Available online at www.sciencedirect.com

ScienceDirect

International Journal for Parasitology 38 (2008) 1435–1444

www.elsevier.com/locate/ijpara

Intra- and interspecific competition among helminth parasites: Effects on *Coitocaecum parvum* life history strategy, size and fecundity

Clément Lagrue [*], Robert Poulin

Department of Zoology, University of Otago, 340 Great King Street, P.O. Box 56, Dunedin 9054, New Zealand

Received 5 February 2008; received in revised form 4 April 2008; accepted 7 April 2008

Abstract

Larval helminths often share intermediate hosts with other individuals of the same or different species. Competition for resources and/or conflicts over transmission routes are likely to influence both the association patterns between species and the life history strategies of each individual. Parasites sharing common intermediate hosts may have evolved ways to avoid or associate with other species depending on their definitive host. If not, individual parasites could develop alternative life history strategies in response to association with particular species. Three sympatric species of helminths exploit the amphipod *Paracalliope fluviatilis* as an intermediate host in New Zealand: the acanthocephalan *Acanthocephalus galaxii*, the trematode *Microphallus* sp. and the progenetic trematode *Coitocaecum parvum*. Adult *A. galaxii* and *C. parvum* are both fish parasites whereas *Microphallus* sp. infects birds. We found no association, either positive or negative, among the three parasite species. The effects of intra- and interspecific interactions were also measured in the trematode *C. parvum*. Both intra- and interspecific competition seemed to affect both the life history strategy and the size and fecundity of *C. parvum*. Firstly, the proportion of progenesis was higher in metacercariae sharing their host with *Microphallus* sp., the bird parasite, than in any other situation. Second, the intensity of intraspecific competition apparently constrained the ability of metacercariae to adopt progenesis and limited both the growth and egg production of progenetic individuals. These results show that the life history strategy adopted by a parasite may be influenced by other parasites sharing the same host.

Keywords: *Coitocaecum parvum*; Progenesis; Life history strategy; Intra and interspecific interactions

1. Introduction

Competition for space and food amongst and within species of free-living organisms sharing the same habitat is an essential factor influencing survival, growth and reproduction (Begon et al., 1996). Parasites occupy habitats (i.e. hosts) that have potentially even more limited available resources depending on the size and life span of the host organism (Poulin, 2007). Each host represents a shared habitat for the parasites it contains, many of which can be at different developmental stages or have different life cycles. As with free-living organisms, both intra- and interspecific competition for resources within a host can influence aspects of individual parasite fitness (Fredensborg and Poulin, 2005; Gower and Webster, 2005).

In the complex life cycles of parasites, the different hosts serve as a food source for both growth and reproduction. Competition between parasites has been widely studied among adult helminths in their vertebrate definitive hosts (see Poulin, 2007 and references therein) and larval trematodes in their snail first intermediate host (Kuris and Lafferty, 1994; Lagrue et al., 2007). However, intermediate hosts are also often used by larval helminths as vehicles of transmission to the definitive host. This transmission mostly relies on the consumption of infected intermediate hosts by the definitive host predator (i.e. trophic transmission; Lafferty, 1999). The difficulty of this strategy has driven the evolution of well documented adaptations serving to facilitate trophic transmission in parasite life cycles

---

[*] Corresponding author. Tel.: +64 3 479 7964; fax: +64 3 479 7584.
E-mail address: lagcl981@student.otago.ac.nz (C. Lagrue).

0020-7519/$34.00 © 2008 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijpara.2008.04.006

(Moore, 2002; Poulin, 2007). For example, many helminths such as acanthocephalans (Moore, 1983; Bakker et al., 1997; Bauer et al., 2000) and microphallid trematodes (Helluy, 1983; Thomas et al., 1997; Hansen and Poulin, 2005) are capable of manipulating their crustacean intermediate hosts in ways that make them more vulnerable to predation by the definitive host (Moore, 2002). However, parasites also need to avoid consumption by non-host predators (Combes et al., 2002; Mouritsen and Poulin, 2003). Interestingly, when several species of helminths share the same intermediate host population, these different parasites may or may not have the same definitive host (Dezfuli et al., 2000). In situations where manipulative parasites of different species share the same intermediate host, mutual or conflicting interests are likely to arise depending on their respective definitive host (Brown, 1999; Thomas et al., 2002a; Poulin et al., 2003). Non-manipulative parasite species might also benefit or incur severe costs from associations with manipulative species as they have no control over the intermediate host alterations and consequently the definitive host species targeted (Thomas et al., 1998; Mouritsen, 2001; Leung and Poulin, 2007). In these situations, the life history strategy of one parasite could adjust to the presence of other heterospecific parasites. Ideally, parasite species should have developed ways of either associating with or avoiding other parasites depending on whether they share a common definitive host (Dezfuli et al., 2000). When different helminth species use the same intermediate host, and at least one is manipulative, selection should favour non-random association or avoidance between these species. If no such mechanism is observed and the association between helminth species is random, parasites may have developed alternative life history strategies adopted in case they share their intermediate host with heterospecific individuals. Sharing an intermediate host can also result in both intra- and interspecific competition among parasites for host resources. Even when parasites share the same definitive host, competitive interactions within the intermediate host can be costly to one or both parasite species (Dezfuli et al., 2001; Fredensborg and Poulin, 2005). For instance, the size of parasite larvae can be influenced by the number of conspecific and/or heterospecific individuals sharing the same host (Brown et al., 2003). In general, the infection success, life history strategy or even later adult size and fecundity can be greatly influenced by the presence of other parasites (Barger and Nickol, 1999; Heins et al., 2002; Fredensborg and Poulin, 2005). However, few studies have investigated the patterns of conspecific and heterospecific associations both in term of occurrence and effects on parasite life history.

