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Otto Seppälä

Host Manipulation by Parasites: Adaptation to Enhance Transmission?

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ABSTRACT

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Trophically-transmitted parasites may predispose infected hosts to predation by altering their phenotype. This can be either an adaptation of the parasites to enhance their transmission to the next hosts in the life cycle or a non-adaptive side-effect of infection. In this thesis, I examined whether the *Diplostomum spathaceum* (Trematoda) eye fluke can manipulate the phenotype of its fish intermediate hosts to increase their susceptibility to predation, and if this could be an adaptation of the parasite to enhance its onward transmission to the bird definitive hosts. In laboratory experiments, I found that anti-predator behaviour of infected fish was reduced compared to uninfected fish. Parasitized fish did not prefer the surface layers of the water column more than control fish, but did show a weaker reaction to an approaching simulated avian predator. Furthermore, their ability to adjust to the environment using cryptic coloration and cryptic behaviour was reduced. These changes led to an increase in the susceptibility of fish to simulated avian predation (capture by dip-net). This result was not reproduced when fish were exposed to predation by wild birds in a field experiment, possibly because the experimental set-up allowed birds to feed on fish in an easy, unnatural manner. Catchability of fish in the laboratory increased with the coverage of parasite-induced cataracts, which suggests that impaired vision may be the definitive mechanism leading to manipulation. Moreover, cataract formation was most intensive after parasites had completed their development, resulting in host manipulation only after parasites had reached infectivity to bird hosts. Furthermore, manipulation was not observed to be costly for the parasite, because it did not predispose fish to predation by non-host piscivorous fish. These findings suggest that manipulation of the fish host may increase the probability of parasite transmission to bird hosts, and thus be a parasite strategy evolved to enhance transmission.

Key words: Cataracts; crypsis; *Diplostomum spathaceum*; host behaviour; *Oncorhynchus mykiss*; parasite–host interactions; predation; Trematoda.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on five original papers, which will be referred to in the text by their Roman numerals I-V. I am the main writer in all papers, and I carried out a large part of the planning and data collection in each paper.

I Seppälä, O., Karvonen, A. & Valtonen, E. T. 2004. Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. Animal Behaviour 68: 257-263.

II Seppälä, O., Karvonen, A. & Valtonen, E. T. 2005. Impaired crypsis of fish infected with a trophically transmitted parasite. Animal Behaviour 70: 895-900.

III Seppälä, O., Karvonen, A. & Valtonen, E. T. 2005. Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. Animal Behaviour 70: 889-894.

IV Seppälä, O., Karvonen, A. & Valtonen, E. T. Host manipulation by parasites and risk of non-host predation: is manipulation costly in an eye fluke–fish interaction? Manuscript (submitted).

V Seppälä, O., Karvonen, A. & Valtonen, E. T. Susceptibility of eye fluke-infected fish to predation by bird hosts. Parasitology, in press.

1 INTRODUCTION

1.1 The parasitic way of life

Parasitic organisms live at least part of their lives in or on other organisms, the hosts, and obtain their resources by utilising host individuals (Price 1980). This way of life offers several benefits. For example, hosts generally are resource-rich and fairly predictable living habitats, which isolate parasites from the adversity of the environment outside the hosts. Moreover, a vast number of potential host species and sites of infection (different organs) offers a wide variety of resources for parasites. These factors have led to the independent evolution of parasitism in several plant and animal taxa, to a high rate of parasite speciation, and to evolution of sophisticated adaptations to exploit certain hosts and organs (e.g. Price 1980, Poulin 1998, Combes 2001). Therefore, parasitism is perhaps the commonest way of life on earth (May 1988, Windsor 1998).

However, parasites also face many challenges in their life histories. For instance, since parasites usually cause harm to their hosts, known as parasite virulence (e.g. Herre 1993, Jaenike et al. 1995, Polak 1996, Fitze et al. 2004), hosts tend to evolve resistance to parasite infections and/or tolerance of their harmful effects (e.g. Wakelin 1996). Especially in vertebrates, highly developed non-specific and specific immune responses replenished by immunological memory form the main physiological barrier against parasite infections (Manning 1994, Jurd 1994, Turner 1994). Furthermore, other defence mechanisms such as avoidance of those circumstances under which infections take place (e.g. Folstad et al. 1991, Hart 1994, Moore 2002, Karvonen et al. 2004a) and group formation to dilute parasite exposure (e.g. Poulin & FitzGerald 1989, Mooring and Hart 1992, Hart 1994) have been described in several parasite–host systems.

