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Copepod reaction to odor stimuli influenced by cestode infection

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The cestode *Schistocephalus solidus* uses copepods as first and sticklebacks as second intermediate hosts. For transmission, an infected copepod has to be preyed upon by a stickleback. We used copepods of the species *Macrocyclops albidus* to test whether infected and uninfected copepods differ in their reaction to two kind of simultaneously presented odors: odors of sticklebacks and odors of sticklebacks and conspecifics. By giving this choice, we attempted to force the copepods to make a trade-off between the benefit of risk dilution and possible predator confusion and the costs of food competition and other disadvantages induced by conspecifics. Within 1–8 h after last feeding, uninfected copepods clearly preferred the odors of conspecifics under the chemically simulated threat of predation. This was in contrast to the infected copepods, who tended to avoid the odor of conspecifics. When the time between experiment and last feeding varied, infected copepods showed an increased preference for fish water only (or avoided conspecifics) with increasing hunger level. This suggests that *S. solidus* benefits from hunger-induced behavioral changes of its copepod host by influencing its microhabitat selection. The same effect could be found in both sexes; however, it was significantly more pronounced in male than in female copepods. We propose several hypotheses that could explain the difference between the sexes in their infection-dependent microhabitat selection. *Key words:* cestodes, copepods, *Macrocyclops albidus*, parasite infection, *Schistocephalus solidus*, sticklebacks. *[Behav Ecol 9:414–418 (1998)]*

Many helminth parasites use intermediate hosts for growth and development. When reaching their infective stage, they depend on their intermediate host being preyed upon by the next intermediate or the final host. Such parasites often alter the intermediate host's biology in a way that favours parasite transmission to the next host (Dobson, 1988; Holmes and Bethel, 1972; Milinski, 1990; Moore, 1984, 1995; Moore and Gotelli, 1990; Poulin, 1994). These alterations can include the host's conspicuousness, its fleeing ability, and its fleeing motivation.

The pseudophillidean cestode *Schistocephalus solidus* is a parasite that has to grow in two intermediate hosts before it can reproduce in the gut of the final host, a fish-eating bird. The first intermediate host is a cyclopoid copepod; the second host is the three-spined stickleback (*Gasterosteus aculeatus*). In both hosts, the larvae grow in the body cavity and can reach relatively large sizes within a short time (e.g., Clarke, 1954). Hence, their resource drawn from the host is obvious and has been demonstrated by several authors (e.g., Pascoe and Mattey, 1977; Walkey and Meakins, 1970; Wedekind, 1997).

Changes in conspicuousness induced by this parasite have been intensely studied in both intermediate hosts. In copepods, infected individuals are more active (Urdal et al., 1995; Wedekind and Milinski, 1996), have a reduced swimming ability and are easier to catch than uninfected individuals (Wedekind and Milinski, 1996). Therefore, sticklebacks preferentially attack and consume parasitized copepods (Wedekind and Milinski, 1996). In the second intermediate host, the stickleback, cestode larvae can become nearly twice as heavy as their host (Clarke, 1954; Wedekind C, personal observation). Therefore, infected fish can be conspicuous to bird predators because of their sometimes enormously distended belly. In some populations, infected sticklebacks turn conspicuously white and swim at the surface near the shore, a behavior that makes them very vulnerable to bird predation (LoBue and Bell, 1993).

In contrast to the parasite induced changes in conspicuousness, changes in antipredator behavior have only been studied in the second intermediate host, the stickleback. Infection induces dietary stress in the fish (Pascoe and Mattey, 1977) and therefore alters foraging activity and risk-taking behavior (i.e., it reduces fright reaction to predators) (Giles, 1983, 1987; Milinski, 1985, 1990; Jakobsen et al., 1988). In copepods, the possibility for parasite-induced changes of antipredator behavior have not been investigated so far.

In this study we concentrated on the copepods' reaction to odors of their fish predators. Freshwater animals and especially zooplankton are known to respond to chemical stimuli released by predators (e.g., Kleiven et al., 1996; Lampert et al., 1994; Larsson and Dodson, 1993). However, there are only few studies in which the possibility of parasite-induced changes in the reaction to predator odors have been investigated. Lefcort and Blaustein (1995) found that the yeast *Candida humicola* does not significantly alter the response of *Rana aurora* tadpoles to predator odors, but Kavaliers and Colwell (1995) found that mice (*Mus musculus*) infected with *Eimeria vermiformis* showed a reduced avoidance of cat odors. The parasites in these two examples are single-host parasites that probably do not benefit from increased predation. This is in contrast to the multihost parasite *S. solidus* that is expected to benefit, if in the infectious stage, from a reduced reaction of its intermediate host to predator odors. Therefore, the aim of the present study was to test under experimental conditions (1) whether infected and uninfected copepods react differently to odor stimuli and choose their feeding sites and/or whereabouts accordingly and (2) whether this reaction correlates with the hunger state of the copepods.

