

The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*

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Abstract

Acanthocephala are parasites with complex life cycles involving arthropod intermediate hosts and vertebrate final hosts. They use predation as a means of transmission, and some species have developed the ability to modify behaviour of their intermediate hosts to enhance the probability of ingestion by the definitive host. Knowledge of how a single parasite species is adapted to modify the behaviour of different intermediate host species is important for the understanding of parasitic transmission in host communities. In Burgundy, the freshwater amphipod crustaceans *Gammarus pulex* (native species) and *Gammarus roeseli* (eastern European invader) are both intermediate hosts for the acanthocephalan *Polymorphus minutus*. The influence of this bird parasite on the geotaxis of *G. roeseli* was evaluated and it was found that when infected, individuals of this host species have a negative geotaxis compared to uninfected individuals. There were two components to the behavioural changes: swimming to the top of the water column, and clinging to surface material. These changes were comparable to those observed in the local host species *G. pulex*, but lower in magnitude. This result contrasts with a previous study on the influence of the fish parasite *Pomphorhynchus laevis*, which is able to alter the native species' behaviour, but not that of the invasive host. Parasite adaptations to local vs invasive intermediate host species are discussed in terms of their dispersal range (i.e. dispersal of their definitive hosts): the wider the dispersal, the greater should be the spectrum of intermediate hosts.

Key words: host–parasite relationships, behavioural modifications, biological invasions, freshwater Crustacea, *Gammarus*

INTRODUCTION

Many parasite taxa have complex life cycles, involving two or more successive hosts, and use predation as a means of transmission from intermediate to definitive hosts. Some of these parasites have developed the ability to modify morphological, physiological or behavioural aspects of their intermediate hosts (Holmes & Bethel, 1972; Moore & Gotelli, 1990; Combes, 1991; Lafferty, 1999; Moore, 2002). These alterations enhance the probability of ingestion by the definitive host, by making infected individuals more susceptible to predation (manipulation or favourization process, Combes, 1991). Among such parasites are the Acanthocephala, a phylum of helminths that depend on predation-mediated transmission from arthropod intermediate hosts to vertebrate definitive hosts to complete their life cycles (Lafferty, 1992). They are known to be particularly

virulent in the behavioural modifications they induce in their intermediate hosts (Dobson, 1988) to increase such trophic transmission (Bethel & Holmes, 1974; Helluy & Holmes, 1990; Maynard, Demartini & Wright, 1996; Lafferty, 1999).

Several studies have recently suggested that parasites may modify the outcome of competition between host species. In particular, parasites modifying host behaviour to increase their trophic transmission may induce a differential in predation in their hosts (Thomas *et al.*, 1995; MacNeil *et al.*, 2003). In the context of biological invasion, where parasites may face new hosts (the invasive species), such a differential may explain to some extent the success of invasive vs local species (Prenter *et al.*, 2004). However, in the first instance, understanding this phenomenon requires fundamental data on how parasites adapt to novel hosts. In Burgundy (eastern France), two species of amphipod crustaceans live in sympatry: the native *Gammarus pulex*, and *Gammarus roeseli*, a recent colonizer of central European origin (Jazdzewski, 1980). They are both intermediate hosts for two species of

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acanthocephalan parasites: *Pomphorhynchus laevis* and *Polymorphus minutus*. The definitive hosts of *P. laevis* are fish (Kennedy, 1996), while those of *P. minutus* are aquatic birds (Holmes & Bethel, 1972). *Pomphorhynchus laevis* is known to alter the reaction of *G. pulex* towards light (Kennedy, Broughton & Hine, 1978; Bakker, Mazzi & Zala, 1997; Cézilly, Grégoire & Bertin, 2000), whereas *P. minutus* modifies its geotactic behaviour (Cézilly *et al.*, 2000).

Bauer *et al.* (2000) showed that *G. roeseli* individuals infected by *P. laevis* do not show an altered reaction to light, and this may result from the parasite's inability to manipulate this invading host species. Kennedy (1996) described *P. laevis* as a poor disperser and colonizer, and suggested that this may have led to rapid local adaptation, as strains diverged according to their preferred definitive host species. The apparent inability of local *P. laevis* to manipulate *G. roeseli* in Burgundy could come from a maladaptation to its non-native intermediate host (Bauer *et al.*, 2000), a result congruent with that obtained on the interaction between this parasite and the immune system of its hosts (Rigaud & Moret, 2003). In this study, we investigated if a similar pattern is found with another acanthocephalan parasite, *P. minutus*, which has a different final host and therefore a different dispersal ability.

