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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.

Keywords: crickets; hairworms; manipulation; *Nemobius sylvestris*; *Notonecta glauca*; notonectids; *Paragordius tricuspidatus*; predation

Very few parasite species have direct predators but most inherit those of their hosts (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