In Lake Waihola, New Zealand, the amphipod *Paracalliope fluviatilis* is an excellent study model as it is used as intermediate host by three different species of helminth parasites, all relying on trophic transmission to reach their definitive host. First, an undescribed species of *Microphallus* (trematode) uses both *P. fluviatilis* and the isopod *Austridotea annectens* as second intermediate hosts (the first intermediate host being the mud-snail *Potamopyrgus antipodarum*), and water birds as definitive hosts. This species is able to alter the behaviour of both its intermediate hosts in ways that are likely to increase their predation by birds (Lefebvre and Poulin, unpublished data; Hansen and Poulin, 2005). The second species using the amphipod as an intermediate host, *Acanthocephalus galaxii*, matures in fish. Although no study has tested the ability of this particular species to manipulate the amphipod, modification of host behaviour appears to be an ancestral trait in acanthocephalan parasites and it is probable that *A. galaxii* possesses the same ability (Moore, 1984). Finally, the trematode *Coitocaecum parvum* uses fish as definitive hosts (the first intermediate host is also the snail *P. antipodarum*). However, *C. parvum* is very different from the two other parasites in two aspects of its biology. Firstly, although it is unable to manipulate its intermediate host, *C. parvum* has evolved a more radical way to counteract the odds of trophic transmission (Poulin, 2001; Lefebvre et al., 2005). When inside the amphipod host, this parasite encysts as a metacercaria and can either await ingestion by a fish in which it will grow, mature and reproduce (the normal three-host cycle), or may keep growing and reach maturity while still inside the amphipod, a strategy known as progenesis (Poulin and Cribb, 2002). Worms that reach maturity in the crustacean intermediate host reproduce by selfing and lay eggs in the amphipod's body cavity (Holton, 1984a; Poulin, 2001). Eggs produced by selfing hatch after the death of the host into larvae that are infective to the snail first host without the need to pass through a fish host (abbreviated cycle). This strategy is preferentially adopted when the opportunities of transmission are limited (Poulin, 2003; Lagrue and Poulin, 2007), but could also be adaptive when sharing an intermediate host with other parasites species. If the co-occurring species is manipulative but does not share the same definitive host, producing eggs quickly before the manipulated host is eaten by the predator would allow *C. parvum* metacercariae to reproduce before a dead-end transmission occurs. Second, in contrast to the other species in which there is usually no more than one conspecific parasite per host, up to six individuals of *C. parvum* can be found sharing the same intermediate host. Because metacercariae are always found in the haemocoel of the amphipod and, fully developed, may fill the host body cavity, co-infecting metacercariae are likely to compete for the same limited resources in term of space and energy (Lefebvre and Poulin, 2005; Michaud et al., 2006). Such competition is unlikely to be without consequences and the number of co-occurring conspecifics could influence the size and fecundity of each individual (Thomas et al., 2002b; Brown et al., 2003; Jäger and Schjørring, 2006). Thus, intense competition among parasites may limit the number of metacercariae that can achieve progenesis (Poulin and Lefebvre, 2006). However, the influence of within-host competition and its density-dependent effects on the growth, life history strategy and egg production of *C. par-*

vum in its amphipod host is currently unknown, as is the case for most parasite communities (Janovy, 2002).

In this study, we used naturally infected hosts to test for possible association patterns between the trematode *C. parvum* and two other co-occurring helminth species within their common amphipod host. First we predicted that the occurrence of *C. parvum* and *A. galaxii* could be positively associated within the amphipod population, these two species sharing the same fish definitive hosts. Second, *C. parvum* and the *Microphallus* sp. should be negatively associated as they have different definitive hosts. An alternative possibility is that no associations exist, positive or negative, in which case alternative life history strategies may be adopted by *C. parvum* according to both the number and identity of co-infecting parasites. To test this hypothesis, we also assessed the effects of interactions within the intermediate host on the life history strategy, and size and fecundity achieved by *C. parvum* in the amphipod. We examined both interspecific effects, with respect to each species' definitive host and the intraspecific effects of sharing a common host. In interspecific infections, we predicted that the proportion of progenetic *C. parvum* will vary according to the co-infecting species: a reduced level of progenesis when there are similar definitive hosts (association with *A. galaxii*) or an increased proportion of progenetic worms when associated with *Microphallus* sp. The size and life history strategy of each individual may also be influenced by the intensity of intraspecific competition (i.e. number of co-occurring *C. parvum* metacercariae).

2. Materials and methods

2.1. Animal collection and dissection

A total of 12 samples of *P. fluviatilis* was collected between January and December 2007, from the same site in Lake Waihola, South Island, New Zealand. Amphipods were captured by dragging a dip net (mesh size 500 μm) through macrophytes (*Myriophyllum triphyllum*). All amphipods were returned alive to the laboratory and kept in aerated lake water with strands of *M. triphyllum*as a food source. Dissections and measurements were completed within 48 h after sampling. Prior to their examination, amphipods were killed in 70% ethanol and rinsed in distilled water to facilitate handling. This method kills the amphipod but not their parasites; therefore it has no effect on parasite measurements.

Amphipods were measured (total length from the anterior tip of the cephalon to the end of the uropods), sexed and then dissected under a dissecting microscope. When parasites were found, they were counted and classified by species. In the case of *C. parvum*, each worm was measured (length and width) and recorded as "normal" (non-egg-producing worm) or "progenetic" (egg-producing worm). Eggs of progenetic worms were also counted, i.e. visually enumerated under the dissecting microscope. These included both eggs released by the worm in its thin-walled cyst and eggs still in utero. Sub-samples of both *Microphallus* sp. and *A. galaxii* were also measured. The body surface of each parasite was then determined and used as a surrogate for body size. This was done using the formula for an ellipsoid, $(\pi LW)/4$, where L and W are the length and width of the parasite (Lagrue and Poulin, 2007), for *C. parvum* and *A. galaxii*. Because *Microphallus*cysts are spherical, their body surface was calculated using the formula for a circle, (πR^2) where R is the radius of the cyst.

After dissection and measurement, we first verified that there was no difference in either composition (males and females) or amphipod mean body length between samples. We also tested for the effects of amphipod sex and size on the occurrence of the three parasite species. We then determined whether helminth associations were more or less likely to be found in amphipods harbouring different numbers of larvae of the different species. Finally, we tested for any difference in the length among amphipods harbouring zero, one or multiple *C. parvum* metacercariae.