Many experiments testing for parasite local adaptation use infectivity (infection success) or virulence (host damage) as parasite fitness measures, and compare migration rates for the parasite and the host (Dybdahl & Storfer, 2003). For *Polymorphus* parasites, inducing changes in the geotaxis of the intermediate host is a reliable estimator of parasite fitness, since it is a component of its transmission (Bethel & Holmes, 1974) and thus its virulence (Poulin & Combes, 1999). To our knowledge, however, the effect of *P. minutus* on the geotactic behaviour of *G. roeseli* has not yet been investigated. Therefore, the geotaxis modification induced by *P. minutus* in the invasive *G. roeseli* was evaluated and compared with that of the local host *G. pulex*. Helluy & Holmes (1990) showed that clinging behaviour is also a component of the behavioural modification induced by a *Polymorphus* parasite in its host *Gammarus lacustris*. While Cézilly *et al.* (2000) measured geotaxis as a component of the behavioural changes induced by *P. minutus* infection in *G. pulex*, they did not investigate this clinging behaviour. Thus, we also aimed to investigate if the clinging behaviour is a component of the behavioural changes in both intermediate host species.

MATERIALS AND METHODS

Gammarus roeseli were collected in the River Ausson (Côte d'Or, France), and *G. pulex* were collected in the River Ouche at Parc de la Colombière (Dijon, Côte d'Or, France), using the kick-sampling technique (Hynes, 1954). Animals were maintained in the laboratory at $16 \pm 2^\circ\text{C}$, and tested at this temperature within 72 h after collection. The following 2 experiments were conducted.

The experimental design used in experiment 1 to evaluate the parasite's influence on its intermediate host geotactic behaviour is described in Cézilly *et al.* (2000). A translucent plastic column (diameter = 11.5 cm) was placed in an aquarium (80 × 40 × 30 cm) filled with oxygenated and filtered water from the sampling zone, and maintained at room temperature. The column was virtually subdivided in 6 zones of equal height (5 cm) by pen lines. Animals were placed individually in the column, and the top of the tank was covered with a black hard top. The underneath of the tank was also kept dark to avoid any confounding phototactic reaction resulting from the room lighting. After an acclimatization period of 5 min in the column, the experiment began: every 30 s during 5 min, the position of the gammarid was reported, and a score was given according to its vertical position in the column (1 at the bottom, up to 6 at the top of the column). The total score for each individual was calculated as the sum of each report, i.e. a gammarid that was always at the bottom of the column for 5 min scored 10, while a gammarid always at the top of the column scored 60.

For experiment 2, the design was changed by placing a piece of netting on the water surface at the top of the column, which the gammarids could cling on to. By comparing experiment 1 with experiment 2, it was possible to determine if clinging behaviour was a component of the behavioural changes induced by *P. minutus* in *G. pulex* and *G. roeseli*.

At the end of each experiment, amphipods were killed in 70% alcohol, measured (linear dimension of the fourth coxal plate, measured from the base to dorsal limit; see Bollache, Gambade & Cézilly, 2000), sexed and dissected to check infection status. Amphipods harbouring immature parasites (acanthella) or > 1 cystacanth were not included in the analyses.

The distribution of geotaxis scores was not normally distributed, and no data transformation technique attempted was successful in enabling the data to meet normality criteria. Therefore data were analysed using non-parametric statistics. Results were considered significant at $P < 0.05$, 2-tailed tests of significance are used throughout. Tests were performed using JMP[®] Software version 5 (SAS Institute, Cary, NC, U.S.A.) and Statistica[®] version 6.0 (StatSoft Inc., Tulsa, OK, U.S.A.).