METHODS

Copepods of the species *Macrocyclops albidus* were caught from an area where *S. solidus* is common (a pond near Bie-

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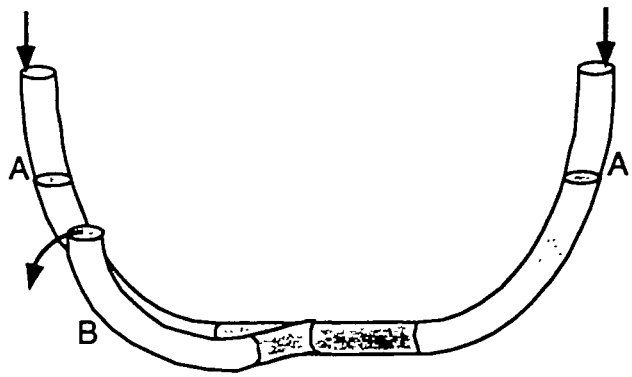

Figure 1
Schematic illustration of the experimental setup. A, flexible-tube side arms; B, basal arm. See text for detailed description and explanations.

lefeld, Germany) and kept in the laboratory under conditions described by Orr and Hopkins (1969). The experiments took place a year later with laboratory-reared copepods.

We cultured *S. solidus in vitro* using a technique modified from Smyth (1954) and described in more detail in Wedekind (1997). The eggs were kept in small petri dishes at 20°C until the coracidia (the infective stage) hatched. Before exposure to the parasite, male and female copepods without egg sacs were filtered from the culture tank and put singly into a well of an ELISA plate (water volume about 2 ml, tap water aged for 2 days). They stayed there for 2 days without being fed to ensure that they were motivated to take up the *S. solidus* coracidia. Then, we added six coracidia each to some randomly chosen copepods wells. Copepods that were not exposed to coracidia served as controls. Thereafter, the copepods were kept in a climate chamber (20° C, 12 h light, 12 h dark) and fed on days 1, 5, and the 9 day after exposure to coracidia with two freshly hatched *Artemia* each. The experiments took place 11, 12, and 13 days after exposure to coracidia.

The behavioral tests were performed in flexible polyethylene tubes fitted together by a T-shaped inflexible connecting piece (Figure 1). The three arms of the connecting piece were 4 cm long each. The flexible tubes that formed the two side arms were 13 cm long each, 1 cm in diameter, and curved upward on both sides to the height of about 11.5 cm so that they could be partly filled with water. This was the tract in which the copepods were observed. The copepods were hindered from entering the basal arm of the T by a 250-μm plankton net. This basal arm was also curved upward to the height of about 6 cm and was filled with water. It served as a drain tube to regulate the water level of the other arms into which water dripped at a rate of about one drop (0.085 ml) per second, regulated by clamps. This water stem from two different source tanks and was transported by small tubes (0.2 cm diam). In each of these tanks another 250-μm plankton net was fixed at the entrance of the tubes that transported the water that dripped into the test tubes. Prior testing with methyl-blue water showed that this created a system with a weak current and some mixing of water in the middle of the T-shaped tube system.

We kept 36 sticklebacks in a 40-l tank (=0.27 g fish/l). The water in this tank was renewed every evening, and the sticklebacks were not fed the days before and during the test runs. Each evening before an experimental day, we filled the two source tanks with 10 l each of water that had been in the stickleback tank for 1 day. In one of these tanks we added 1.4 g live copepods (=0.14 g copepods/l). These stimulus copepods were mostly adult females (46 adult females without egg sacs had an average fresh weight of 0.115 mg, measured in 4 batches with a Satorius MC210P balance (i.e., we had added about 1000 to 1500 copepods/l). These copepods were not fed as long as they were in the source tank. The plankton net fixed at the entrance of the drain tubes prevented them from being transported to the observation tubes.

In the morning of each experimental day (between 0845 and 0915 h), four *Artemia* nauplii were given to each experimental copepod in the ELISA plates. The behavioral test began at the earliest 1 h after this feeding. One copepod per trial was pipetted randomly into one of the two side arms of the observation tube system and allowed to settle for 15 min before we recorded its preference as the side where it was found outside the connecting middle part, or the side it first entered coming from this middle part of the system. If the copepod did not enter one of the flexible arms within 30 min it was removed from the system (n = 8). Five systems were used in parallel, and the water from the two source tanks dropped in different sides into each test systems.

