Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts?

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Abstract

Several anecdotal reports in the literature have suggested that insects parasitized by hairworms (Nematomorpha) commit 'suicide' by jumping into an aquatic environment needed by an adult worm for the continuation of its life cycle. Based on 2 years of observations at a swimming pool in open air, we saw this aberrant behaviour in nine insect species followed by the emergence of hairworms. We conducted field and laboratory experiments in order to compare the behaviour of infected and uninfected individuals of the cricket *Nemobius sylvestris*. The results clearly indicate that crickets infected by the nematomorph *Paragordius tricuspidatus* are more likely to jump into water than uninfected ones. The idea that this manipulation involved water detection from long distances by infected insects is not supported. Instead, our observations suggest that infected insects may first display an erratic behaviour which brings them sooner or later close to a stream and then a behavioural change that makes them enter the water.

Introduction

Parasite-induced alterations of host phenotype are now known from a wide range of host–parasite associations (Combes, 1991; Poulin, 1998; Poulin & Thomas, 1999). These changes are often adaptive for the parasite as they enhance host-to-host transmission, ensure the parasite or its propagules are released in an appropriate location, or increase parasite survival (but see Poulin, 1995). For instance, many tropically transmitted parasites alter the phenotype of their intermediate hosts in a way that increases their likelihood of being eaten by predatory definitive hosts (Moore, 1984; Thomas & Poulin, 1998; Lafferty, 1999; Berdoy *et al.*, 2000; Brown *et al.*, 2001; Hurd *et al.*, 2001). Several fungus species have been termed 'enslaver' parasites because they make their

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insect hosts die perched in an optimal position for the dispersal of fungal spores by the wind (Maitland, 1994). Some trematodes drive their mollusc intermediate hosts toward ideal sites for the release of cercariae (Curtis, 1987). Parasitic wasps can make their host seek protection within curled leaves to protect themselves from hyperparasitoids (Brodeur & McNeil, 1989), or can even make the host weave a special cocoon-like structure to protect the wasp pupae against heavy rain (Eberhard, 2000). These few impressive examples suggest that host manipulation represents the sophisticated products of parasite evolution rather than simply accidental side-effects of infection.

The Nematomorpha is a relatively unknown taxon which contains about 300 species distributed around the world and commonly called hairworms (Schmidt-Rhaesa, 1997). Adult males and females are free-living in aquatic environments and gather to mate in tight masses (i.e. a 'gordian knot'). Unlike adults, juveniles are parasitic in arthropods. Hosts (mainly terrestrial insects) become infected with hairworms when they ingest parasitic larvae (directly or indirectly through a paratenic

host, see Hanelt & Janovy, 1999; Schmidt-Rhaesa, 2001). During their development, nematomorphs grow from a microscopic larva to a large worm whose size exceeds the length of the host by a considerable amount. Indeed, when the parasitic development has been completed, the worm occupies most of the host cavity with the exception of the head and the legs. Worms are only ready to emerge once they reach this stage. Based on several anecdotal observations, it has often been hypothesized that mature nematomorphs manipulate the behaviour of their terrestrial insect host making them seek water and jumping into it (see Blunk, 1922; Thorne, 1940; Dawkins, 1990; Poinar, 1991; Begon *et al.*, 1996; Schmidt-Rhaesa, 1997).

The aim of this study was to determine whether hairworms altered the behaviour of their host in order to reach an aquatic environment needed for their emergence and reproduction. Based on field observations made during two consecutive summers, we first provide a list of the insects for which we observed the infected host entering water in order to release a worm. We then conducted a field and a laboratory experiment to compare the behaviour of the cricket *Nemobius sylvestris* when uninfected and when infected by the nematomorph *Paragordius tricuspidatus*. We discuss our results in relation with current ideas on the adaptiveness of parasite-induced phenotypic changes in their hosts.

Materials and methods

Study area and field observations

Our study area was a private swimming pool (15 × 10 m) in open air located in Avènes les Bains (Southern France, 70 km north from Montpellier). This swimming pool was located near a forest largely criss-crossed by small streams in which adult nematomorphs were commonly found during the summer. Between this swimming pool and the forest, a concrete area 5 m wide allowed direct observations of insects arriving from the forest in the direction of the swimming pool. Observations were made almost every night over two consecutive summers (2000 and 2001). When not captured for the experiments (see below), all insects detected on the concrete area were visually followed without disturbing them until they entered the swimming pool itself. Then, the host and the worm emerging from its body were captured and then preserved in alcohol (70%) to be identified.