Hosts are also relatively short-lived habitats compared to those utilised by many free-living organisms. Therefore, because parasites are dependent on their hosts, transmission of parasite individuals between hosts is an essential process. This is a very uncertain stage in parasite life histories, because hosts are usually patchily distributed in the environment. Especially in parasites with

multihost complex life cycles, an individual parasite has only a small probability of surviving and completing its life cycle because of high parasite mortality during transmission (e.g. Dobson et al. 1992). Thus, natural selection favours parasite genotypes that can compensate for losses by producing more offspring (e.g. Price 1974), or by being better at infecting the target hosts. A wide variety of adaptations to enhance parasite transmission have been described in parasite–host relationships. These include production of phenotypically dissimilar offspring to reduce the risk of transmission failure in unpredictable environments, known as 'bet hedging' (Fenton & Hudson 2002, Hakalahti et al. 2004), and release of parasite eggs or infective stages at the time when successful transmission is most likely to occur (e.g Théron 1984, Shostak & Dick 1989, Combes et al. 1994, Karvonen et al. 2004b).

1.2 Trophic transmission and host manipulation

Several complex parasite life cycles include at least one stage at which the infected host has to be ingested by the target host for successful transmission. Trophic transmission has been suggested to evolve when addition of a new host into a cycle increases the probability of the parasite reaching a definitive host. This may be, for instance, if the parasite has higher contact probability with the prey of the target host than the target host itself (Choisy et al. 2003). Complex life cycles can also serve to mix parasite genotypes ending up to a definitive host, and thus may have evolved to avoid inbreeding (Rauch et al. 2005). In trophic transmission, parasites are directly dependent on their hosts. Therefore, according to the theory of the evolution of parasite virulence, when virulence is measured as direct parasite-induced host mortality, trophically-transmitted parasites should evolve to be more benign to their hosts than parasites with several other host exploitation strategies (Jokela et al. 1999, Hurd et al. 2001). However, to maximise transmission probability, it would be beneficial for the parasite to alter host behaviour or other phenotypic traits to make infected hosts easier prey for target hosts (Rothschild 1962, Holmes & Bethel 1972).

Phenotypic alterations in infected hosts have been described in several parasite–host interactions (reviewed by Moore 2002), and the ability of parasites to cause such alterations has usually been considered as an evolutionary adaptation to increase parasite transmission efficiency. For example, terrestrial isopods infected with *Plagiorhynchus cylindraceus* (Acanthocephala) spend more time in exposed microhabitats (no leaf coverage, light background coloration) than uninfected individuals, which leads to increased avian predation and transmission efficiency (Moore 1983). Similarly, *Microphallus* (Trematoda) parasites cause aquatic snails to stay on the upper sides of rocks in the early morning hours when they are exposed to intensive predation by waterfowl definitive hosts, and to hide for the rest of the day when predation risk by non-host fish is increased (Levri & Lively 1996, Levri 1998). Moreover, altered snail

behaviour takes place only when parasites are fully developed and thus infective to target hosts (Levri & Lively 1996). This is beneficial for the parasite because predation of uninfective larvae always leads to death of the parasite.

However, not all parasite-induced alterations in host phenotype necessarily enhance parasite transmission (e.g. Webster et al. 2000, Edelaar et al. 2003). For example, *Tenebrio molitor* beetles infected with the cestode *Hymenolepis diminuta* are less concealed than their uninfected counterparts, but this does not increase their susceptibility to predation by the rat definitive host, at least under laboratory conditions (Webster et al. 2000). Furthermore, in addition to increased host susceptibility to predation by target hosts, manipulation can also predispose infected hosts to capture by predator species which are unsuitable hosts for the parasite. For instance, *Curtuteria australis* (Trematoda) parasites reduce the ability of their cockle hosts to burrow into the substrate (Thomas & Poulin 1998). This predisposes infected cockles to predation by bird definitive hosts, but also to whelk and fish non-host predators (Mouritsen & Poulin 2003, Tompkins et al. 2004), which may override the benefits of manipulation. Therefore, it is difficult to distinguish parasite–host interactions in which parasite-induced alterations in host phenotype have evolved to increase transmission efficiency. Phenotypic changes can also be caused by traits such as host exploitation that have probably evolved for other purposes yet still affect host phenotype. Furthermore, some alterations can even be adaptations of the hosts to defend themselves against parasites. For example, behavioural fever and chill may help to kill parasite individuals or reduce host mortality (e.g. Louis et al. 1986, Müller & Schmid-Hempel 1993, Watson et al. 1993). Similarly, increased host feeding rate can compensate for energetic losses caused by parasites (e.g. Milinski 1985, 1990, Godin & Sproul 1988).

