Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission?

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Trophically transmitted parasites often alter their intermediate host's phenotype, thereby predisposing the hosts to increased predation. This is generally considered a parasite strategy evolved to enhance transmission to the next hosts. However, the adaptive value of host manipulation is not clear as it may be associated with costs, such as increased susceptibility to predators that are unsuitable next hosts for the parasites. We examined the ratio between the benefits and costs of host manipulation for transmission success of *Acanthocephalus lucii* (Acanthocephala), a parasite that alters the hiding behaviour and pigmentation of its isopod hosts. We experimentally compared the susceptibility of infected and uninfected isopods to predation by perch (*Perca fluviatilis*; definitive host of the parasite) and dragonfly larvae (dead end). We found that the parasite predisposed the isopods to predation by both predators. However, the increased predation vulnerability of the infected isopods was higher towards perch. This suggests that, despite the costs due to non-host predation, host manipulation may still be advantageous for the parasite.

Keywords: parasite–host interactions; host phenotype; non-host predation; Acanthocephala; *Acanthocephalus lucii*; *Asellus aquaticus*

1. INTRODUCTION

Parasites with complex life cycles (which include a definitive host and at least one intermediate host) typically have only a small probability of surviving and reaching maturity because mortality of parasite individuals during transmission between hosts is often high (Dobson *et al.* 1992). Therefore, natural selection may favour parasite genotypes that can compensate for these losses with higher rates of reproduction (e.g. Price 1974) or by improving transmission success to the target hosts (the next host in the life cycle). Several complex parasite life cycles include at least one stage where the infected host has to be ingested by the target host for successful transmission. In such systems, parasites may gain a selective advantage if they can increase their probability of transmission by altering host phenotype (e.g. behaviour, appearance) to make infected hosts easier prey for target hosts (Rothschild 1962; Holmes & Bethel 1972). Indeed, the ability of trophically transmitted parasites to manipulate intermediate host phenotype is widely documented (reviewed by Moore 2002), and in many cases, it has been shown to predispose hosts to predation (e.g. Bethel & Holmes 1973, 1977; Moore 1983; Poulin *et al.* 1992; Lafferty & Morris 1996; Pulkkinen *et al.* 2000).

However, the adaptive value of host manipulation is still uncertain because the potential costs associated with manipulation are poorly understood. One possible cost related to host manipulation is that infected hosts may not

only be predisposed to predation by target hosts but can also be more easily caught by predators that are unsuitable next hosts for the parasites. Such predators represent dead ends in parasite life cycles, and can thus counterbalance the benefits of manipulation. This can significantly erode the adaptive value of manipulation because in nature prey are typically exposed to several different predator species, not only to the target hosts of the parasites. For example, Mouritsen & Poulin (2003) estimated that only 2.5% of *Curtuteria australis* (Trematoda) metacercariae inducing surfacing in their cockle intermediate hosts are transmitted successfully to bird definitive hosts, whereas 17.1% are lost to fishes, which are non-host predators that take advantage of manipulation. However, the risk of non-host predation as a cost of manipulation has been considered only in very few other study systems (*Microphallus* sp.–snail: Levri & Lively 1996, Levri 1998; *Diplostomum spathaceum*–fish: Seppälä *et al.* 2006; *Pomphorhynchus laevis*–gammarid: Lagrue *et al.* 2007), and its consequences for parasite transmission success have been investigated experimentally only in the eye fluke (*D. spathaceum*)–fish interaction (Seppälä *et al.* 2004, 2005, 2006). Therefore, empirical studies examining the ratio between the benefits and costs of manipulation for parasite transmission are in high demand.

In this study, we examined the adaptive value (effect on transmission success) of host manipulation by a trophically transmitted parasite, *Acanthocephalus lucii* (Acanthocephala). The parasite has a two-host life cycle (see Schmidt 1985), in which freshwater fishes, especially

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European perch (*Perca fluviatilis*), serve as definitive hosts (Bratley 1988; Karvonen *et al.* 2005). Parasites reproduce in the fish's intestine and eggs are released into the water with the host's faeces. Freshwater isopods of the species *Asellus aquaticus* become infected through ingestion of eggs and the parasites develop in isopods to an infective stage called a cystacanth. For successful parasite transmission, the infected isopod needs to be eaten by an appropriate fish definitive host. The parasite is known to alter isopod phenotype; the respiratory opercula of infected isopods become darkly pigmented (Bratley 1983) leading to overall darkening of abdominal pigmentation (Benesh *et al.* 2008), and the infected isopods hide less (spend less time under shelters) compared to the uninfected individuals (Benesh *et al.* 2008). These alterations predispose isopods to predation by fish hosts (Bratley 1983), suggesting that host manipulation may enhance parasite transmission. However, in the wild, these phenotypic changes may also predispose isopods to predators that are unsuitable next hosts for the parasite (e.g. aquatic insects). Thus, the aim of this study was to compare the effect of infection on host susceptibility to predation both by the target hosts and a dead-end species, and thereby evaluate the value of host manipulation as a parasite strategy to enhance its onward transmission.