Field experiment (July 2000)

The aim of this experiment was to compare the behaviour of the cricket *N. sylvestris* when uninfected and when harbouring a mature hairworm (*P. tricuspidatus*). We collected at night 33 individuals in the forest (100 m from the swimming pool) and 38 individuals near the edge of the swimming pool. The forest sampling area and

the swimming pool are parallel to the same stream and are consequently at an equal distance from it (7 m). All these insects were kept for one night in the laboratory, in a terrarium containing wood and leaves from their natural habitat. The next night, we studied their behaviour for a maximum of 15 min by placing them on the concrete area at 2 m from the edge of the swimming pool. Test individuals were deposited inside an opaque plastic tumbler for 3 min before we raised the tumbler. We simultaneously placed four crickets (two from the forest and two from the concrete area) with a distance of 3 m between them. When a cricket entered the water, the experiment was completed for this individual. After 15 min, all the insects were preserved in alcohol (70%). In the laboratory, crickets were sexed and dissected to confirm their parasitic status.

Laboratory experiment (July 2001)

To investigate whether the presence of water is an attractive stimulus for infected crickets, we conducted a choice experiment in an Y-maze made of transparent plexiglas (arm length: 1.5 m). The end of each arm consisted of a trough but only one was filled with water (1 L). In order to avoid positional biases, the arms with water and no water were randomized throughout the experiments. To increase the possibility of water detection by crickets, we supplied a small air current generated by an aquarium air pump in each arm of the maze. The air speed in both the humid and the dry arms was kept equal. Temperature was 23 °C and light level was adjusted to the minimum required for an observer to locate the cricket in the maze. Crickets were captured in the first part of the night (before 1 AM) both in the forest and around the swimming pool, and were kept as before (see Field Experiment) in the laboratory until being tested the next night. Each cricket was tested individually in the Y-maze and only once. Test individuals were gently deposited in the end of the tail of the maze inside an opaque plastic tumbler. After 3 min we raised the tumbler allowing the cricket free access in the maze for a maximum of 30 min. If the cricket fell in a trough before 30 min, the experiment was stopped. When a cricket did not fall in a trough within the 30 min, we noted its final position (i.e. humid or dry arm). All crickets tested were then preserved in alcohol, measured, sexed and dissected to confirm their parasitic status.

Data were analysed using logistic regressions with S-PLUS 2000 Professional Release 2© (MathSoft, Inc., Seattle, WA, USA). A first logistic regression was conducted to analyse the decisions made by crickets when given the choice between the two branches of the Y-maze. Explanatory variables used in the analysis were the parasitic status, the sex of the cricket, its age (larvae or nymph), its size, and the side of the trough containing water (left or right). Then, we performed where necessary other logistic regressions to analyse the behaviour of

crickets when engaged in a given branch (i.e. whether they fell in the trough or stayed in the corridor). Independent variables used in the analysis were the same as before except the side of the trough with water. All the independent variables were entered into logistic regression models, permitting one to control for the effect of the other independent variables on a given descriptor variable. The total model considered all main effects and two-way interactions. We then proceeded a backward elimination procedure in order to identify the best models according to their Akaike information criterion (from the lowest to the highest). The deviance analyses were performed using χ^2 tests.

Results

Field observations

After two summers of observations, we saw nine species of insects coming from the forest toward the swimming pool, entering the water and releasing one or several worms belonging to the two species *P. tricuspidatus* (Dufour, 1828) and *Spinochordodes tellinii* (Camerano, 1888) (Table 1). Additionally, three spiders (two individuals of *Pistius truncatus* and one individual of *Olios argelasius*) were observed to jump into the swimming pool and each releases one large undetermined mermithid nematode. The majority of hosts entered water during the first part of the night (i.e. before 1–2 AM). The two most common species entering water were the crickets *N. sylvestris* in July and *Meconema thalassinum* in August. A movie showing the aberrant behaviour of infected *N. sylvestris* is provided as supplementary material (see 'Supplementary material' section).

Except for the species *Antaxius pedestris* and *M. thalassinum* for which uninfected individuals could sometimes be found on the concrete area, all the insects in Table 1, when found around the swimming pool, were infected by a hairworm and sooner or later took to the water of the swimming pool. Insects entered the water by jumping into it or by entering gradually. After the host had

entered the water, the emergence of the worm could be immediate (e.g. *S. tellinii* emerging from *M. thalassinum*) or could take several minutes, i.e. after the host had drowned (e.g. frequent for *P. tricuspidatus* emerging from *N. sylvestris*). In the latter case, however, we always saw just after the host has jumped into the water and was thus in contact with a liquid medium, the worm emerging 1–2 cm and returning inside the host, presumably because the end of the cricket abdomen was not directly in contact with water (the worm was always seen to emerge fully 2–5 min after). A few seconds after the emergence from the host, the worm actively swims away and leaves its host (see the movie).

