Do parasites adopt different strategies in different intermediate hosts? Host size, not host species, influences *Coitocaecum parvum* (Trematoda) life history strategy, size and egg production

R. RUIZ DANIELS[1,2], S. BELTRAN[1], R. POULIN[2] *and* C. LAGRUE[2]*

[1] *Université de Poitiers, UMR CNRS 7267, Laboratoire EBI Ecologie & Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Poitiers, France*
[2] *University of Otago, Department of Zoology, Dunedin, New Zealand*

(*Received 2 July 2012; revised 24 July 2012; accepted 21 August 2012; first published online 15 October 2012*)

SUMMARY

Host exploitation induces host defence responses and competition between parasites, resulting in individual parasites facing highly variable environments. Alternative life strategies may thus be expressed in context-dependent ways, depending on which host species is used and intra-host competition between parasites. *Coitocaecum parvum* (Trematode) can use facultative progenesis in amphipod intermediate hosts, *Paracalliope fluviatilis*, to abbreviate its life cycle in response to such environmental factors. *Coitocaecum parvum* also uses another amphipod host, *Paracorophium excavatum*, a species widely different in size and ecology from *P. fluviatilis*. In this study, parasite infection levels and strategies in the two amphipod species were compared to determine whether the adoption of progenesis by *C. parvum* varied between these two hosts. Potential differences in size and/or egg production between *C. parvum* individuals according to amphipod host species were also investigated. Results show that *C. parvum* life strategy was not influenced by host species. In contrast, host size significantly affected *C. parvum* strategy, size and egg production. Since intra-host interactions between co-infecting parasites also influenced *C. parvum* strategy, size and fecundity, it is highly likely that within-host resource limitations affect *C. parvum* life strategy and overall fitness regardless of host species.

Key words: life cycle abbreviation, intermediate hosts, *Coitocaecum parvum*, *Paracalliope fluviatilis*, *Paracorophium excavatum*.

INTRODUCTION

Phenotypic plasticity is often seen as an adaptive strategy in highly variable environments (Anurag, 2001). Host exploitation by parasites generally results in rapid evolutionary responses of hosts for defence purposes, resulting in a situation where parasites face a constantly changing environment and have to co-evolve with their hosts (Van Valen, 1974). Furthermore, the fitness of a parasite completely depends upon host-parasite interactions; infection and reproduction rates in a particular host-determining parasite success (Paterson and Piertney, 2011). Parasite phenotypic plasticity and alternative growth strategies should thus be expressed in precise context-dependent ways, depending on the parasite's environment/host (Thomas *et al*. 2002*a*; Parker *et al*. 2003).

The environment of a parasite is often not restricted to a single host since multiple species, parasites and/or hosts, are frequently encountered in a parasite life cycle (Fredensborg and Poulin, 2005; Gower and Webster, 2005; Rauch *et al*. 2005). This is especially true for trematodes, their complex life cycles involving up to 4 widely different hosts and countless potential intra- and interspecific parasite competitors (Poulin, 2007). Although in the majority of trematodes a three-host life cycle is maintained, several species have lost a host from their cycle (Poulin and Cribb, 2002). This life-cycle abbreviation is often realized by dropping the definitive host, maturing and reproducing in the second intermediate host through progenesis and self-fertilization. In some species, life-cycle abbreviation remains facultative and both strategies are observed concurrently in parasite populations (Poulin and Cribb, 2002).

For example, the digenean trematode *Coitocaecum parvum* is able to adaptively adopt progenesis or the normal 3-host life cycle in response to different environmental factors (MacFarlane, 1939; Holton, 1984*a*,*b*; Lagrue and Poulin, 2007, 2008*a*). Adult *C. parvum* colonize the digestive tract of fish definitive hosts where they sexually reproduce. Eggs are released with fish feces and hatch into free-swimming miracidiae. After entering the first intermediate host, the snail *Potamopyrgus antipodarum*, they develop into cercariae-producing sporocysts.

---

* Corresponding author: Department of Zoology, University of Otago, 340 Great King Street, PO Box 56, Dunedin 9054, New Zealand, Tel: +64 3479 7986. Fax: +64 3479 7584. E-mail: clement.lagrue@gmail.com

Parasitology (2013), **140**, 275–283.
doi:10.1017/S0031182012001564

Cercariae leave the snail and must infect an amphipod second intermediate host where they encyst as metacercariae. The parasite can then adopt one of two alternative life strategies; either wait for a fish definitive host to consume the infected amphipod host or undergo progenesis while still inside the amphipod intermediate host and reproduce by selfing (Poulin and Cribb, 2002; Lefèbvre and Poulin, 2005). Progenetic individuals produce viable eggs released into the environment upon the death of the amphipod host. A previous study showed no evidence for heritability of progenesis or negative effects of selfing on offspring fitness (Lagrue and Poulin, 2009*a*). However, many factors influence adoption of the abbreviated life cycle by individual *C. parvum*. For example, progenesis rate increases when fish definitive hosts are absent from the environment (Lagrue and Poulin, 2007). Time spent in the amphipod host also increases the likelihood of progenesis adoption (Lagrue and Poulin, 2009*b*). Finally, co-infecting parasites and intra-host competition in the second intermediate host (the amphipod *Paracalliope fluviatilis*) strongly affect the adoption of progenesis in *C. parvum* (Lagrue and Poulin, 2008*a*; Lagrue *et al*. 2009).