1.3 Aims of the study

In this study, I examined host manipulation using an eye fluke of fish (*Diplostomum spathaceum*; Trematoda) as a model species. My first aim was to investigate whether this trophically-transmitted parasite can manipulate phenotypic traits of its fish intermediate hosts to predispose them to predation by bird definitive hosts. My second aim was to study the adaptive value of manipulation by investigating the susceptibility of fish to both host and non-host predators, and by examining manipulative effort in relation to parasite infectivity to birds. In laboratory experiments, I studied the effect of the parasite on the preference of fish for the surface layers of the water column (I), on the fish escape response to predators (I) and on fish crypsis (II). I also examined host susceptibility to predation by exposing them to avian predation simulated with a dip-net (I, III), and investigated parasite transmission to non-host piscivorous fish as a cost of manipulation (IV). Furthermore, in a field experiment, I examined the susceptibility of fish to predation by wild birds (V).

2 STUDY SYSTEM

The taxonomy of the genus *Diplostomum* is not completely resolved and different species can be distinguished reliably only as adult stages. Fish in northern Finland carry two different morphological forms of *Diplostomum* metacercariae in their eye lenses, and according to Niewiadomska (1986), most of them resemble *D. spathaceum* (Valtonen & Gibson 1997). Cercariae of *D. spathaceum* can be distinguished from several other furcocercariae according to their resting position, where they hang down with furcae spread at an angle of 180° and tail bent at an angle of 90°. However, these morphological traits are closely similar to cercariae of *D. pseudospathaceum* (Niewiadomska 1986). In this study, I consider parasites with previous descriptions as *D. spathaceum*, but recognise that other species may also have been present.

Diplostomum spathaceum has a three-stage life cycle with bird definitive host, and snail and fish intermediate hosts (Fig. 1, Chappell et al. 1994). The parasite matures in the intestine of fish-eating birds, where it reproduces sexually. Several bird species are suitable hosts for the parasite, but gulls (Laridae) and terns (Sternidae) are probably most common. After sexual reproduction, eggs of the parasite are released to water with the bird's faeces, where they hatch into free-swimming miracidia. Miracidia infect aquatic snails (first intermediate host) mainly of the genus *Lymnaea*. Infection in a snail gives rise to sporocysts in which cercariae larvae are produced through asexual reproduction. The development of patent infection takes 4-10 weeks depending on the water temperature (Chappell et al. 1994), after which an individual snail can produce thousands of cercariae per day for several weeks (Karvonen et al. 2004b). Cercariae infect a wide variety of fresh water and brackish water fish species (second intermediate host) (Valtonen & Gibson 1997, Valtonen et al. 1997) by penetrating the gills and skin (Whyte et al. 1991, Höglund 1995). In fish, parasites migrate to the eye lenses where they develop to metacercariae. Parasites must establish in the lenses within 24 hours after penetration, and individuals not succeeding in this are killed by host defences (Erasmus 1959, Whyte et al. 1991). For successful transmission to the avian definitive host, an infected fish has to be eaten by a fish-eating bird. In the lenses of fish,

metacercarial stages of the parasite reduce host vision by inducing cataract formation (Rushton 1937, 1938, Shariff et al. 1980, Karvonen et al. 2004c) and disrupting lens structure (Shariff et al. 1980). Therefore, by injuring an important sensory organ, *D. spathaceum* has the potential to alter fish behaviour and other phenotypic traits such that their vulnerability to predation could be increased. Earlier, the parasite has been suggested to manipulate fish by inducing surface seeking behaviour, which could predispose them to predation by birds (Crowden & Broom 1980).

