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Research note

Species associations among larval helminths in an amphipod intermediate host

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Abstract

Larval helminths that share the same intermediate host may or may not also share the same definitive hosts. If one or more of these helminth species can manipulate the phenotype of the intermediate host, there can be great advantages or severe costs for other helminths resulting from co-occurring with a manipulator, depending on whether they have the same definitive host or not. Among 2372 specimens of the amphipod *Echinogammarus stammeri* collected from the river Brenta, northern Italy, there was a positive association between two acanthocephalan species with the same fish definitive hosts, the relatively common *Pomphorhynchus laevis* and the much less prevalent *Acanthocephalus clavula.*The number of cystacanths of *P. laevis* per infected amphipod, which ranged from one to five, did not influence the likelihood that the amphipod would also host *A. clavula*. A third acanthocephalan species, *Polymorphus minutus,*which matures in birds, showed no association with either of the two other species. These results show that associations among helminth species in intermediate hosts are not random, and are instead the product of selection favouring certain pathways of transmission. © 2000 Australian Society for Parasitology Inc. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Acanthocephalans; *Echinogammarus stammeri*; Host manipulation; Life cycles; Transmission strategy

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In the complex life cycles of helminth parasites, intermediate hosts often serve mainly as vehicles whose purpose is to take the parasite to its definitive host via predation [1]. An interesting situation arises when two or more species of helminths share the same intermediate host population. These parasites may or may not also share the same definitive host species, and thus they may or may not have the same preferred direction for their intermediate host vehicle. Many larval helminths, in particular acanthocephalans [2], are capable of altering the phenotype of their intermediate hosts in ways that make them more susceptible to predation by the definitive host [3]. When two of more manipulative parasite species share an intermediate host population, cases of mutual or conflicting interests become more acute: intermediate host vehicles are in the hands of several drivers that can favour one direction (one definitive host) over another [4,5]. In these situations, the transmission strategy of one parasite must adapt to the presence of other sympatric parasites. The simplest type of evolutionary response would be for a helminth species to develop ways of either associating with other larval parasites in the intermediate host (when they have a common definitive host) or avoiding other parasites (when their definitive hosts differ). Very few investigations have focused on patterns of host use by assemblages of larval helminths transmitted by predation, but the little evidence available suggests that such positive or negative associations may be common [5].

Populations of the amphipod *Echinogammarus stammeri* in the river Brenta, northern Italy, provide an ideal system to study associations between larval helminths. *Echinogammarus stammeri* serves as intermediate host to four species of helminths, all transmitted by predation to vertebrate definitive hosts. *Pomphorhynchus laevis* (Acanthocephala) is the most abundant larval helminth in the amphipod population [6]. Both this parasite and the less common *Acanthocephalus clavula* (Acanthocephala) use fish as definitive hosts, in particular chub, *Leuciscus cephalus*. The third species using the amphipod as intermediate host, *Polymorphus minutus* (Acanthocephala), matures in aquatic birds [7]. Finally, *Cyathocephalus truncatus* (Cestoda) is the least common of the four parasites of *E. stammeri*; it uses brown trout, *Salmo trutta*, as definitive host [8]. All these helminths occur in the haemocoel of their amphipod host, and except in the case of *P. laevis,* there is usually no more than one conspecific parasite per amphipod.

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The three acanthocephalan parasites have the potential of manipulating the phenotype of their amphipod intermediate host. *Pomphorhynchus laevis* increases the phototaxis and activity levels of *E. stammeri* from the river Brenta [9]. *Pomphorhynchus laevis* is also known to alter the behaviour [10–12], colouration [12] and physiology [13,14] of other gammarid amphipods. The consequence of these changes is that amphipods harbouring *P. laevis* cysts are more likely to drift in streams [9,15] and be ingested by fish [10,12]. Changes in the behaviour and colouration of crustacean hosts are also frequently induced by acanthocephalans of the genera *Polymorphus* [16,17] and *Acanthocephalus* [18–20]. Thus all three acanthocephalans may modify the phenotype of their hosts in ways that may facilitate their own transmission to their definitive hosts. No information is available regarding the manipulative ability of *Cyathocephalus truncatus*, but it is worth noting that the larvae of other cestodes are capable of altering the behaviour of their crustacean intermediate hosts (e.g. [21,22]).

For a larval helminth, the cost of associating in the intermediate host with a manipulator parasite that uses a different definitive host is potentially high: the manipulator will bias transmission toward its own definitive host. On the other hand, the benefits of associating with a manipulator when both parasites share the same definitive host are substantial; if both species are capable of manipulation, their effects may even be synergistic and result in greatly increased transmission success [5]. We predicted that among the *E. stammeri* amphipods of the river Brenta, there should be a positive association between the occurrence of *P. laevis* and *A. clavula*, the only two helminth species sharing the same definitive host. Among all other pairs of larval helminths, we expect negative associations, or at the very least no positive associations. We tested these predictions using data on infections in field-collected amphipods. Since *P. laevis* was the only moderately prevalent parasite in our study system, here we only test associations involving this parasite.

