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Life cycle abbreviation in trematode parasites and the developmental time hypothesis: is the clock ticking?

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Abstract

The typical multi-host life cycle of many parasites, although conferring several advantages, presents the parasites with a highly hazardous transmission route. As a consequence, parasites have evolved various adaptations increasing their chances of transmission between the different hosts of the life cycle. Some trematode species like the opecoelid *Coitocaecum parvum* have adopted a more drastic alternative strategy whereby the definitive host is facultatively dropped from the cycle, resulting in a shorter, hence easier to complete, life cycle. Like other species capable of abbreviating their life cycle, *C. parvum* does so through progenetic development within its intermediate host. Laboratory-reared *C. parvum* can modulate their developmental strategy inside the second intermediate host according to current transmission opportunities, though this ability is not apparent in natural *C. parvum* populations. Here we show that this difference is likely due to the time *C. parvum* individuals spend in their intermediate hosts in the natural environment. Although transmission opportunities, i.e. chemical cues of the presence of definitive hosts, promoted the adoption of a truncated life cycle in the early stages of infection, individuals that remained in their amphipod host for a relatively long time had a similar probability of adopting progenesis and the abbreviated cycle, regardless of the presence or absence of chemical cues from the predator definitive host. These results support the developmental time hypothesis which states that parasites capable of facultative life cycle abbreviation should eventually adopt progenesis regardless of transmission opportunities, and provide further evidence of the adaptive plasticity of parasite transmission strategies.

Introduction

Parasites with complex life cycles, perhaps more than any other organisms, face considerable odds against the successful completion of successive generations. Indeed, for the many taxa of parasites with developmental strategies that consist of several different life stages, hosts and transmission events, there is a great risk of failing to complete the whole cycle (Choisy *et al.*, 2003; Parker *et al.*, 2003a; Poulin, 2007). For example, trematode parasites typically have a three-host life cycle. First, eggs produced by adult worms in the vertebrate definitive host are released into the environment with host faeces, and hatch into free-living larvae that must find a suitable mollusc first intermediate host. Second, the free-living cercariae emerging from the first intermediate host must locate a suitable second intermediate host, in which they encyst as metacercariae. Finally, metacercariae must be ingested, along with the second intermediate host, by the definitive host to complete the cycle. This last step, involving trophic transmission, is widespread among parasites with complex life cycles (Brown *et al.*, 2001; Moore, 2002). At this point, the parasite (and, consequently, its host) needs 'to be at the right place and right time' to achieve transmission to the definitive host. The

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probability that an individual parasite finds the required suite of three hosts is likely to be low and highly unpredictable (Fenton & Hudson, 2002). Through natural selection, trematodes have evolved various adaptations facilitating the completion of their life cycle. High adult fecundity, asexual multiplication within the molluscan host and host-finding mechanisms in free-living larvae are among those (Poulin & Cribb, 2002; Thomas *et al.*, 2002a; Cribb *et al.*, 2003).

The adoption of a vertebrate predator as definitive host is also likely to offer several advantages for parasites such as longer lifespan, greater body size, higher fecundity (Parker *et al.*, 2003a) or greater access to sexual partners as large definitive hosts accumulate large numbers of parasites (Brown *et al.*, 2001; Rauch *et al.*, 2005). However, trophic transmission to the definitive host remains a very hazardous step in the life cycle of trematodes as passage from the second intermediate to the definitive host necessitates high predation rate to maintain a sufficient transmission rate (Parker *et al.*, 2003a). As a result, some parasites relying on the consumption of their intermediate host by a specific predator have evolved the ability to alter the behaviour and/or appearance of this host to increase their chances of transmission (Combes *et al.*, 2002; Moore, 2002; Lagrue *et al.*, 2007a). Alternatively, several distantly related parasites have independently adopted a more drastic strategy where the definitive host is simply dropped from the cycle, making the latter easier to complete (Font, 1980; Barger & Esch, 2000; Poulin & Cribb, 2002; Lefebvre & Poulin, 2005a). Those parasites produce viable eggs while still inside their intermediate host, via progenesis (i.e. early maturation). Eggs are produced through self-fertilization, trematodes being hermaphroditic, and released in the environment after host death (Poulin, 2001). Although some parasites have obligatory truncated life cycles, most species display facultative life cycle abbreviation with both the normal and abbreviated cycles used as alternative strategies and simultaneously present in the parasite population.

Facultative life cycle truncation is likely to be advantageous for parasites using definitive host species that fluctuate in abundance and are periodically absent (Poulin & Cribb, 2002); it gives the parasite the ability to switch between different reproductive strategies as a phenotypic response to a variable environment, allowing offspring production even under unfavourable conditions (i.e. definitive host absent). Progenesis as an adaptive strategy was first proposed by Holton (1984a) to explain the high frequency of shorter cycles in New Zealand trematodes using freshwater fish as definitive hosts; because of migration or seasonal periods of low abundance, most of these fish are regularly unavailable as hosts (Holton, 1984a; McDowall, 1990). In this case, the timing of maturation and reproduction ('early' progenesis or 'late' normal cycle) and the propensity to initiate progenesis should be influenced by the environmental conditions.