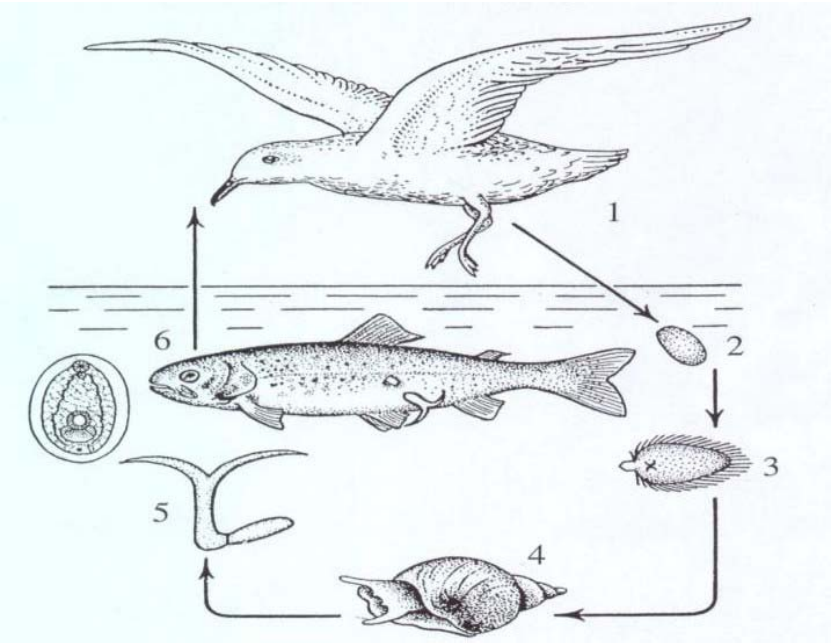

FIGURE 1 The life cycle of *Diplostomum spathaceum*; 1 = bird definitive host, 2 = egg, 3 = miracidia, 4 = snail first intermediate host, 5 = cercariae, 6 = fish second intermediate host (modified from Dogiel et al. 1961).

In this study, I used rainbow trout (*Oncorhynchus mykiss*) as a model host species. From the variety of fish species suitable for the parasite, I selected rainbow trout because it is relatively susceptible to infection (Betterton 1974) and easy to maintain under laboratory conditions. Fish were obtained from a commercial fish farm where they had been reared in indoor tanks supplied with ground water, which ensured that they had no eye flukes or other helminth parasites. I recognize that the behaviour of farmed fish may not be fully comparable to that of free-living fish. However, wild fish are commonly infected with several parasite species, which favours the use of fish farmed in ground water. Furthermore, rainbow trout is not a native fish species in Finland, and therefore it has only a short coevolutionary history with local parasite populations. Rainbow trout was introduced to Europe from North America in the nineteenth century and, although *D. spathaceum* is common also in America, lack of direct coevolution between the parasite and its host in Europe may have affected the results because European and American strains of the parasite may differ genetically.

3 RESULTS AND DISCUSSION

3.1 Phenotypic alterations

Several complex parasite life cycles include at least one stage at which the infected host has to be ingested by the target host for successful parasite transmission. Since the probability of an individual parasite to complete its life cycle is typically very small (e.g. Dobson et al. 1992), transmission success is a major determinant of overall parasite fitness (Anderson & May 1991). Therefore, ability of trophically-transmitted parasites to predispose infected hosts to predation by target hosts may increase their transmission efficiency and thus be favoured by natural selection (Rothschild 1962, Holmes & Bethel 1972). Indeed, several parasite species have been shown to alter behaviour or other phenotypic traits of infected hosts (reviewed by Moore 2002). In most cases, these changes have been suggested to be adaptations of the parasites to enhance their transmission.

Diplostomum spathaceum is one parasite that has been proposed to manipulate its fish hosts to increase transmission efficiency. By locating in the eye lenses of fish and inducing cataract formation (Rushton 1937, 1938, Shariff et al. 1980, Karvonen et al. 2004c) it impairs fish vision and thus has the potential to alter fish phenotype. Crowden & Broom (1980) found that dace (*Leuciscus leuciscus*) infected with *D. spathaceum* spend more time near the water surface compared to uninfected fish. This was suggested to predispose fish to predation by gull hosts and in this way to enhance parasite transmission. However, the experiment was conducted using a water depth of only 30 cm. In my study, when fish were allowed to select their location from a water layer with a depth of two meters, infected fish did not prefer surface layers of the water column more than controls (I). Instead, I found that eye fluke infection impaired fish anti-predator behaviour by reducing the intensity of their escape reaction to an approaching simulated avian predator (I). This can be considered as a potential mechanism to predispose fish to predation and thus to enhance parasite transmission.