Although *C. parvum* uses 2 different amphipod species as intermediate hosts, how its life-history strategy (abbreviated or normal 3-host cycle) may differ between these two hosts remains unknown. The two amphipod species often co-exist in the field despite profound ecological differences. While *Paracalliope fluviatilis* is demersal and free-swimming, *Paracorophium excavatum* is a purely benthic, sedentary, burrow-dwelling amphipod; this may increase its exposure to the non-swimming cercariae of *C. parvum* (Holton, 1984*a*). *Paracorophium excavatum* is also larger, may provide more resources to trematode parasites and thus affect the adoption of progenesis by *C. parvum* and/or egg production in progenetic individuals. Most studies have only considered *P. fluviatilis* as *C. parvum* intermediate host even though *P. excavatum* was early documented as a potential host in which progenesis occurred (Holton, 1984*a*; Luque *et al*. 2007). However, potential differences in progenesis rates and egg production between *C. parvum* individuals infecting co-existing *P. fluviatilis* and *P. excavatum* are still unknown.

While intra-host (intra- and interspecific) competition among parasites is known to affect both life strategy and egg production in *C. parvum*, these effects have been studied only in *P. fluviatilis* (Lagrue and Poulin, 2008*a*). Intraspecific competition between co-infecting *C. parvum* individuals strongly affects progenesis rates and decreases egg production in *P. fluviatilis*, the smaller of the two amphipod host species (Lagrue and Poulin, 2008*a*). It is possible that such effects are dampened by the larger size and higher available resources of *P. excavatum* hosts. Similarly, *Microphallus* sp. is known to infect both amphipods species (Luque *et al*. 2007). It is also capable of manipulating amphipod host behaviour to enhance its transmission to bird definitive hosts (Hansen and Poulin, 2005; Coats *et al*. 2010). Because *C. parvum* and *Microphallus* sp. do not share definitive hosts, conflicting interests over transmission routes and/or developmental strategy are likely to arise (Brown, 1999; Thomas *et al*. 2002*b*; Poulin *et al*. 2003). *Coitocaecum parvum* might thus benefit from adopting progenesis in the presence of *Microphallus* sp.; its transmission probabilities being likely reduced by *Microphallus* sp. host manipulation. *Microphallus* sp. prevalence and abundance are also much higher in *P. excavatum* than *P. fluviatilis* (Luque *et al*. 2007). Furthermore, *P. excavatum* is host to a large fish nematode (*Hedruris spinigera*) while *P. fluviatilis* is not (Luque *et al*. 2007; Lagrue and Poulin, 2008*a*, *b*). Metacercariae of *C. parvum* infecting *P. excavatum* are consequently more likely to compete with other parasites than conspecifics in *P. fluviatilis*. Natural prevalence and/or abundance variations in parasitic infections between *P. excavatum* and *P. fluviatilis* may consequently influence progenesis adoption in *C. parvum* in its two intermediate host species.

In this study, we examined parasite prevalences and abundances in the two amphipod host species and gave particular attention to the frequency of progenesis in *C. parvum* infecting *P. excavatum* and *P. fluviatilis* to detect possible species-specific intermediate host-induced effects on the parasite strategy. For *C. parvum* individuals that adopted progenesis, we determined whether parasite size and/or egg production was higher in *P. excavatum* than in the smaller *P. fluviatilis*. Finally, we examined potential effects of interactions with co-infecting parasites on size and egg production of progenetic *C. parvum*.

MATERIALS AND METHODS

Study site and animal sampling

Naturally infected *Paracalliope fluviatilis* and *Paracorophium excavatum* amphipods were collected in Lake Waihola (46°01′S, 170°05′E), South Island, New Zealand, using dip nets (500 μm nylon mesh). All amphipods were collected during February and April 2012 and both species were collected at the same time and location to avoid potential spatial or temporal effects on parasite prevalences and/or abundances. *Paracorophium excavatum* were captured by dredging the fine surficial sediment where this species burrows. The retained sediment with associated organisms was placed in sorting trays from which *P. excavatum* individuals were collected (Schnabel *et al*. 2000). *Paracalliope fluviatilis* were collected from the same area by dragging dip nets in patches of macrophytes (*Elodea canadensis*).

Amphipods were separated from macrophytes using a 5 mm mesh sieve and collected in sorting trays. All amphipods were kept alive in plastic containers filled with local water and brought back to the laboratory for later dissection. Amphipods were dissected within 3 days of capture.

Parasite prevalences and abundances, and Coitocaecum parvum *strategy, size and egg production*

Amphipods were killed in 70% ethanol and rinsed with distilled water to allow for easy manipulation (measurement and dissection) without affecting data collection on parasites (Lefèbvre and Poulin, 2005). Before dissection, amphipods were identified (species) and measured (total body length) to the closest 0·1 mm under a binocular microscope (×20 magnification) as host size can influence parasite prevalence and abundance in naturally infected amphipods (Lagrue and Poulin, 2008*b*). For each amphipod, the species (*C. parvum, Microphallus* sp. and/or *H. spinigera*) and number of parasites were recorded to determine parasite prevalences (proportion of infected hosts) and abundances (number of parasites per host). Metacercariae of *C. parvum* were also classified as either normal (non-egg producing) or progenetic (egg producing) according to the presence of eggs in the cyst or still *in utero*. Each metacercaria was measured (total length) and its eggs counted.