Generally, an organism's timing of reproduction is a fundamental life-history variable and inappropriate timing can have severe fitness costs (Simons & Johnston, 2000). Several organisms can adjust their age at maturity or the timing of their reproduction according to environmental variation in abiotic (Reed *et al.*, 2009) or biotic (Fredensborg & Poulin, 2006) factors. For example, monocarpic plants that die following a single episode of reproduction (semelparous) can delay or accelerate growth and flowering as a phenotypic response to changes in their environment, such as density and/or resource availability (Hesse *et al.*, 2008). Plants that delay flowering (i.e. reproduction) grow larger and produce more seed (i.e. higher fecundity) than plants that reproduce early at a smaller size, but increase their risk of dying before reproduction (Metcalf *et al.*, 2003, 2008; Burd *et al.*, 2006); trematodes with facultative progenesis are in the same situation. Similarly, in the Trinidadian guppy (*Poecilia reticulata*), adult size and age at maturity vary among populations in response not only to the presence of predators but also to the type of predator (Reznick, 1982; Reznick *et al.*, 1990; Rodd *et al.*, 1997; Gosline & Rodd, 2008). Guppies under predation from small, gape-limited predatory fishes grow larger and mature at an older age than guppies co-occurring with larger predators. As there is always some trade-off between growth, survival and fecundity, monocarpic plants, guppies and also progenetic parasites need to be judicious gamblers, in terms of strategic decisions, in their variable environment (Metcalf *et al.*, 2003). In trematodes with facultative progenesis, the co-existence of the two developmental strategies could be due to the shorter cycle being a conditional life strategy adopted in response to unfavourable conditions in their uncertain world (i.e. seasonally low host densities; Poulin & Cribb, 2002; Poulin & Lefebvre, 2006), thus serving as a reproductive insurance (Wang & Thomas, 2002). Although the normal three-host cycle may be preferable when definitive hosts are abundant, a switch to the abbreviated life cycle should be favoured when these hosts are rare.

For example, experiments under controlled conditions have shown that the New Zealand trematode *Coitocaecum parvum*, in which life cycle abbreviation is facultative, can adjust its life-history strategy in response to chemical cues from the fish definitive host (Poulin, 2003; Lagrue & Poulin, 2007). Metacercariae grow faster and preferentially adopt progenesis and the shorter life cycle when chemical cues from the definitive host are absent. On the other hand, *C. parvum* individuals infecting amphipods exposed to chemical cues from the definitive host (common bully, *Gobiomorphus cotidianus*) showed significantly lower rates of progenesis (Poulin, 2003; Lagrue & Poulin, 2007). Seasonal changes in common bully densities are a normal feature of the fish biology owing to the periodicity of spawning and related ontogenic shifts in habitat use as juvenile fish develop (Rowe, 1994, 1999;

Rowe *et al.*, 2001; Kattel & Closs, 2007). However, in the field, there is no correlation between the abundance of fish definitive hosts and the proportion of progenetic metacercariae (Lagrue & Poulin, 2008a).

Nevertheless, both theory and empirical evidence suggest that parasite transmission success depends on local host density (Roberts *et al.*, 2002). *Coitocaecum parvum*'s second intermediate host, the amphipod *Paracalliope fluviatilis*, occurs at very high densities but it is short-lived (< 1 year): the probability of a given amphipod being eaten by fish may thus be very low (Poulin, 2001; Lefebvre *et al.*, 2005; Hansen & Poulin, 2006). If a *C. parvum* metacercaria has been encysted in its amphipod host for a relatively long time, some internal developmental clock may cause it to mature and start producing eggs inside the second intermediate host, a phenomenon proposed as 'the developmental time hypothesis' by Poulin & Cribb (2002). This would be independent from the absence of definitive hosts: the real cause would be the time spent by the parasite inside its second intermediate host regardless of its transmission probability (Poulin & Cribb, 2002). Previous studies showed that the presence of definitive hosts has a direct effect on *C. parvum* life-history strategy: after 5 weeks, metacercariae exposed to chemical cues from the fish host were less likely to become progenetic (Poulin, 2003; Lagrue & Poulin, 2007). However, little is known of the fate of parasites that remain in their amphipod intermediate hosts for extended periods of time and whether they all eventually become progenetic, regardless of the presence of fish hosts.

Here, using experimental infections of *C. parvum*, we tested the developmental time hypothesis of Poulin & Cribb (2002). We hypothesized that even if the trematode *C. parvum* can adjust its strategy according to its transmission opportunities (Poulin, 2003; Lagrue & Poulin, 2007), some internal developmental clock might cause metacercariae to mature and start producing eggs inside the amphipod host regardless of whether fish definitive hosts are present. We expected that *C. parvum* would quickly adopt progenesis when predator scent is absent whereas metacercariae in amphipods exposed to common bully chemical cues should mostly adopt the normal three-host cycle at first. Over time, we expected that metacercariae remaining in amphipods would be more likely to mature and produce eggs regardless of the treatment; the ageing of both host and parasite triggering developmental attempts to abbreviate the life cycle, to ensure the production of at least a few eggs.

Material and methods

Life cycle of *C. parvum*

Coitocaecum parvum (Trematoda, Opecoelidae) is a common parasite of freshwater fish in New Zealand, mostly the common bully, *G. cotidianus* (MacFarlane, 1939; Holton, 1984b). Eggs produced by adult worms inside the fish gut are released in host faeces and hatch into free-swimming miracidia. These penetrate mud snails (Hydrobiidae, *Potamopyrgus antipodarum*) in which they mature and asexually produce cercariae, which are the next infective stage. Cercariae actively leave the snail host and enter the amphipod *P. fluviatilis* where they encyst as metacercariae in the body cavity. At this stage, metacercariae can either await ingestion by a fish where they will mature and reproduce, or keep growing and reach maturity via progenesis while still inside the amphipod. Worms that reach maturity in amphipods reproduce by selfing and lay eggs within their cyst (Holton, 1984a; Poulin, 2001). Eggs are released after host death and decomposition, and their hatching success is very similar to that of eggs produced by adult parasites inside the fish (Lagrue & Poulin, 2009). Both alternative life cycles occur simultaneously in natural *C. parvum* populations (Poulin, 2001, 2003). Also, it is worth noting that neither strategy (progenesis or the normal three-host cycle) is heritable, so the strategy adopted by offspring is independent of that used by their parents (Lagrue & Poulin, 2009).

