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Interspecific interactions between acanthocephalan species in the intestine of stone loach and minnow

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Research Paper

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Abstract

Interspecific interactions between parasites sharing the same host are often antagonistic; the presence of one species decreases the number of individuals or negatively affects both the distribution and reproduction of the other species. Antagonistic interactions between co-infecting parasites may translate into direct competition or interactive segregation, but elements of both may be present. Potential interactions between two acanthocephalan species, *Pomphorhynchus laevis* and *Acanthocephalus anguillae*, were studied in the field in two of their natural fish definitive hosts. There was no evidence for competitive exclusion between *P. laevis* and *A. anguillae*. However, a negative interaction was found for the first time in the field between these two species. Based on the analysis of parasite abundance and total biomass using a static regression approach, I found that the abundance and total biomass of parasite was also limited by host characteristics. These results are consistent with previous laboratory studies on competition between *P. laevis* and *A. anguillae*.

Introduction

Interspecific interactions among parasites co-infecting the same host have been well documented in both laboratory and field settings (Holmes, 1962; Holland, 1984; Sousa, 1993). Several types of interactions are possible. Interspecific interactions can be positive when initial infection by one parasite species decreases the host's immune response, thus facilitating establishment and exploitation by other parasites (Sousa, 1994). More often, however, interactions are antagonistic; the presence of one parasite species decreases the number and has a negative effect on the distribution and reproduction of other species (Poulin, 1998). Antagonistic interactions between different parasite species in the same host may be expressed as direct competition or as interactive segregation, but elements of both may be present (Holmes, 1973). In the former case, selection would be expected to lead to an adaptively superior competitor, capable of excluding inferior competitors. In the latter case, selection would be expected to stabilize the segregation within hosts, leading to narrower niches for both parasites. Competitive exclusion among parasites appears to be fairly common and is frequently unilateral, with one species markedly affected by the presence of the second, but not *vice versa* (Schad, 1966; Chappell, 1969; Holmes, 1973; Bates & Kennedy, 1990). Competition between parasites can be seen as a potential regulator of parasite population densities and is an important factor structuring parasites communities (Grey & Hayunga, 1980; Holmes & Price, 1986; Kuris & Lafferty, 1994; Sousa, 1994).

In the British Isles, the distributions of two acanthocephalan species, *Pomphorhynchus laevis* and *Acanthocephalus anguillae* are local, restricted and discontinuous, and, when both species occurred in the same river systems, their distributions tended to be discrete, with little or no overlap (Kennedy *et al.*, 1989). In addition, where distribution overlap did occur, there was no record of both species occurring in the same fish host, such that the geographical and intestinal (i.e. within host) distributions of *A. anguillae* and *P. laevis* are mutually exclusive. Kennedy *et al.* (1989) thus suggested that interspecific competition between these two acanthocephalans species was one of the factors limiting their distribution.

Bates & Kennedy (1990, 1991) investigated the possibility of interspecific competition using an elegant series of laboratory experiments designed to study the establishment, growth, maturation, fecundity and within-host site selection of the two species when alone and when co-occurring. The results, however, were contradictory. In the first experiment, rainbow trout (*Onchorhynchus mykiss*) were used as a laboratory host. Results showed that both the survival of *A. anguillae* and its location in the fish intestine of were affected by *P. laevis*, especially at high abundance of mixed infections. In contrast, *P. laevis* remained unaffected by the presence of *A. anguillae*, suggesting that the interspecific competition was unilateral (Bates & Kennedy, 1990). In a second study using eels (*Anguilla anguilla*) as final hosts, Bates &

Kennedy (1991) found no evidence of interspecific competition between *P. laevis* and *A. anguillae*. These two parasites utilize different crustacean species as intermediate hosts. *Pomphorhynchus laevis* relies on the amphipod *Gammarus pulex*, whereas *A. anguillae* exploits the isopod *Asellus aquaticus*. Both parasites, however, are capable of infecting a wide range of definitive hosts. Although interactions between these two species have been well studied in the laboratory, little attention has been devoted to the nature of their interactions in the field.