2. MATERIAL AND METHODS

(a) Experimental animals

Isopods for this study originated from Lake Jyväsjärvi in Central Finland (62°14' N, 25°44' E). In September 2006, we collected over 200 infected and 200 uninfected isopods from the wild to be used in the experiment. We collected the isopods by hand from the litter and rocks in the lake littoral. We measured the body length of the isopods to the nearest 0.1 mm and identified individuals infected with *A. lucii* cystacanths from their darkened respiratory opercula (Bratley 1983). This method has proven to be reliable in our earlier studies (Benesh *et al.* 2008). Because we used isopods infected in the wild instead of experimentally infected individuals, parasite infection was not a randomly assigned treatment in the study. Therefore, it is possible that some pre-existing differences (not induced by the parasite) between infected and uninfected isopods may have affected the results. However, natural infections are advantageous in this system because experimental infections typically lead to unnaturally high parasite intensities (Bratley 1986; Benesh & Valtonen 2007; Hasu *et al.* 2007; intensity indicates the number of parasites in an infected host; Bush *et al.* 1997), and thus isopod phenotypes probably not encountered by predators in the field.

We used perch (target host (Bratley 1988; Karvonen *et al.* 2005); $n=10$; body length 8.8–11.8 cm) and dragonfly (Anisoptera, Odonata) larvae (dead end (O. Seppälä, E. T. Valtonen & D. P. Benesh 2006, personal observations); $n=10$; body length 1.7–2.6 cm) as predators in the study. In August 2006, we collected the perch with fish traps from Lake Konnevesi in Central Finland (62°37' N, 26°21' E). This was done four weeks before the experiment to acclimate fish to laboratory conditions. We maintained the perch in a 920 l tank and fed them daily with frozen chironomid larvae. We collected dragonfly larvae from Lake Jyväsjärvi during isopod collections. We did not identify the dragonfly species because identification of early instar larvae is not possible based on morphology (Askew 1988).

(b) Experimental design

We conducted the experiment in 10 glass aquaria (40×25 cm, water depth 14 cm). To provide shelter for animals, we piled up three rocks (each with a diameter of 7–9 cm) in the middle of each aquarium so that approximately 15% of the bottom area was covered. Shelter was provided because both isopods and dragonfly larvae are commonly observed to hide under objects in nature. We illuminated the aquaria with fluorescent lamps (True Light 36 W) placed 3 m above them, and set the light intensity to 80 lx measured at the water surface. We aerated the aquaria continuously with aquarium pumps and used a water temperature of 17.0°C in the experiment.

We conducted the experiment in two consecutive rounds. In each round, we placed 10 infected and 10 uninfected isopods into each aquarium. We size matched the isopods so that within each group body length distributions of infected and uninfected isopods were similar. We allowed the isopods to recover from the transfer and to become familiar with the experimental arena for four hours before starting the experiment. In the experiment, we placed either one perch or one dragonfly larva into each aquarium, and then let them feed on isopods for 36 hours. In both rounds, we used different prey and predator individuals and randomly determined which aquaria received perch and which dragonfly larvae (five perch and five dragonfly larvae at each round). After the experiment, we collected the remaining isopods from the aquaria, determined the number of infected and uninfected individuals, and measured their body length to the nearest 0.1 mm. Three predation trials (two perch and one dragonfly larva) were excluded from the data as predators did not eat enough isopods to assess differential predation susceptibility of infected and uninfected isopods. Furthermore, three isopods died during the experiments without showing any signs of predatory attacks. We excluded these individuals from the data, which reduced the number of animals in the beginning of the predation trials by one in three groups of isopods. The exclusion of these individuals was important for the analysis of data.

(c) Statistical analyses

We calculated the values of Manly's α (Manly 1974) for each group of isopods to assess the relative predation susceptibility of infected and uninfected individuals,

$$\alpha = \frac{\ln[(n_i - r_i)/n_i]}{\ln[(n_i - r_i)/n_i] + \ln[(n_u - r_u)/n_u]},$$

where n_i is the number of infected isopods at the beginning of the experiment and r_i is the number of infected isopods eaten during the experiment. Correspondingly, n_u represents the number of uninfected isopods at the beginning of the experiment and r_u the number of uninfected isopods eaten. The values of α range between 0 and 1, and values higher than 0.5 indicate that infected isopods were eaten more often than uninfected individuals. The preference index takes into account the variation in availability of different prey types and is robust to changes in prey densities during the experiment (Manly 1974).