Animal collection

Naturally infected snails (*P. antipodarum*) and amphipods (*P. fluviatilis*) were collected in Lake Waihola, South Island, New Zealand, using fine-meshed (500 μm) dip nets. Five hundred *C. parvum* infected snails were obtained by selectively choosing individuals that displayed an altered shell shape, a definite sign of infection by *C. parvum* (Lagrue *et al.*, 2007b), and kept as a source of parasite larvae. Uninfected amphipods were obtained by inspecting individuals under a dissecting microscope and discarding those that showed signs of infection: an opaque mass in the body cavity corresponding to a metacercaria. This method allows the selection of only uninfected individuals with an accuracy of about 95% (Lefebvre & Poulin, 2005b). Because host condition (age, size, sex) can influence the growth of larval helminths in their intermediate host (Parker *et al.*, 2003b), we used only male amphipods of similar size (around 3 mm body length), and therefore age, in our experimental infections, minimizing the variation in body condition among hosts. Furthermore, fresh amphipods were collected every week to control for any delay before infection. Snails and amphipods were kept separately in aerated lake water and fed macrophytes from the lake. Twelve common bullies were captured in Lake Waihola using a set net, kept alive in a 5-L tank filled with aerated and filtrated lake water, and fed commercial fish food.

Experimental infections

Coitocaecum parvum cercariae were obtained from naturally infected snails under controlled conditions to ensure

that the cercariae used to experimentally infect amphipods were freshly released (< 20 min) and therefore more likely to penetrate the amphipod. Infected snails were transferred from the stock tank to Petri dishes filled will lake water and then incubated at 25 °C for 20 min under constant light, conditions known to induce cercarial release (Hay *et al.*, 2005). Cercariae released by the snails were transferred to 500 μL Eppendorf tubes using a 20 μL micropipette. Two cercariae were placed in each tube with 2.5 μL of filtrated lake water and an amphipod was then added. Amphipods were left in the tube along with the two cercariae for 5 h, after which time unsuccessful cercariae stop moving and die (Lagrue & Poulin, 2007). Amphipod survival, at this stage, approaches 100% (Lagrue & Poulin, 2007). Amphipods were then haphazardly separated into groups of about 35 individuals. Each group was placed in a plastic container filled with 400 mL of aged lake water (water collected at least 1 week before the experiment to allow any chemical cue to deteriorate; Poulin, 2003); strands of macrophytes (*Elodea canadensis*) were added for food. Given that a total of 3278 amphipods were experimentally infected for the purpose of this study, controlled infections and subsequent dissections were conducted over several weeks between late May and late July 2008, and mid-June and late August 2008 respectively.

Treatment

Bully-scented water was obtained directly from the tank containing the fish. Control water was taken from a second similar tank containing only water to standardize the treatment. Every 2 days and for 3, 5, 7 or 9 weeks depending on the treatment duration, 20 mL of water was removed from each amphipod container and replaced with either control or bully-scented water. The same treatment was applied to a given container for the whole experiment. In total, 1651 (294, 376, 449 and 532 for 3, 5, 7 and 9 weeks respectively) amphipods served as control and 1627 (353, 305, 473 and 496 for 3, 5, 7 and 9 weeks respectively) received bully-scented water.

Measures and statistical analyses

At the end of the treatment, 1116 (68%) amphipods [267 (91%), 297 (79%), 263 (59%) and 289 (54%) after 3, 5, 7 and 9 weeks respectively] from the control treatment and 916 (56%) amphipods [259 (73%), 187 (61%), 249 (53%) and 221 (45%) after 3, 5, 7 and 9 weeks respectively] from the bully treatment had survived. All surviving amphipods were killed in 70% ethanol to facilitate dissections and measurements, rinsed in distilled water, and immediately measured (body length) and dissected under a dissecting microscope. Any *C. parvum* metacercaria they contained was measured (length and width) under a compound microscope, and recorded as 'normal' (nonegg-producing worm) or 'progenetic' (egg-producing worm); in the case of progenetic parasites, eggs free within the cyst were also counted. The surface (i.e. body area) 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 respectively.

Of the 2032 surviving amphipods (1116 from the control and 916 from the bully treatment), 1036 (51%) were infected. Because it was impossible to determine the proportion of parasitized individuals in the amphipods that died during the experiment, we could not determine the actual percentage of individuals successfully infected through controlled infections. However, *C. parvum* prevalence did not significantly vary between treatment durations (51.3%, 52.9%, 50.0% and 49.0% after 3, 5, 7 and 9 weeks respectively; Fisher's exact tests for pair-wise comparisons, all $P > 0.05$) and, by extrapolation, the initial success of experimental infections probably revolved around 50%. As amphipods were initially exposed to two cercariae, individuals contained either no (996), one (743) or two (296) metacercariae for a total of 1335 *C. parvum* metacercariae. Because competition among individual parasites sharing the same host is an important factor influencing life-history strategies and growth of parasites (Thomas *et al.*, 2002b; Parker *et al.*, 2003b), including *C. parvum* (Lagrue & Poulin, 2007, 2008b; Lagrue *et al.*, 2007b), metacercariae recovered during dissections were divided into two different classes under 'infection status': single infections (one *C. parvum* metacercaria per amphipod) and double infections (two *C. parvum* per amphipod).

First, effects of treatment, treatment duration and infection status (single or double infection) on parasite strategy were tested using Fisher's exact tests; the proportions of parasites (progenetic or not) were compared in a pair-wise manner. Note that no progenetic metacercariae were found after 3 weeks in neither of the two treatments (Figs 1 and 2), so no pair-wise comparison between treatments and infection status could be performed at 3 weeks treatment duration. Also, because all series of pair-wise comparisons consisted of multiple Fisher's exact tests, a sequential Bonferroni approach for multiple comparisons was used (Rice, 1989). The Bonferroni-adjusted alpha levels consequently used for the P-values generated by the Fisher's exact tests was $\alpha = 0.017$ for the effects of treatment and infection status as only three treatment durations were compared; i.e. no test could be run at 3 weeks treatment duration. For the effects of treatment duration, $\alpha = 0.0125$ was used as pair-wise comparisons were performed for all four treatment durations (3, 5, 7 and 9 weeks). Note that the standard $\alpha = 0.05$ was used for the rest of the analyses.