Statistical analysis

Potential differences in parasite prevalences and/or proportion of progenetic *C. parvum* between the two amphipod species were tested in a pair-wise manner using Fisher's exact tests. Effects of intermediate host species (*P. fluviatilis* and *P. excavatum*) on specific parasite abundances (*C. parvum, Microphallus* sp. and *H. spinigera*) and possible differences in host size between the two amphipod species and between infected and uninfected hosts were examined using non-parametric tests (Mann-Whitney U test), as data did not follow normal distributions. Potential relationships between host size, parasite abundance, and *C. parvum* size and egg production were also tested using linear regressions. Non-parametric tests and linear regressions described above were performed using STATISTICA Software 6.0 (StatSoft Inc., France).

General Linear Models (GLM) were used to test for the effects of multiple factors on the probability of *C. parvum* metacercariae adopting progenesis, and on the size and/or egg production of progenetic metacercariae. Factors potentially affecting the probability of progenesis in *C. parvum* and included in the models were host species, host size, number of co-infecting *Microphallus* sp., number of co-infecting non-egg producing *C. parvum* metacercariae and/or presence of the fish nematode *H. spinigera*. The dependent variable was treated in a binomial manner with *C. parvum* metacercariae coded either as 1 (progenetic/egg-producing) or 0 (non-egg producing). GLMs were also performed to determine the factors affecting the size and egg production of progenetic metacercariae. Host species, host size, number of co-infecting *Microphallus* sp., number of co-infecting non-egg producing *C. parvum* metacercariae and/or presence of *H. spinigera* were included as factors in those GLMs. The size of progenetic metacercariae was also included as a factor when egg production was the response variable. In GLMs testing for the factors affecting *C. parvum* egg production, only amphipod hosts containing egg-producing *C. parvum* were included. Log-transformation before analyses was used when necessary to normalize the data. All factors were initially included in the GLMs to determine the most appropriate model using the Akaike Information Criterion (AIC), with the model awarded the lowest AIC having the highest explanatory power for the observed data. Only factors remaining in the best models are presented in the GLM result tables. In all models, effect size was determined using t-values and degrees of freedom. GLMs were performed using R Software 2.15.0 (R Development Core Team, 2011).

RESULTS

Host species, host size and parasite prevalences and abundances

Overall, 812 amphipods were measured and dissected, 219 *P. excavatum* and 593 *P. fluviatilis*. Parasite prevalences were significantly higher in *P. excavatum* than *P. fluviatilis* (Fisher's exact tests, $\chi^2 = 57{\cdot}64$, 47·65 and 224·54, all $P < 0{\cdot}0001$ for *C. parvum, H. spinigera* and *Microphallus* sp., respectively; see Table 1 for details). Similarly, parasite abundances were significantly higher in *P. excavatum* than *P. fluviatilis* (Mann-Whitney U test, $Z = 8{\cdot}284$, 7·056 and 17·396, all $P < 0{\cdot}0001$ for *C. parvum, Microphallus* sp. and *H. spinigera* respectively; Table 1).

Host size was significantly different between the two amphipod species (Mann-Whitney U test, $Z = 15{\cdot}916$, $P < 0{\cdot}0001$); *P. excavatum* being larger than *P. fluviatilis* (Table 2). Amphipod size was also different between infected and uninfected individuals. In *P. excavatum*, infected individuals were significantly larger than their uninfected conspecifics for the three parasites species (Mann-Whitney U test, $Z = -3{\cdot}479$, $-2{\cdot}42$ and $-3{\cdot}046$, $P = 0{\cdot}0005$, 0·016 and 0·002 for *C. parvum, Microphallus* sp. and *H. spinigera*, respectively; see Table 2 for details). The same trend was observed in *P. fluviatilis* with *C. parvum* and *Microphallus* sp. infected amphipods

Table 1. Prevalences (proportion of infected amphipods) and abundances (mean number of parasites ± S.E. per individual amphipod) for the three species of parasites (*Coitocaecum parvum, Microphallus* sp. and *Hedruris spinigera*) in the two amphipod host species (*Paracorophium excavatum* and *Paracalliope fluviatilis*) sampled in Lake Waihola

(When prevalence was 0, parasite abundance could not be calculated and is shown as not available (na).)

Host	*P. fluviatilis*		*P. excavatum*	
Parasite	Prevalence (%)	Abundance (± S.E.)	Prevalence (%)	Abundance (± S.E.)
C. parvum	18·2	0·30 ± 0·09	44·3	1·42 ± 0·25
Microphallus sp.	22·8	0·46 ± 0·14	80·4	8·66 ± 0·96
H. spinigera	0·0	na	8·2	0·08 ± 0·06

Table 2. Host size (mean amphipod body length in mm ± S.E.) for the two amphipod species (*Paracorophium excavatum* and *Paracalliope fluviatilis*) sampled in Lake Waihola according to their infection status (infected or uninfected (i.e. uninf.))

(Data are shown for the three species of parasites, *Coitocaecum parvum, Microphallus* sp. and *Hedruris spinigera*. When parasite prevalence was 0, mean infected host size is shown as not available (na).)