Trials were run with a time lag of up to 8 h from last feeding. Hence, time from last feeding varied between trials and was recorded. Infection of the copepods were handled by C.W. The experimenter, P.J., was unaware of which copepod had been exposed to coracidia. After all the behavioral tests were done (i.e., on the 14th day postinfection), each copepod was anaesthesized with carbonated water and examined under a microscope to determine sex and developmental stage (3.8% of the copepods could not be examined because they had died for unknown reasons between the experimental day and the day of microscopic examination; we assume that this mortality was unbiased by infection; see Wedekind, 1997). If infected, the copepod and its procercoids (cestode larvae) were videotaped with a system connected to the microscope. Further measurements from these recordings were performed on a Macintosh computer using a public domain NIH Image program (developed at the U.S. National Institutes of Health, Bethesda, Maryland, and available on the Internet at http://rsb.info.nih.gov/nih-image/). We measured the length of the overall part of the copepod "body" including the fourth thoracic segment (i.e., the distance from the base of first antenna to the end of the fourth thoracic segment) to the nearest 0.01 μm and used it to estimate copepod volume with the formula

$$\text{Copepod volume} = e^{-4.486} \times \text{length}^{3.383} \quad (1)$$

(Wedekind et al., in preparation). We estimated procercoid volume (including cercomer) by measuring the maximal area of the longitudinal section of its body (excluding the cercomer) and using the formula

$$\text{Procercoid volume} = e^{0.533} \times \text{area}^{1.363}. \quad (2)$$

For the few procercoids that had not yet developed their cercomer, we used the formula

$$\text{Procercoid volume} = e^{0.279} \times \text{area}^{1.385} \quad (3)$$

(Wedekind et al., in preparation). The percentage of the volume of all parasites in a copepod relative to the body volume of its host ("parasite index") was used as a measure for severity of infection. We analysed the data using Systat (Systat, 1992).

RESULTS

The two types of noninfected copepods [the nonexposed (control) and the exposed but noninfected ones] did not differ significantly in their reaction to the two types of odor stimuli (Fisher's Exact test, p = .83). They both preferred to move toward or to stay in the arm receiving water from the fish+copepods tank (all noninfected copepods pooled, test

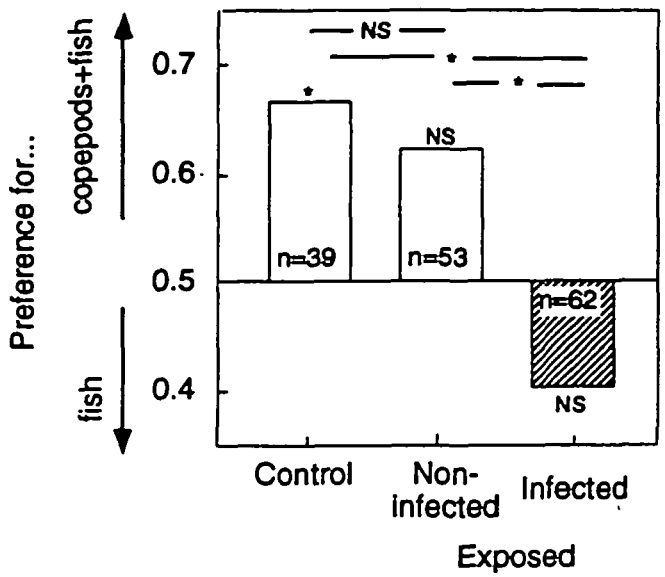

Figure 2
Preference of nonexposed (control), exposed but noninfected, and infected copepods for the odor of fish-only or fish+copepods shown as the deviation from the null hypothesis. The scale of the y-axis gives the frequency of copepods that chose the side receiving water from the copepod tank. Asterisks above bars mean that the observation differs from the 0.5 null hypothesis (Z test, $p < .05$); asterisks between bars mean that the two frequencies differ from each other (Fisher's exact test, $p < .05$); NS, nonsignificant. The numbers of copepods per group are given in the bars.