RESULTS

There was no effect of sex on geotaxis in *G. pulex* (Wilcoxon two-sample test: uninfected: $Z = 0.57$, $P = 0.57$; infected: $Z = 0.71$, $P = 0.48$) or in *G. roeseli* (uninfected: $Z = -1.25$, $P = 0.21$; infected: $Z = 1.37$, $P = 0.17$). Neither was there an effect of body size on geotaxis for both *G. pulex* (Spearman's correlation: uninfected: $\rho = 0.006$, $P = 0.93$; infected: $\rho = 0.08$, $P = 0.54$) and *G. roeseli* (uninfected: $\rho = -0.05$, $P = 0.43$, infected: $\rho = -0.10$, $P = 0.29$). Therefore, for each species, individuals were pooled and compared for the effect of *P. minutus* infection on geotaxis.

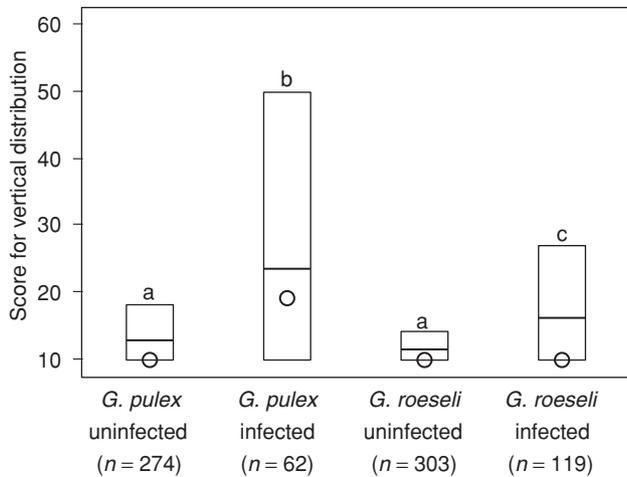


Fig. 1. Vertical distribution of uninfected and *Polymorphus minutus*-infected *Gammarus pulex* and *G. roeseli* in experiment 1. Vertical rectangles, 10–90% interquartile; circles, median; horizontal bars, mean. Different letters above bars denote series that differ significantly in their geotaxis score, after the non-parametric post-hoc test of Siegel & Castellan (1988).

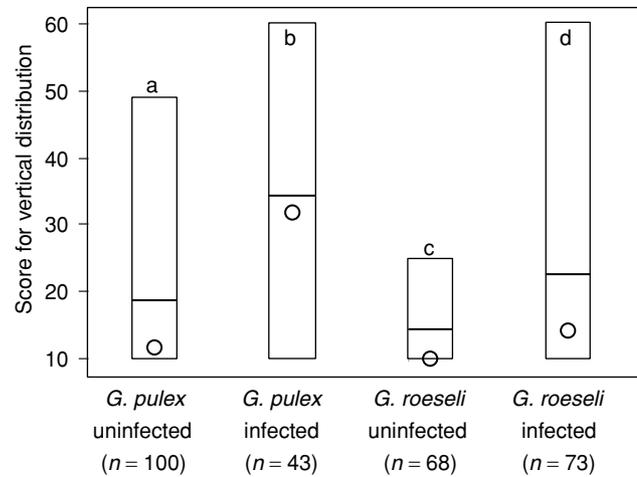


Fig. 2. Vertical distribution of uninfected and *Polymorphus minutus*-infected *Gammarus pulex* and *G. roeseli* in experiment 2. Vertical rectangles, 10–90% interquartile; circles, median; horizontal bars, mean. Different letters above bars denote series that differ significantly in their geotaxis score, after the non-parametric post-hoc test of Siegel & Castellan (1988).

In experiment 1 (no net added at the top of the water column), *G. pulex* individuals infected by *P. minutus* had significantly higher geotaxis scores than uninfected animals (Wilcoxon two-sample test: $Z=7.46$, $P<0.0001$) (Fig. 1). The same result was found in *G. roeseli* ($Z=4.56$, $P<0.0001$) (Fig. 1). A multiple comparison between all groups of infection revealed significant differences, except between uninfected *G. roeseli* and *G. pulex* (Kruskal–Wallis: $H=85.96$, $P<0.0001$; Fig. 1). Animals infected by *P. minutus* had, on average, a higher position in the water column compared to uninfected ones, although the behavioural modification was significantly greater for *G. pulex* than *G. roeseli*. In experiment 2 (with a net added at the top of the water column), infection status also had a highly significant effect on geotaxis, both for *G. pulex* (Wilcoxon two-sample test: $Z=3.42$, $P=0.0006$) and *G. roeseli* ($Z=-3.68$, $P=0.0002$) (Fig. 2): infected animals had higher scores for vertical distribution. A multiple comparison revealed significant differences between all groups of infection (Kruskal–Wallis: $H=31.07$, $P<0.0001$; Fig. 2).