2.2. Interspecific associations and effects on C. parvum life history strategy

Associations between the three 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 (presence–absence data; Janson and Vegelius, 1981). It accounts for the number of uninfected hosts, as well as the number of hosts infected by each parasite species or both species. Then, to determine whether *A. galaxii* and *Microphallus* sp. influence *C. parvum* life history strategy, we used Fisher's exact tests to compare the frequency of progenetic metacercariae in amphipods with either only *C. parvum* (one or two individuals) or *C. parvum* associated with another parasite species (*A. galaxii* or *Microphallus* sp.). We compared the frequency of progenetic metacercariae in interspecific infections with both single and double infections of *C. parvum* because within host competition for resources, both intra- and interspecific, can influence parasite life history strategy regardless of the parasites' definitive hosts (Thomas et al., 2002b). We also compared, using Fisher's exact tests, the proportion of progenetic *C. parvum* individuals between interspecific co-infections with *A. galaxii* and *Microphallus* sp. Effects of infection status (*C. parvum* associated with either *Microphallus* sp. or *A. galaxii*, or *C. parvum* alone) and life history strategy (progenetic or normal) on parasite body size were tested using a two-way ANOVA with the size of metacercariae used as the dependent variable. Parasite body size was log-transformed to normalize the data. The effect of infection status on egg production was also tested using a non-parametric test (Kruskal–Wallis ANOVA) with the number of eggs used as the dependent variable and the infection status as the independent variable; only parasites that had produced at least one egg were considered in this analysis. A linear regression between the size of metacercariae and the number of eggs produced was also used to assess the effect of parasite size on egg production.

2.3. Intraspecific competition and effects on C. parvum life history strategy

To determine whether within-host intraspecific competition influences the life history of individual *C. parvum*, we used Fisher's exact tests to compare the frequency of progenetic individuals in amphipods with one or more metacercariae; excluding amphipods containing *C. parvum*and *Microphallus* sp. or *A. galaxii*. We also tested for the effects of intraspecific competition on individual body size and egg production. Effects of infection status (single or multiple *C. parvum* infection) and life history strategy (progenetic or normal) on parasite body size were tested using a two-way ANOVA with the size of metacercariae used as the dependent variable. Again, parasite body size was log-transformed to normalize the data and least significant difference post-hoc tests (i.e. Fisher's LSD) were used when appropriate. Effects of the number of co-infecting metacercariae on egg production were also tested using a non-parametric test (Kruskal–Wallis ANOVA) with the number of eggs used as the dependent variable and the infection status (single or multiple *C. parvum* infection) as the independent variable; again, only parasites that had produced at least one egg were considered in this analysis. Finally, a linear regression between the size of metacercariae and the number of eggs produced was used to assess the effect of parasite size on egg production. In all of the above tests, P values < 0.05 were considered statistically significant.

3. Results

3.1. Animal collection and dissection

3.1.1. Effects of host sex and size on parasite prevalence

A total of 11,077 amphipods was measured and dissected for this study (see Table 1 for details). While the composition (males and females) of the different amphipod samples was variable, there was no significant effect of sample date on amphipod size (Table 1; ANOVA, $F_{11,11053} = 1.0$, $P = 0.418$). Amphipod sex seemed to have an effect on the occurrence of *C. parvum* as a higher proportion of male (12.5%) than female (6.7%) amphipods was infected (Fisher's exact test, $\chi^2 = 67.90$, $P < 0.0001$). However, females were significantly smaller than males (mean ± SEM: 2.30 ± 0.00 and 3.18 ± 0.00 mm, respectively; ANOVA, $F_{1,11075} = 26468.5$, $P < 0.0001$) and, overall, *C. parvum*-infected amphipods were on average larger than their uninfected counterparts (3.15 ± 0.01 and 2.93 ± 0.01 mm, respectively; ANOVA, $F_{1,11075} = 218.1$, $P < 0.0001$; Table 2). When males and females of similar sizes were compared, we found no difference in the occurrence of *C. parvum* (7.3 and 6.7%, respectively; Fisher's exact test, $\chi^2 = 0.91$, $P = 0.34$). It is therefore more likely

Table 1
Numbers of males (M) and females (F), mean length (±SEM), and prevalence (% hosts infected) of helminth infections in the different samples of the amphipod *Paracalliope fluviatilis* collected from Lake Waihola, New Zealand in 2007

Month	Numbers (M/F)	Length (mm)	*Coitocaecum parvum*	*Microphallus* sp.	*Acanthocephalus galaxii*
January	581/409	2.85 ± 0.02	4.14	17.78	0.10
February	563/315	2.90 ± 0.02	3.53	16.63	0.68
March	717/154	3.08 ± 0.02	6.20	11.60	4.25
April	824/50	3.11 ± 0.01	9.60	17.51	4.35
May	818/73	3.05 ± 0.01	9.65	6.40	1.68
June	761/264	2.97 ± 0.01	9.66	5.85	1.27
July	738/261	2.95 ± 0.01	10.00	8.61	1.10
August	764/144	3.03 ± 0.01	8.40	5.84	2.20
September	702/238	2.97 ± 0.01	8.62	8.62	1.81
October	742/172	3.01 ± 0.01	16.90	3.39	1.31
November	647/216	2.95 ± 0.01	27.10	2.32	1.39
December	624/300	2.86 ± 0.01	21.30	10.50	1.08
Total	8481/2596	2.98 ± 0.00	11.16	9.58	1.73

Table 2
Numbers, mean (±SEM), minimum and maximum sizes (body length) of amphipods in each "infection status" class

Infection status	Number of amphipods[a]	Size (mm)		
		Mean ± SEM	Min.	Max.
Coitocaecum parvum	1,236	3.15 ± 0.01	2.0	4.7
Microphallus sp.	1,061	3.12 ± 0.01	2.0	4.7
Acanthocephalus galaxii	192	3.17 ± 0.03	2.1	4.3
Uninfected	8,812	2.93 ± 0.01	2.0	4.7

[a] Note that amphipods infected by combinations of parasite species were included in each "infection status" class.

that host size, rather than sex, affects the prevalence of parasites.

