

Progenesis and reduced virulence as an alternative transmission strategy in a parasitic trematode

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SUMMARY

The complexity of the life-cycle of many parasitic helminths has driven the evolution of several well-documented adaptations serving to facilitate the completion of each difficult step in the cycle. In some trematode taxa, selection has even favoured the truncation of the life-cycle: progenetic larval stages (metacercariae) are capable of producing eggs inside their usual intermediate hosts, eliminating the need to be transmitted by predation to a definitive host. In some species, progenesis is shown by all individuals, whereas in other species both the normal and truncated life-cycles coexist in the same populations. Here, the strategies chosen by normal and progenetic metacercariae of the trematode *Coitocaecum parvum* are investigated and compared. Normal metacercariae, i.e. metacercariae that only develop into adults following ingestion by a suitable fish definitive host, were not capable of manipulating the behaviour of their amphipod intermediate host in ways that could facilitate their capture by fish. These metacercariae were associated with increased mortality in amphipods. Progenetic metacercariae, on the other hand, showed lower virulence levels than normal metacercariae. At the time of their death, amphipods harbouring progenetic metacercariae contained close to the maximum number of eggs that can be produced by progenetic metacercariae, suggesting that the parasite lowers its virulence to keep its host alive long enough to maximize egg output. The maintenance of the 2 strategies in the *C. parvum* population suggests that they may have equal fitness payoffs: progenesis and low virulence guarantee the production of at least some eggs, whereas the normal life-cycle and its associated higher virulence in the intermediate host provides a low probability of much higher fecundity.

Key words: amphipod, *Coitocaecum parvum*, host manipulation, *Paracalliope fluviatilis*, truncated life-cycle.

INTRODUCTION

The life-cycle of many parasitic helminths consists of a series of unlikely events for which parasites have evolved a range of adaptations (Poulin, 1998). For example, the complex life-cycle of a typical digenean trematode involves 3 different host species and 3 improbable transmission events (Kearn, 1998). First, microscopic miracidia hatched from eggs must locate and penetrate a suitable molluscan first intermediate host. Then, the cercariae that develop within the mollusc must leave this host to seek and infect the second intermediate host in the life-cycle. Finally, encysted metacercariae within the second intermediate host must be ingested by a suitable vertebrate definitive host for the parasite to become an egg-producing adult. High adult fecundity and asexual multiplication within the molluscan first intermediate host are seen as adaptations serving to counter the odds faced by the parasite during the first 2 transmission events (Poulin, 1998). The third step in the cycle i.e., the ingestion of metacercariae by the definitive host, can be facilitated in at least 2 ways (Poulin, 1998). First, many parasitic helminths, including trematodes, are capable of altering the

phenotype of their intermediate hosts such that their conspicuousness or vulnerability to predatory definitive hosts is increased (Moore & Gotelli, 1990; Combes, 1991; Poulin, 1995, 1998). Second, some parasites adopt patience as their transmission strategy, producing long-lived larval stages in their intermediate hosts that can wait for rare acts of predation, i.e. rare transmission opportunities (Poulin, 1998).

In some systems, however, neither of the above two strategies provides an advantage to the parasite. If the second intermediate host of a trematode is short-lived and if its risk of predation by a definitive host cannot be enhanced, then producing long-lived metacercariae and the manipulation of the intermediate host's phenotype are not features that will be favoured by selection. In these cases, deleting this transmission step from the life-cycle is one possible solution. Progenesis, or the precocious development of the reproductive system in juvenile stages, is observed in some taxa of parasitic helminths (Combes, 1995; Kearn, 1998). Progenetic trematodes display a truncated life-cycle, in which the second intermediate host also serves as definitive host: cercariae infect this host and develop straight into adults. Usually, all members of a species are progenetic, and even all species within a monophyletic clade. The trematode family Schisto-