Second and prior to testing the effects of treatment and treatment duration on parasite size, a two-way ANOVA was applied to test for any effect of the treatment (control

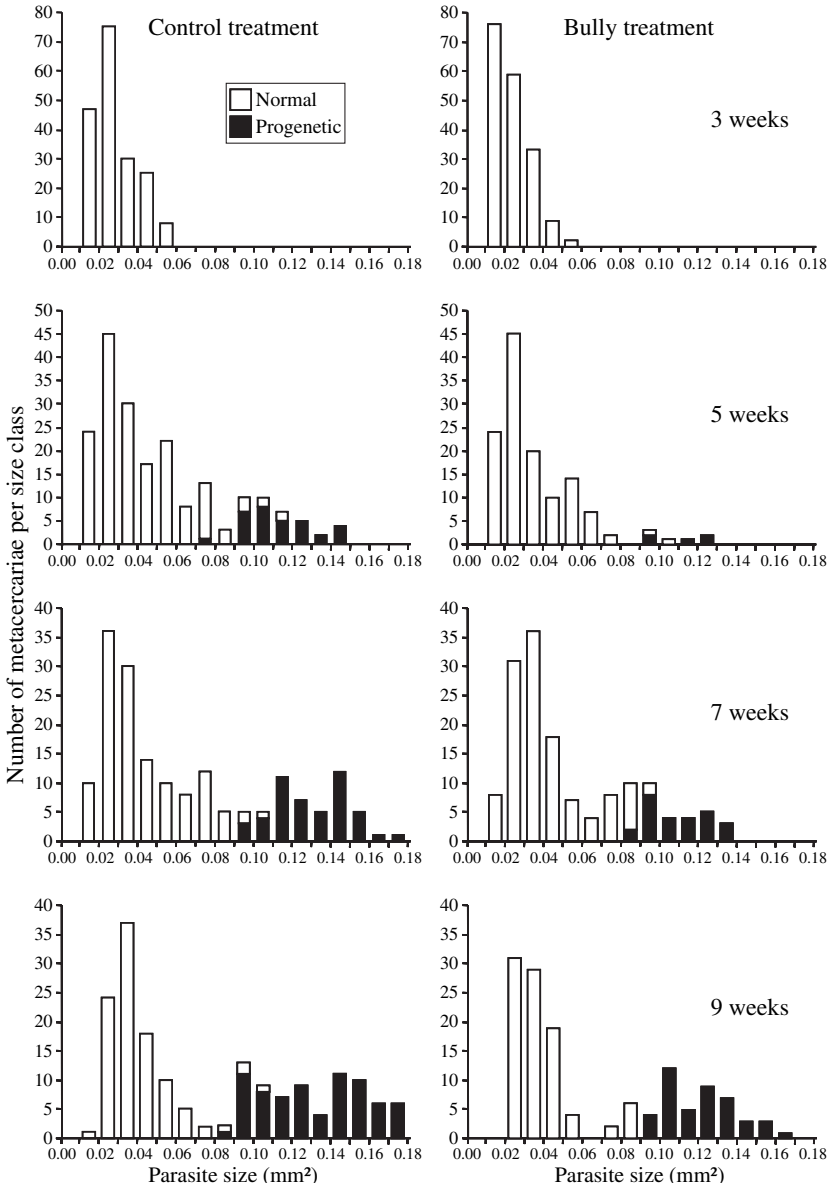

Fig. 1 Size distribution of normal and progenetic *Coitocaecum parvum* metacercariae in the control and bully treatments after 3, 5, 7 and 9 weeks of treatment.

or bully) and treatment duration (3, 5, 7 or 9 weeks) on amphipod size (body length); the appropriate linear regressions were then applied to test for the effect of amphipod size on the size of *C. parvum* metacercariae (body surface). Although only amphipods of similar size (around 3 mm body length), and therefore age, were used at the beginning of the experiment, amphipod mean size significantly increased with treatment duration ($F_{3,1024} = 401.9$, $P < 0.0001$). However, amphipod lengths did not differ between treatments ($F_{1,1024} = 0.2$, $P = 0.663$) and no interaction between the two factors was detected ($F_{3,1024} = 0.3$, $P = 0.815$). Thus, all infected amphipods were used to test for the effect of amphipod size on the size of the parasites, regardless of the treatment they were submitted to, but distinct linear regressions were run for each treatment duration, parasite strategy (normal or progenetic) and infection status (single or double) combination.

Coitocaecum parvum metacercariae showed a clear bimodal distribution in size with progenetic parasites being significantly larger than normal ones (0.125 ± 0.002 and 0.036 ± 0.001 mm^2 respectively; ANOVA, $F_{1,1327} = 1919.4$, $P < 0.0001$; Fig. 1). Furthermore, no metacercaria had produced eggs after 3 weeks of treatment (Fig. 1). Consequently, the effects of the different treatments, treatment durations and infection status on parasite body size were tested using two distinct three-way ANCOVAS, one for 'normal' and one for 'progenetic'

metacercariae, with *C. parvum* body area used as the dependent variable and amphipod length as a covariate. Parasite body area was log transformed before analyses to normalize the data. The effects of the different factors on egg production were tested using nonparametric tests with the number of eggs used as the dependent variable and either the type of treatment (control or bully), treatment duration (5, 7 or 9 weeks) or infection status (single or double) as the independent variable; only parasites that had produced at least one egg were included in these analyses. Finally, a linear regression between the size of the parasite and the number of eggs produced was used to assess the effect of parasite size on egg production.