Parasite		*C. parvum*		*Microphallus* sp.		*H. spinigera*	
Host size (mm ± S.E.)	Total	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.
P. excavatum	4·0 ± 0·1	4·2 ± 0·1	3·8 ± 0·1	4·1 ± 0·1	3·7 ± 0·1	4·6 ± 0·2	3·9 ± 0·1
P. fluviatilis	2·8 ± 0·0	3·1 ± 0·1	2·7 ± 0·0	2·9 ± 0·1	2·8 ± 0·0	na	na

being significantly larger than uninfected individuals (Mann-Whitney U test, Z = − 5·393 and −3·059, $P < 0·0001$ and $P = 0·002$, respectively; Table 2); note that *H. spinigera* was never found infecting *P. fluviatilis*. There was also a significant effect of host size on parasite abundances (number of parasites per individual host). Larger *P. excavatum* individuals tended to be more heavily infected than their smaller conspecifics ($r = 0·338$, 0·436 and 0·207, $n = 219$, $P < 0·0001$, $P < 0·0001$ and $P = 0·002$ for *C. parvum, Microphallus* sp. and *H. spinigera*, respectively; Fig. 1). Again, similar trends were detected in *P. fluviatilis*; parasite abundances were significantly correlated to amphipod host size ($r = 0·208$ and 0·180, $n = 593$, both $P < 0·0001$ for *C. parvum* and *Microphallus* sp., respectively; Fig. 1).

Coitocaecum parvum *strategy, size and egg production*

A total of 488 *C. parvum* metacercariae were recovered; 311 found in *P. excavatum* and 177 in *P. fluviatilis*. The proportion of progenetic metacercariae was not significantly different between the two host species (12·8% and 14·7% for *P. excavatum* and *P. fluviatilis*, respectively; Fisher's exact test, $\chi^2 = 0·32$, $P = 0·570$). This is consistent with results from GLMs showing that only host size had a significant effect on *C. parvum* strategy (Table 3). Host species and the number of co-infecting non-progenetic *C. parvum* were included in the model with the lowest AIC score although their effect size was small and not statistically significant (Table 3). The presence of *Microphallus* sp. metacercariae had no detectable effect on *C. parvum* strategy.

Similarly, host species did not influence the size of progenetic metacercariae (Table 4). The only factor significantly influencing *C. parvum* size was the host size; larger amphipod hosts tending to harbour larger egg-producing *C. parvum* metacercariae, regardless of host species ($r = 0·559$, $n = 57$, $P < 0·0001$; Fig. 2). Again, host species had no effect on *C. parvum* egg production. The number of eggs produced by progenetic *C. parvum* was clearly influenced by the size of the metacercaria (Table 5); egg production increasing with *C. parvum* size, regardless of host species ($r = 0·826$, $n = 57$, $P < 0·0001$; Fig. 3). Intra-host interactions between parasite species seemed to play a major role on *C. parvum* egg production (Table 5). Furthermore, these effects are likely to be species-specific. Progenetic *C. parvum* egg production was clearly higher in the presence of *Microphallus* sp. (mean egg production ± S.E. = 306·9 ± 50·2 and 151·7 ± 38·4 with and without co-infecting *Microphallus* sp., respectively; Mann-Whitney U test, Z = − 1·993, $P = 0·046$). However, there was no significant effect of the presence of non-progenetic *C. parvum* (241·4 ± 50·4 and 219·9 ± 41·9 with and without co-infecting non-progenetic *C. parvum*, respectively; Mann-Whitney U test, Z = 0·176, $P = 0·868$), possibly because of a density-dependent effect, rather than just presence or absence (Table 5; Lagrue and Poulin, 2008*a*). Contrastingly, co-infection with *H. spinigera* significantly reduced *C. parvum* egg production in