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somatidae is a good example of a taxon in which all species display a derived 2-host life-cycle (Combes, 1995). Progenesis occurs in other trematode taxa as well. For instance, the trematode *Aphalloides coelomicola* (Cryptogonimidae) also has an abbreviated life-cycle: cercariae penetrate the skin of gobiid fish, go through a metacercarial stage before maturing into adults within the body cavity of the fish (Maillard, 1973). As in other taxa, the progenetic adult trematode is not located in the host's gut, and has no easy way of releasing its eggs in the external environment. In the case of *A. coelomicola*, eggs can only reach the external aquatic habitat following the death and disintegration of the fish host. Recent evidence suggests that *A. coelomicola* has evolved a level of virulence higher than that of related trematodes, and that it causes its host to die as a means of achieving egg dispersal (Pampoulie *et al.* 1999, 2000). Thus the adoption of progenesis and a truncated life-cycle allows trematodes to skip an improbable transmission event (the predation of an intermediate host by a definitive host), but it may necessitate changes in how the parasite exploits its host.

An ideal system to address these issues would be one in which progenesis is facultative, i.e. only adopted by certain individuals in a trematode species while others go through the 'normal' 3-host life-cycle. *Coitocaecum parvum* (Opecoelidae) is such a species (Macfarlane, 1939; Holton, 1984*a*). This trematode uses the common freshwater snail *Potamopyrgus antipodarum* as its first intermediate host; cercariae produced asexually in snails then encyst in the next intermediate host, the amphipod *Paracalliope fluviatilis* (Amphipoda: Eusiridae). Normally, the life-cycle is completed when infected amphipods are eaten by suitable definitive hosts, which include several fish species: bullies *Gobiomorphus* spp. (Eleotridae), *Galaxias* spp. (Galaxiidae), and eels *Anguilla* spp. (Anguillidae). However, progenetic metacercariae are commonly found in amphipod intermediate hosts (Macfarlane, 1939; Holton, 1984*a*). Progenetic metacercariae reproduce by self-fertilization within their cyst (trematodes are hermaphroditic); following the death of their host, they excyst and the eggs (up to 200 per worm; Macfarlane, 1939) they release in the water hatch within days into functional miracidia capable of infecting the snail host (Holton, 1984*b*). Presumably, progenetic metacercariae could also release their eggs if their amphipod host were ingested by a fish, but this is not a requirement for their success as it is for 'normal' metacercariae. The interesting question here is: why are both the normal and truncated life-cycles maintained within the same population of *C. parvum*? An obvious answer would be that transmission rates to the definitive host (i.e. predation rates of fish on amphipods) must be low or highly variable to select for an abbreviation of the

life-cycle. In other trematode species using amphipods as intermediate hosts, this is sometimes solved by manipulation of amphipod behaviour to increase their susceptibility to predation by the definitive host (e.g. Helluy, 1984; McCurdy, Forbes & Boates, 2000). Various factors may limit the potential effectiveness of host manipulation in the case of *C. parvum*. Its amphipod host, *P. fluviatilis*, is a small crustacean common in coastal lakes of New Zealand. It feeds by grazing on epiphytic diatoms and fine detritus attached to macrophytic plants such as *Nitella* spp. or the introduced *Elodea canadensis*. Two obvious features of its behaviour are its mild photophobia, which keeps it in poorly-lit waters, and its positive thigmotaxis, i.e. its tendency to cling to macrophytes or other substrates with only infrequent swimming bouts (Macfarlane, 1939). These behavioural responses can serve to reduce the risk of predation on amphipods by small fish, and would be good targets for manipulation by parasites. However, the densities of *P. fluviatilis* in coastal lakes are extremely high (more than 15 individuals per litre; F. Wilhelm, personal communication), and fish predators ingest only a tiny fraction of the population. In addition, *P. fluviatilis* is short-lived (< 1 year), and thus is only available to predators for a short period of time, whether manipulated or not. In such circumstances, host manipulation should be advantageous, but other strategies, such as progenesis, may be even more advantageous.