Here, some new results on the interactions between *P. laevis* and *A. anguillae* in two of their natural hosts in the River Tille (Burgundy, eastern France) are presented. Distribution patterns of each parasite species are assessed and results discussed in relation to previous considerations on their potential interactions. I specifically tested the exclusion hypothesis between *P. laevis* and *A. anguillae* to account for the lack of records of the two parasite co-occurring within the same host.

Materials and methods

This work followed the University of Burgundy guidelines for the treatment of animals in research. Information about individuals' origin, collection, housing conditions and killing are described below. Transport between sampling site and laboratory was devised to reduce stress and maximize animals' welfare.

A total of 195 individual fish were collected in the River Tille (Burgundy, France) between March and June 1999, using a hand net. The number of fish sampled was 104 *Noemacheilus barbatulus* (stone loach) and 91 *Phoxinus phoxinus* (minnow). After capture, fish were returned alive to the laboratory where they were immediately anesthetized with tricaine (MS-222, 300 mg/l, Sigma), killed by cervical dislocation, the fork length measured to the nearest 1.0 mm and weighted with a precision scale (±0.01 mg) (Precisa 262 SMA-FR). Fish were then dissected. The digestive tract, from stomach to anus, was removed and examined for parasites. Each parasite was identified based on the arrangement of the proboscis armature, number of hook rows and the size, shape and number of hooks by row (Brown *et al.*, 1986). For each parasite species, prevalence, mean abundance and mean intensity were calculated as described by Bush *et al.* (1997), as well as the variance-to-mean abundance ratio as a measure of over dispersion (Anderson & Gordon, 1982). In addition, parasite biomass was taken as the total combined biomass of all parasites per individual host. The competition exclusion hypothesis was tested by comparing the observed number of concurrent infection to that expected on the basis of the prevalence of each parasite species using phi coefficient of association (Siegel & Castellan, 1988). Because competition is both a spatial and temporal process; ideally, single habitat patches would be followed through time in a field. However, for endoparasites systems, this is clearly not possible because the host must be killed to count parasites. Detecting competition in the field is particularly difficult in this case where data are collected from a single sample. Thus, interaction coefficients between parasite species were estimated using a static regression approach to census data at one point in time over many sites (Schoener, 1974; Pfister, 1995). Separate regression analyses were performed on the abundance and total biomass of each parasite species (excluding uninfected fish), with fish size or fish biomass as the independent variable, respectively. Residuals from the regression for each parasite species were used in correlations for each parasite species against one another as an estimate of interspecies interactions (Rosenzweig *et al.*, 1984). Thus, prevalence, the variation in parasite species abundance and biomass due to fish characteristics are explained in the first regression, and only the variance unexplained by this is tested when assessing patterns potentially due to heterospecific parasite competition. In all cases, residuals were inspected for normality and for constant variance. Differences in the prevalence between parasites species and between fish species were tested using Fisher's exact test. Differences in mean biomass between parasite species were tested using the Mann–Whitney *U*-test. All results were considered significant at the 5% level.

Results

Pomphorhynchus laevis was found in both fish species (table 1). The prevalence of *P. laevis* was not significantly different between fish species (Fisher's exact test, $P = 0.518$; table 1). While *A. anguillae* was also found to occur in both *P. phoxinus* and *N. barbatulus*, parasite prevalence was significantly different between fish species (Fisher's exact test, $P < 0.001$; table 1). In all cases, the degree of overdispersion was >1, showing an aggregated distribution of each parasite species. A positive correlation was found between number of parasites and host body size in *N. barbatulus* ($r_s = 0.267$, $n = 104$, $P < 0.01$) and *P. phoxinus* ($r_s = 0.508$, $n = 91$, $P < 0.0001$). Similarly, parasite biomass and fish biomass were positively correlated in *N. barbatulus* ($r_s = 0.223$, $n = 104$, $P < 0.05$) and *P. phoxinus* ($r_s = 0.507$, $n = 91$, $P < 0.001$). There was no difference in the number of observed and expected concurrent infections based on the prevalences of *P. laevis* and *A. anguillae* in *P. phoxinus* (Phi coefficient of association, $\chi^2 = 2.35$, df = 2, $P = 0.125$) or *N. barbatulus* ($\chi^2 = 0.24$, df = 2, $P = 0.623$).