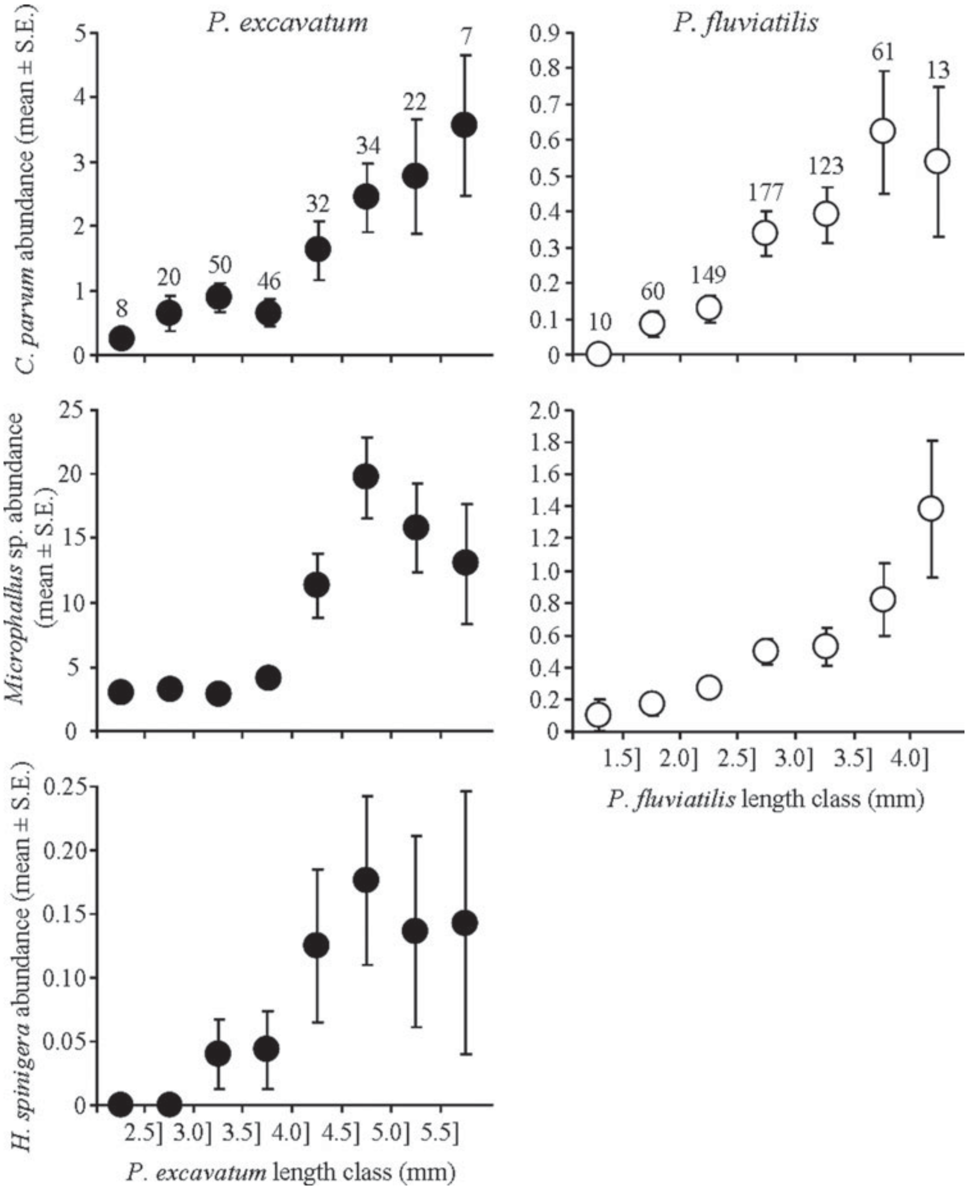

Fig. 1. Abundances (mean ± S.E.) of *Coitocaecum parvum* (top graphs), *Microphallus* sp. (middle) and *Hedruris spinigera* (bottom graph) in relation to host size (amphipod length class) in *Paracorophium excavatum* (left graphs) and *Paracalliope fluviatilis* (right). Sample size (i.e. number of amphipods) per host size class is indicated above each data point on the top graphs. Note that *P. fluviatilis* was never infected by *H. spinigera*.

P. excavatum (61·2 ± 19·1 and 367·2 ± 54·3 with and without co-infecting *H. spinigera*, respectively; Mann-Whitney U test, Z = 2·724, $P = 0{\cdot}004$); *P. fluviatilis* was not considered here since it was never infected by *H. spinigera*.

DISCUSSION

The main goal of the study was to explore potential factors influencing the frequency of progenesis in *C. parvum* metacercariae in their amphipod second intermediate hosts. In particular, we tested whether the parasite adjusted its life strategy to the species of amphipod host. Results show that host species *per se* did not influence *C. parvum*'s strategy; the proportion of progenetic metacercariae was similar in *P. excavatum* and *P. fluviatilis*, the two amphipod host species. Furthermore, intermediate host species *per se* had no effect on progenetic *C. parvum* size or egg production.

Overall, *P. excavatum* was more heavily infected than *P. fluviatilis*. Both prevalence and abundance of the three parasite species were higher in *P. excavatum*. Whether this difference was due to the larger size of this species compared to *P. fluviatilis* or different biological characteristics between the two amphipod species (benthic versus demersal and/or sedentary burrowing versus free-swimming, respectively) cannot be determined with certainty from our study. However, since larger individuals tend to be more heavily infected than smaller individuals in both amphipod species and *P. excavatum* is significantly

Table 3. General Linear Model testing for the effects of host species (*Paracorophium excavatum* and *Paracalliope fluviatilis*), host size (total body length), number of co-infecting parasites (*Microphallus* sp. and/or non-progenetic *Coitocaecum parvum* metacercariae) and presence of the nematode *Hedruris spinigera* on *C. parvum* life strategy (occurrence of progenetic individuals) in amphipod hosts

(Results shown originate from the model awarded the lowest AIC (Akaike Information Criterion) score. Significant *P* values are indicated by *.)

Factors	Estimate	s.e.	*t*-value	*P*	Effect size
Host species	0·562	0·404	1·390	0·165	0·049
Host size	1·348	0·217	6·221	< 0·0001*	0·213
Non-progenetic *C. parvum*	0·093	0·067	1·379	0·168	0·048

Table 4. General Linear Model testing for the effects of host species (*Paracorophium excavatum* and *Paracalliope fluviatilis*), host size (total body length), number of co-infecting parasites (*Microphallus* sp. and/or non-progenetic *Coitocaecum parvum* metacercariae) and presence of the nematode *Hedruris spinigera* on the size of progenetic *C. parvum* metacercariae (body length)

(Results shown originate from the model awarded the lowest AIC (Akaike Information Criterion) score. Significant *P* values are indicated by *.)