Here, I investigate several questions relating to the use of progenesis as a transmission strategy of the trematode *C. parvum* in the amphipod *P. fluviatilis*, its second intermediate host. First, are either or both normal and progenetic metacercariae capable of manipulating amphipod behaviour? Second, is the occurrence of progenesis related to the size (or age) of the amphipod host? Third, do normal and progenetic metacercariae show different levels of virulence (defined here as parasite-induced host mortality) in amphipods? Fourth, since egg production by progenetic metacercariae stops when their amphipod host dies, is there evidence that these metacercariae accumulate eggs before causing any harm to the host? Taken together, answers to those questions will allow the first test of progenesis as an adaptive strategy for transmission in parasitic trematodes.

MATERIALS AND METHODS

Amphipods were obtained in November 2000 from Lake Waihola, south of Dunedin, South Island, New Zealand. Suitable definitive hosts in the lake include the common bully, *Gobiomorphus cotidianus*, smelt, *Retropinna retropinna*, and the inanga, *Galaxias maculatus*. Amphipods were collected from a boat by dragging a dipnet, less than 1 m below the water surface, through a bed of submerged macro-

phytes (mainly *E. canadensis*). Amphipods were returned to the laboratory, where they were maintained with shoots of *E. canadensis* in lake water, at 16 ± 1 °C and under a natural photo-period, for at least 3 days before the beginning of the experiments.

Behavioural responses

Individual amphipods ($N = 318$) were used in 2 successive but distinct behavioural tests, with the order of the tests being randomized and a 1 h interval between tests. These tests were performed in complete ignorance of the infection status of individual amphipods, which was determined subsequently. All tests were carried out at 16 ± 1 °C and using aerated lake water.

First, the response of amphipods to light was measured using a protocol similar to that used in previous studies on the effects of parasites on amphipod behaviour (Kennedy, Broughton & Hine, 1978; Bakker, Mazzi & Zala, 1997; Cézilly, Gregoire & Bertin, 2000). An individual amphipod was placed in a glass tube (10 cm long, 2.5 cm diameter) filled with water, which was then sealed with a stopper and placed horizontally. Half of the tube was covered with dark, opaque plastic, to provide the amphipod with a choice between a light zone and a dark zone of identical volumes. Illumination was provided by a 60 W fluorescent tube mounted 1 m above the experimental glass tube. After an acclimation period of 5 min, the position of the amphipod was recorded at 30 s intervals for 5 min. At the end of a trial, the behaviour of the amphipod was scored from the number of times it was observed in the light zone, ranging from 0 (highly photophobic) to 10 (highly photophilic).

Second, the tendency of an amphipod to leave a strand of macrophyte to which it clings and swim to another macrophyte was tested experimentally. An individual amphipod was placed in a circular container (10 cm diameter, 3 cm water depth) illuminated by a 60 W fluorescent tube mounted 1 m above. Two small strands (3 cm long) of fresh *E. canadensis* were attached to the wall of the container, on opposite sides. Typically, an amphipod would be clinging to one of these strands within 1 min after being introduced into the container. Following a 5 min acclimation period, the number of times the amphipod left a macrophyte strand to swim freely, over a 5 min observation period, was recorded. Thus, for each amphipod tested, a behavioural score was obtained: its minimum value was 0, for amphipods that never left their initial attachment site, and the greater its value, the more active the amphipod.

At the conclusion of the 2 behavioural tests, each amphipod was measured (total body length, from the anterior tip of the cephalon to the posterior tip of the telson) and sexed. In the case of brooding females,