Fish body size and fish biomass explained a significant amount of the variation in the parasite abundance and parasite biomass of *A. anguillae* but not of *P. laevis* (table 2). There was a significantly negative interaction between *P. laevis* and *A. anguillae* for the parasite biomass in both *P. phoxinus* and *N. barbatulus*, and for the abundance of parasite only in *N. barbatulus* (table 3). In addition, mean parasite biomass differed significantly between *A. anguillae* and *P. laevis* in the *N. barbatulus* host (Mann–Whitney *U*, $z = 7.78$, $P < 0.001$) and in *P. phoxinus* (Mann–Whitney *U*, $z = 3.39$, $P < 0.001$) (see table 3).

Discussion

The exclusion hypothesis based on competition between *P. laevis* and *A. anguillae* was proposed to account for the lack of records of the two parasites co-occurring within the same individual fish, even when the two species coexisted in the same river (Kennedy *et al.*, 1989). Contrastingly, the present data show no evidence for competitive exclusion between *P. laevis* and *A. anguillae* in the River Tille within the two natural final hosts, the stone loach and the minnow. However, a negative interaction between the two parasite species was detected in both abundance and biomass in *N. barbatulus*, and, to a lesser extent, in biomass in *P. phoxinus*.

The fact that the biomass analysis makes it easier to detect the negative interaction between the two parasites than the simple parasite number analysis could be explained by the difference in body size between *P. laevis* and *A. anguillae*. Indeed, from the host perspective, parasite biomass is known to be a more relevant measure of parasite abundance than the total number of parasite

Table 1. Occurrence of parasites among fish species in the River Tille (France).

	Fish species	
Parasite species	*Noemacheilus barbatulus* *n* = 104	*Phoxinus phoxinus* *n* = 91
Pomphorhynchus laevis		
Number of fish infected	30	22
Prevalence	28.8%	24.2%
Mean intensity + standard deviation	1.8 ± 1.1	1.8 ± 1.1
Range of intensities	1–5	1–5
Mean abundance + standard deviation	0.5 ± 1.0	0.4 ± 0.9
Overdispersion	1.9	2.1
Mean biomass	1.01 ± 0.58 mg	1.69 ± 1.01 mg
Acanthocephalus anguillae		
Number of fish infected	49	22
Prevalence	47.1%	24.2%
Mean intensity + standard deviation	2.5 ± 1.5	2.9 ± 2.7
Range of intensities	1–6	1–11
Mean abundance + standard deviation	1.2 ± 1.7	0.7 ± 1.8
Overdispersion	2.3	4.6
Mean biomass	2.27 ± 1.24 mg	2.49 ± 1.21 mg
Number of co-infections		
P. laevis–A. anguillae	13	8
Prevalence of co-infection	12.5%	8.8%

Table 2. Simple regression coefficients with their associated probabilities, for fish body size and parasite abundance relationship, and fish biomass and parasite biomass relationship.

	Dependent variable			
Independent	Fish body size/parasite abundance		Fish biomass/parasite biomass	
Variable	*Pomphorhynchus laevis*	*Acanthocephalus anguillae*	*Pomphorhynchus laevis*	*Acanthocephalus anguillae*
Noemacheilus barbatulus	0.049	0.304	0.198	0.246
	$P = 0.696$	$P < 0.05$	$P = 0.110$	$P < 0.05$
Phoxinus phoxinus	0.012	0.319	0.230	0.328
	$P = 0.945$	$P < 0.05$	$P = 0.177$	$P < 0.05$

individuals, simply because body sizes of individual parasites can vary between parasite genus and species (Poulin & George-Nascimento, 2007). This hypothesis is confirmed in our study since the mean biomass of *A. anguillae* parasites were always more important than the mean biomass of *P. laevis*. In addition, this confirms that the biomass of parasite was limited, at least partly, by the host's characteristics (i.e. size and biomass).

