. Ecol. Sociobiol.* **49**, 296-303.

Bollache, L., Rigaud, T. and Cézilly, F. (2002). Effects of two acanthocephalan parasites on the fecundity and pairing status of female *Gammarus pulex* (Crustacea: Amphipoda). *J. Invertebr. Pathol.* **79**, 102-110.

Boze, B. G. V., Hernandez, A. D., Huffman, M. A. and Moore, J. (2012). Parasites and dung beetles as ecosystem engineers in a forest ecosystem. *J. Insect Behav.* **25**, 352-361.

Briffa, M., Rundle, S. D. and Fryer, A. (2008). Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proc. Biol. Sci.* **275**, 1305-1311.

Cézilly, F. and Perrot-Minnot, M.-J. (2005). Studying adaptive changes in the behaviour of infected hosts: a long and winding road. *Behav. Processes* **68**, 223-228.

Cézilly, F. and Perrot-Minnot, M.-J. (2010). Interpreting multidimensionality in parasite-induced phenotypic alterations: panselectionism versus parsimony. *Oikos* **119**, 1224-1229.

Cézilly, F., Grégoire, A. and Bertin, A. (2000). Conflict between co-occurring manipulative parasites? An experimental study of the joint influence of two acanthocephalan parasites on the behaviour of Gammarus pulex. *Parasitology* **120**, 625-630.

Cézilly, F., Thomas, F., Médoc, V. and Perrot-Minnot, M.-J. (2010). Host-manipulation by parasites with complex life cycles: adaptive or not? *Trends Parasitol.* **26**, 311-317.

Coats, J., Poulin, R. and Nakagawa, S. (2010). The consequences of parasitic infections for host behavioural correlations and repeatability. *Behaviour* **147**, 367-382.

Combes, C. (2005). Manipulations: variations on the themes of signalling and exaptation. *Behav. Processes* **68**, 211-213.