In addition to escaping, fish also use other defence mechanisms, such as crypsis and shoaling, in predator avoidance (e.g. Sumner 1935, Magurran 1990, Pitcher & Parrish 1993, Johnsen & Sosik 2003). In this study, I investigated the effect of the eye flukes on the crypsis of fish. I found that the ability of infected fish to adjust themselves to the environment using cryptic coloration and cryptic behaviour was reduced compared to uninfected fish (II). Both infected and uninfected fish were able to match their coloration to a black substrate, but when maintained against lighter backgrounds, the contrast between fish colour and background increased. The increase was higher in infected fish that remained darker than control fish (II). Furthermore, when fish were allowed to select between black and white bottom colorations, the preference of infected fish for the dark background was reduced (II). This indicates that the camouflage of fish is reduced by the infection. Therefore, infected fish should also be more conspicuous and thus more easily observed by avian predators (see LoBue & Bell 1993).

3.2 Susceptibility to predation

To enhance parasite transmission, manipulation should predispose infected hosts to predation by target hosts. In laboratory experiments, I found that fish infected with eye flukes were more prone to artificial predation, which simulated attacks by surface feeding birds such as gulls and terns (I, III). In paper I, when fish were caught with a dip-net from shoals containing both heavily infected fish and control fish, infected fish were caught more often compared to controls. Similarly, in paper III, when predation vulnerability of fish was determined by counting the number of attempts required to catch individual fish from the tanks, the number of attempts needed was lower in infected fish. These results suggest that infected fish should be easier prey for bird hosts. However, in a field experiment where wild birds were allowed to feed on fish from cages placed in a lake, I found no difference in the vulnerability of fish to predation (V). Thus, it is possible that the laboratory experiments may have overestimated to some extent the effect of altered fish phenotype on their susceptibility to predation. On the other hand, the latter result was likely affected by the experimental set-up. Most of the fish that were eaten in the experiment were caught by gulls and only a minority by terns. In nature, however, except for lesser black-backed gull (*Larus fuscus*), fish caught by the birds themselves generally constitute only a low proportion in the diet of Finnish gull species (reviewed by Götmark 1984). Usually gulls use mainly terrestrial food (e.g. earthworms and other invertebrates) or refuse and fish offal from human activities (Götmark 1984). Therefore, it is possible that other bird species such as terns, loons and mergansers, which eat mainly fish (e.g. McCaw et al. 1996, Granadeiro et al. 2002, Jackson 2003), may act as 'required' (*sensu* Holmes 1979) hosts in maintaining parasite populations. Perhaps only in

circumstances where gulls are able to feed on considerable amounts of fish, for example, in fish docks or in fish farms, may their role in maintaining parasite life cycle be locally important. In this study, cages attracted gulls probably because they allowed them to feed on fish in an unnatural manner and thus provided an easy food source for them. Usually gulls stood on the edges of the cages (ca. 15 cm above the water surface) and waited until the fish swam close to them before attack. With this technique, fish were more easily caught than when birds attacked by plunging into the water from the air. This may have contributed to equal predation susceptibility of infected and control fish. Therefore, the controlled laboratory experiments described in papers I and III are likely to give a more reliable estimate of the effect of the parasite on the vulnerability of fish to predation. However, the overall effect of manipulation on parasite transmission efficiency in the wild can not be evaluated without experiments considering predation also by other bird species than gulls.

In many natural ecosystems, organisms are concurrently exposed to several predator species. Therefore, host manipulation may not only predispose infected individuals to predation by target hosts, but also to predator species which are unsuitable hosts for parasites (e.g. Mouritsen & Poulin 2003, Tompkins et al. 2004). In that case, parasite transmission may not be enhanced through manipulation (see below). In this study, eye fluke infection did not predispose fish to predation by pike (*Esox lucius*), which represents a dead-end predator for the parasite (IV). Reasons for the difference in the susceptibility of fish to different type of predators are unclear, but possibly differences in predator behaviour are involved. Fish-eating birds typically actively search for prey contrary to pike, which ambush prey by staying motionless. Therefore, the effect of phenotypic alterations on the susceptibility of fish to predation may differ between predator species. If, for instance, activity of infected fish is reduced, it may lead to a lower contact probability with pike. Furthermore, when avoiding underwater attacks, fish are able to use not only their vision but also their lateral line and olfaction to detect predators. Hence the role of vision may be particularly important when avoiding aerial avian predators.

3.3 Adaptiveness of manipulation

Host manipulation has usually been considered as an evolutionary adaptation of the parasites to enhance their transmission to target hosts. However, in those parasite–host relationships where altered host phenotype has been described, parasite transmission efficiency does not always increase through manipulation (e.g. Webster et al. 2000, Edelaar et al. 2003). Furthermore, several non-trophically-transmitted parasites also increase host vulnerability to predation even though this always leads to death of the parasites (e.g. Hudson et al. 1992, Murray et al. 1997, Vance & Peckarsky 1997, Steen et al. 2002). Therefore, it is difficult to distinguish parasite–host interactions in which the ability of

parasites to manipulate host phenotype has evolved to increase their transmission efficiency from those where manipulation is a result of traits that have evolved for other purposes yet still affect host phenotype (e.g. host exploitation).