the number of offspring in the marsupium was also recorded. Each amphipod was then dissected and the occurrence of parasites in its haemocoel was recorded. The juveniles of *C. parvum* in their amphipod intermediate hosts can be separated into 3 developmental stages (Macfarlane, 1939; Holton, 1984a). Recently-encysted cercariae are small, enclosed within a delicate and transparent cyst, usually 0.25–0.30 mm in maximum length; these are not infective to fish hosts. Fully developed metacercariae are longer, ranging from 0.5 to 1.0 mm in length, still enclosed within the delicate cyst. Progenetic metacercariae attain larger sizes, usually between 1.5 and 2.0 mm in length; well-formed, pinkish eggs are visible in their ovaries, and very often they have been released to fill the cyst, or even the host's haemocoel if the thin cyst has been ruptured. Numbers of each of these developmental stages present in each amphipod were recorded separately. In addition, 2 other parasites also occurred in the amphipods. Metacercarial cysts of another, unidentified species of trematode were also found in a few amphipods, along with various combinations of the 3 developmental stages of *C. parvum*; since it was impossible to distinguish which parasite may have caused a behavioural change in the host, these amphipods were excluded from further analyses. Also, some amphipods harboured acanthella stages of the acanthocephalan parasite *Acanthocephalus galaxii* in their haemocoel. This parasite uses amphipods as its only intermediate host, in which the acanthella reaches a size between 1 and 2 mm in length; it matures in a range of freshwater fish species (Hine, 1977). *A. galaxii* always occurred singly in the amphipods used in this experiment, except for 1 acanthella that co-occurred with a recently-encysted *C. parvum* cercaria. Data on amphipods harbouring *A. galaxii* were included in the analyses for comparative purposes.

Data on amphipod behaviour and infection levels did not conform to a normal distribution. Therefore, these data were analysed using non-parametric statistical tests.

Survival experiment

To determine how infection by different stages of *C. parvum* affected amphipod survival, 8 groups of 100 amphipods were randomly chosen from the stock population (see earlier) and placed in 8 separate plastic containers (30 × 12 cm, 6 cm water depth). All amphipods chosen were ≥ 2 mm in body length. The water used was aerated lake water, and all containers were placed in incubators at 16 ± 1 °C and under a natural photo-period. Food was provided by placing three 10-cm long strands of *E. canadensis* in each container, replaced every 3 days with fresh ones. Every day, each container was carefully scanned for dead amphipods. Most amphipods

readily swam after gentle probing with a plastic pipette; those showing absolutely no response to this probing were considered to be dead and were removed from the container. Because amphipods collected in November with a body length of ≥ 2 mm are unlikely to live longer than 4–6 weeks at 16 °C (F. Wilhelm, personal communication), the experiment was stopped after 18 days. At that time, all surviving amphipods were retrieved from the containers. Each amphipod that died during the experiment or that was still alive after 18 days was measured, sexed, and dissected, and the occurrence of parasites in its haemocoel was recorded as described above. In addition, the number of eggs released by progenetic metacercariae was counted (eggs still *in utero* were not counted). Amphipods from 3 of the containers were excluded from the analyses because signs of a fungal infection were observed in them. Of the remaining 500 amphipods in the experiment, those harbouring the unidentified trematode or the acanthocephalan were also excluded, and a few were not found again, so that the total number of amphipods recovered during the whole experiment was 473.

Because amphipod size is likely to influence both infection levels and survival time during the experiment, it was taken into account in the analyses. Amphipods were separated into 3 size classes, ≤ 3.0 mm, 3.1–3.4 mm, and ≥ 3.5 mm (very few amphipods attain sizes greater than 4.0 mm). A 3-dimensional contingency table test (Zar, 1984) was used to determine whether the proportions of infected and uninfected amphipods differed among size classes and more importantly between amphipods that died within 18 days and survived until the end of the experiment. This was repeated separately for the 3 main developmental stages of *C. parvum*, i.e. recently encysted cercariae, fully developed metacercariae, and progenetic metacercariae.

RESULTS

Infection parameters

Amphipods used in the behavioural response experiments also provided data on infection levels and their association with host size or sex. Of the 318 amphipods examined, 164 (51.6%) were infected with the trematode *C. parvum* and 13 (4.1%) were infected with the acanthocephalan *A. galaxii*. A total of 324 individual trematodes were found (1–6 per infected host), of which 234 were recently-encysted cercariae, 55 were fully developed metacercariae, and 35 were progenetic metacercariae.