Coppens, C. M., de Boer, S. F. and Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philos. Trans. R. Soc. Lond. B* **365**, 4021-4028.
Cornet, S., Franceschi, N., Bauer, A., Rigaud, T. and Moret, Y. (2009a). Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: consequences for the risk of super-infection and links with host behavioural manipulation. *Int. J. Parasitol.* **39**, 221-229.
Cornet, S., Biard, C. and Moret, Y. (2009b). Variation in immune defence among populations of *Gammarus pulex* (Crustacea: Amphipoda). *Oecologia* **159**, 257-269.
Cornet, S., Sorci, G. and Moret, Y. (2010). Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*. *Parasitology* **137**, 137-147.
Dalziel, A. C., Rogers, S. M. and Schulte, P. M. (2009). Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. *Mol. Ecol.* **18**, 4997-5017.
David, M., Auclair, Y. and Cézilly, F. (2011). Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. *Anim. Behav.* **81**, 219-224.
Dawkins, R. (1982). *The Extended Phenotype: The Long Reach of the Gene*. Oxford, UK: Oxford University Press.
Dezfuli, B. S., Maynard, B. J. and Wellnitz, T. A. (2003). Activity levels and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis*. *Folia Parasitol.* **50**, 129-134.
Dezfuli, B. S., Castaldelli, G., Bo, T., Lorenzoni, M. and Giari, L. (2011). Intestinal immune response of *Silurus glanis* and *Barbus barbus* naturally infected with *Pomphorhynchus laevis* (Acanthocephala). *Parasite Immunol.* **33**, 116-123.
Djikanovic, V., Gacic, Z. and Cakic, P. (2010). Endohelminth fauna of barbell *Barbus barbus* (L. 1758) in the Serbian section of the Danube River, with dominance of acanthocephalan *Pomphorhynchus laevis*. *Bull. Eur. Assoc. Fish Pathol.* **30**, 229-236.
Dubois, F., Cézilly, F. and Pagel, M. (1998). Mate fidelity and coloniality in waterbirds: a comparative analysis. *Oecologia* **116**, 433-440.
Dudinák, V. and Spakulová, M. (2003). The life cycle and seasonal changes in the occurrence of *Pomphorhynchus laevis* (Palaeacanthocephala, Pomphorhynchidae) in a small isolated lake. *Parasite* **10**, 257-262.
Dzieweczynski, T. L. and Crovo, J. A. (2011). Shyness and boldness differences across contexts in juvenile three-spined stickleback *Gasterosteus aculeatus* from an anadromous population. *J. Fish Biol.* **79**, 776-788.
Eberhard, W. G. (2010). Recovery of spiders from the effects of parasitic wasps: implications for fine-tuned mechanisms of manipulation. *Anim. Behav.* **79**, 375-383.
Escamilla-Chimal, E. G., Hiriart, M., Sánchez-Soto, M. C. and Fanjul-Moles, M. L. (2002). Serotonin modulation of CHH secretion by isolated cells of the crayfish retina and optic lobe. *Gen. Comp. Endocrinol.* **125**, 283-290.
Fermer, J., Culloty, S. C., Kelly, T. C. and O'Riordan, R. M. (2011). Manipulation of *Cerastoderma edule* burrowing ability by *Meiogymnophallus minutus* metacercariae? *J. Mar. Biol. Assoc. U. K.* **91**, 907-911.
Forber, P. (2009). Introduction: a primer on adaptationism. *Biol. Philos.* **24**, 155-159.
Franceschi, N., Rigaud, T., Moret, Y., Hervant, F. and Bollache, L. (2007). Behavioural and physiological effects of the trophically transmitted cestode parasite, *Cyathocephalus truncatus*, on its intermediate host, *Gammarus pulex*. *Parasitology* **134**, 1839-1847.
Francová, K., Ondrackova, M., Polacik, M. and Jurajda, P. (2011). Pararsite fauna of native and non-native populations of *Neogobius melanostomus* (Pallas, 1814) (Gobiidae) in the longitudinal profile of the Danube River. *J. Appl. Icthyol.* **27**, 879-886.
Fuller, C. A., Rock, P. and Philips, T. (2003). Behavior, color changes and predation risk induced by acanthocephalan parasitism in the Caribbean termite *Nasutitermes acajutlae*. *Caribb. J. Sci.* **39**, 128-135.
Glass, G. V. (1976). Primary, secondary, and meta-analysis of research. *Educ. Res.* **5**, 3-8.
Gould, S. J. and Lewontin, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B* **205**, 581-598.
Gould, S. J. and Vrba, E. S. (1982). Exaptation – a missing term in the science of form. *Paleobiology* **8**, 4-15.
Guo, Z. and Nelson, J. C. (2008). Multiple-trait quantitative trait locus mapping with incomplete phenotypic data. *BMC Genet.* **9**, 82.
Haine, E. R., Boucansaud, K. and Rigaud, T. (2005). Conflict between parasites with different transmission strategies infecting an amphipod host. *Proc. Biol. Sci.* **272**, 2505-2510.
Hatcher, M. J., Dick, J. T. A. and Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: linking interdependent processes. *Front. Ecol. Environ* **10**, 186-194.
Hernandez, A. D. and Sukhdeo, M. V. K. (2008). Parasite effects on isopod feeding rates can alter the host's functional role in a natural stream ecosystem. *Int. J. Parasitol.* **38**, 683-690.
Hoek, R. M., van Kesteren, R. E., Smit, A. B., de Jong-Brink, M. and Geraerts, W. P. M. (1997). Altered gene expression in the host brain caused by a trematode parasite: neuropeptide genes are preferentially affected during parasitosis. *Proc. Natl. Acad. Sci. USA* **94**, 14072-14076.
Höjesjö, J., Adriaenssens, B., Bohlin, T., Jonsson, C., Hellstrom, I. and Johnsson, J. I. (2011). Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family variation and performance in the wild. *Behav. Ecol. Sociobiol.* **65**, 1801-1810.
Jirsa, F., Konecny, R., Frank, C. and Sures, B. (2011). The parasite community of the nase *Chondrostoma nasus* (L. 1758) from Austrian rivers. *J. Helminthol.* **85**, 255-262.
Jones, K. A. and Godin, J.-G. J. (2010). Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proc. Biol. Sci.* **277**, 625-632.