We also found a significant difference in the mean size of amphipods infected by the three species of parasites (ANOVA, $F_{2,2486} = 3.59$, $P = 0.028$). Amphipods infected by *Microphallus* sp. were on average slightly smaller than those infected by *C. parvum* and *A. galaxii* (Table 2; Fisher's LSD, degrees of freedom (df) = 2486, $P = 0.017$ and 0.066). However, the size range of infected amphipods was similar for the three parasites and to that of uninfected individuals (Table 2). All individuals of *P. fluviatilis* were thus included in the analyses. We were consequently able to pool all samples for analyses when necessary.

3.1.2. Occurrences of single and multiple infections

Of the 1236 amphipods found to be infected with *C. parvum*, 984 (79.6%) harboured a single metacercaria; the others harboured mostly two (15.2%) but amphipods sometimes contained up to six metacercariae. No significant difference in length was found between amphipods infected by one, two, three or $\geqslant 4$ *C. parvum* metacercariae (ANOVA, $F_{2,1011} = 2.256$, $P = 0.105$). *Microphallus* sp. was recovered from 1061 amphipods: 879 (82.9%) contained only one cyst while the others harboured between two and 12 cysts. Only 192 amphipods were found to be infected by *A. galaxii*, among which a majority (90.6%) had only one cystacanth. However, a few individuals contained two (16) or three (two) cystacanths.

The frequency of co-occurrence of different parasite species in the same amphipod host was not affected by the number of larvae of each species per amphipod, in any of the possible helminth combinations (Fisher's exact tests, all $P > 0.05$); therefore all infected amphipods were included in the estimation of r_θ. Amphipod numbers and sizes given above include individuals infected by combinations of parasite species and/or several larvae of the same species. These intra- and interspecific combinations are subsequently described and analyzed.

3.2. Interspecific associations and effects on C. parvum life history strategy

3.2.1. Coefficients of biological association between parasite species

Although the overall prevalence of *C. parvum*and *Microphallus* sp. were similar (11.2 and 9.6%, respectively), prevalence within individual samples was highly variable (Table 1). The association between these two species was therefore tested both within individual samples and overall. The overall prevalence of *A. galaxii* was too low (1.7%) to detect species associations within individual samples so all samples were pooled when estimating r_θ between the acanthocephalan and the two other parasite species. Ten amphipods (0.09%) were found to be infected simultaneously by the three parasite species and 31 (0.28%) contained combinations of several *C. parvum* metacercariae associated with larvae of one of the other parasite species. These individuals were included in the estimation of the coefficient of biological association (r_θ) but subsequently discarded from analyses on the effect of intra- and interspecific competition on the life history strategy of *C. parvum*. Overall, there was no significant association between any of the three helminth species (Table 3). However, we found a significant positive association between *C. parvum* and the microphallid parasite in five individual samples (see Table 3 for details) and there was an overall weak trend towards a positive association between these two species.

3.2.2. Coitocaecum parvum life history strategy

Of the 130 amphipods infected by a combination of one *C. parvum* metacercariae and *Microphallus* sp., 20 (15.4%) harboured more than one *Microphallus* cyst. However, the number of cysts had no effect on the proportion of *C. parvum* adopting progenesis: 53.6% of metacercariae found in association with a single *Microphallus*cyst adopted progenesis whereas 60% of those found with two or more *Microphallus* cysts adopted progenesis (Fisher's exact test, $\chi^2 = 0.28$, $P = 0.599$); thus all amphipods were used in analyses. The proportion of *C. parvum* metacercariae adopting progenesis when sharing an amphipod host with *Microphallus* sp. was significantly higher than that of *C. parvum* found alone (Fisher's exact test, $\chi^2 = 17.22$, $P < 0.0001$; Fig. 1). This difference was not significant when comparing *C. parvum* individuals sharing hosts with either *Microphallus* sp. or a conspecific (Fisher's exact test, $\chi^2 = 2.54$, $P = 0.110$; Fig. 1).

There was no significant difference in the proportion of progenetic individuals between *C. parvum* found in co-infection with *A. galaxii* and in metacercariae found alone or with a conspecific (Fisher's exact tests, $\chi^2 = 0.21$ and 0.38, $P = 0.647$ and 0.538, respectively; Fig. 1). Again, there was no difference between *C. parvum* sharing a host with either *Microphallus* sp. or *A. galaxii* (Fisher's exact test, $\chi^2 = 1.80$, $P = 0.180$; Fig. 1). However, this could be due to the low number of amphipods infected by combinations of *C. parvum* and *A. galaxii*.

3.2.3. Coitocaecum parvum size and fecundity

Overall, progenetic metacercariae were significantly larger than non-egg producing individuals (0.158 ± 0.001 and 0.053 ± 0.001 mm^2, respectively; ANOVA, $F_{1,948} = 302.249$, $P < 0.0001$). *Acanthocephalus galaxii* cystacanths were on average seven times larger than *Microphallus* sp. cysts (0.236 ± 0.003 and 0.034 ± 0.000 mm^2, respectively; Mann–Whitney *U*-test, $Z = -10.644$, $P < 0.0001$) and the size of both species was not affected by the co-occurrence of *C. parvum* metacercariae (Mann–Whitney *U*-tests, $Z = 0.699$ and 0.700, respectively, both $P = 0.484$). Accordingly, *C. parvum* metacercariae sharing their host with *A. galaxii* were on average slightly smaller than metacercariae alone or in co-infection with *Microphallus* sp. (Fig. 2), although no significant effect of infection status was detected (ANOVA, $F_{2,948} = 0.911$, $P = 0.402$), and

Table 3
Infected and uninfected amphipods collected from Lake Waihola, New Zealand in 2007

