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Commentary

Parasitic manipulation: going beyond behaviour

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1. Introduction

Parasites are capable of altering a large range of phenotypic traits in their host, including morphology, physiology and behaviour (see de Jong-Brink et al., 1997). They induce these changes to continue their life cycle with the ultimate aim of transmission to the next host. To secure transmission parasites face several tasks. First, they need to find a suitable host in time and – in case of endoparasites – they have to be able to disrupt the epithelial lining of the host (e.g., skin, intestine) in order to enter. During this penetration process and once inside, the parasites immediately have to cope with the host's innate immune system (IS) and – in case of reinfection – also with the adaptive IS. Subsequently, they can actively or passively move to their favoured site within the host's body. As soon as they have reached that location it becomes important to have enough space and energy for increasing their numbers, either asexually or sexually, while not killing their host untimely. Finally, the parasites' condition, position within the host and numbers determine the success of transmission to the next host (see e.g., de Jong-Brink, 1995; de Jong-Brink et al., 1997, 2001; Shaldoum, 2002).

As most important tools to manipulate their hosts, parasites have excretion/secretion (E/S) products. Such manipulative substances that parasites use to influence their hosts can be seen as allomones (Brown et al., 1970, see also Koene and Ter Maat, 2001). Because the E/S products are essential for the parasite's transmission, many researchers focus on the effects and identification of these products. However, results from such studies are rarely put into the broader context of ecology and evolution. This topic is addressed in the review by Thomas, Adamo and Moore in the current issue of Behavioural Processes (Thomas et al., 2005). We welcome this review very much, but have a few comments that we will address in this commentary.

2. Phenotypic changes versus behavioural changes

In their very interesting paper, Thomas et al. (2005) focus on the behavioural changes parasites bring about

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in their host. They review the discussion about whether changes in host behaviour are adaptive or non-adaptive for either parasite or host. Non-adaptive changes in host behaviour have been proposed to occur accidentally as 'by-products' of other phenotypic changes (Minchella, 1985). Like the authors, we doubt whether such accidentally caused behavioural changes will persist during co-evolution of parasites and hosts if they do not somehow favour the parasite-host interaction. Namely, coincidentally beneficial changes persist when they are (indirectly) adaptive. Therefore, the behavioural change induced by the eye fluke *Diplostomum* (Poulin, 1995) should be seen as a manipulation of feeding behaviour of the host, which at the same time makes the host more visible. This manipulation of the host's behaviour achieves two goals of the parasite, it favours energy intake of the present host as it is important that the host's life continues until transmission and enhances transmission to the next host, a predatory bird.

For several parasites (e.g., schistosomes), it has been shown that they use multiple strategies to manipulate processes in their host (see de Jong-Brink et al., 2001). Hence, it seems unlikely that natural selection will favour a parasitic strategy that is accompanied by non-adaptive behavioural changes. This means that we suppose that all behavioural changes caused by parasites in their hosts are adaptive in the long run.

By focussing on behavioural changes, Thomas et al. (2005) make a – sometimes rather artificial – separation between parasite-induced changes in host behaviour and changes of other phenotypic traits of the host. Moreover, it is not entirely clear what the authors mean when they refer to 'novel behaviour' exhibited by parasitized hosts. This seems like an unfortunate choice of words because we are convinced that the authors are aware that any 'novel' behaviour elicited by a parasite in a host ultimately depends on the existing behavioural repertoire of which some components may be unknown to the investigators. An apparently new behaviour is therefore simply an exaggeration, a modification or an assembly of already existing behavioural components. In this way, hairworms are able to get their hosts into the water although the host would normally not do so (Thomas et al., 2002), *Ligula intestinalis* is able to make fish easier to be caught (Loot et al., 2002), and *Wolbachia* is able to make the host produce only female offspring (e.g., Hurst and Jiggins, 2000).