Factors	Estimate	s.e.	*t*-value	*P*	Effect size
Host size	0·151	0·044	3·409	0·001*	0·415
Microphallus sp.	− 0·003	0·003	1·122	0·267	0·148

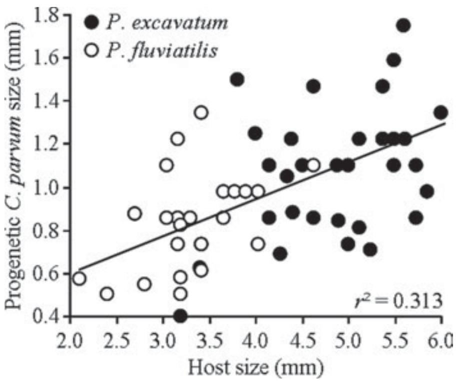

Fig. 2. Relationship between amphipod host size (body length in mm) and the size of progenetic *Coitocaecum parvum* metacercariae (body length in mm) in the two amphipod species, *Paracorophium excavatum* and *Paracalliope fluviatilis*. Line of best fit and coefficient of determination are shown on the figure.

larger than *P. fluviatilis*, it is likely that host size plays a significant role in parasite infection levels. Nevertheless, *C. parvum* cercariae cannot swim and instead crawl on the substrate to find a suitable intermediate host while *H. spinigera* is transmitted through the ingestion of the parasite eggs by amphipod hosts. It is thus possible that the benthic and detritivore life style of *P. excavatum* leads to higher exposure to infections in this species compared to *P. fluviatilis*. Information on *Microphallus* sp. transmission mode is lacking and interpretation of our results is difficult but similar trends are plausible.

Larger host size may also be linked to higher levels of within-host resources availability to parasites allowing for higher parasite abundance, growth and/or egg production (Saad-Fares and Combes, 1992; Grutter and Poulin, 1998; Johnson *et al.* 2005). Although host species *per se* did not affect the likelihood of *C. parvum* adopting progenesis, our results show that host size and thus possibly within-host space/resources significantly affected parasite abundances and *C. parvum* strategy, size and egg production. The size of *C. parvum* progenetic metacercariae, and subsequently egg production, are known to be correlated with intermediate host size (Lagrue and Poulin, 2007). Since *P. excavatum* is significantly larger than *P. fluviatilis, C. parvum* metacercariae adopting progenesis in *P. excavatum* are on average larger and produce more eggs, a result consistent with an effect of host resource availability on *C. parvum* size and egg production.

Alternatively, parasite infections may induce differential mortality rates in the two host species. Interspecific differences in parasite prevalences and abundances between the two amphipod hosts may be explained by a higher parasite-induced mortality in *P. fluviatilis* following infection and/or parasite accumulation. Our results tend to show that differential mortality is unlikely since parasite abundances increased with host size, indicating that both species accumulate parasites as they grow with no apparent increased mortality (Thomas *et al.* 1995; Rousset *et al.* 1996). Larger, hence older, individual hosts thus contain higher parasite abundances, because of

Table 5. General Linear Model testing for the effects of host species (*Paracorophium excavatum* and *Paracalliope fluviatilis*), host size (total body length), number of co-infecting parasites (*Microphallus* sp. and/or non-progenetic *Coitocaecum parvum* metacercariae), presence of the nematode *Hedruris spinigera* and *C. parvum* size (body length) on the number of eggs produced by progenetic metacercariae in amphipod hosts

(Results shown originate from the model awarded the lowest AIC (Akaike Information Criterion) score. Significant *P* values are indicated by *.)

Factors	Estimate	S.E.	*t*-value	*P*	Effect size
Host species	−0·635	0·327	−1·705	0·094	0·222
H. spinigera	0·136	0·061	−2·225	0·031*	0·285
Microphallus sp.	0·023	0·010	2·195	0·033*	0·282
Non-progenetic *C. parvum*	−0·141	0·055	−2·556	0·014*	0·323
Progenetic *C. parvum* size	2·424	0·469	5·169	<0·0001*	0·57

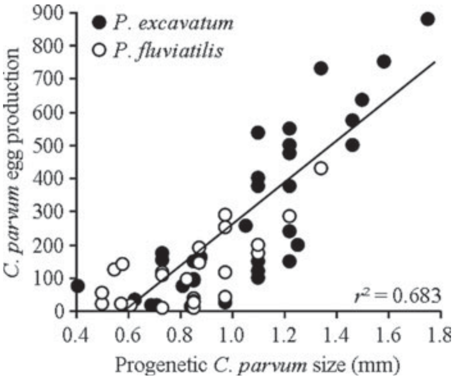

Fig. 3. Relationship between the size of progenetic *Coitocaecum parvum* metacercariae (body length in mm) and egg production in the two amphipod host species, *Paracorophium excavatum* and *Paracalliope fluviatilis*. Line of best fit and coefficient of determination are shown on the figure.

longer time for infections to accumulate (Read and Taylor, 2001). They may also contain older progenetic metacercariae that have had more time for growth and/or egg production.

Several factors tend to indicate that host size rather than host age influences *C. parvum* growth and egg production. First, there is no seasonal variation in *C. parvum* prevalence, proportion of progenetic metacercariae or size/age distribution in amphipod hosts (Lagrue and Poulin, 2008*b*). Infection by *C. parvum* is most likely constant over time and host age/size; large/old amphipod hosts should not necessarily contain older metacercariae. Second, growth and egg production can be very rapid in *C. parvum*; individuals adopting progenesis can quadruple in size in 5 weeks, having produced over 20 eggs by that time and egg production then increasing exponentially with parasite size, unless limited by within-host resources (Lagrue and Poulin, 2007, 2009*b*). *Coitocaecum parvum* metacercariae of the same age also show a clear bimodal distribution in size, progenetic individuals being much larger than normal ones while individuals of intermediate sizes are rare (Lagrue and Poulin, 2009*b*). Size and/or egg production of *C. parvum* metacercariae is thus unlikely to depend solely on the size of individual parasites. Finally, for an equivalent size, female amphipods are older than males but *C. parvum* prevalence, proportion of progenetic metacercariae, as well as egg production, are similar in these hosts (Lagrue and Poulin, 2008*a*). Amphipod host size, rather than age, likely drives *C. parvum* strategy, size and egg production although host age may eventually have some influence on *C. parvum* strategy (Lagrue and Poulin, 2009*b*).