A total of 12 samples of *E. stammeri* were collected between January 1999 and April 2000, from the same site in the river Brenta, near Grantorto, Province of Padua, northern Italy. Amphipods were obtained with a dip net (mesh size 3 mm) in shallow water near the river bank. All specimens were fixed in 6% formaldehyde and returned to the laboratory for examination within two weeks following their capture. Prior to their examination, they were measured, sexed, and cleared in lactic acid to facilitate the identification and counting of the parasite cysts in their haemocoel.

Amphipod sex has no effect on the occurrence of *P. laevis*, the most common larval parasite in the population, and there is no difference in length among amphipods harbouring between zero and three *P. laevis* cysts [6]. We thus pooled all samples for further analyses, because the low prevalence of the other helminth species made it impossible to detect species associations within individual samples. Associations between pairs of helminth species in their amphipod hosts were tested using r_θ, a modification of the standard Pearson's product-moment correlation coefficient adapted to binary data, i.e. presence-absence data [23]. It takes into account the number of uninfected hosts, as well as the number of hosts harbouring each parasite species or both parasite species. To determine whether *A. clavula* is more or less likely to be found in amphipods harbouring different numbers of cysts of *P. laevis*, we used a Fisher's exact test to compare the frequency of *A. clavula* infections in amphipods with either one or more than one *P. laevis* cysts.

The composition (males and females) and average body length of the different amphipod samples were roughly similar (Table 1). The overall prevalence of *P. laevis* was 18.5%. Of the 440 amphipods found to be infected with *P. laevis*, 356 (80.9%) harboured a single cyst; the others harboured

Table 1

Numbers of males and females, mean length, and prevalence (% hosts infected) of helminth infections in the different samples of the amphipod *E. stammeri* collected from the river Brenta, Italy

Date	Numbers (M, F)	Length, mean ± SD (mm)	*Pomphorhynchus laevis*	*Polymorphus minutus*	*Acanthocephalus clavula*	*Cyathocephalus truncatus*
Jan. 1999	92,108	7.7 ± 1.3	31.50	3.00	1.50	0
Feb. 1999	111,53	7.8 ± 1.0	27.44	0.61	0.61	0
Apr. 1999	99,109	7.6 ± 1.3	27.88	0	0.96	0
July 1999	82,118	7.3 ± 1.2	7.00	1.00	1.00	0
Aug. 1999	87,113	6.6 ± 0.8	30.00	1.00	0.50	0
Sept. 1999	93,107	7.0 ± 0.9	26.50	1.00	4.00	0
Nov. 1999	94,106	8.1 ± 1.6	19.00	1.50	2.00	0
Dec. 1999	81,119	7.4 ± 1.1	14.00	0.50	1.50	0
Jan. 2000	91,109	7.1 ± 1.0	6.00	0	1.00	0.50
Feb. 2000	108,92	7.7 ± 1.4	8.50	2.00	1.00	0
Mar. 2000	63,137	7.4 ± 1.4	17.00	0	1.00	0
Apr. 2000	95,105	7.8 ± 1.5	9.00	0.5	0	0
Total	1096,1276	7.5 ± 1.3	18.55	0.93	1.26	0.04

between two and five cysts. The prevalence of the other two acanthocephalan species was much lower (Table 1). The intensity of infection by *P. minutus* was always one cyst per host, whereas 27 of the 30 amphipods infected by *A. clavula* harboured a single cyst (the others harboured two or three cysts). Only one amphipod was infected with the cestode *Cyathocephalus truncatus*, harbouring a single larva of this species and no other parasites; because of its rarity, *C. truncatus* was excluded from further analyses.

Among the 2372 amphipods from all 12 samples pooled, there was a significant, positive association between the acanthocephalans *P. laevis* and *A. clavula* ($r_\theta = 0.131$, $P < 0.001$). In other words, cysts of *A. clavula* were significantly more likely to be found in amphipods also harbouring *P. laevis* than in amphipods not infected with *P. laevis* (Fig. 1). The only other association that could be tested statistically was that between *P. laevis* and *P. minutus*, and no significant association was found between these two species ($r_\theta = 0.022$, $P > 0.10$; Fig. 1). Finally, *A. clavula* and *P. minutus* were never observed together; based on the product of their respective low prevalences and the sample size, these two species were not even expected to co-occur in a single amphipod, so our results are not evidence for an avoidance strategy.

The frequency of co-occurrence of *P. laevis* and *A. clavula* in the same amphipod host was not influenced by the number of *P. laevis* cysts per amphipod. Overall, 3.93% of the 356 amphipods infected by a single *P. laevis* cyst also harboured *A. clavula*, whereas 5.95% of the 84 amphipods infected by two-to-five *P. laevis* cysts harboured *A. clavula* as well (Fisher's exact test, $P > 0.10$).

