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Hairworm response to notonectid attacks

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Very few parasite species are directly predated but most of them inherit the predators of their host. We explored the behavioural response of nematomorph hairworms when their hosts are preyed upon by one of the commonest invertebrate predators in the aquatic habitat of hairworms, notonectids. The hairworm *Paragordius tricuspidatus* can alter the behaviour of its terrestrial insect host (the cricket *Nemobius sylvestris*), causing it to jump into the water; an aquatic habitat is required for the adult free-living stage of the parasite. We predicted that hairworms whose hosts are captured by a notonectid should accelerate their emergence to leave the host before being killed. As predicted, the emergence length of the worm was significantly shortened in cases of notonectid predation, but the exact reason of this response seems to be more complex than expected. Indeed, experimental manipulations revealed that hairworms are remarkably insensitive to a prolonged exposure to predator effluvia which notonectids inject into prey, so accelerated emergence is not a protective response against digestive enzymes. We discuss other possibilities for the accelerated exit observed, ranging from unspecific stress responses to other scenarios requiring consideration of the ecological context.

Very few parasite species have direct predators but most inherit those of their host (Thomas et al. 2002a). This peculiar ecological context has favoured the evolution of diverse adaptations by parasites to avoid succumbing to predation upon their host. The principal and most common response is to reduce the encounter rate with potential predators by altering the behaviour of the host (Brodeur & McNeil 1994; Levri 1998; Lafferty et al. 2000; Thomas et al. 2002b; Haine & Rigaud 2005). Where predation is unavoidable, certain parasites have developed the capacity to encyst in the predator until a new favourable event occurs (e.g. Robert et al. 1988; Pampoulie et al. 2000) or resist a complete transit in the predator gut (McFarland et al. 2003). Over evolutionary time, parasites have also evolved the capacity to colonize and exploit the predators of their host, thereby evolving complex life cycles (Poulin 1998; Lafferty 1999; Parker et al. 2003; see Choisy et al. 2003 for review).

Recently, a novel antipredator strategy by parasites was found in the hairworm *Paragordius tricuspidatus* (Nematomorpha: Gordiida) parasitizing orthoptera. The larval stages of this parasite develop in the cricket *Nemobius sylvestris*, which is terrestrial, but the adult phase is free living and aquatic in ponds and streams of southern France. To exit the cricket and enter the water, the mature parasite alters the behaviour of the insect host, making it seek out and jump into water (i.e. induced host 'suicide'; Thomas et al. 2002c). These water areas are frequently inhabited by both vertebrate and invertebrate predators. Ponton et al. (2006a, b) showed that if the crickets that enter the water are eaten by fish or frogs then the hairworm is able to escape not only from its insect host but also from the digestive tract of the predator. The worm emerges alive from the mouth, gills or nose of the predators and continues its life cycle without any fitness costs. This escape response was the first example of a parasite, or any organism, surviving predation in this way (Ponton et al. 2006a).

In the forest ponds of southern France, predators include not only vertebrates but also several predatory

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invertebrates, the most common of which are notonectids or backswimmers (Hemiptera, Notonectidae, *Notonecta glauca*). Notonectids are voracious generalist predators that attack just about any prey that they can overpower ranging from mosquito larvae to pike fry. Notonectids are known to structure ecological communities (Murdoch & Scott 1984; Murdoch et al. 1984; Geddes 1986; Arner et al. 1998; Blaustein 1998; Pace et al. 1999) and influence the oviposition behaviour of mosquitoes (Chesson 1984). They will attack orthoptera that accidentally fall into water (F. Thomas, personal observations). Like all hemipterans, notonectids lack chewing mouthparts and feed using a rostrum, or 'sucking-beak'. These predatory hemipterans inject digestive juices down one canal of the rostrum and suck up the digested prey through another canal. The significance of such feeding for any parasite of the prey item is two-fold. First it means that, unlike vertebrate predation, the body of the prey is not physically ingested inside the predator; instead it stays outside and is released when empty. Second, for parasites occupying the host's haemocoel (as hairworms do), it means that digestive juices will be encountered immediately.

The aim of this study was to determine whether hairworms display antipredator behaviour against notonectids and, if so, to examine how it differs from antipredator behaviour against vertebrates. We predicted that hairworms would avoid notonectid predation by means of a more rapid emergence from the cricket host when the host was attacked. We also wanted to assess the cost of predation by notonectids on hairworms so we experimentally prevented worms from escaping their host following a notonectid attack. We discuss the relevance of the hairworm response in the context of antipredator strategies.