Sample month	Numbers of amphipods in each category					
	C. parvum + *Microphallus*	*Microphallus*	*C. parvum*	Uninfected	r_0	*P*-value
Association between Coitocaecum parvum and Microphallus sp.						
January	11	165	30	784	0.049	>0.10
February	5	141	26	706	−0.003	>0.50
March	10	91	44	726	0.056	>0.10
April	23	130	61	660	0.085	<0.02*
May	10	47	75	759	0.071	<0.05*
June	8	52	91	874	0.031	>0.20
July	23	63	77	836	0.171	<0.001*
August	8	45	68	787	0.060	>0.05
September	15	66	66	793	0.108	<0.002*
October	9	22	145	738	0.061	>0.05
November	8	12	226	617	0.045	>0.10
December	34	63	163	664	0.115	<0.001*
Overall	164	897	1072	8944	0.044	>0.10
	C. parvum + *A. galaxi*	*A. galaxii*	*C. parvum*	Uninfected	r_0	*P*-value
Association between Coitocaecum parvum and Acanthocephalus galaxii						
Overall	41	151	1195	9690	0.043	>0.10
	Microphallus + *A. galaxii*	*Microphallus*	*A. galaxii*	Uninfected	r_0	*P*-value
Association between Microphallus sp. and Acant hocephalus galaxii						
Overall	28	1033	164	9852	0.023	>0.20

Numbers for each helminth combination, coefficient of biological association r_0 and statistical significance (*P*) of r_0 are given in the table. Rows marked with an (∗) show significant association between helminth species.

there was no interaction between the two factors (ANOVA, $F_{2,948} = 0.351$, $P = 0.704$).

Again, while progenetic metacercariae found in co-infection with *A. galaxii* produced on average fewer eggs than *C. parvum* metacercariae alone or with *Microphallus* sp. (Fig. 3), egg production was not significantly influenced by infection status (Kruskal–Wallis ANOVA, $H_{2,365} = 2.216$, $P = 0.330$) but increased with the size of the parasite ($r = 0.786$, $n = 365$, $P < 0.001$).

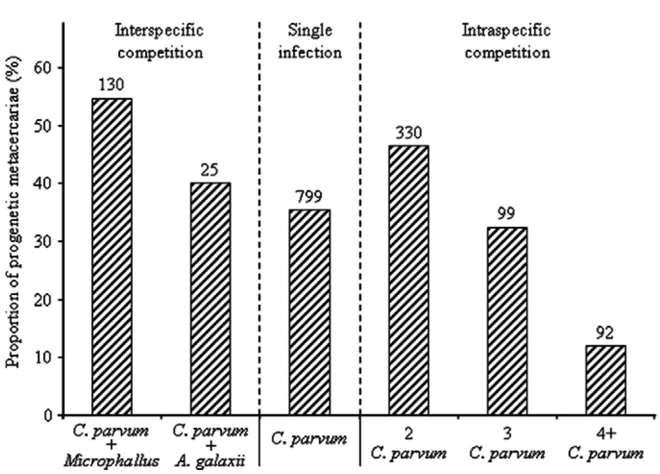

Fig. 1. Proportion (%) of progenetic *Coitocaecum parvum* metacercariae within each combination category. Numbers above bars are sample sizes (i.e. total number of metacercariae per category).

3.3. Intraspecific competition and effects on C. parvum life history strategy

As indicated above, interspecific competition is likely to affect the life history strategy of *C. parvum* and, therefore, amphipods infected by combinations of different helminth species were discarded from further analyses. Because there was no difference in the proportion of progenetic *C. parvum* between amphipods infected by four, five or six metacerca-

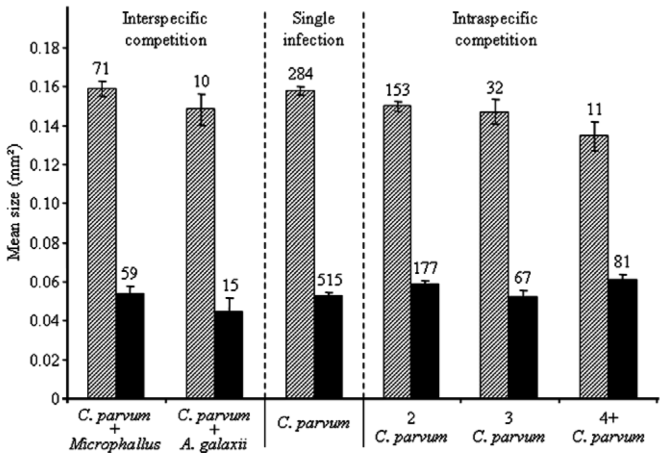

Fig. 2. Mean body size (±SEM) of progenetic (▨) and normal (■) *Coitocaecum parvum* metacercariae within each combination category. Numbers above bars are sample sizes (i.e. total number of metacercariae per category).

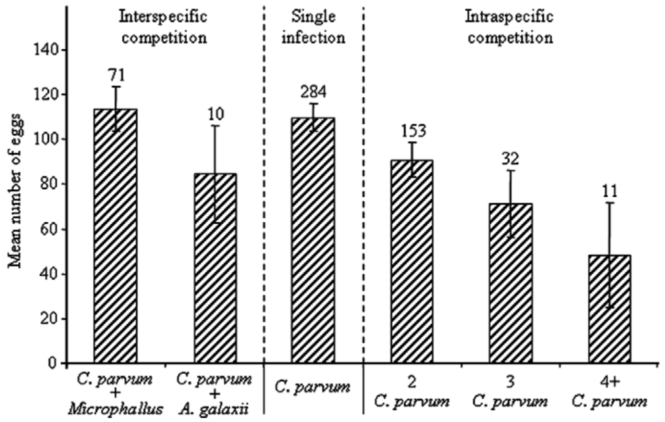

Fig. 3. Mean number of eggs (±SEM) produced by progenetic *Coitocaecum parvum* metacercariae within each combination category. Numbers above bars are sample sizes (i.e. total number of metacercariae per category).

riae (13.0%, 13.0% and 0.0%, respectively; Fisher's exact tests, all $P > 0.05$), these were pooled together to increase sample size.