Negative interactions between *P. laevis* and *A. anguillae* are not surprising and have been found previously by Bates & Kennedy (1990) in laboratory experiments in rainbow trout and by Dezfuli *et al.* (2001) in brown trout (*Salmo truta*), although not strong enough to lead to an exclusion of one species from hosts harbouring a second species. Comparison with results from these previous studies seems to show a variation in the strength of these interactions according to biological processes and environment context.

In my study, the two species of fish hosts used are not the preferred hosts of either species of parasites (Bates & Kennedy, 1991), and are rather small species compared to other fish species used in previous studies (Bates & Kennedy, 1990, 1991; Dezfuli *et al.*, 2001). Thus, differences in the negative interaction between *P. laevis* and *A. anguillae* detected among studies within the different final hosts might merely reflect different host suitability for the two parasites.

Table 3. Correlation coefficients between residuals from the regression between fish size and fish biomass and either abundance or biomass of each parasite species.

	Acanthocephalus anguillae	
	Abundance	Biomass
Pomphorhynchus laevis		
Noemacheilus barbatulus	−0.373**	−0.416***
Phoxinus phoxinus	0.175	−0.366*

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Furthermore, Dezfuli *et al.* (2001) pointed out that negative interactions between A. *anguillae* and *P. laevis*, and more generally between helminth species, were not significant in all streams, and could only be detectable under certain conditions of abundance and dispersion of parasite species among host individuals (Dobson, 1985). In this study, *P. laevis* and *A. anguillae* are characterized by a complex, two-host life cycle, and are known to induce modifications of their host's behaviour in ways that may increase their susceptibility to predation by final hosts (Lagrue *et al*., 2007). The intermediate hosts of *P. laevis* are crustacean amphipod belonging to the genus *Gammarus* (Lagrue *et al*., 2007; Moret *et al*., 2007), whereas *Asellus aquaticus* is the intermediate host of *A. anguillae* (Dezfuli *et al*., 1994). These two intermediate hosts can show different densities according to water pollution level (Galli *et al*., 1998; MacNeil *et al*., 2002), or predator–prey relationship (MacNeil *et al*., 2002), which may, in turn, influence the probability of infection for the definitive hosts.

Although competition often leads to exclusion, there are numerous mechanisms whereby coexistence can occur. Niche partitioning is, for example, one of the best-understood mechanisms of coexistence (Karvonen *et al*., 2006). Thus, competitive interactions within the host can shape the evolution of parasite phenotypes and can even facilitate the coexistence of multiple parasite types and increase parasite diversity (Bashey, 2015). Competition between *P. laevis* and *A. anguillae* in naturally infected hosts seems to lead to site segregation in the gut. An analysis of the situation in other and preferred hosts, like chub (*Leuciscus cephalus*) or barbel (*Barbus barbus*), and a comparison between interspecific and intraspecific competition could permit a better understanding of the population regulation and the maintenance of parasite diversity.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Anderson RM and Gordon DM (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Bashey F (2015) Within-host competitive interactions as a mechanism for the maintenance of parasite diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140301.

Bates RM and Kennedy CR (1990) Interactions between the acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in rainbow trout: testing an exclusion hypothesis. *Parasitology* **100**, 435–444.

Bates RM and Kennedy CR (1991) Potential interaction between *Acanthocephalus anguillae* and *Pomphorhynchus laevis* in their natural hosts chub, *Leuciscus cephalus* and the European eel, *Anguilla anguilla*. *Parasitology* **102**, 289–297.

Brown AF, Chubb JC and Veltkamp CJ (1986) A key to the species of Acanthocephala parasitic in British freshwater fishes. *Journal of Fish Biology* **28**, 327–334.

Bush AO, Lafferty KD, Lotz JM and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.

Chappell LH (1969) Competitive exclusion between two intestinal parasites of the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Parasitology* **55**, 775–778.

Dezfuli BS, Rosetti E and Fano EA (1994) Occurrence of larval *Acanthocephalus anguillae* (Acanthocephala) in *Asellus aquaticus* (Crustacea, Isopoda) from the River Brenta. *Italian Journal of Zoology* **61**, 77–81.