In the *D. spathaceum*–fish interaction, the parasites lodge themselves in the lenses of fish eyes, and induce cataract formation through their movements and metabolic wastes (Rushton 1937, 1938, Shariff et al. 1980, Karvonen et al. 2004c). Since the susceptibility of fish to predation (capture by dip-net) increased with the coverage of parasite-induced cataracts (III), impaired vision of fish may be the definitive mechanism leading to manipulation in this system. Therefore, it is possible that the eye as the site of infection has been favoured by natural selection if it increases parasite transmission efficiency. On the other hand, selection may have favoured parasites escaping the host immune defence by locating in the lenses, which have been suggested to be immunologically naïve because of the lack of blood vessels (see Szidat 1969). Moreover, since cataract formation is probably tightly connected to parasite metabolic rate, increased susceptibility to predation could be just a side effect of selection favouring faster parasite growth and development in fish. Whatever the original reason for locating in the eyes and inducing cataract formation, natural selection may still act on all parasite features that influence parasite transmission efficiency. In this study, I found that cataract formation was slow when metacercariae were growing, but fast after they had completed development (III). This led to an increase in the susceptibility of fish to predation only after parasites were infective to bird hosts (III). Delayed timing of manipulation has also been observed in several other parasite–host interactions (e.g. Bethel & Holmes 1974, Hurd & Fogo 1991, Poulin et al. 1992, Tierney et al. 1993, Levri & Lively 1996), and it may be highly advantageous under selection favouring higher parasite transmission efficiency, because predation of uninfective larvae always leads to death of the parasite. These results suggest that precise timing of cataract induction *after* metacercariae have reached infectivity to birds leads to increased vulnerability of fish to predation, and thus may be a parasite strategy evolved to increase transmission efficiency in this system.

Furthermore, independence of host manipulation from intensity of infection is generally interpreted as support for adaptive host manipulation in the systems where the number of parasites in infected hosts is typically low (Poulin 1994). However, this is not the case in the eye fluke–fish interaction, where parasites usually live with other conspecifics, and thus do not have to be able to induce manipulation on their own. Indeed, trematodes are known to affect their hosts in an intensity-dependent manner (e.g. Lafferty & Morris 1996), and in *D. spathaceum*, cataract formation also depends on parasite intensity. Karvonen et al. (2004c) showed that coverage of parasite-induced cataracts increased with the intensity of infection in rainbow trout, and that individuals harbouring on average over 20 metacercariae per eye had intensive cataracts (whole lens occluded), which is likely to reduce fish vision. In this study, however, parasite intensity did not affect catchability of fish (III). This is

likely to be caused by the use of small fish, which have small lenses, and high parasite intensities (range 11-109 per fish). However, in paper I, control fish had a very low level of *D. spathaceum* infection and, since their vulnerability to predation was much lower compared to heavily infected fish, it is likely that host manipulation does depend on parasite intensity in this system too. Thus, because *D. spathaceum* parasites are typically aggregated into a small portion of fish populations (e.g. Burrough 1978, Karvonen et al. 2004d), only the most intensively infected individuals may experience manipulation and thus maintain the parasite life cycle in nature.

In addition to increased susceptibility to predation by target hosts, manipulation may also be associated with costs that reduce parasite fitness by counterbalancing the benefits of manipulation. In many systems, prey are exposed to several predator species, and therefore manipulation can also increase host susceptibility to predators which are unsuitable hosts for the parasites. Since phenotypic changes induced by *D. spathaceum* are probably caused by impaired vision of fish (III), manipulation could predispose fish not only to predation by avian hosts but also to non-host predator species such as piscivorous fish. In this study, however, infection did not predispose fish to predation by pike, which represents a dead-end predator for the parasite (IV). This suggests that by manipulating fish phenotype, *D. spathaceum* parasites may increase their probability of successful transmission to the definitive hosts, and manipulation can be favoured by natural selection. In other systems, infected hosts have also been reported to be more vulnerable to predation by non-host species (Mouritsen & Poulin 2003, Tompkins et al. 2004), which may override the benefits of manipulation. However, increased susceptibility to non-host predators does not necessarily mean that manipulation cannot enhance parasite transmission. This depends on the likelihood of successful parasite transmission in the absence of manipulative effort. Even if most parasite individuals are in hosts killed by dead-end predators, the overall probability of successful parasite transmission may still be enhanced through manipulation. Current studies in several systems have compared only the susceptibility of infected and uninfected individuals to predation, but to examine the actual effect of manipulation on parasite transmission, quantitative comparisons with differing manipulative efforts are needed (see also Poulin et al. 2005).