3. How does a parasite change host behaviour?

We agree with Thomas et al. (2005) that in order to fully understand parasite-host interactions, including the way in which parasites alter host behaviour, we have to unravel the underlying mechanisms. In their review, the authors (like many before them) make the distinction between effects that are caused in a direct and an indirect way. The latter case refers to secondarily altered host behaviour.

Direct effects are exerted on neurons or muscles. It is, however, impossible to conclude that parasites have either a direct or an indirect effect on neurons involved in regulating a certain behaviour, based on immunohistochemical staining. Activity of neurons can not be deduced from their immunostaining as already demonstrated by Wendelaar Bonga (1971). The release of, for example, serotonin does not necessarily coincide with a decline of immunostaining because this depends on the turn-over rate of secretory material.

By using a combination of parameters we have demonstrated that parasite-induced changes at the host's neuronal level are sometimes established in a rather complicated way. Gene expression (in situ hybridization) and the size and number of motor neurons (measured and counted in histological sections) controlling copulation behaviour in the snail *L. stagnalis* clearly reflect the inhibited development (small size) of their target, the male copulation organ, in parasitized snails. These effects on the innervating neurons appeared to depend on the connection with this target organ (de Lange et al., 2001). Organ culture experiments have shown that the development of the copulation organ, on the other hand, is inhibited in a direct way: by means of parasitic E/S products added to the culture medium (de Jong-Brink et al., 1999).

The authors also state that parasitic alteration of host's behaviour is usually an indirect effect of the parasite (Adamo, 2002). They suggest that this possibly also holds for the effect of the schistosome *Trichobilharzia ocellata* on the expression of the gene encoding neuropeptide Y in the central nervous system of the snail host *Lymnaea stagnalis* (LyNPY; de Jong-Brink et al., 1999). It has, however, not yet been investigated

whether the parasite or their E/S products interfere directly with LyNPY gene expression or indirectly via by example schistosomin derived from cells belonging to the internal defence system of the snail host.

New approaches are necessary to determine whether behavioural changes are direct or indirect effects of parasite manipulation, as indicated by Thomas et al. (2005). They point out that proteomics is one of the ways forward in studying the mechanisms underlying manipulation of hosts by parasites and mention that several studies are currently being undertaken using this approach. Obviously, more or less the same applies to peptidomics. Here, we would like to note that the use of microarrays will be a second extremely powerful tool (e.g., Mallo et al., 2002; Couillault et al., 2004). The prerequisite for this approach is that (a large part of) the genome of the species of interest needs to have been sequenced. But then, using such a DNA microchip, one can directly compare the differential expression of a whole slew of genes in parasitized and non-parasitized animals.

If microarrays are available for both host and parasite, this will allow for a differentiation between direct and indirect effects of infection, which seems much more difficult in proteomics or peptidomics. But besides using fancy techniques, proper observations and experimental design can also provide clear evidence for whether an effect is direct or indirect. There are good examples of studies where parasites have been shown to directly affect the host's behaviour (e.g., Franz and Kurtz, 2002; Brown et al., 2002). whereas in other cases the effect of the parasite turned out to be indirect (e.g., Edelaar et al., 2003).

Whether a parasitic manipulation is direct or indirect may also have implications for the costs of the manipulation for the parasite. The authors mention that the parasite's fitness costs for host manipulation are often simply deduced from the occurrence of manipulation. Obviously, there will normally be a cost for the parasite to bring the manipulation about. Therefore, the authors rightly suggest to take the underlying mechanisms into account in order to asses the costs for the parasite. But it should also be noted that the parasite's investment has to be outweighed by the gained benefit, otherwise the manipulation could not be maintained.

An important point that is only addressed very briefly is that the costs for the host are also often assumed. In cases like male-killing (Hurst and Jiggins, 2000) or castration (de Jong-Brink et al., 1999) the fitness costs for the host are evident, because they limit lifetime reproductive output. However, when organisms have more subtle effects on their hosts, a cost needs to be demonstrated. Several studies have revealed that increased immune function of infected animals can be costly and is traded-off against investment in reproduction and predator avoidance (e.g., Sheldon and Verhulst, 1996; Webster and Woolhouse, 1999; Rigby and Jokela, 2000). Additionally, potential benefits from the infection for the host need to be excluded. That there exists a very fine distinction between parasitism and symbiosis has been revealed in cleaner fish and red-billed oxpeckers (resp. Grutter and Bshary, 2003; Weeks, 2000).