To investigate the effect of netting on geotactic behaviour, we compared the proportion of individuals that were always at the bottom of the column (total score = 10) or always at the top (total score = 60), between experiments 1 and 2 (Fig. 3). The variation in the proportion of animals staying at the bottom of the water column was not influenced by the experimental design: infection by *P. minutus* was associated with a lower proportion of animals always staying at the bottom (Fig. 3a, Table 1). The host species also influenced this proportion: more *G. roeseli* always stayed at the bottom compared to *G. pulex* (Fig. 3a, Table 1). This suggests that the active swimming to the surface is

stronger in *G. pulex* than in *G. roeseli*. The variation in the proportion of animals staying at the top of the water column was influenced by parasitic infection (more infected animals spent all their time at the top), but not by host species (Fig. 3b, Table 1). The experimental design strongly influenced the behaviour of gammarids: a higher proportion of individuals stayed at the top of the water column in experiments with a net (Fig. 3b, Table 1). This proportion increased for both infected and uninfected individuals (no significant interaction between experiment and infection, Table 1). However, while the increase was not significant for uninfected animals in *G. pulex* (Fisher exact test: $P=0.09$) or *G. roeseli* (Fisher exact test: $P=0.08$), it was significant for infected animals (Fisher exact test: $P=0.02$ for *G. pulex*, $P=0.006$ for *G. roeseli*).

DISCUSSION

Our results show that *Gammarus roeseli* individuals parasitized by *Polymorphus minutus* exhibited a different geotactic behaviour to uninfected individuals. This behavioural alteration in parasitized animals is comparable to the one observed in the native host *Gammarus pulex* infected by the same parasite species, but the magnitude of the effect is different between the species. Changes in behaviour were stronger in *G. pulex* than in *G. roeseli*. Amphipods with natural infections were used in our experiments, and therefore it cannot be determined whether the infection is responsible for the behavioural change, or if the behavioural change led to an increased likelihood of infection by the acanthocephalan. Previous studies of similar host–parasite systems (e.g. Bethel & Holmes, 1974), however, have shown that modified

Table 1. Logistic regression testing the effect of host species, infection status by *Polymorphus minutus* and the experiment type on two components of the geotaxis behaviour of the gammarid hosts: (a) the proportion of individuals always at the bottom of the water column (score 10, see Fig. 3a); (b) the proportion of individuals always at the top of the water column (score 60, see Fig. 3b)

Source	(a)			(b)		
	d.f.	Wald χ^2	<i>P</i>	d.f.	Wald χ^2	<i>P</i>
Species	1	25.31	< 0.0001	1	0.01	0.91
Experiment	1	0.01	0.91	1	18.08	< 0.0001
Infection	1	44.57	< 0.0001	1	26.51	< 0.0001
Species \times experiment	1	2.27	0.13	1	0.04	0.85
Species \times infection	1	2.03	0.15	1	0.46	0.50
Experiment \times infection	1	0.19	0.66	1	0.06	0.81
Whole model	6	75.03	< 0.0001	6	63.03	< 0.0001