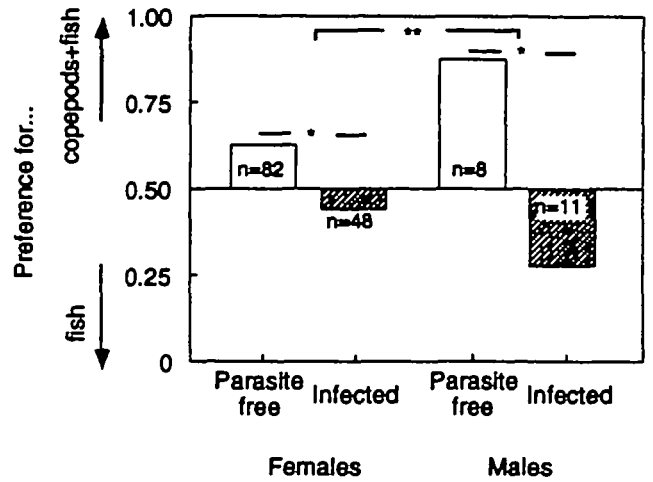

Figure 3
Preference of male and female copepods that were infected or parasite-free (controls and exposed but noninfected ones pooled) for the odor of fish-only or fish+copepods shown as the deviation from the null hypothesis. Data are plotted as in Figure 2. Differences in the sample sizes of Figures 2 and 3 are due to the five individuals that could not be sexed because they were in the fourth copepodite stage. The scale of the y-axis gives the frequency of copepods that chose the side receiving water from the copepod tank. *Parasite-free and infected copepods differ in their choice (Fisher's Exact test, $p < .05$); **Males and females differ in the strength of their response to the test situation (Mantel test, $\chi^2 = 8.01$, $p = .005$). The numbers of copepods per group are given in or near the bars.

against 0.5: $Z = 3.13$, $p = .002$, two-tailed; see Figure 2). This was in contrast to the infected copepods, which preferred the fish-only water (Figure 2).

This difference between infected and noninfected copepods in their reaction toward the two types of odors could be found in both sexes (Figure 3). However, males and females differed in the strength of their response to the test situation. The difference between infected and uninfected males was much greater than that between infected and uninfected females (Figure 3).

The "parasite index" we used to estimate severity of infection (i.e., the parasite volume as percentage of copepod volume) did not significantly correlate with the choice of the infected copepods in the test apparatus (analyzed for each experimental day separately: Mann Whitney U tests, all $p > .15$, two-tailed). However, these parasite indices tend to be higher in infected males ($n = 11$, median = 2.3%, range = 1.1–6.5%) than in infected females ($n = 49$, median = 1.4%, range = 0.4–6.5%; Mann Whitney $U = 173$, $p = .077$, two tailed), especially so in copepods that preferred the fish-only water (Mann Whitney $U = 56$, $p = .04$, two tailed). The different durations between behavioral experiment and procercoid measurement were neglected in these analyses for sex effects because the proportion of infected males was similar in all 4 experimental days (range = 10–22%).

A comparison of times at which noninfected and infected copepods showed the preference for one of the odors revealed that infected copepods increasingly preferred to move toward or stay in fish-only water instead of fish+copepod water as time after last feeding increased (Figure 4). There was no such difference detectable in noninfected copepods (Figure 4). The number of days since exposure to coracidia (i.e., 11, 12, or 13 days) did not correlate with the copepods' preferences in the test runs ($\chi^2 = 0.66$, df = 2, $p = .72$).

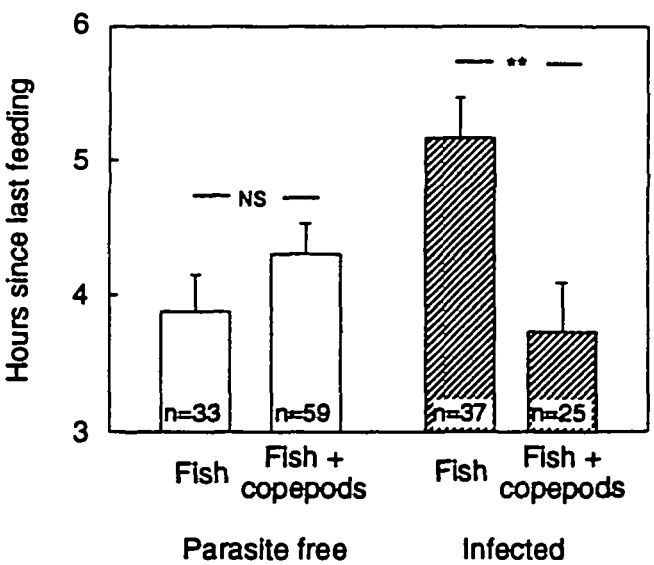

Figure 4
Comparison of mean hours since last feeding (+SE) at which parasite-free or infected copepods choose either the side receiving fish-only water or the side receiving water from fish+copepods. **The two means differ from each other ($t = 2.95$, $p = .004$); NS, nonsignificant ($t = 1.14$, $p = .26$). The numbers of copepods per group are given in the bars. The longer an infected copepod remained without food, the more likely it was to choose the fish-only water.