Furthermore, host adaptations to resist parasites and their harmful effects may also affect manifestation of host manipulation. Generally parasites are expected to be ahead in the coevolutionary arms race with their hosts (see Barnard 1990). This is mainly because parasites generally have a higher cost of failure, shorter generation times, higher population densities and higher mutation rates than their hosts (Hafner et al. 1994). A similar asymmetry also exists in predator–prey interactions, where the prey usually outruns the predator (Dawkins & Krebs 1979). However, manipulative parasites cause high costs to their hosts and, in the case of *D. spathaceum*, in addition to increased host susceptibility to predation, the parasite also has a negative effect on fish food intake (Crowden & Broom 1980, Owen et al. 1993). Moreover, the parasite

is very common in many fish populations (e.g. Valtonen & Gibson 1997, Marcogliese et al. 2001), and therefore, natural selection may strongly favour higher parasite resistance and tolerance in fish. Eye fluke infection leads to acquired resistance against further infections (e.g. Höglund & Thuvander 1990, Whyte et al. 1990, Karvonen et al. 2004d, 2005), but, at least in rainbow trout, this alone is not sufficient to protect fish against harmful cataract formation (Karvonen et al. 2004a). However, fish species are known to differ in their susceptibility to *D. spathaceum* infection (e.g. Betterton 1974, Sweeting 1974, Speed & Pauley 1984), and thus susceptibility to cataract formation may also be species-specific. Rainbow trout is highly susceptible to parasite-induced cataract formation (Karvonen et al. 2004a, 2004c), and I have observed intensive cataracts also in farmed whitefish (*Coregonus lavaretus*). However, roach (*Rutilus rutilus*) appear more able to resist cataract formation and intensive cataracts are observed only in individuals with very high parasite intensities (personal observations). Therefore, the ability of the parasite to manipulate a fish phenotype may be determined by the susceptibility of that fish both to parasite infection and to cataract formation. In that case, host manipulation may be species-specific and only certain fish species may experience manipulation in natural populations, although this suggestion needs to be verified.

4 CONCLUSIONS

Parasites may predispose infected hosts to predation by altering their phenotype, and, in cases of trophic transmission, this can be a parasite strategy evolved to enhance transmission to the next hosts in the life cycle (Rothschild 1962, Holmes & Bethel 1972). On the other hand, host manipulation can also be a side effect of selection favouring other traits (e.g. host exploitation) that have probably evolved for other purposes yet still affect the host phenotype. In this thesis, by using the eye fluke (*Diplostomum spathaceum*) of fish as a model species, I have presented empirical evidence supporting the hypothesis that host manipulation can be an adaptation of the parasite to predispose infected hosts to predation by target hosts. I have shown that both anti-predator behaviour and crypsis of fish were reduced as a consequence of infection so that infected fish should be more easily observed and caught by bird definitive hosts (I, II). Furthermore, I demonstrated that the susceptibility of infected fish to simulated avian predation was higher compared to controls (I, III), although increased vulnerability to predation by wild birds was not observed in a field study (V). The latter result was likely affected by the experimental set-up, which allowed birds to feed on fish in an easy and unnatural manner. Susceptibility of fish to simulated predation increased with the coverage of parasite-induced cataracts, and cataract formation was most intensive after parasites were fully developed and thus capable to get transmitted (III). This led to host manipulation only after parasites had reached infectivity to birds (III). These results suggest that impaired vision may be the definitive mechanism leading to manipulation, and that exact timing of cataract formation may have evolved to enhance parasite transmission. However, manipulation could also be associated with costs such as increased susceptibility of infected hosts to predator species which are unsuitable hosts for the parasite (Mouritsen & Poulin 2003, Tompkins et al. 2004). In this study, host manipulation did not predispose fish to predation by non-host piscivorous fish, implying that manipulation may increase the probability of parasite transmission to bird hosts, and thus be a parasite strategy evolved to enhance transmission.

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YHTEENVETO

Loisten kyky manipuloida isäntiään: sopeuma transmission tehostamiseen?