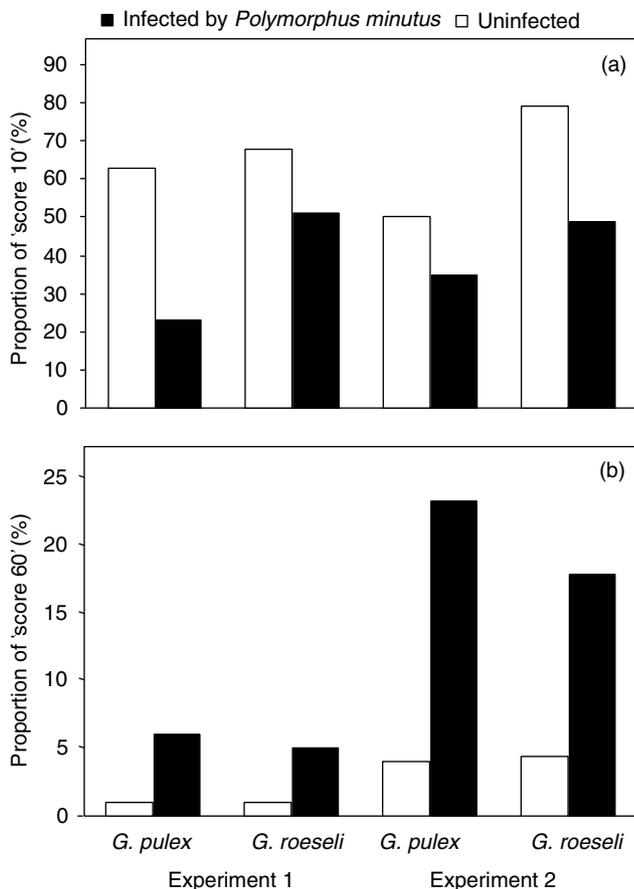


Fig. 3. Experiments measuring geotaxis as a function of the experimental design and the infection status of the individuals (see Material and methods). Proportion of *Gammarus pulex* and *G. roeseli*: (a) always at the bottom of the water column (score = 10); (b) always at the top of the water column (score = 60).

behaviours are observed only after the acanthocephalan cystacanths have become infective to the definitive host, indicating that the observed alterations in infected host behaviour are the consequence, and not the cause, of infection. In the field, the modified vertical distribution of infected *G. roeseli* should make them more prone to predation by water birds compared to uninfected individuals, as previously concluded for *G. pulex* (Cézilly

et al., 2000), or in another *Polymorphus* parasite (Helluy & Holmes, 1990).

Our two experimental designs allowed us to identify two components of the behavioural changes associated with *P. minutus* infection. Experiment 1 showed that more infected individuals swim upwards in the water column. In addition, the netting used in experiment 2 allowed the infected gammarids to hold on and therefore to stay at the top of the water column for longer. A similar, so-called 'skimming and clinging behaviour', has been reported for *Gammarus lacustris* infected by *Polymorphus paradoxus* (Bethel & Holmes, 1974; Helluy & Holmes, 1990; Maynard *et al.*, 1996), where infected amphipods firmly clung on to floating material in a characteristic position. Thus, our second experiment confirms that the clinging behaviour is an additional component of the behavioural changes induced by *P. minutus*.

The host behavioural change associated with *P. minutus* infection contrasts with that induced by another acanthocephalan parasite, *Pomphorhynchus laevis*, which also infects *G. pulex* and *G. roeseli* in the same geographic area. Infection by *P. laevis* was not associated with any change in phototaxis in *G. roeseli*, while reaction to light was altered in infected *G. pulex* (Bauer *et al.*, 2000). This difference can be discussed in terms of local adaptation of parasites to their hosts. The local adaptation hypothesis is classically tested between sympatric and allopatric populations of one single host species (Gandon *et al.*, 1996; Dybdahl & Storfer, 2003), but we propose that this can be extended to a difference between a local species and an allopatric invading species. Parasites are usually found to be less well-adapted and more virulent to newly colonizing host species (Sasal *et al.*, 2000). *Polymorphus minutus* clearly has an effect on behaviour of both native and colonizing hosts, which might suggest that the specificity of this parasite species is low. The differential influence observed between *P. laevis* and *P. minutus* could be linked to the migratory capacities of their definitive hosts (fish and birds, respectively). Birds, more than any other group of organisms, migrate over large distances, and their role in the dispersal of aquatic organisms has been widely recognized (Clausen *et al.*, 2002; Green, Figuerola & Sanchez, 2002). Thus, as the definitive hosts for *P. minutus*, their dispersal range may overlap with both the native and newly colonized range

of *G. roeseli*, and they may be vectors for long-distance acanthocephalan dispersal. This may not be the case for fish definitive hosts for *P. laevis*. This hypothesis relies on the model of Gandon *et al.* (1996) showing that local adaptation patterns are strongly influenced by the differential migration rates of hosts and parasites. Comparative data on the behavioural effect of the parasite on *G. roeseli* in other areas of its geographic range, and reciprocal cross-infection experiments (as stated in classical local adaptation tests; see Gandon *et al.*, 1996; Dybdahl & Storfer, 2003), are clearly needed to investigate these issues further.

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