Male amphipods ($N = 190$, prevalence = 58.4%) harboured more trematodes on average than females ($N = 128$, prevalence = 58.6%) (Mann–Whitney *U*-test: $Z = 4.298$, $P = 0.0001$). Excluding the 3 females with acanthocephalans, 40 (54.1%) of the 74 females uninfected by trematodes carried offspring, com-

pared with 27 (52.9%) of the 51 infected females; there was no difference between the number of offspring (range 1–12 per female) carried by infected and uninfected females ($Z = 0.628$, $P = 0.5297$).

The generally higher numbers of trematodes harboured by male amphipods compared with females may be explained by their slightly larger sizes (males: 3.09 ± 0.03 mm (S.E.), females: 2.48 ± 0.03 mm; *t*-test: $t = 15.541$, D.F. = 316, $P = 0.0001$). Amphipod size correlated positively with the number of trematode per amphipod, whether across all amphipods (Spearman rank correlation: $r = 0.319$, $N = 318$, $P = 0.0001$) or among infected amphipods only ($r = 0.376$, $N = 164$, $P = 0.0001$). When amphipods were separated into groups based on what type of parasites they harboured, differences in amphipod sizes emerged among the groups (ANOVA: $F_{6,311} = 5.258$, $P = 0.0001$), with uninfected amphipods and those harbouring only progenetic metacercariae being the smallest, and amphipods harbouring both normal and progenetic metacercariae being the largest (Table 1). Excluding amphipods with acanthocephalans, there was a difference in the number of normal metacercariae (either recently-encysted or fully developed) harboured by amphipods with and without progenetic metacercariae ($Z = 3.739$, $P = 0.0002$): amphipods with progenetic metacercariae were more likely to also contain normal metacercariae than amphipods without progenetic worms (Fig. 1). Thus, progenetic metacercariae were found either singly in small amphipods, or more commonly, co-occurring with normal metacercariae in large amphipods.

Behavioural responses

There were no differences between male and female amphipods in either photophilia (number of times seen in the light zone) or activity (number of times they left macrophytes to swim) (Mann–Whitney *U*-tests, both $P > 0.62$). Most amphipods were photophobic and rarely swam away from the macrophyte to which they initially clung (Table 1). Amphipods were separated into groups based on what type of parasites they harboured, and comparisons of behavioural scores were made across these groups. No significant differences were found for either photophilia (Kruskal–Wallis test: $H = 6.524$, D.F. = 6, $P = 0.367$) or activity ($H = 6.705$, D.F. = 6, $P = 0.349$). Subsequent pairwise comparisons between the behavioural scores of infected amphipods and those of uninfected amphipods confirmed that there were no significant differences in behaviour for any of the infected groups (Table 1; multiple comparison tests, Zar (1984) p. 199: all $P > 0.13$). There is thus no evidence that either normal or progenetic metacercariae are capable of manipulating the behaviour of their amphipod intermediate host.

Table 1. Host body length and behaviour in infected and uninfected amphipods

(Means (\pm s.e.) are given for body length, whereas medians and interquartile ranges are shown for the 2 behavioural measures. Normal metacercariae refer to either the newly-encysted or fully-developed metacercariae that are not producing eggs.)

Infection status	Number of Amphipods	Body length (mm)	Photophilia	Activity
Uninfected	147	2.72 \pm 0.04	2, 0-4	0, 0-6
With acanthocephalans	13	3.01 \pm 0.07	0, 0-4	3, 0-10
With newly-encysted metacercariae only	94	2.94 \pm 0.05	1, 0-4	0, 0-4
With fully-developed metacercariae only	22	2.92 \pm 0.09	3, 0-9	0, 0-1
With both newly-encysted and fully-developed metacercariae	15	2.91 \pm 0.12	0, 0-3	0, 0-3
With progenetic metacercariae only	6	2.60 \pm 0.11	3, 0-3	0, 0-7
With both normal and progenetic metacercariae	21	3.15 \pm 0.04	3, 0-7	0, 0-9