Results

In single infections (one metacercaria per amphipod), the proportion of progenetic parasites in the control treatment was higher than in the bully treatment after 5 weeks of treatment (Fig. 2a); however, that difference decreased with time and was not statistically significant following Bonferroni adjustment after 7 or 9 weeks of treatment (Table 1a, Fig. 2a). For parasites in double infections (two metacercariae per amphipod), no difference in the proportion of progenetic individuals was found at any of the treatment durations (Table 1a, Fig. 2b). Within each treatment (control or bully), the infection status (single or double infection) had clear effects on the occurrence of progenesis (Table 1b, Fig. 2). In the control treatment, the proportion of progenetic parasites appeared higher in single than in double infections after 5 and 7 weeks of treatment (Table 1b, Fig. 2); however, due to the Bonferroni-adjusted alpha level used, the difference was statistically significant only after 7 weeks. Again, no difference in the level of progenesis was found after 9 weeks (Table 1b, Fig. 2). In the bully treatment, the proportion of progenetic parasites was significantly higher, although only marginally, in single than in double infections only in the 7-week treatment duration (Table 1b, Fig. 2). These differences between treatments and treatment durations seem due to differences in the temporal increase of the proportion of egg-producing individuals within each of these treatment combinations (Table 2, Fig. 2). In the control treatment, the proportion of progenetic metacercariae in single infections significantly increased between 3 and 5 weeks, and 5 and 7 weeks and then stabilized after 7 weeks of treatment (Table 2, Fig. 2). In double infections, the occurrence of progenesis increased

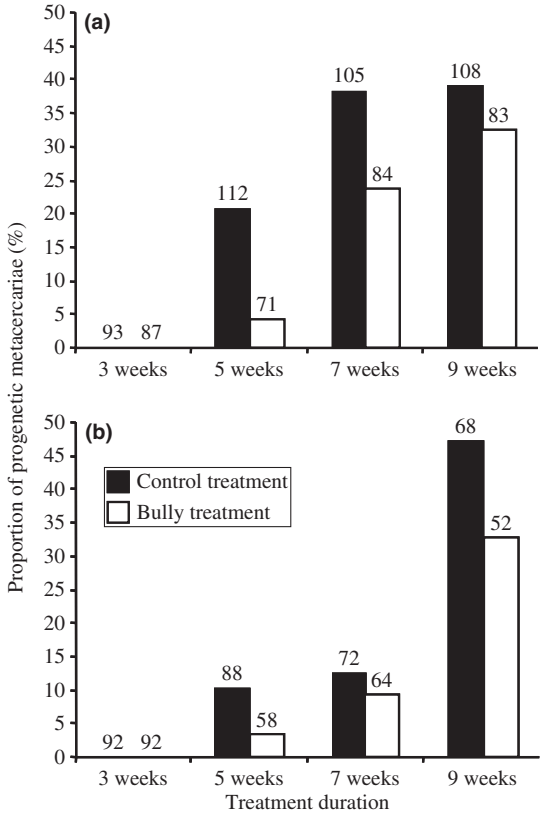

Fig. 2 Proportion of progenetic *Coitocaecum parvum* metacercariae in the control and bully treatments after 3, 5, 7 and 9 weeks of treatment in (a) single infections and (b) double infections. Numbers above bars are sample sizes.

Table 1 Results of Fisher's exact tests for pair-wise comparisons of the proportion of egg-producing (i.e. progenetic) parasites at each treatment duration (5, 7 and 9 weeks) between (a) treatments (control vs. bully) for parasites in single and double infections and (b) infection status (single vs. double) in the control and bully treatments. *P*-values significant after Bonferroni adjustment for multiple comparisons ($\alpha = 0.017$) are shown in bold in the table. No test was run at 3 week treatment duration as no progenetic parasite was found.

	χ^2	*P*-value
(a) Control vs. bully treatments		
Single infections		
5 weeks	9.48	**0.002**
7 weeks	4.40	0.042
9 weeks	0.82	0.448
Double infections		
5 weeks	2.29	0.201
7 weeks	0.34	0.596
9 weeks	2.52	0.135
(b) Single vs. double infections		
Control treatment		
5 weeks	3.90	0.036
7 weeks	13.98	**0.0001**
9 weeks	1.14	0.181
Bully treatment		
5 weeks	0.05	0.596
7 weeks	5.23	**0.017**
9 weeks	0.03	0.565

Table 2 Results of Fisher's exact tests for pair-wise comparisons of the proportion of egg-producing parasites between treatment durations (3, 5, 7 and 9 weeks) in each treatment (control and bully) and for each infection status (single and double). *P*-values significant after Bonferroni adjustment for multiple comparisons ($\alpha = 0.0125$) are shown in bold in the table.

	χ^2	*P*-value
Control treatment		
Single infections		
3 weeks vs. 5 weeks	21.51	**< 0.0001**
5 weeks vs. 7 weeks	8.11	**0.005**
7 weeks vs. 9 weeks	0.01	0.983
Double infections		
3 weeks vs. 5 weeks	9.90	**0.001**
5 weeks vs. 7 weeks	0.20	0.802
7 weeks vs. 9 weeks	20.17	**< 0.0001**
Bully treatment		
Single infections		
3 weeks vs. 5 weeks	3.75	0.089
5 weeks vs. 7 weeks	11.68	**0.001**
7 weeks vs. 9 weeks	1.57	0.232
Double infections		
3 weeks vs. 5 weeks	3.22	0.145
5 weeks vs. 7 weeks	1.74	0.277
7 weeks vs. 9 weeks	9.81	**0.002**

significantly between 3 and 5 week treatment duration, then remained constant between 5 and 7 weeks and showed a sharp increase between 7 and 9 weeks (Table 2, Fig. 2). In the bully treatment, the same trend was observed for metacercariae in double infections, although the difference was not statistically significant between 3 and 5 weeks (Table 2, Fig. 2). In single infections, contrary to the control treatment, the proportion of progenetic metacercariae from the bully treatment did not significantly increase before 5 weeks of exposure to bully odour. However, again, a significant increase in the occurrence of progenesis was detected between 5 and 7 weeks. Interestingly, contrary to the control treatment, a slight increase in the proportion of progenetic metacercariae was also found after 7 weeks of treatment, though again the difference was not statistically significant (Table 2, Fig. 2).