To examine whether the infected and uninfected isopods differed in their susceptibility to predation, we compared the observed values of α to a situation of equal susceptibility ($\alpha=0.5$) using one-sample t -tests. We conducted the analysis separately for perch and dragonfly larvae. Furthermore, to quantitatively evaluate the ratio between the benefits and

Figure 1. Relative susceptibility of *A. lucii*-infected and uninfected isopods to predation by perch (target host of the parasite) and dragonfly larvae (dead end) in mixed groups of prey presented as values of Manly's α ($0 \leq \alpha \leq 1$). Reference line ($\alpha = 0.5$) shows the level above which infected isopods were eaten more often than uninfected individuals. Number next to the circle refers to two overlapping observations.

costs of host manipulation, we compared the values of α for different predator types using an independent-samples *t*-test. Despite the robustness of Manly's α , it could be affected to some degree by the number of prey individuals eaten during the experiment. This is because the ratio between prey types may change when the number of prey individuals decreases. Therefore, to examine if the values of α could be confounded by different total numbers of prey consumed by different predator types, we compared the proportions of isopods eaten by perch and dragonfly larvae using an independent-samples *t*-test. We also investigated whether size-specific predation by perch and/or dragonfly larvae could have confounded the results by comparing the body length distributions of isopods available in the beginning of the study and those left after the experiment using two-sample Kolmogorov–Smirnov tests.

3. RESULTS

Infected isopods were more likely to be eaten both by perch and dragonfly larvae (one-sample *t*-test for index $\alpha = 0.5$; perch: $t_7 = 5.909$, $p = 0.001$; dragonfly larvae: $t_8 = 3.184$, $p = 0.013$; figure 1). However, the increased susceptibility of infected isopods to predation was more pronounced towards perch definitive hosts (independent-samples *t*-test for index α : $t_{15} = 3.123$, $p = 0.007$; figure 1). On average, 6.4 infected and 3.1 uninfected isopods were eaten by perch and 5.9 infected and 4.4 uninfected isopods were eaten by dragonfly larvae. Across all predation trials, 25–75% of isopods available were eaten during the experiment, and the proportion of prey eaten did not differ between predator types (independent-samples *t*-test: $t_{15} = -0.424$, $p = 0.678$). Body length of the isopods used in the study varied between 4.0 and 7.8 mm, and length distributions did not differ between individuals available in the beginning of the experiment and those left after predation trials (two-sample Kolmogorov–Smirnov test: perch/uninfected isopods: $Z = 0.584$, $p = 0.885$, $n_1 = 80$, $n_2 = 55$; perch/infected isopods: $Z = 0.653$, $p = 0.787$, $n_1 = 80$, $n_2 = 27$; dragonfly

larvae/uninfected isopods: $Z = 0.454$, $p = 0.986$, $n_1 = 90$, $n_2 = 50$; dragonfly larvae/infected isopods: $Z = 0.491$, $p = 0.970$, $n_1 = 90$, $n_2 = 37$). Thus, differences in predatory behaviour, i.e. overall prey consumption and size-dependent prey choice, between perch and dragonfly larvae are unlikely to have affected the results.

4. DISCUSSION

The ability of trophically transmitted parasites to manipulate the phenotype of their intermediate hosts is widely documented and generally considered a parasite adaptation to enhance their between-host transmission (reviewed by Moore 2002). However, the adaptive value of host manipulation is not clear because the relationship between phenotypic alterations and parasite transmission success remains poorly understood. This is because not all parasite-induced changes in host phenotype necessarily result in higher susceptibility to predation (Webster *et al.* 2000), and/or because host manipulation may be exploited by non-host predators, which unavoidably leads to transmission failure and may thus override the benefits of manipulation (see Mouritsen & Poulin 2003; Tompkins *et al.* 2004). Here, we examined the adaptive value (effect on transmission success) of host manipulation by a trophically transmitted parasite *A. lucii* that is known to alter the pigmentation (Bratley 1983; Benesh *et al.* 2008) and hiding behaviour (Benesh *et al.* 2008) of its isopod hosts. When we exposed infected and uninfected isopods to predation by either perch (definitive host of the parasite) or dragonfly larvae (dead end), we found that infected isopods were more susceptible to predation by both predator types. This suggests that host manipulation is associated with both benefits and costs in this system. However, the increase in the vulnerability to predation due to parasitism was greater towards perch, the parasite's definitive host. Thus, in spite of the increased risk of being captured by dead-end predators, host manipulation may increase parasite transmission success and could be considered adaptive.