Dezfuli BS, Giari L, De Biaggi S and Poulin R (2001) Associations and interactions among intestinal helminths of the brown trout, *Salmo trutta*, in northern Italy. *Journal of Helminthology* **75**, 331–336.

Dobson AP (1985) The population dynamics of competition between parasites. *Parasitology* **91**, 317–347.

Galli P, Mariniello L, Crosa G, Ortis M, Ambrogi AO and D'Amelio S (1998) Populations of *Acanthocephalus anguillae* and *Pomphorhynchus laevis* in rivers with different pollution levels. *Journal of Helminthology* **72**, 331–335.

Grey AJ and Hayunga EG (1980) Evidence for alternative site selection by *Glaridacris larvei* (Cestoda: Caryophyllidae) as a result of interspecific competition. *Journal of Parasitology* **66**, 371–372.

Holland C (1984) Interactions between *Moniliformis* (Acanthocephala) and *Nippostrongylus* (Nematoda) in the small intestine of laboratory rats. *Parasitology* **88**, 303–315.

Holmes JC (1962) Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocaphala). II. Effects on growth. *Journal of Parasitology* **48**, 87–96.

Holmes JC (1973) Site selection by parasite helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology* **51**, 333–347.

Holmes JC and Price PW (1986) Communities of parasites. pp. 187–213 *in* Anderson DJ and Kikkawa J (*Eds*) *Community ecology: pattern and process*. Oxford, UK, Blackwell Scientific Publications.

Karvonen A, Terho P, Seppälä O, Jokela J and Valtonen ET (2006) Ecological divergence of closely related *Diplostomum* (Trematoda) parasites. *Parasitology* **133**, 229–235.

Kennedy CR, Bates RM and Brown AF (1989) Discontinuous distributions of fish acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in Britain and Ireland: an hypothesis. *Journal of Fish Biology* **34**, 607–619.

Kuris AM and Lafferty KD (1994) Community structure: larval trematodes in snail hosts. *Annual Review of Ecology, Evolution, and Systematics* **25**, 189–217.

Lagrue C, Kaldonski N, Perrot-Minnot MJ, Motreuil S and Bollache L (2007) Modification of hosts' behavior by a parasite: field evidence for adaptive manipulation. *Ecology* **88**, 2839–2847.

MacNeil C, Dick JTA, Bigsby E, Elwood RW, Montgomery WI, Gibbins CN and Kelly DW (2002) The validity of the *Gammarus: Asellus* ratio as an index of organic pollution: abiotic and biotic influences. *Water Research* **36**, 75–84.

Moret Y, Bollache L, Wattier R and Rigaud T (2007) Is the host or the parasite the most locally adapted in an amphipod–acanthocephalan relationship? A case study in a biological invasion context. *International Journal for Parasitology* **37**, 637–644.

Pfister CA (1995) Estmating competition coefficients from census data: a test with field manipulations of tidepool fishes. *The American Naturalist* **146**, 271–291.

Poulin R (1998) *Evolutionary ecology of parasites*. London, UK, Chapman and Hall.

Poulin R and George-Nascimento M (2007) The scaling of total parasite biomass with host body mass. *International Journal for Parasitology* **37**, 359–364.

Rosenzweig ML, Abramsky Z and Brand S (1984) Estimating species interactions in heterogenous environments. *Oikos* **43**, 329–340.

Schad GA (1966) Immunity, competition, and natural regulation of helminth populations. *The American Naturalist* **100**, 259–264.

Schoener TW (1974) Competition and the form of habitat shift. *Theoretical Population Biology* **6**, 265–307.

Siegel S and Castellan NJ (1988) *Nonparametric statistics for behavioral sciences*. 2nd edn. New York, Mc Graw-Hill.

Sousa WP (1993) Interspecific antagonism and species coexistence in a diverse guild of larval trematode parasites. *Ecological Monograph* **63**, 103–128.

Sousa WP (1994) Patterns and processes in communities of helminth parasites. *Trends in Ecology and Evolution* **9**, 52–57.