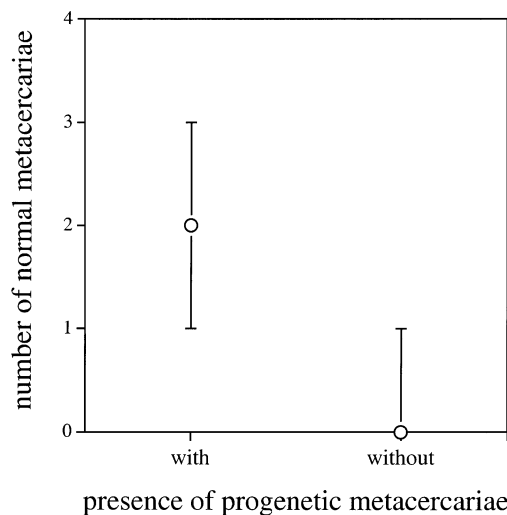


Fig. 1. Number (median and interquartile range) of 'normal' metacercariae in amphipods with ($N = 27$) and without ($N = 278$) progenetic metacercariae of the trematode *Coitocaecum parvum*.

Survival experiments

Amphipods that died during the experiment (within 18 days) were larger than those still alive at the end of the experiment (3.31 ± 0.05 mm (s.e.) versus 2.90 ± 0.02 mm; t -test: $t = 8.594$, D.F. = 471, $P = 0.0001$). This may simply reflect age- or size-related mortality. More importantly, amphipods that died during the experiment were more likely to harbour fully-developed metacercariae than amphipods that survived the experiment, independent of amphipod size (3-dimensional contingency table test: $\chi^2 = 17.42$, D.F. = 5, $P < 0.005$; Fig. 2). No such difference was found for either recently-encysted cercariae or progenetic metacercariae (same test, both $P > 0.5$; Fig. 2). Amphipods that died during the experiment harboured on average more fully-developed metacercariae than those that survived the full 18 days (Mann-Whitney U -test: $Z = 3.459$, $P = 0.0005$); this did not apply to the other two types of metacercariae (both $P > 0.25$).

Overall, the number of eggs produced by progenetic metacercariae did not correlate with the

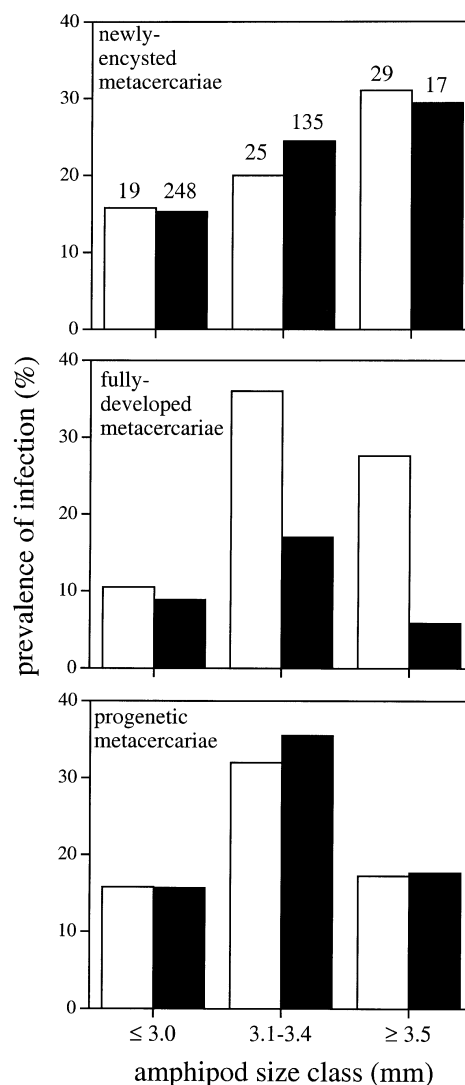


Fig. 2. Prevalence of infection by the 3 types of metacercariae of the trematode *Coitocaecum parvum* among amphipod hosts that died within 18 days (\square) and amphipods that survived the whole duration of the experiment (\blacksquare). Results are shown separately for 3 amphipod size classes; sample sizes i.e. numbers of amphipods examined (shown above the bars in the top graph), are the same for the 3 graphs. Note: some amphipods were infected by more than 1 type of metacercariae.