Coitocaecum parvum and *Microphallus* sp. were also found in higher prevalence and abundance in *P. excavatum* than in *P. fluviatilis* and the nematode *H. spinigera* was found to infect *P. excavatum* only. Intra-host competition is thus more common and probably more intense in *P. excavatum*. The presence of co-infecting parasites can have variable effects on *C. parvum* life strategy, size and/or egg production, possibly due to species-specific and abundance related intra-host competition effects. Intra- and interspecific parasite competition can apply to transmission routes and/or host resources (Lagrue and Poulin, 2008*a*). In *P. fluviatilis*, the presence of *Microphallus* sp. did not induce higher progenesis rates but clearly increased egg production in *C. parvum*. *Microphallus* sp. manipulates intermediate host behaviour to reach bird definitive hosts while *C. parvum* must infect a fish host or use progenesis to reproduce (Hansen and Poulin, 2005; Lagrue and Poulin, 2008*a*). Conflicts over transmission pathways may thus induce progenetic *C. parvum* to produce as many eggs as possible, eventually killing its host, before *Microphallus* sp. host manipulation succeeds. Our results suggest that this trend is similar and probably exacerbated since *Microphallus* sp. abundance, and hence host manipulation (Brown *et al.* 2003), is much higher in *P. excavatum*. In contrast, co-infection with *H. spinigera* or non-progenetic *C. parvum*, both needing to reach a fish definitive host, seemed to reduce *C. parvum* size and/or egg production. Since there is no conflict over transmission in these situations, host resource limitations most likely induce reductions in size and egg production in progenetic individuals (Lagrue and Poulin, 2008*a*).

Although all parasites probably use similar host resources, results indicate that intra-host competition with *H. spinigera* induces the most drastic reduction in *C. parvum* egg production. This is not surprising since *H. spinigera* larvae are very large compared to progenetic *C. parvum* (around 8 times larger) and host size (Luque *et al*. 2007, 2010). *Hedruris spinigera* likely uses more host resources than the proportionally smaller non-progenetic *C. parvum* or *Microphallus* sp. metacercariae (around 33% and 20% of progenetic *C. parvum* size, respectively; Lagrue and Poulin, 2008*b*), thus exerting stronger competition pressure on co-infecting progenetic *C. parvum*. *Coitocaecum parvum*'s strategy, size and egg production seemed to be influenced by the presence of other parasites in species- and intensity-specific ways. Resource availability in general, be it within-host space or energy, is likely to have strong influences on the size and egg production of progenetic metacercariae.

Overall, the frequency of progenesis did not vary between amphipod host species. However, host size had a small but significant effect on the probability of metacercariae becoming progenetic, regardless of host species. Similarly, host species *per se* did not strongly influence egg production compared to host size, and thus possibly host age. Disentangling host size and host age effects on *C. parvum* strategy, growth and/or egg production could be achieved by experimentally infecting amphipod hosts of equivalent age but different size, either males and females of the same species or individuals of the two species used in our study. Alternatively, amphipods of similar size and thus contrasting age could be infected using the same methods (Lagrue and Poulin, 2007).

Within-host resource availability is probably an important factor influencing parasite life strategy, size and egg production and this is largely determined by host size. Since *P. excavatum* hosts are significantly larger than *P. fluviatilis*, apparent host species effects on *C. parvum* strategy, size and egg production are most likely due to interspecific size differences between intermediate host species. However, further experiments should look at the relative effects of within-host resource (i.e. energy, nutrients) and space (host size) limitations on *C. parvum* metacercariae. This may be tested by experimentally infecting amphipod hosts of similar sizes that are subsequently fed with diets of contrasting nutritive value, or hosts of contrasting sizes fed on an energetically equivalent diet.

In conclusion, results suggest that factors other than host species have more important effects on *C. parvum*'s strategy. These include previously documented factors such as definitive host presence or time since infection, and factors tested herein like intermediate host size or intra-host interactions between co-infecting parasites. Overall, the plasticity offered by facultative progenesis may allow parasites to adjust their developmental strategy in a context-dependent way in response to an array of environmental variables (host size/resources, species-specific intra-host competition, transmission probability) but regardless of host species. Since progenesis acts as a reproductive insurance, allowing individuals to produce at least a few eggs, what maintains progenesis as a facultative strategy is not yet known and would deserve further investigation.

ACKNOWLEDGEMENTS

This project was funded by an Erasmus Mundus Program 'European Master in Applied Ecology' (EMAE) grant to R.R.D. The authors would also like to thank Richard Sydney Taylor (University of Bristol) for his help during the process and Mathieu Sicard (University of Poitiers) for his administrative assistance. We thank an anonymous reviewer for constructive comments on a previous version of the manuscript.

REFERENCES

Anurag, A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science* **294**, 321–326.

Brown, S. P. (1999). Cooperation and conflict in host-manipulating parasites. *Proceedings of the Royal Society of London, B* **266**, 1899–1904.

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

Coats, J., Nakagawa, S. and Poulin, R. (2010). The consequences of parasitic infections for host behavioural correlations and repeatability. *Behaviour* **147**, 367–382.