In the earlier studies examining the risk of non-host predation as a cost of host manipulation, the susceptibility of infected and uninfected hosts to different predator types have been investigated experimentally only in the eye fluke (*D. spathaceum*)–fish interaction (Seppälä *et al.* 2004, 2005, 2006). In those studies, eye fluke-infected fish were more susceptible to artificial predation (capture by a dip net), imitating predation by gulls and terns (definitive hosts of the parasite; Seppälä *et al.* 2004, 2005), but infection did not predispose fish to predation by pike, a non-host predaceous fish species (Seppälä *et al.* 2006). Those findings also suggest that host manipulation may be advantageous for the parasite through increased transmission probability. However, those studies could not estimate quantitative differences in the effect of infection on the vulnerability of fish to different predators as the experimental set-ups used in different studies were not comparable. Thus, to our knowledge, the present work is the first to show that host manipulation may enhance parasite transmission although it is also associated with costs due to an increased risk of non-host predation. The exact mechanisms underlying the different susceptibilities of infected isopods to predation by perch and dragonfly larvae are not clear, but are likely to involve differences in

behaviour between predator types. For example, reduced hiding of infected isopods is likely to increase their susceptibility mainly to predation by perch. This is because perch typically look for prey from the open water, whereas dragonfly larvae are often observed under litter and rocks in the wild.

However, it is important to note that in nature several ecological factors may modify the ratio between the benefits and costs of host manipulation for the parasites. First, in natural systems, the structure of predator communities is generally more complex than in our experiment. Thus, isopods are likely to be exposed also to other non-host predator species than dragonfly larvae (e.g. other invertebrates, waterfowl). These predators may differ from dragonfly larvae in their predatory behaviour, and thus the effect of parasite infection on isopod vulnerability to those species cannot be evaluated. However, in the present study, we focused on dragonfly larvae as they are important predators in several aquatic systems and amenable to laboratory experiments that are fully comparable to those possible with fish. Second, the structure of predator communities can be temporally and spatially variable in nature. This may lead to a mosaic of evolutionary sites (see Thompson 1994; Tompkins *et al.* 2004), in which the selective advantage of host manipulation varies. For example, in water bodies with more fish and fewer non-host predators, the benefits of manipulation may be substantial, perhaps favouring high manipulative effort. On the other hand, in communities with high proportion of non-host predators, the value of manipulation may decrease because the relative increase in probability of successful transmission due to manipulation is likely to be smaller. Furthermore, in addition to ecological costs, possible physiological/energetic costs of host manipulation (e.g. production of chemical compounds necessary to alter host phenotype; see Poulin 1994) also need to be considered in order to determine the relative profitability of a certain manipulation strategy.

To conclude, our results suggest that host manipulation may increase parasite transmission probability even in systems where manipulation is exploited by non-host predator species. Therefore, host manipulation can be considered a parasite strategy evolved to enhance between-host transmission. However, in some other systems, a larger proportion of parasites is killed by non-host predators than are actually transmitted successfully to target hosts (Mouritsen & Poulin 2003). That, however, does not necessarily mean that host manipulation cannot be favoured by natural selection also in such systems (Cézilly & Perrot-Minnot 2005). Even if manipulation predisposes hosts mainly to dead-end predators, it is still possible that it has evolved to increase the probability of parasite transmission. This is because the value of manipulation depends only on how it affects the likelihood of successful parasite transmission, not on the ways in which transmission could fail. In other words, the actual cause of transmission failure (e.g. dead-end predation or death within an intermediate host before transmission) is not essential for parasite fitness. Therefore, further studies are needed, for example, to quantitatively examine the net fitness outcome of host manipulation by parasite strains differing in manipulative effort (see also Poulin *et al.* 2005). This is especially important because in several parasite–host systems involving host manipulation,

dead-end predators may kill most parasite individuals, but manipulation may still ensure that at least some parasites are transmitted successfully.

This research followed the laws and ethical guidelines of Finland.

We are grateful to the anonymous referees for their helpful comments on the manuscript. Konnevesi Research Station provided the facilities for the experiment, and the study was funded by Emil Aaltonen Foundation (OS), the Academy of Finland (OS) and the Biological Interactions Graduate School at the University of Turku (DPB).

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