METHODS

Sampling

As in Thomas et al. (2002c), infected *N. sylvestris* were captured at night (between 2200 and 0100 hours) around a private swimming pool (15 × 10 m) and on a parking area located in Avènes les Bains (southern France, 70 km north of Montpellier). All specimens were collected during July 2006. The swimming pool and the parking area are beside a forest that is crisscrossed by small streams in which adult *P. tricuspidatus* were commonly found during the summer. Paved areas allowed direct observation and capture of infected crickets moving from the forest. Previous observations (Thomas et al. 2002c) revealed that crickets detected on the concrete area were always infected by at least one worm.

Notonectids in surrounding ponds were sampled on the same date using a net. Captured individuals were kept singly in plastic bottles (8 cm diameter, 20 cm height) that were placed in aquaria (60 cm length, 30 cm height, 30 cm width) and filled with constantly aerated water. The bottoms of the bottles were covered by a net (2 mm mesh size), allowing water from the tank to circulate freely through all the compartments. Notonectids were acclimatized for a period of 4 days during which no food was provided to induce a fast attack response required for the

experiment. Crickets were collectively kept in aquaria (30 × 25 cm, height 16 cm) provided with ad libitum food (in equal proportions: cereals, fish food Tetra Ani Min, dry gammarids and dry tubifex) and humidified cotton. All individuals, notonectids and crickets, were placed in undisturbed rooms which had a 16:8 h light:dark cycle that mimicked the natural photoperiod at capture period. The analysis was based on 34 infected crickets in the first experiment (14 tested in presence of a notonectid and 20 without predator) and 35 in the second experiment (20 crickets in presence of predator and 15 without predator).

Experimental Procedure

We presented infected crickets to a notonectid to determine the hairworm's response to predation. Experiments were performed during the afternoon (between 1400 and 2000 hours). Infected crickets were gently placed into a tank of water containing a notonectid. Control infected crickets were placed into a tank without a notonectid. We considered a predation test valid only if the notonectid attacked the cricket immediately after its entrance in the water. In no cases had worms begun to emerge at the moment the cricket was attacked; that is, the parasite was fully inside the cricket.

To assess whether there were negative effects on notonectids because of predation on their host we experimentally prevented hairworm emergence by covering the terminal part of the cricket's abdomen with superglue (i.e. openings were blocked). Once the attack had finished (the notonectid released the dead cricket) we gently opened the abdomen to liberate the worm inside and examine its state. We determined whether the worm was dead or alive. If alive we determined whether it could still swim and whether it was able to reproduce (lay eggs for females and donate a spermatophore for males). Worms from crickets treated with superglue in the absence of predators were used as a control. To avoid confounding effects of multiple infection, only individuals singly infected were used for the analysis.

Statistical Analysis

Statistical tests were performed following Sokal & Rohlf (1981) and Siegel & Castellan (1988). Homogeneity of variance between groups was tested using the Levene statistic. Since variance heterogeneity between the attacked and the not-attacked groups was one of the predictions concerning the length of the emergence (the time that it takes to emerge), we used a Welch ANOVA on untransformed and ln-transformed data (Welch 1951) to compare groups. Welch ANOVA is suitable since it allows comparisons when variances are unequal and the data are approximately normalized. All tests were two tailed.

RESULTS

As predicted, worms that were inside cricket hosts that were predated by notonectids emerged significantly faster than controls (110 versus 380 s; Welch

ANOVA: $F_{1,19.38} = 8.34$, $P = 0.0093$; Fig. 1). The variance (Levene test: $P < 0.0001$) was significantly shorter when the host was attacked by a notonectid than when there was no predator (Fig. 1). Once it had emerged, the adult hairworm was never directly attacked by the notonectid.

The second experiment revealed that the use of superglue prevented hairworm emergence so that the worm was constrained to remain inside the cricket host throughout the entire predation event (approximately 5 min). Once the dead cricket was released we examined the worm and found that all worms in the predated group were alive (as were those in the control). There was no significant size difference between worms from the two groups (Student's t test: $t_{27} = -0.34$, $P = 0.74$) nor observable difference in their swimming ability. Finally, there was no significant difference between the two groups in the proportion of worms mating (80% for predated worms, 67% for control; Fisher exact test: $P = 0.405$).

DISCUSSION

The role of predation in the evolutionary ecology of animal communities has long been a focus of ecological research but, to date, sparse attention has been given to the responses of parasites when their hosts are victims of predation. Because half of life on earth may be parasitic (Price 1980) and because parasite virulence is shaped by predation (Read 1994), this is a serious oversight. Previous work (Ponton et al. 2006a, b) showed that hairworms have evolved an original solution to the predation of their host by vertebrates: they crawl out from the stomach of the predator. Since invertebrate predators also inhabit the

Figure 1. Mean ± SE time taken to emerge for worms exposed to notonectid predation and those used as a control (in absence of predators).

aquatic habitat needed by adult hairworms, we predicted that evolving antipredator responses to other kinds of natural enemies should also be a part of the wider strategy of hairworms for the completion of their life cycle.