When different helminth species use the same intermediate host, and when at least one of them is capable of manipulating the phenotype of that host, selection should favour the establishment of non-random associations between these species [5]. Here we found that two relatively rare acanthocephalan species had different patterns of association with the more common *P. laevis* in amphipod intermediate hosts. Larvae of *A. clavula* were positively associated with those of *P. laevis*, with whom they share the same definitive hosts. In contrast, larvae of *P. minutus,* which mature in avian definitive hosts, showed no association with those of *P. laevis*, which mature in fish definitive hosts. These patterns can be explained in terms of the similarities and differences in the life cycles of the three species, but also in terms of their respective prevalences. The costs and benefits of avoiding or associating with another parasite species depend on prevalence [4]. For instance, *P. minutus* may be more likely to die in a fish gut if it co-occurs with either of the two other acanthocephalan species. In addition, its ability to manipulate amphipods to its own benefit is reduced when it co-occurs with *P. laevis* [24]. However, even by infecting amphipods at random, *P. minutus* has only one chance out of five of co-occurring with *P. laevis* and one chance out of a hundred of co-occurring with *A. clavula* (based on the product of the observed prevalences). Given those odds, it is probably not advantageous to evolve an avoidance mechanism.

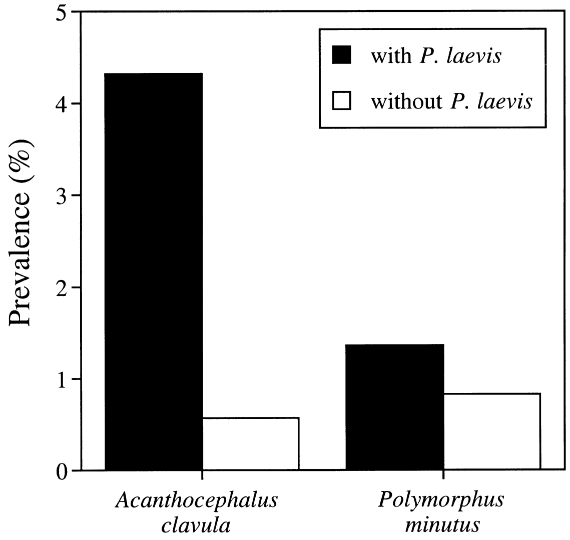

Fig. 1. Prevalence of *Acanthocephalus clavula* and *Polymorphus minutus* infections in 440 *Echinogammarus stammeri* amphipods also infected with *Pomphorhynchus laevis*, and in 1932 amphipods not infected with *P. laevis*, from the river Brenta, Italy.

The situation is different for *A. clavula*. Since only about one amphipod out of five harbours cysts of *P. laevis*, choosing hosts at random will not often result in benefits for *A. clavula*. The benefits it can obtain by sharing an amphipod host with *P. laevis* are of at least two types. First, if both species manipulate amphipod phenotype in ways that make it more susceptible to fish predation, and if their effects are additive, both acanthocephalan species may achieve a greater transmission rate to definitive hosts when they co-occur in amphipods than when they occur alone. Second, if manipulating host phenotype involves costly secretions or other mechanisms, it may be possible for the two species to share these costs when they co-occur. For these reasons, actively seeking amphipods harbouring *P. laevis* cystacanths is more advantageous for *A. clavula* than random host choice. Our results suggest it has adopted this strategy. Given that *P. laevis* can alter the behaviour of amphipods in ways that could cause a spatial segregation of infected and uninfected amphipods [9–12], it may be relatively easy for *A. clavula* to target specifically the portion of the amphipod population that is infected with *P. laevis.* In a system involving digenean parasites sharing an amphipod host, the motile cercariae of one species actively swam toward the spatially segregated amphipods harbouring cysts of the other species [25]. In acanthocephalans, infection of amphipods occurs via the ingestion of eggs: there is no motile larva at this stage of the life cycle. However, the presence or absence of filaments on the eggs, or their specific density, can determine in which microhabitat they will accumulate and which part of the amphipod population they are more likely to infect [26]. It is probably a mechanism of this sort that allows *A. clavula* to associate with *P. laevis.*

Although this positive association must benefit one or both species in terms of transmission success, it may also result in costs. The cystacanths of *A. clavula* are larger than those of *P. laevis* (Dezfuli, unpublished data). The presence of cystacanths of one species may interfere with the establishment of larvae of a second species. The prevalence of *A. clavula* infections was the same in amphipods harbouring one or many *P. laevis* cystacanths, but the development of these larvae may be influenced by the presence of others. Because of space or nutrient restrictions, there could be reductions in development rates or levels of infectivity of cystacanths that share the intermediate host with other species. In an experimental study focusing on a different pair of acanthocephalan species in amphipods [27], the presence of one species slowed down the development of the other and prevented it from becoming infective to the definitive host. It would be interesting to determine whether there are also antagonistic interactions in the *P. laevis* – *A. clavula* system.

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