Loiset elävät muiden eliöiden sisällä tai pinnalla ottaen näistä käyttämänsä ravinnon ja ovat tästä syystä riippuvaisia isännistään. Koska yksittäiset isännät ovat kuitenkin usein varsin lyhytikäisiä, loisten jälkeläisten onnistunut transmissio eli siirtyminen uusiin isäntiin on välttämätön vaihe loisten elinkierroissa. Loisilla, joilla on epäsuora, useita eri isäntiä sisältävä elinkierto, todennäköisyys saavuttaa uusi pääisäntä on kuitenkin hyvin pieni, mikä johtuu elinkierron eri vaiheissa tapahtuvasta suuresta kuolleisuudesta. Tästä syystä luonnonvalinnan tulisi suosia loisgenotyyppejä, jotka pystyvät kompensoimaan kuolleisuuden joko tuottamalla suuria määriä jälkeläisiä tai kasvattamalla transmission onnistumisen todennäköisyyttä. Väitöskirjatyössäni tutkin ravintoketjuissa etenevien loisten kykyä manipuloida isäntiensä fenotyyppiä (ulkonäkö, käyttäytyminen) mahdollisena sopeumana transmission tehostamiseen. Tällöin loisten aiheuttamien fenotyyppisten muutosten tulisi altistaa isäntä elinkierrossa seuraavana olevan isännän saalistukselle. Vaihtoehtoisesti loisten vaikutukset voivat kuitenkin olla pelkkiä loisinfektioiden patologisia sivuvaikutuksia, joilla ei ole adaptiivista merkitystä.

Tutkimuksessa käytin mallilajina *Diplostomum spathaceum* -imumatoloista, joka käyttää elinkierrossaan lintu-, kotilo- ja kalaisäntiä. Loisen siirtyminen linnuista kotiloihin ja edelleen kotiloista kaloihin tapahtuu vedessä vapaasti uivien toukkien avulla. Siirtyminen kaloista lintuihin tapahtuu sen sijaan ainoastaan, kun jokin kalaa syövä lintu käyttää loisitun kalan ravintonaan. Kaloissa *D. spathaceum* -loiset elävät silmien linsseissä ja heikentävät kalojen näkökykyä aiheuttamalla kaihia. Tämä tarjoaa loiselle mahdollisuuden manipuloida kaloja siten, että niiden alttius joutua lintujen saaliiksi kasvaa ja loisen transmissio siten tehostuu.

Laboratoriotutkimuksissa havaitsin loisten heikentävän kalojen pakoreaktiota lähestyvään lintupetomalliin sekä kykyä sulautua ympäristöön suojavärityksen avulla. Nämä muutokset johtivat loisittujen kalojen suurempaan alttiuteen joutua saaliiksi kokeissa, joissa lintujen saalistusta simuloitiin pyytämällä kaloja altaista haavilla. Vastaavaa ei kuitenkaan havaittu kenttäkokeessa, jossa lokit ja tiirat saalistivat kaloja järveen asetetuista sumpuista. Tässä tapauksessa sumput kuitenkin mahdollistivat lintujen ruokailun epäluonnollisella tavalla sumppujen reunoilta, mikä on voinut vaikuttaa tulokseen. Laboratoriokokeissa kalojen alttius joutua saaliiksi kasvoi kaihin voimakkuuden lisääntyessä, mikä viittaa siihen, että kalojen näkökyvyn heikkeneminen on manipulaatioon johtava tekijä. Lisäksi kaihin muodostuminen oli voimakkainta, kun loiset olivat saavuttaneet kyvyn tarttua lintuun. Samaan aikaan kalojen saalistusalttiudessa havaittiin selvä nousu, mikä tukee adaptiivisen isäntämanipulaation teoriaa. Manipulaatiosta voi kuitenkin koitua myös kustannuksia, joihin kuuluu mm. isän-

tien lisääntynyt alttius joutua väärien, loiselle seuraavaksi isännäksi sopimattomien petojen saaliiksi. Tutkin asiaa kokeellisesti altistamalla kaloja loiselle pääisännäksi sopimattoman pedon, hauen, saalistukselle. Tulokset osoittivat, että loisitut kalat eivät olleet alttiimpia hauen saalistukselle verrattuna loisettomiin. Tutkimukseni tulokset osoittavat, että *D. spathaceum* -loisen kykyä manipuloida kalaisäntiään voidaan pitää loisen mahdollisena sopeumana tehostaa transmissiota lintuihin.

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