Crickets (*N. sylvestris*) that had been rescued ($n = 10$) immediately returned to the edge of the swimming pool and jumped in again. Finally, in five occasions, we saw individuals of *N. sylvestris* leaving the swimming pool after having released their worm at the surface of the water. This phenomenon is probably rare in natural conditions because of the current in streams.

Field experiment (July 2000)

The prevalence of infection by *P. tricuspidatus* was very different between *N. sylvestris* collected in the forest (5/33, i.e. 15%) and those collected around the swimming pool (36/38, 95%) (Fisher's exact test, $P < 0.00001$). Thus, the field experiment was conducted from 41 infected and 30 uninfected individuals. None of these insects were adults (i.e. all were larvae or nymphs). The sex ratio was not significantly different between infected (16 males and 25 females) and uninfected individuals (18 males and 12 females) (Fisher's exact test, $P = 0.10$).

Among the 41 individuals harbouring a worm, 20 (i.e. 48.7%) entered the water within 15 min whereas only four uninfected individuals among 30 (i.e. 13.3%) entered the water (Fisher's exact test, $P = 0.002$). Individuals which did not enter the water explored the concrete area in no particular direction, tried to hide themselves by entering a fissure in the concrete or went toward the forest. Among these 41 infected insects, 36

Table 1 List of the host–hairworm associations for which we saw the host entering water and the emergence of the worm.

Host species	Nematomorph species	Observations
Gryllidae		
Nemobius sylvestris	*Paragordius tricuspidatus*	Exclusively on July (more than 70 observations)
Tettigoniidae		
Meconema thalassinum	*Spinochordodes tellinii*	Almost exclusively on August (30 observations)
Pholidoptera griseoptera	*S. tellinii*	August (11 observations)
Uromenus rugosicollis	*S. tellinii*	August (five observations)
Ephippiger cunii	*S. tellinii*	August (one observation)
Barbitistes sericauda	*S. tellinii*	August (one observation)
Leptophyes punctatissima	*S. tellinii*	August (one observation)
Antaxius pedestris	*S. tellinii*	August (one observation)
Yersinella raymondi	*S. tellinii*	August (seven observations)

harboured one worm, four harboured two worms and one individual harboured four worms.

Laboratory experiment (July 2001)

As in July 2000, the difference of prevalence was highly significant between *N. sylvestris* collected in the forest (0/17, 0%) and those collected around the swimming pool (16/17, 94%) (Fisher's exact test, $P < 0.00001$). Sex ratio was not significantly different between infected (11 males and five females) and uninfected individuals (11 males and seven females) (Fisher's exact test, $P = 0.73$).

Among infected crickets, three males did not leave the base of the Y-maze and were excluded from the analysis (we kept these individuals in the laboratory and they were dead the day after, suggesting that they were in a poor condition when tested). Both infected and uninfected crickets explored the Y-maze but sample sizes observed in each branch were not significantly different from those expected under the null hypothesis of a random choice (infected crickets: $\chi_1^2 = 0.69$, n.s.; uninfected crickets: $\chi_1^2 = 2.0$, n.s). The logistic regression showed that only the size of the cricket has a slight effect (and only when other variables were kept constant) to explain the branch choices made by crickets (Table 2; mean ±SD, humid branch: 9.2 ± 1.1 mm, $n = 20$; dry branch: 8.5 ± 1.3 mm).

All crickets (i.e. infected and uninfected) entering the dry arm walked straight and jumped into the dry trough within the 30 min. However, in the humid branch, all infected crickets jumped into the trough with water whereas only one of 12 uninfected crickets found in this branch did so (Fisher's exact test, $P = 0.00007$). A logistic regression revealed that among predictor variables, only the parasitic status was significant to explain the probability of entering water (Table 3).

In summary, it seems that crickets, infected or not, chose their branch irrespective of water presence but once they encounter water, infected individuals were more likely to enter it.

Discussion

This is the first study to document the behavioural change of insects infected by nematomorphs. Indeed,

Table 2 Results obtained from logistic regression for predicting branch choice (interaction terms were not significant).