4. Parasite-host interactions in an ecological context

Thomas et al. (2005) indicate that studies under laboratory or semi-natural conditions may be a poor approximation of the field situation. Although controlled infections in the laboratory may exaggerate the situation in the field, they are essential for detailed studies of the effect of a parasite on its host, including the underlying mechanisms. Nevertheless, we agree that assessing the field situation will provide an additional piece of the manipulation puzzle.

In recent years, several studies have used exactly the approach suggested by Thomas et al. (2005) to study parasite-host interactions in an ecological context. For example, it has been shown that different natural strains of *Caenorhabditis elegans* cope differently with a potential parasite (Schulenburg and Müller, 2004). Also, a field study on the reef fish *Thalassoma bifasciatum* demonstrated that the level of parasite infection influences the size at which sex change occurs (Scharer and Vizoso, 2003). Another parasite-host interaction that has been investigated in detail in the laboratory is the interaction between the pond snail *L. stagnalis* and the trematode *T. ocellata* (e.g., de Jong-Brink et al., 2001). Although the majority of snails can be infected under laboratory conditions, in the field infections with *Trichobilharzia* are rare (0.17%: Loy and Haas, 2001; 0.7–4.8%: Zbikowska, 2004). Nonetheless, all year round almost 50% of the snails collected in the field are infected with one ore more species of trematodes (Loy

and Haas, 2001; Koene et al., unpublished). Among these are *T. ocellata, Echinostoma revolutum, Opisthioglyphe ranae*, *Hypoderaeum conoidum*, *Diplostomum spathaceum*, and *Pseudoechinoparyphium echinatum*.

The above findings illustrate the importance of considering host-parasite interactions in a metapopulation context, as clearly put forward by Thomas et al. (2005). That this idea is not new, becomes evident from studies that have investigated differences in parasite resistance between different populations of a host species. For instance, for freshwater snails it has been demonstrated that resistance is dependent on infection risk within a population: resistance increases with higher numbers of parasites (Wiehn et al., 2002; Kristt et al., 2000). Likewise, infection with more strains of the same species of parasites has been shown to reduce survival of the host snails (Davies et al., 2002). And, the genetic diversity of sticklebacks has been shown to play a key role in how well they cope with infections by multiple species of parasites (Wegner et al., 2003).

For several species, it has been established that, by exposing a host population to certain compatible parasites, one can select for susceptible and resistant genotypes (Gutierrez et al., 2003; Webster et al., 2003). It has also been shown that resistant *Biomphalaria* distinguish between infected and non-infected mating partners, and have a preference for the latter (Webster et al., 2003). Likewise, *Lymnaea* prefers to inseminate not-infected mating partners, presumably to avoid wasting gametes on a castrated host (de Jong-Brink, 1990). These examples of mate choice provide an additional process through which parasites can affect host populations. We therefore propose that mate choice should be added to the three processes mentioned by the authors (namely interference with competition phenomena, effect on the predator community and interference with ecosystem engineers).

While the host is developing resistance, parasites are co-evolving to overcome their host's resistance. This was elegantly demonstrated in the prosobranch snail *Potamopyrgus antipodarum*, where the trematode *Microphallus* sp. was shown to be better adapted to infecting the local snail genotype than a genotype from a different population (Lively and Dybdahl, 2000). Thus, within a species local selection pressures cause adaptations in hosts and parasites, resulting in co-evolution. Comparisons between species also uncover such arms races between hosts and parasites, thus explaining the strict host specificity of many parasites (Lockyer et al., 2004). As recently pointed out by Woolhouse et al. (2002), understanding these co-evolutionary processes is essential for fully understanding the impact of pathogens on their hosts, and should provide novel insights for medical and veterinary research.

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