In single infections, the body size of normal worms was not related to amphipod length for any of the treatment duration ($r = 0.039$, 0.143, 0.066 and 0.129, $n = 180$, 157, 129 and 122, $P = 0.602$, 0.074, 0.460 and 0.158 for 3, 5, 7 and 9 weeks respectively). Similarly, in double infections, no significant relationship between host and parasite sizes was found in the three shorter treatment durations ($r = 0.062$, 0.113 and 0.062, $n = 184$, 135 and 121, $P = 0.400$, 0.193 and 0.497 for 3, 5 and 7 weeks respectively). However, after 9 weeks, the size of normal parasites found in double infections was significantly correlated with host size ($r = 0.331$, $n = 70$, $P = 0.005$). The body size of progenetic metacercariae was not significantly related to amphipod length in either single ($r = 0.111$, 0.039 and 0.211, $n = 26$, 60 and 69, $P = 0.588$, 0.766 and 0.081 for 5, 7 and 9 weeks respectively) or double infections ($r = 0.219$, 0.170 and 0.124, $n = 11$, 15 and 50, $P = 0.518$, 0.544 and 0.392 for 5, 7 and 9 weeks respectively); note that no progenetic metacercariae were found after the 3-week treatment duration. Overall, regardless of treatment duration, the body size of normal *C. parvum* metacercariae was weakly but positively linked to host size in both single ($r = 0.235$, $n = 588$, $P < 0.0001$) and double infections ($r = 0.238$, $n = 511$, $P < 0.0001$). A similarly weak trend, although not significant, was observed in progenetic individuals ($r = 0.136$ and 0.182, $n = 155$ and 76, $P = 0.091$ and 0.116 for single and double infections respectively).

Regardless of host size, the growth achieved by *C. parvum* was significantly higher in the control than in the bully treatment in both normal (0.038 ± 0.001 and 0.033 ± 0.001 mm^2 for control and bully treatment respectively; Table 3) and progenetic parasites (0.129 ± 0.002 and 0.117 ± 0.002 mm^2; Table 3). Infection status (single or double) had a significant but opposite effect on the size of normal (0.035 ± 0.001 and 0.037 ± 0.001 mm^2 in single and double infections respectively; Table 3) and progenetic parasites (0.130 ± 0.002 and 0.114 ± 0.002 mm^2; Table 3) with normal metacercariae being significantly larger in double than in single infections and vice versa for progenetic individuals. Treatment duration also influenced metacercarial size (Table 3). The size of normal parasites significantly increased between 3 and 5, and 5 and 7 weeks (0.026 ± 0.001, 0.039 ± 0.001 and 0.043 ± 0.001 mm^2 respectively; Fisher's LSD, d.f. = 1081, $P < 0.0001$ and $P = 0.0006$). Similarly,

Table 3 Results of the three-way ANCOVAS using treatment, treatment duration and infection status as independent variables, amphipod host length as a covariate and the size of the parasites as the dependent variable for normal and progenetic metacercariae.

Main effects	d.f.	*F*	*P*-value
Normal metacercariae			
Treatment	1	15.870	< 0.0001
Treatment duration	3	41.832	< 0.0001
Infection status	1	14.275	0.0002
Treatment × treatment duration	3	2.263	0.080
Treatment × infection status	1	12.138	0.0005
Treatment duration × infection status	3	2.512	0.057
Treatment × treatment duration × infection status	3	0.848	0.468
Progenetic metacercariae			
Treatment	1	7.803	0.006
Treatment duration	3	5.163	0.006
Infection status	1	6.272	0.013
Treatment × treatment duration	3	4.775	0.009
Treatment × infection status	1	2.423	0.121
Treatment duration × infection status	3	2.220	0.111
Treatment × treatment duration × infection status	3	4.657	0.010

progenetic metacercariae were significantly larger after 7 weeks of treatment than after only 5 weeks (0.114 ± 0.003 and 0.124 ± 0.002 mm^2 respectively; Fisher's LSD, d.f. = 218, $P = 0.012$). However, in both types of parasite (normal and progenetic), there was no significant size difference between individuals from the 7 and 9 week treatment durations [Fisher's LSD, d.f. = 1081 and 218, $P = 0.444$ and 0.092 for normal (0.040 ± 0.001 mm^2 at 9 weeks) and progenetic (0.129 ± 0.002 mm^2 at 9 weeks) respectively]. In normal parasites, there was a significant interaction between the treatment and infection status (Table 3). Although there was no size difference between metacercariae found in single and double infections in the control treatment (Fisher's LSD, d.f. = 1081, $P = 0.681$), in the bully treatment, normal parasites found in single infections were significantly smaller than those in double infection (Fisher's LSD, d.f. = 1081, $P < 0.0001$). In progenetic parasites, we found a significant interaction between the type of treatment and the treatment duration (Table 3). Although no size difference was found between the two treatments after 5 weeks (Fisher's LSD, d.f. = 218, $P = 0.87$), progenetic metacercariae in the control treatment were significantly larger than those in the bully treatment at 7 and 9 week treatment durations (Fisher's LSD, d.f. = 218, $P < 0.0001$ and $P = 0.005$ respectively). There was also a significant interaction between the three factors in progenetic parasites but not in normal parasites, although trends were noticeable (Table 3, Fig. 3). The mean size of progenetic parasites found in single infections increased significantly between 5 and 7, and 7 and 9 week treatment duration in the control treatment (Fisher's LSD, d.f. = 218, $P = 0.009$ and 0.002 respectively; Fig. 3). A similar but weaker trend was detected in the bully treatment (Fisher's LSD, d.f. = 218, $P = 0.501$ and 0.042 respectively); the difference was not significant between 5 and 7 weeks probably due to the small sample size at 5 weeks (Fig. 3). In double infections, no clear pattern was found for either control or bully treatment (Fig. 3). Finally there were no other significant interactions (Table 3).

The mean number of eggs produced per progenetic parasite was significantly influenced by the type of treatment (42.2 ± 3.7 and 23.6 ± 3.2 for control and bully treatment respectively; Mann–Whitney U-test, $Z = 2.612$, $n = 231$, $P = 0.009$; Fig. 4), treatment duration (12.5 ± 2.5, 31.0 ± 3.6 and 46.7 ± 4.5 for 5, 7 and 9 weeks treatment durations respectively; Kruskal–Wallis ANOVA, $H_{2,231} = 15.120$, $P < 0.0001$; Fig. 4) and infection status (45.6 ± 3.7 and 17.0 ± 2.7 for single and double infections respectively; Mann–Whitney U-test, $Z = 5.594$, $n = 231$, $P < 0.0001$; Fig. 4). Finally, as found in a previous study (Lagrue & Poulin, 2007), egg production was highly related to the size of progenetic metacercariae, increasing significantly with parasite body size ($r = 0.861$, $n = 231$, $P < 0.0001$).