3.3.1. *Coitocaecum parvum life history strategy*

The number of *C. parvum* metacercariae sharing the same amphipod host had a significant effect on the proportion of progenetic individuals (Fig. 1). Metacercariae found in double infections were significantly more likely to adopt progenesis than individuals in single infections or other multiple infections (Fig. 1; Fisher's exact tests; all $P < 0.02$) while the proportion of progenetic metacercariae in amphipods infected with four or more *C. parvum* was significantly lower than in any other category (Fig. 1; Fisher's exact tests; all $P < 0.001$). There was no difference in the proportion of progenesis between amphipods infected by one or three *C. parvum* metacercariae (Fig. 1; Fisher's exact test, $\chi^2 = 0.40$, $P = 0.527$).

3.3.2. *Coitocaecum parvum size and fecundity*

Again, progenetic metacercariae were significantly larger than non-egg producing individuals (0.154 ± 0.002 and 0.055 ± 0.001 mm^2, respectively; ANOVA, $F_{1,1312} = 499.33$, $P < 0.0001$; Fig. 2). We found no effect of infection status on metacercarial size (ANOVA, $F_{3,1312} = 1.01$, $P = 0.389$) but there was a significant interaction between infection status and parasite strategy (ANOVA, $F_{3,1312} = 4.00$, $P = 0.008$). Although the size of progenetic metacercariae seemed to decrease with the number of co-infecting individuals, there was no significant effect of infection status on the size of progenetic individuals (Fig. 2; Fisher's LSD, df = 1312, all $P > 0.05$). This could be due to the small sample sizes for multiple infections. Non-egg-producing metacercariae were generally smaller in single infections than in multiple infections (Fig. 2; Fisher's LSD, df = 1312, all $P < 0.05$) although there was no difference between single and triple infections (Fig. 2; Fisher's LSD, df = 1312, $P = 0.846$). There was no significant difference in the size of non-egg-producing metacercariae found in multiple infection combinations (double, triple or more; Fisher's LSD, df = 1312, all $P > 0.05$).

We found a significant effect of the number of co-infecting metacercariae on mean egg production (Fig. 3; Kruskal–Wallis ANOVA, $H_{3,480} = 9.975$, $P = 0.018$). Individual egg production tended to decrease when the number of co-infecting metacercariae increased (Fig. 3). Generally, the mean number of eggs produced per progenetic parasite increased significantly with the size of the parasite both in single ($r = 0.800$, $n = 284$, $P < 0.001$) and multiple infections ($r = 0.751$, $n = 196$, $P < 0.001$).

3.4. *Summary of results*

1. Although the frequency of interspecific infections was not negligible and potential conflicts existed between co-infecting parasites species, there were no overall biological associations, positive or negative, between the different helminth species (Table 3).
2. Interspecific interactions had specific effects on *C. parvum* life history strategy: metacercariae sharing a host with *Microphallus* sp., the bird parasite, were significantly more likely to adopt progenesis than when alone (Fig. 1). No such difference was found in *C. parvum* metacercariae co-occurring with *A. galaxii*, the fish parasite.
3. Intraspecific competition affected *C. parvum* life history strategy, size and fecundity. The proportion of progenetic individuals was higher in double compared with single infections but subsequently decreased with the number of co-infecting metacercariae (Fig. 1). Size and fecundity of progenetic metacercariae were intensity-dependent and decreased with the intensity of infection (Figs. 2 and 3).

4. Discussion

It is hypothesized that when different helminth species use the same intermediate host, and when at least one of them is capable of manipulating that host in a way that increases its predation risk by a specific predator, selection should favour non-random associations between these species (Dezfuli et al., 2000; Lafferty et al., 2000). We would then expect helminth species with different definitive hosts to have a negative association pattern and species sharing the same definitive host to positively associate in their second intermediate host. Here we found no clear association, positive or negative, between three different species of helminth parasites. Whether they share the same definitive host and whether they can manipulate their intermediate hosts' phenotype did not influence the association patterns. A positive association with *A. galaxii* could benefit *C. parvum*, assuming that *A. galaxii* can efficiently manipulate the amphipod host, in terms of transmission success. However, the prevalence of the acanthocephalan is so low that, even by actively seeking amphipods harbouring *A. galaxii* cystacanths, *C. parvum* cercariae are likely to die before finding such a host. *Coitocaecum parvum* cercariae have a very limited dispersion ability; they do not swim but crawl on the substrate, and their life expectancy is very short (MacFarlane, 1939; Holton, 1984b; Lagrue and Poulin, 2007). For these reasons, it is probably not advantageous for *C. parvum* to evolve a search mechanism. Overall, there was no significant association between *Microphallus* sp., a manipulative bird parasite, and *C. parvum*, a non-manipulative fish parasite. However, we detected a positive association in five out of 12 individual samples. Whether amphipods already infected by one species are more vulnerable to infection by the other cannot be determined from our study. Nevertheless, these results do not support our hypothesis. While this pattern cannot be explained in terms of similarities in life cycles, the cost and benefits of avoiding or associating with other parasite species also depend on their respective prevalence (Thomas et al., 1998). Although it clearly seems costly for *C. parvum* to associate with a manipulative bird parasite such as *Microphallus* sp., even by infecting amphipods at random, chances of co-infecting a host with *Microphallus* sp. are only about 9%. In the case of *A. galaxii*, the odds of co-occurring with *Microphallus* sp. are even lower (around one in 100). Thus, evolving an avoidance mechanism might be more costly than advantageous for these parasites.

Instead, *C. parvum* could use alternative life history strategies in response to specific associations with other parasite species. The highest rate of progenesis was observed among *C. parvum* metacercariae sharing their host with *Microphallus* sp., the manipulative bird parasite. Although the difference was not significant, *C. parvum* individuals co-occurring with *A. galaxii*, the other fish parasite, showed a much lower rate of progenesis. This result is consistent with our hypothesis that *C. parvum* sharing a host with different parasite species should adapt its strategy according to the other species' definitive host. However, the proportion of progenesis was higher in *C. parvum* metacercariae co-occurring with *A. galaxii* or a second *C. parvum* than in *C. parvum* individuals found alone in their host, even though they all have the same definitive host. Intra-host competition between parasites could also influence *C. parvum* life history strategy.