Fredensborg, B. L. and Poulin, R. (2005). Larval helminths in intermediate hosts: does competition early in life determine the fitness of adult parasites? *International Journal for Parasitology* **35**, 1061–1070.

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

Grutter, A. S. and Poulin, R. (1998). Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. *Marine Ecology Progress Series* **164**, 263–271.

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

Holton, A. L. (1984*a*). A redescription of *Coitocaecum parvum* Crowcroft, 1945 (Digenea: Allocreadiidae) from crustacean and fish hosts in Canterbury. *New Zealand Journal of Zoology* **11**, 1–8.

Holton, A. L. (1984*b*). 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.

Johnson, K. P., Bush, S. E. and Clayton, D. H. (2005). Correlated evolution of host and parasite body size: tests of Harrison's rule using birds and lice. *Evolution* **59**, 1744–1753.

Lagrue, C. and Poulin, R. (2007). Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? *Journal of Evolutionary Biology* **20**, 1189–1195.

Lagrue, C. and Poulin, R. (2008*a*). Intra- and interspecific competition among helminth parasites: effects on *Coitocaecum parvum* life history strategy, size and fecundity. *International Journal for Parasitology* **38**, 1435–1444.

Lagrue, C. and Poulin, R. (2008*b*). Lack of seasonal variation in the life-history strategies of the trematode *Coitocaecum parvum*: no apparent environmental effect. *Parasitology* **135**, 1243–1251.

Lagrue, C. and Poulin, R. (2009*a*). Heritability and short-term effects of inbreeding in the progenetic trematode *Coitocaecum parvum*: is there a need for the definitive host? *Parasitology* **136**, 231–240.

Lagrue, C. and Poulin, R. (2009*b*). Life cycle abbreviation in trematode parasites and the developmental time hypothesis: is the clock ticking? *Journal of Evolutionary Biology* **22**, 1727–1738.

Lagrue, C., Poulin, R. and Keeney, D. B. (2009). Effects of clonality in multiple infections on the life-history strategy of the trematode *Coitocaecum parvum* in its amphipod intermediate host. *Evolution* **63**, 1417–1426.

Lefèbvre, F. and Poulin, R. (2005). Progenesis in digenean trematodes: a taxonomic and synthetic overview of species reproducing in their second intermediate hosts. *Parasitology* **130**, 587–605.

Luque, J. L., Bannock, L. M., Lagrue, C. and Poulin, R. (2007). Larval *Hysterothy-lacium sp*. (Nematoda, Anisakidae) and trematode metacercariae from the amphipod *Paracorophium excavatum* (Corphiidae) in New Zealand. *Acta Parasitologica* **52**, 146–150.

Luque, J. L., Vieira, F. M., Herrmann, K., King, T. M., Poulin, R. and Lagrue, C. (2010). New evidence on a cold case: trophic transmission, distribution and host specificity in *Hedruris spinigera* (Nematoda: Hedruridae). *Folia Parasitologica* **57**, 223–231.

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

Parker, G. A., Chubb, J. C., Roberts, G. N., Michaud, M. and Milinski, M. (2003). Optimal growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionary Biology* **16**, 47–54.

Paterson, S. and Piertney, S. B. (2011). Frontiers in host-parasite ecology and evolution. *Molecular Ecology* **20**, 869–871.

Poulin, R. (2007). *Evolutionary Ecology of Parasites*. 2nd Edn, Princeton University Press, Princeton, NJ, USA.

Poulin, R. and Cribb, T. H. (2002). Trematode life cycles: short is sweet? *Trends in Parasitology* **18**, 176–183.

Poulin, R., Nichol, K. and Latham, A. D. M. (2003). Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict? *International Journal for Parasitology* **33**, 425–433.

Rauch, G., Kalbe, M. and Reusch, T. B. H. (2005). How a complex life cycle can improve a parasite's sex life. *Journal of Evolutionary Biology* **18**, 1069–1075.

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. Vienn, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Read, A. F. and Taylor, L. H. (2001). The ecology of genetically diverse infections. *Science* **292**, 1099–1102.

Rousset, F., Thomas, F., de Meeüs, T. and Renaud, F. (1996). Inference of parasite-induced mortality from distributions of parasite loads. *Ecology* **77**, 2203–2211.

Saad-Fares, A. and Combes, C. (1992). Abundance/host size relationship in a fish trematode community. *Journal of Helminthology* **66**, 187–192.

Schnabel, K. E., Hogg, I. D. and Chapman, M. A. (2000). Population genetic structure of two New Zealand corophiid amphipods and the presence of morphologically cryptic species: implications for the conservation of diversity. *New Zealand Journal of Marine and Freshwater Research* **34**, 637–644.

Thomas, F., Brown, S. P., Sukhdeo, M. and Renaud, F. (2002*a*). Understanding parasite strategies: a state-dependent approach? *Trends in Parasitology* **18**, 387–390.

Thomas, F., Fauchier, J. and Lafferty, K. D. (2002*b*). Conflict of interest between a nematode and a trematode in an amphipod host: test of the "sabotage" hypothesis. *Behavioural and Ecological Sociobiology* **51**, 296–301.

Thomas, F., Renaud, F., Rousset, F., Cézilly, F. and de Meeüs, T. (1995). Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Zoological Society of London* **260**, 349–352.

Van Valen, L. (1974). Molecular evolution as predicted by natural selection. *Journal of Molecular Evolution* **3**, 89–101.