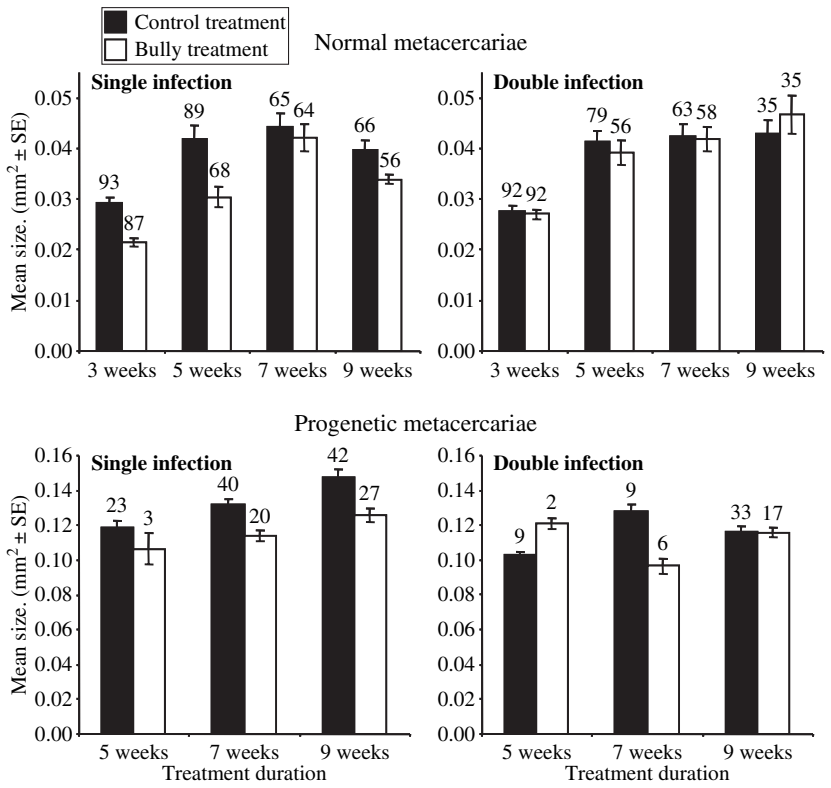

Fig. 3 Mean size (i.e. body surface area in mm^2 ± SE) of normal and progenetic *Coitocaecum parvum* metacercariae in the control and bully treatments after 3, 5, 7 and 9 weeks of treatment and in single infections and double infections. Numbers above bars are sample sizes. Note that no progenetic metacercaria was found after 3 weeks of treatment.

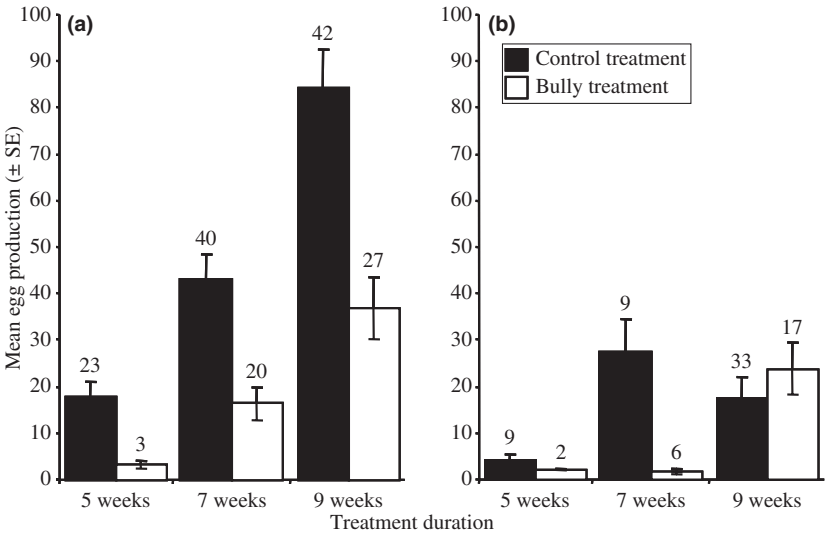

Fig. 4 Mean egg production (± SE) in the control and bully treatments after 5, 7 and 9 weeks of treatment in (a) single infections and (b) double infections. Numbers above bars are sample sizes. Note that no progenetic metacercaria was found after 3 weeks of treatment.

Discussion

Transmission success is often thought to depend mostly on local host density (Roberts *et al.*, 2002), and low parasite transmission rates between hosts is often considered as the main force acting on the evolution of abbreviated life cycles (Poulin & Cribb, 2002). For example, facultative progenesis gives parasites an alternative life-history strategy and a wider range of transmission options in situations where predation rates are highly variable. Consequently, parasites with plastic life-history strategies and the ability to evaluate their immediate transmission opportunities should adopt progenesis and the shorter life cycle in the absence of definitive hosts, and vice versa. Laboratory studies have shown that the trematode *C. parvum* is indeed more likely to adopt progenesis in the absence of chemical cues from its fish definitive host (Poulin, 2003; Lagrue & Poulin, 2007). However, in the natural environment, no seasonal correlation between the abundance of fish definitive hosts and the proportion of progenetic *C. parvum* was detected (Lagrue & Poulin, 2008a). Therefore, it is likely that other factors influence *C. parvum*'s life-history strategy.