Factors such as space and nutrients, in limited supply within hosts, may constrain the possibility for any given *C. parvum* metacercaria to adopt progenesis. This strategy is associated with increased size and egg production is probably demanding in terms of nutrients. Sharing a given host with other parasites should impose costs that are expected to vary with the co-occurring species (Dezfuli et al., 2001). For example, fully grown cystacanths of *A. galaxii* and *Microphallus* sp. cysts may not use much energy from the host as they only wait for ingestion by the definitive host, but the former is on average 2.5 times larger than *C. parvum* metacercariae while the latter is three times smaller. Their size also appeared to be less affected by within-host competition than that of *C. parvum*. Different levels of interspecific competition could therefore explain the difference in progenesis rates observed between *C. parvum* metacercariae sharing their host with *A. galaxii* or *Microphallus* sp., regardless of each species' definitive host. Intra-host competition also seemed to influence both the size and egg production of *C. parvum*. Metacercariae sharing their host with the much larger acanthocephalan were on average slightly smaller and produced fewer eggs than those found alone or co-occurring with the smaller *Microphallus* sp.

Intraspecific competition among *C. parvum* metacercariae may also affect the strategy, size and egg production of each individual. Competition among *C. parvum* metacercariae appeared to be density-dependent. The level of progenesis significantly increased from single to double infections but subsequently decreased with the number of co-infecting metacercariae. It is possible that *C. parvum* responds to intraspecific competition by adopting progenesis more rapidly than when alone. Within-host competition between parasites of the same species is known to favour increased virulence (Thomas et al., 2002b; Parker et al., 2003). In the case of *C. parvum*, it seems to translate into the adoption of progenesis. Individuals adopting progenesis will use most if not all of the space and resources available in the host to grow swiftly and produce as many eggs as possible, to the host's detriment. The intense competition for host resources among co-occurring parasites is likely to limit the number of individuals that can achieve progenesis, hence the decreased proportion of progenesis observed in amphipods containing three or more metacercariae. Therefore, in multiple infections, individual of *C. parvum* should adopt progenesis as soon as possible before another metacercaria does. Intraspecific competition may also affect both the size and egg production of *C. parvum* metacercariae. Normal metacercariae tended to be larger in multiple than in single infections. This could be explained by the fact that, when in multiple infections, more metacercariae will grow faster than in single infections but only a few will actually reach a sufficient size to achieve progenesis and produce eggs. Due to the intense intraspecific competition, especially for space, metacercariae that could not achieve progenesis have to wait for ingestion by a fish definitive host. Individuals that succeed in becoming progenetic must still compete with co-infecting metacercariae for the space and nutrients needed for egg production. Our results show that the size and egg output of progenetic worms decreased with the number of metacercariae sharing the same host, and therefore the intensity of within-host competition. Overall, the size that *C. parvum* metacercariae can achieve within their amphipod host is a key factor influencing both their ability to reach progenesis and the total egg production achieved by each individual.

We cannot determine from our study whether the life history strategy of *C. parvum* in interspecific infections is influenced primarily by the identity of the definitive host of each co-infecting species or simply by the intensity of within-host competition for resources, but it seems clear that different helminth species can each affect *C. parvum* in particular ways. Intraspecific competition in *C. parvum* limited the number of individuals that could achieve progenesis but also reduced the average egg production of progenetic worms. These results and those of previous studies emphasize that a multitude of factors is influencing the life history strategies of parasites (Poulin, 2003; Poulin and Lefebvre, 2006). Some might be unique to particular species while others, such as within-host competition, are likely to act on a wide variety of species. Whether they are specific or general, determining the factors that drive the evolution of alternative life history strategies is a vital step in the understanding of the evolution of parasite life cycles in general.

Acknowledgements

This research was supported by a grant from the Marsden Fund (The Royal Society of New Zealand) to Robert Poulin. We are grateful to A. Besson, A. Thierry, A. Lewis and H. Sturrock for field assistance.

References

Bakker, T.C.M., Mazzi, D., Zala, S., 1997. Parasite-induced changes in behavior and colour make *Gammarus pulex* more prone to fish predation. Ecology 78, 1098–1104.

Barger, M.A., Nickol, B.B., 1999. Effects of coinfection with *Pomphorhynchus bulbocolli* on development of *Leptorhynchoides thecatus* (Acanthocephala) in amphipods (*Hyalella azteca*). J. Parasitol. 85, 60–63.

Bauer, A., Trouvé, S., Grégoire, A., Bollache, L., Cézilly, F., 2000. Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. Int. J. Parasitol. 30, 1453–1457.

Begon, M., Harper, J.L., Townsend, C.R., 1996. Ecology: Individuals, Populations and Communities, 3rd ed. Blackwell, Oxford.

Brown, S.P., 1999. Cooperation and conflict in host-manipulating parasites. Proc. R. Soc. Lond. B 266, 1899–1904.

Brown, S.P., De Lorgeril, J., Joly, C., Thomas, F., 2003. Field evidence for density-dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. J. Parasitol. 89 (4), 668–672.

Combes, C., Bartoli, P., Théron, A., 2002. Trematode transmission strategies. In: Lewis, E.E., Campbell, J.F., Sukhdeo, M.V.K. (Eds.), The behavioural Ecology of Parasites, CAB International. Wallingford, pp. 1–12.

Dezfuli, B.S., Giari, L., Poulin, R., 2000. Species associations among larval helminths in an amphipod intermediate host. Int. J. Parasitol. 30, 1143–1146.

Dezfuli, B.S., Giari, L., Poulin, R., 2001. Costs of intraspecific and interspecific host sharing in acanthocephalan cystacanths. Parasitology 122, 483–489.

Fredensborg, B.L., Poulin, R., 2005. Larval helminths in intermediate hosts: does competition early in life determine the fitness of adult parasites?. Int. J. Parasitol. 35 1061–1070.

Gower, C.M., Webster, J.P., 2005. Intraspecific competition and the evolution of virulence in a parasitic trematode. Evolution 59, 544–553.

Hansen, E.K., Poulin, R., 2005. Impact of a microphallid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance? Parasitol. Res. 97, 242–246.