DISCUSSION

In our experiment the copepods could choose the "lesser of two evils." Sticklebacks are a main predator of copepods, and both sides of our test system contained stickleback-conditioned water. By adding the odor of conspecifics to one of the sides, we attempted to force the copepods to make a trade-off between the antipredator benefit of risk dilution or possible predator confusion and the costs of food competition and other disadvantages induced by conspecifics. Noninfected copepods that were fed ad libitum 1–8 h before the experiment (i.e., that were probably not hungry at the time the experiment took place) showed a preference for the side that smelled like conspecifics. This preference was clearly distinct from the preference of copepods that were infected by procercoids of the cestode *S. solidus*, although they had also been fed ad libitum at the same time as uninfected copepods. Infected copepods appeared to avoid conspecifics and to prefer the side that smelled like sticklebacks only, although this trend was not statistically significant.

Nine to 13 days after infection, most of the procercoids are expected to be in an infective stage under the conditions we kept them (Wedekind, 1997). Accordingly, when we checked the copepods the day after the last experiment had taken place, nearly all the procercoids had developed their cercomer (111 of 117 procercoids). Because it is obligatory for *S. solidus* to reach its second intermediate host, the three-spined stickleback, the tendency of infected copepods to move toward higher risk of being preyed upon by a stickleback is clearly in the interest of the parasite.

There are at least two explanations for changes in host behavior that favor transmission of its parasite (e.g., Milinski, 1990; Moore and Gotelli, 1990; Poulin, 1994): (1) the parasite has evolved a way to manipulate its host, which may include that it has to pay a cost for its manipulation effort, or (2) the changed host behavior is a side effect of parasitization which is by chance in favor of the parasite's fitness (i.e., the parasite does not have to pay the extra cost for manipulation effort to achieve high transmission). Parasites of relatively large size that draw significant amounts of resources from their hosts may especially benefit from hunger-induced behavioral changes of their hosts.

The different reaction to odor stimuli of parasite-free and infected copepods was more pronounced in males than it was in females. This could have several explanations that may be connected to the different life histories of the sexes of *M. albidus*. Male longevity is shorter than that of females, which could explain the paucity of males in our sample (at 20°C the difference in longevity is about 50%; Laybourn-Parry et al., 1988), and males develop slightly faster than females (Laybourn-Parry et al., 1988). The sexes are also size dimorphic with males being clearly smaller than females (Laybourn-Parry et al., 1988; Jakobsen and Wedekind, personal observations). These differences are likely to be influenced by their reproductive strategies: in contrast to males who show no parental care, females invest heavily in their offspring (they carry their eggs in relatively large egg sacs for some days). As a consequence, males are probably under higher inter- or intrasexual selection (Clutton-Brock and Parker, 1992). This is potentially a reason that males show a stronger preference than females for the odor of conspecifics when they are parasite-free and satiated. However, when infected they may suffer more from infection than females (Poulin, 1996; Zuk and McKean, 1996). In another study with standardized exposure, infected males carried, on average, more procercoids than infected females (Wedekind and Jakobsen, 1998). The same trend, although statistically not significant, could be observed in this study (infected males had, on average, 2.2 procercoids; infected females on average 1.7 procercoids; Mann Whitney $U = 229$, $p = .29$, directed). Moreover, in this study the infected males tended to carry more parasite biomass for their given body size than infected females did. This could explain a tentatively stronger preference for fish-only water in infected male copepods than in infected females.

We found a correlation between the time since last feeding and the reaction of the infected copepods to odor stimuli. This suggests that the more hungry the infected copepods are, the more they are willing to risk predation in order to avoid food competition by conspecifics (see also Giske et al., 1997). It seems that the changed host behavior is mainly caused by the energy drain from the parasite and therefore by the hunger level of the host. This would be analogous to the behavior of infected and noninfected sticklebacks: individuals that were infected with *S. solidus* were less risk sensitive when foraging than those that were not (Giles, 1983, 1987; Jakobsen et al., 1988; Milinski, 1985, 1990).

Our tests do not exclude the possibility of active manipulation by *S. solidus*. However, such active manipulation is probably not needed in this case. The parasite might have to pay a cost for its manipulation effort (Poulin, 1994), while the benefit of such a manipulation would only add to the hunger-correlated effects we found here.

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