Source	d.f.	Deviance	Pr (Chi)
Parasitic status	1	0.086	0.77
Side of the water trough	1	0.176	0.67
Cricket sex	1	0.328	0.57
Cricket size	1	3.728	0.05
Cricket age	2	0.786	0.37
Residual	25	35.22	

Table 3 Results obtained from logistic regression for predicting the probability of entering water once inside the humid branch (interaction terms were not significant).

Source	d.f.	Deviance	Pr (Chi)
Parasitic status	1	20.64	0.000005
Side of the water trough	1	1.48	0.22
Cricket sex	1	0.91	0.34
Cricket size	1	1.72	0.19
Cricket age	1	0.0007	0.98
Residual	14	2.77	

despite several anecdotal reports in the literature of insects entering water to release a worm, no previous attempt has been made to determine how widespread it is among arthropods harbouring such parasites.

Field observations, as well as experiments conducted in the field and in the laboratory, clearly indicate a behavioural difference between infected and uninfected individuals of *N. sylvestris*. As a result of this behavioural difference, infected insects are more likely to finish in water, where adult nematomorphs must emerge. The results of our two experiments on *N. sylvestris* do not support the idea that infected crickets detect the presence of water from long distances. Our observations are also in accordance with another, and probably more realistic, hypothesis given the ecological context. In both the field and the laboratory experiments, only 50% of the infected crickets (*N. sylvestris*) went toward the water and entered it. First, it is possible that infected individuals which did not enter water were simply under stress or in a poor condition when tested, and/or that our experiments did not last for long enough. Conversely, we cannot exclude the possibility that infected crickets which did not enter water were not manipulated when tested. The absence of manipulated crickets during the day or after 2–3 AM (F. Thomas, field observations) indeed suggests that manipulation is not permanent even when the worm is mature. In addition, we must keep in mind that the necessity of water detection in this manipulation becomes questionable when we consider the ecological conditions in which this host–parasite system has evolved. A behavioural alteration induced by nematomorphs could just be the induction of an erratic behaviour: infected crickets would leave their microhabitat but in no particular direction. Given the abundance of streams in their native forest, this would undoubtedly bring the cricket close to a stream. Alternatively, if insects routinely encounter water during a time scale appropriate to worm development, there may be no need at all to induce erratic or water seeking behaviour. In accordance with the former idea all the crickets that we found in atypical habitats (two *N. sylvestris* on a car park and ten in a hotel in Avènes les Bains) harboured a worm.

Once infected crickets encounter water, there is an important behavioural difference compared with uninfected individuals. Crickets harbouring a worm often jumped into the water whereas uninfected crickets most of the time were reluctant to enter it. This behavioural difference is a key step in the manipulative process as it allows the hairworm to emerge immediately after its host enters water. Whether infected crickets are attracted by the liquid, or whether they simply do not perceive the danger linked to the presence of water (e.g. anxiolytic action of the parasite, see for instance Berdoy *et al.*, 2000) is not currently known. We cannot exclude that infected crickets do not react to a number of outside cues, including water, and therefore end up falling into it, rather than avoiding it. Alterations in host behaviour following parasitic infection are often exactly what we would expect to see if the host were to start acting in a way that benefits the parasite (Poulin, 1998). For this reason, they appear to be adaptations rather than mere pathological side-effects. Changes observed may also lead to improved parasite fitness because they increase its probability of reaching a mating place.

Adaptations can also be recognized at the macroevolutionary scale when different parasite lineages evolving under similar selective pressures have independently evolved the ability to cause identical alterations in host behaviour (Poulin, 1995, 1998; Thomas & Poulin, 1998). Although we did not compare the behaviour of infected and uninfected individuals in the eight other insect species harbouring nematomorphs, our field observations suggest that a similar behavioural change occurs. Whether these behavioural changes derive from the same or different proximal mechanisms among these different systems cannot be determined from these data. Further investigations, particularly in physiology and neurobiology, would be necessary to clarify this point, and to determine if these changes are legacies from a common ancestor, or conversely independent adaptations. The aberrant behaviour of the spiders harbouring mermithids is in accordance with previous anecdotes (e.g. Maeyama *et al.*, 1994). Mermithids are phylogenetically unrelated to nematomorphs but have a similar biology, suggesting an evolutionary convergence between nematomorphs and mermithids in their effect on host behaviour.

Supplementary material

An interactive online version of this model can found at the following web address: http://www.blackwell-science.com/products/journals/suppmat/JEB/JEB410/JEB410sm.htm

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