Kaldonski, N., Perrot-Minnot, M.-J. and Cézilly, F. (2007). Differential influence of two acanthocephalan parasites on the anti-predator behaviour of their common intermediate host. *Anim. Behav.* **74**, 1311-1317.
Kaldonski, N., Perrot-Minnot, M. J., Dodet, R., Martinaud, G. and Cézilly, F. (2009). Carotenoid-based colour of acanthocephalan cystacanths plays no role in host manipulation. *Proc. Biol. Sci.* **276**, 169-176.
Kaplan, J. and Pigliucci, M. (2001). Genes 'for' phenotypes: a modern history view. *Biol. Philos.* **16**, 189-213.
Kirk, R. E. (1996). Practical significance: a concept whose time has come. *Educ. Psychol. Meas.* **56**, 746-759.
Kortet, R., Hedrick, A. V. and Vainikka, A. (2010). Parasitism, predation and the evolution of animal personalities. *Ecol. Lett.* **13**, 1449-1458.
Lagrue, C., Kaldonski, N., Perrot-Minnot, M.-J., Motreuil, S. and Bollache, L. (2007). Modification of hosts' behavior by a parasite: field evidence for adaptive manipulation. *Ecology* **88**, 2839-2847.
Lange, C. and Whittaker, J. C. (2001). Mapping quantitative trait Loci using generalized estimating equations. *Genetics* **159**, 1325-1337.
Leung, T. L. F. and Poulin, R. (2006). Effects of the trematode *Maritrema novaezealandensis* on the behaviour of its amphipod host: adaptive or not? *J. Helminthol.* **80**, 271-275.
Libersat, F. and Moore, J. (2000). The parasite *Moniliformis moniliformis* alters the escape response of its cockroach host *Periplaneta americana*. *J. Insect Behav.* **13**, 103-110.
Lihoreau, M., Brepson, L. and Rivault, C. (2009). The weight of the clan: even in insects, social isolation can induce a behavioural syndrome. *Behav. Processes* **82**, 81-84.
Makkapan, W., Maikaeo, L., Miyazaki, T. and Chotigeat, W. (2011). Molecular mechanism of serotonin via methyl farnesoate in ovarian development of white shrimp: *Fenneropenaeus merguiensis* de Man. *Aquaculture* **321**, 101-107.
McCahon, C. P., Maund, S. J. and Poulton, M. J. (1991). The effect of the acanthocephalan parasite (*Pomphorhynchus laevis*) on the drift of its intermediate host (*Gammarus pulex*). *Freshw. Biol.* **25**, 507-513.
McCurdy, D. G., Forbes, M. R. and Boates, J. S. (1999). Testing alternative hypotheses for variation in amphipod behaviour and life history in relation to parasitism. *Int. J. Parasitol.* **29**, 1001-1009.
Médoc, V. and Beisel, J.-N. (2011). When trophically-transmitted parasites combine predation enhancement with predation suppression to optimize their transmission. *Oikos* **120**, 1452-1458.
Messick, G. A., Overstreet, R. M., Nalepa, T. F. and Tyler, S. (2004). Prevalence of parasites in amphipods *Diporeia* spp. from Lakes Michigan and Huron, USA. *Dis. Aquat. Org.* **59**, 159-170.
Moore, J. (2002). *Parasites and the Behaviour of Animals*. Oxford, UK: Oxford University Press.
Nakagawa, S. and Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev. Camb. Philos. Soc.* **82**, 591-605.
Oetinger, D. F. and Nickol, B. B. (1981). Effects of acanthocephalans on pigmentation of freshwater isopods. *J. Parasitol.* **67**, 672-684.
Pagel, M. and Meade, A. (2006). Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* **167**, 808-825.
Parker, G. A., Ball, M. A., Chubb, J.C., Hammerschmidt, K. and Milinski, M. (2009). When should a trophically transmitted parasite manipulate its host? *Evolution* **63**, 448-458
Pellegrini, A. F. A., Wisenden, B. D. and Sorensen, P. W. (2010). Bold minnows consistently approach danger in the field and lab in response to either chemical or visual indicators of predation risk. *Behav. Ecol. Sociobiol.* **64**, 381-387.
Perrot-Minnot, M.-J. (2004). Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of *Pomphorhynchus laevis* (Acanthocephala). *Int. J. Parasitol.* **34**, 45-54.
Perrot-Minnot, M.-J. and Cézilly, F. (2013). Investigating candidate neuromodulatory systems underlying parasitic manipulation: concepts, limitations and prospects. *J. Exp. Biol.* **216**, 134-141.
Perrot-Minnot, M.-J., Kaldonski, N. and Cézilly, F. (2007). Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *Int. J. Parasitol.* **37**, 645-651.
Perrot-Minnot, M.-J., Maddaleno, M., Balourdet, A. and Cézilly, F. (2012). Host manipulation revisited: no evidence for a causal link between altered photophobia and increased trophic transmission of amphipods infected with acanthocephalans. *Funct. Ecol.* **26**, 1007-1014.
Pigliucci, I. and Kaplan, I. (2000). The fall and rise of Dr Pangloss: adaptationism and the Spandrels paper 20 years later. *Trends Ecol. Evol.* **15**, 66-70.
Pigliucci, M. (2003). Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecol. Lett.* **6**, 265-272.
Plaistow, S. J., Troussard, J. P. and Cézilly, F. (2001). The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host *Gammarus pulex*. *Int. J. Parasitol.* **31**, 346-351.
Ponton, F., Biron, D. G., Joly, C., Duneau, D. and Thomas, F. (2005). Ecology of parasitically modified populations: a case study from a gammarid–nematode system. *Mar. Ecol. Prog. Ser.* **299**, 205-215.
Popper, A. N., Salmon, M. and Horch, K. W. (2001). Acoustic detection and communication by decapod crustaceans. *J. Comp. Physiol. A* **187**, 83-89.
Poulin, R. (1994). Metaanalysis of parasite-induced behavioral changes. *Anim. Behav.* **48**, 137-146.
Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: a critical review. *Int. J. Parasitol.* **25**, 1371-1383.
Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. *Adv. Stud. Behav.* **41**, 151-186.
Poulin, R., Fredensborg, B. L., Hansen, E. and Leung, T. L. F. (2005). The true cost of host manipulation by parasites. *Behav. Processes* **68**, 241-244.