Our experiments indicated that the time taken by the worm for full emergence from its host was significantly reduced in case of notonectid attack. Such an accelerated exit might be interpreted as an antipredator response from the worm. Ponton et al. (2006a) showed that time was indeed a key component in the antipredator response of hairworms towards vertebrates: if a worm was not observed coming out the fish within 5 min, it never exited, presumably because it died in the hostile environment of the predator's stomach. Notonectids do not ingest crickets as fish do but instead pump digestive juices into the host, and this, we speculate, would be hazardous to the hairworm that occupies the cricket's abdomen. We reasoned a priori that the harsh environment would impair the viability and/or the reproductive capacities of the worm. Against our expectations, the results of the second experiment showed that notonectid attack itself does not appear to harm the worm. Hairworms from attacked hosts were not only alive and able to swim as well as controls but they also reproduced. At the adult stage, hairworms are simply a mobile bag of gametes. The length of the worm is highly correlated with the number of gametes and hence to fitness (Hanelt et al. 2005). Therefore, in our assessment of fitness we measured worm length and general vigour. The conclusion that notonectid attack has no significant effect on worm fitness is based on the measurement of the relatively few fitness surrogates that we are able to measure. Arguably there are potentially other aspects of fitness that may be negatively impacted by the predation event which we were not able to measure. For example, the biology of this parasite system does not lend itself to other measurements such as lifetime fecundity or longevity because egg release from this mobile bag of eggs may occur even when the worm is apparently dead, and ascertaining the moment of death is problematic since worms can remain completely inactive for weeks (F. Thomas & A. Schmidt-Rhaesa, personal observations). Egg shedding might even occur from dead worms.

It would appear that the accelerated exit of the worm when its host is attacked by a notonectid is not a response to the physiological damage posed by exposure to digestive juices. This might be due to the highly resilient cuticle that adult hairworms possess. The physical and chemical resistance capabilities of the skin lie in its ultrastructural organization and biochemical composition (Swanson 1970; Schmidt-Rhaesa 1996; Brivio et al. 2000). The cuticle is multilayered and extremely complex (Schmidt-Rhaesa 2004), which may provide effective protection from the harsh environment of the notonectids' effluvia. The rapid emergence, despite no obvious mortality risks, may occur because worms cannot discriminate between host attacks that are dangerous and those that are not. If no proximate factor permits the worm to make such a distinction, it is undoubtedly better to accelerate the exit in all cases, especially if this has only trivial fitness costs as suggested by our data and by Ponton et al (2006b). It is also possible that the predation of crickets by notonectids increases the visibility
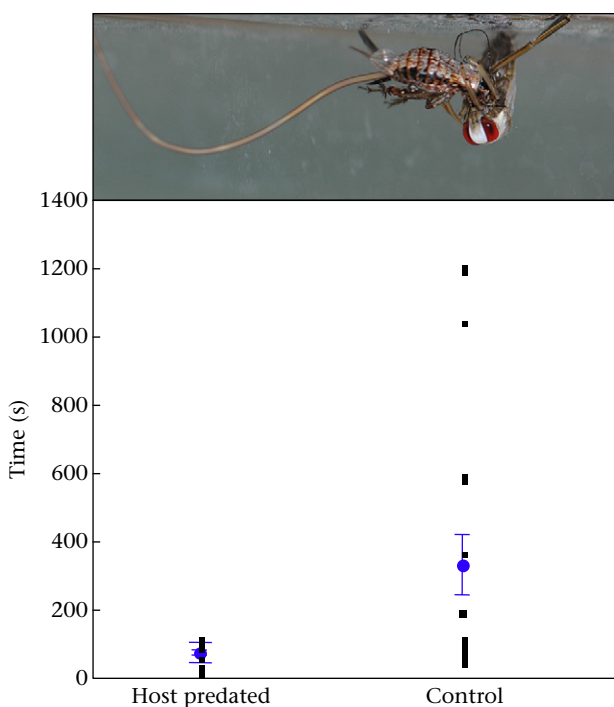
of the cricket to visually hunting vertebrates such as fish. In this case more rapid emergence would be advantageous; although worms can survive predation by vertebrates, it is fatal in many cases (Ponton et al. 2006a). Finally, because the contortion performed by emerging worms (Fig. 1) make them more visible to generalist predators that could also consume both notonectid and cricket, we cannot fully exclude the possibility that notonectids induce the rapid emergence of the worm to reduce their own predation risk. The proximate mechanisms that would sustain such a scenario remain to be determined; it is of interest that the cuticle of hairworms has sensory capabilities (Schmidt-Rhaesa 2004).

Acknowledgments

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