Heins, D.C., Baker, J.A., Hillery, C.M., 2002. The “crowding effect” in the cestode *Schistocephalus solidus*: density-dependent effects on plerocercoid size and infectivity. J. Parasitol. 88, 302–307.

Helluy, S., 1983. Relations hôtes-parasites du trématode *Microphallus papillorobustus* (Rankin 1940). II. Modifications du comportement des Gammarus hôtes intermédiaires et localisation des métacercaires. Ann. Parasitol. Hum. Comp. 58, 1–17.

Holton, A.L., 1984a. Progenesis as a mean of abbreviating life histories in two New Zealand trematodes, *Coitocaecum parvum* Crowfton, 1945 and *Stegodexamene anguillae* MacFarlane, 1951. Mauri Ora 11, 63–70.

Holton, A.L., 1984b. A redescription of *Coitocaecum parvum* Crowcroft, 1945 (Digenea: Allocreadiidae) from crustacean and fish hosts in Canterbury. N.Z.J. Zool. 11, 1–8.

Jäger, I., Schjørring, S., 2006. Multiple infections: relatedness and time between infections affect the establishment and growth of the cestode *Schistocephalus solidus* in its stickleback host. Evolution 60, 616–622.

Janovy, J., 2002. Concurrent infections and the community ecology of helminth parasites. J. Parasitol. 88, 440–445.

Janson, S., Vegelius, J., 1981. Measures of ecological association. Oecologia 49, 371–376.

Kuris, A.M., Lafferty, K.D., 1994. Community structure: larval trematodes in snail hosts. Annu. Rev. Ecol. Syst. 25, 189–217.

Lafferty, K.D., 1999. The evolution of trophic transmission. Parasitol. Today 15, 111–115.

Lafferty, K.D., Thomas, F., Poulin, R., 2000. Evolution of host phenotype manipulation by parasites and its consequences. In: Poulin, R., Morand, S., Skorping, A. (Eds.), Evolutionary Biology of Host–Parasite Relationships: Theory Meets Reality. Elsevier Science, Amsterdam, pp. 117–127.

Lagrue, C., Poulin, R., 2007. Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? J. Evol. Biol. 20, 1189–1195.

Lagrue, C., McEwan, J., Poulin, R., Keeney, D.B., 2007. Co-occurences of parasite clones and altered host phenotype in a snail-trematode system. Int. J. Parasitol. 37, 1459–1467.

Lefebvre, F., Poulin, R., 2005. Alternative reproductive strategies in the progenetic trematode *Coitocaecum parvum*: comparison of selfing and mating worms. J. Parasitol. 91, 93–98.

Lefebvre, F., Fredensborg, B.L., Armstrong, A., Hansen, E., Poulin, R., 2005. Assortative pairing in the amphipod *Paracalliope fluviatilis*: a role for parasites? Hydrobiologia 545, 65–73.

Leung, T.L.F., Poulin, R., 2007. Interactions between parasites of the cockle *Austrovenus stutchburyi*: hitch-hikers, resident-cleaners, and habitat-facilitators. Parasitology 134, 247–255.

MacFarlane, W.V., 1939. Life cycle of *Coitocaecum anaspidis* Hickman, a New Zealand digenetic trematode. Parasitology 31, 172–184.

Michaud, M., Milinski, M., Parker, G.A., Chubb, J.C., 2006. Competitive growth strategies in intermediate hosts: experimental tests of a parasite life-history model using the cestode, *Schistocephalus solidus*. Evol. Ecol. 20, 39–57.

Moore, J., 1983. Responses of an avian predator and its isopod prey to an acanthocephalan parasite. Ecology 64, 1000–1015.

Moore, J., 1984. Altered behavioral responses in intermediate hosts: an acanthocephalan parasite strategy. Am. Nat. 123, 572–577.

Moore, J., 2002. Parasites and the behaviour of animals. Oxford University Press, Oxford.

Mouritsen, K.N., 2001. Hitch-hiking parasite: a dark horse may be the real rider. Int. J. Parasitol. 31, 1417–1420.

Mouritsen, K.N., Poulin, R., 2003. Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator’s nightmare. Int. J. Parasitol. 33, 1043–1050.

Parker, G.A., Chubb, J.C., Roberts, G.N., Michaud, M., Milinski, M., 2003. Optimal growth of larval helminths in their intermediate host. J. Evol. Biology 16, 47–54.

Poulin, R., 2001. Progenesis and reduced virulence as an alternative transmission strategy in a parasitic trematode. Parasitology 123, 623–630.

Poulin, R., 2003. Information about transmission opportunities triggers a life-history switch in a parasite. Evolution 57, 2899–2903.

Poulin, R., 2007. Evolutionary Ecology of Parasites, 2nd ed. Princeton University Press, Princeton.

Poulin, R., Cribb, T.H., 2002. Trematode life cycles: short is sweet? Trends Parasitol. 18, 176–183.

Poulin, R., Nichol, K., Latham, A.D.M., 2003. Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict? Int J. Parasitol. 33, 425–433.

Poulin, R., Lefebvre, F., 2006. Alternative life-history and transmission strategies in a parasite: first come, first served? Parasitology 132, 135–141.

Thomas, F., Mete, K., Helluy, S., Santalla, F., Verneau, O., de Meeus, T., Cézilly, F., Renaud, F., 1997. Hitch-hiker parasites or how to benefit from the strategy of another parasite. Evolution 51, 1316–1318.

Thomas, F., Renaud, F., Poulin, R., 1998. Exploitation of manipulators: hitch-hiking as a parasite transmission strategy. Anim. Behav. 56, 199–206.

Thomas, F., Fauchier, J., Lafferty, K.D., 2002a. Conflict of interest between a nematode and a trematode in an amphipod host: test of the "sabotage" hypothesis. Behav. Ecol. Sociobiol. 51, 296–301.

Thomas, F., Brown, S.P., Sukhdeo, M., Renaud, F., 2002b. Understanding parasite strategies: a state-dependent approach? Trends Parasitol. 18, 387–390.