Here, we provide evidence for an age-dependent strategy in this trematode. Even though transmission opportunities perceived by the parasite did not change over time in the treatments as fish chemical cues were renewed regularly, after 9 weeks the same proportion of metacercariae had become progenetic in the control and fish treatments. This is consistent with the developmental time hypothesis suggested by Poulin & Cribb (2002) whereby trematode metacercariae that have been in their intermediate host for a relatively long time will mature precociously and start producing eggs in response to some internal developmental clock, regardless of their transmission opportunities. Nonetheless, as in previous studies (Poulin, 2003; Lagrue & Poulin, 2007), we found that the presence or absence of chemical cues from fish definitive hosts had an immediate effect on *C. parvum* life-history strategy. Indeed, 5 and 7 weeks post-infection, *C. parvum* metacercariae were significantly less likely to adopt progenesis within amphipod hosts constantly exposed to fish chemical cues, although the difference in the proportion of progenetic individuals was less pronounced after 7 weeks of treatment. However, after 9 weeks, that difference no longer existed and the proportion of *C. parvum* metacercariae adopting the shorter life cycle was similar between individuals kept with or without fish odour.

The same trends were observed for parasites in double infections. Although interspecific competition between *C. parvum* metacercariae sharing the same amphipod host had an effect on their life-history strategy early in their development (5–7 weeks), with fewer individuals adopting progenesis, after 9 weeks there was no difference in the proportion of progenetic individuals either between treatments (control or bully) or between infection status (single or double). Again, *C. parvum* metacercariae are more likely to adopt progenesis as they are ageing regardless of interspecific competition.

Although neither treatment nor infection status seemed to have a lasting effect on parasite strategy, both of these factors influenced the size and egg production of *C. parvum* metacercariae (Figs 3 and 4). Metacercariae in single infections tend to be larger when their amphipod host is not submitted to stress from fish chemical cues, even after 9 weeks of development. When in double infection, the size difference between co-infecting metacercariae seems to be smaller; this could be explained by the intra-host competition between metacercariae sharing the same resources

(space and nutrients; Lagrue & Poulin, 2007, 2008b). Similarly, although metacercariae found in double infections were eventually as likely to adopt progenesis as those in single infections, the size and egg production achieved by progenetic individuals sharing their amphipod host with another metacercaria were clearly affected by intra-host competition (Figs 3 and 4); for example, intra-host competition significantly lowered egg production in metacercariae found in double infections (Fig. 4).

Altogether, our results confirm some previous findings on this species but also shed some new light on the rate of progenesis in *C. parvum* from natural populations. First, they confirm the observation that *C. parvum* can adjust its life-history strategy according to its immediate transmission opportunities, a result similar to those found in previous studies (Poulin, 2003; Lagrue & Poulin, 2007). This phenotypic plasticity allows the parasite to quickly respond to the presence or absence of the fish definitive host and accurately adjust its life-history strategy. Second, intra-host competition between individual parasites had an effect on metacercarial size and egg production as found earlier (Fig. 3; Lagrue & Poulin, 2007, 2008b). Furthermore, these new data seem to show long-lasting effects, as size and egg production differences were still detected after 9 weeks of treatment. However, although intraspecific competition between *C. parvum* metacercariae did delay the adoption of progenesis in co-infecting individuals, our results show that eventually, after 9 weeks, the rate of progenesis was similar in parasites found in single infections and double infections. Hence, contrary to size and fecundity, intraspecific competition did not have long-term effects on the parasite strategy. Finally, although the rate of progenesis was influenced by the perceived transmission opportunities at first, after 9 weeks, metacercariae exposed to scent from the fish definitive host were almost as likely to adopt progenesis as those reared without definitive host chemical cues. This is consistent with the 'developmental time hypothesis' mentioned previously whereby a parasite metacercaria encysted in its intermediate host for a relatively long time might use progenesis as a reproductive insurance against failed transmission (Poulin & Cribb, 2002). Thus, nearing the end of the parasite's life and/or that of its intermediate host could trigger a switch in developmental strategy and a late attempt at progenesis. The subsequent adoption of the shorter cycle would then result from ageing of the parasite and/or the host, which would mean that the window of opportunity for transmission to fish is closing rapidly. For example, the older the amphipod host, the less likely it is to be eaten before dying. In this context, it is interesting to notice that abbreviated life cycles in facultatively progenetic trematodes are often more common in ageing intermediate hosts (Grabda-Kazubska, 1976; Poulin & Cribb, 2002). The 'developmental time hypothesis' could, at least partly, explain some cases in which progenesis is facultative in trematode parasites.

In extreme cases, it is plausible that this phenomenon eventually drove the evolution of the permanent and obligate progenesis and abbreviated life cycles observed in several trematode species. However, in most cases, other forces are likely to select against progenesis and self-fertilization (Poulin & Cribb, 2002; Lefebvre & Poulin, 2005a). Although producing eggs inside the second intermediate host can act as a reproductive insurance when transmission to the definitive host fails, there may be costs associated with progenesis and self-fertilization both in terms of offspring quantity and quality. First, it is likely that progenetic metacercariae in their second intermediate hosts are not as fecund as adults could be in their definitive host (Lefebvre & Poulin, 2005a). In *C. parvum*, egg numbers in progenetic individuals average around 100 per worm, and rarely exceed 200, whereas adult worms inside the fish definitive host can produce thousands of eggs over their lifespan (MacFarlane, 1939; Poulin, 2001, 2003; Lagrue & Poulin, 2008b). Second, egg formation in progenetic parasites relies on self-fertilization, which represents the most severe case of inbreeding and produces offspring with dramatically reduced genetic heterogeneity. The deleterious effects of inbreeding are commonly assumed to explain why progenesis is not more widespread (Font, 1980; Lefebvre & Poulin, 2005a). In *C. parvum*, although inbreeding and heterozygote deficiencies have been documented in wild populations (Lagrue *et al.*, 2007c, 2009), no deleterious effect of inbreeding was found in offspring produced through progenesis and self-fertilization (Lagrue & Poulin, 2009). Generally, in species where progenesis is facultative, it is very likely that, as observed in *C. parvum*, a multitude of factors influence the adoption of progenesis by individual parasites capable of facultative progenesis and modulate the costs and benefits of such strategy (Poulin & Cribb, 2002; Lefebvre & Poulin, 2005a; Lagrue & Poulin, 2008a). It would be interesting to test the relative importance of all these different factors in some of the other trematode species using facultative progenesis as an alternative life-history strategy (Lefebvre & Poulin, 2005a).

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