Rigaud, T. and Moret, Y. (2003). Differential phenoloxidase activity between native and invasive gammarids infected by local acanthocephalans: differential immunosuppression? *Parasitology* **127**, 571-577.
Rumpus, A. E. and Kennedy, C. R. (1974). The effect of the acanthocephalan *Pomphorhynchus laevis* upon the respiration of its intermediate host, *Gammarus pulex*. *Parasitology* **68**, 271-284.
Salwiczeck, L. H. and Wickler, W. (2009). Parasites as scouts in behaviour research. *Ideas Ecol. Evol.* **2**, 1-6.
Sathyanandam, S., Vasudevan, S. and Natesan, M. (2008). Serotonin modulation of hemolymph glucose and crustacean hyperglycemic hormone titers in *Fenneropenaeus indicus*. *Aquaculture* **281**, 106-112.
Sato, T., Egusa, T., Fukushima, K., Oda, T., Ohte, N., Tokuchi, N., Watanabe, K., Kanaiwa, M., Murakami, I. and Lafferty, K. D. (2012). Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. *Ecol. Lett.* **15**, 786-793.
Scharsack, J. P., Koch, K. and Hammerschmidt, K. (2007). Who is in control of the stickleback immune system: interactions between *Schistocephalus solidus* and its specific vertebrate host. *Proc. Biol. Sci.* **274**, 3151-3158.
Shirakashi, S. and Goater, C. P. (2005). Chronology of parasite-induced alteration of fish behaviour: effects of parasite maturation and host experience. *Parasitology* **130**, 177-183.
Sih, A., Bell, A. and Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol. Evol.* **19**, 372-378.
Skorping, A. (1985). Parasite-induced reduction in survival and fecundity – the effect of the nematode *Elasphostrongylus rangiferi* on the snail intermediate host. *Parasitology* **91**, 555-562.
Tain, L., Perrot-Minnot, M.-J. and Cézilly, F. (2006). Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proc. Biol. Sci.* **273**, 3039-3045.
Tain, L., Perrot-Minnot, M.-J. and Cézilly, F. (2007). Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric host species. *Biol. Lett.* **3**, 69-72.
Takeuchi, N. (2009). Ontogenetic changes in foraging tactics of the piscivorous cornetfish *Fistularia commersonii*. *Ichthyol. Res.* **56**, 18-27.
Thomas, F., Crivelli, A., Cézilly, F., Renaud, F. and deMeeus, T. (1997). Parasitism and ecology of wetlands: a review. *Estuaries* **20**, 646-654.
Thomas, F., Adamo, S. A. and Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behav. Processes* **68**, 185-199.
Thomas, F., Poulin, R. and Brodeur, J. (2010a). Host manipulation by parasites: a multidimensional phenomenon. *Oikos* **119**, 1217-1223.
Thomas, F., Poulin, R. and Brodeur, J. (2010b). Infection syndrome and multidimensionality: two terms for two different issues. *Oikos* **119**, 1230.
Thomas, F., Rigaud, T. and Brodeur, J. (2012). Evolutionary routes leading to host manipulation by parasites. In *Host Manipulation by Parasites* (ed. D. P. Hughes, J. Brodeur and F. Thomas), pp. 16-33. Oxford, UK: Oxford University Press.
Tikkanen, P., Muotka, T. and Huhta, A. (1994). Predator detection and avoidance by lotic mayfly nymphs of different size. *Oecologia* **99**, 252-259.
Wagner, G. P. and Zhang, J. (2011). The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. *Nat. Rev. Genet.* **12**, 204-213.
Westram, A. M., Jokela, J., Baumgartner, C. and Keller, I. (2011). Spatial distribution of cryptic species diversity in European freshwater amphipods (*Gammarus fossarum*) as revealed by pyrosequencing. *PLoS ONE* **6**, e23879.
Wickler, W. (1976). Evolution-oriented ethology, kin selection, and altruistic parasites. *Z. Tierpsychol.* **42**, 206-214.
Wudkevich, K., Wisenden, B. D., Chivers, D. P. and Smith, R. J. F. (1997). Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. *J. Chem. Ecol.* **23**, 1163-1173.