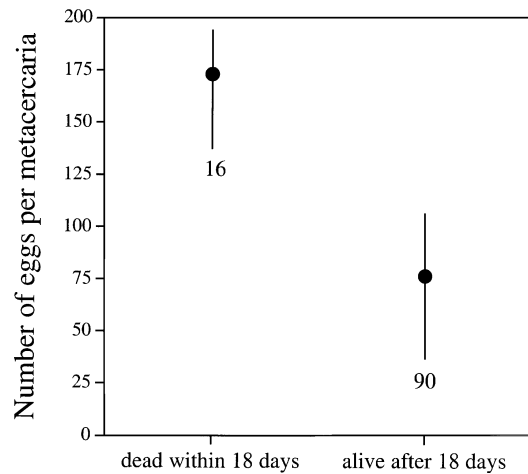


Fig. 3. Number (median and interquartile range) of eggs produced by progenetic metacercariae of *Coitocaecum parvum* in amphipod hosts that either died during or survived the 18-day survival experiment. Sample sizes are indicated below the ranges.

size of the amphipod host (Spearman rank correlation: $r_s = 0.151$, $N = 106$, $P = 0.122$). However, the number of eggs produced by progenetic metacercariae in amphipods that died early was significantly higher than the number of eggs produced by progenetic worms in amphipods that survived the full 18 days of the experiment ($Z = 5.679$, $P = 0.0001$). The difference in egg output was more than 2-fold (Fig. 3). Thus hosts of progenetic metacercariae died at a time when the egg output of the parasite approached the maximum value of 200 eggs per worm recorded by Macfarlane (1939).

DISCUSSION

Finding alternative life-history strategies among members of the same species provides ideal situations to study the determinants of life-history evolution (Stearns, 1992). Here, I investigated the features associated with the 2 life-cycle pathways of the trematode *C. parvum*: the normal 3-host cycle, and the truncated 2-host cycle. Although experimental infections of laboratory-bred hosts would ultimately be needed, the results presented here still provide strong answers to some fundamental questions about the evolution of abbreviated life-cycles. One of these questions is whether the two strategies are fixed or conditional. Amphipod hosts harbouring progenetic metacercariae were more likely to also harbour normal metacercariae than amphipods not carrying progenetic worms. This may simply be due to the fact that they co-occurred in large amphipods, which tend to harbour more metacercariae of all kinds. When found alone, progenetic metacercariae occurred in amphipods that were much smaller than all other types of amphipods. At first glance, there is therefore no

obvious sign that the onset of progenesis in this trematode is strictly linked to external factors such as host age or size, or whether or not the host is shared with conspecific parasites. There may be an explanation for these observations, however, that would be consistent with progenesis being a conditional strategy adopted under certain conditions. Small amphipods in the lake studied are prey to the crustacean *Tenagomysis* spp., whereas because of size-selective predation fish definitive hosts are more likely to feed on medium-sized and large amphipods. Thus progenesis would be preferable when the amphipod host is small, with little chance of being ingested by fish, or relatively large and approaching the end of its natural life-span. This would explain the patterns observed. The alternative strategies (3-host versus host 2-host life-cycle) may then be conditional on host size and its influence on the probability of transmission to fish, although the possibility that they are genetically predetermined cannot be excluded at this stage.

The most interesting results of this study are those that compare the traits associated with the 2 strategies. Neither normal or progenetic metacercariae are capable of altering the 2 host behaviours investigated here, photophobia and the tendency to remain attached to macrophytes; changes in either of these behaviours could increase the susceptibility of amphipod hosts to predation by fish. These behaviours were chosen because they are often altered by parasitism in other amphipod species (Helluy, 1984; Bakker *et al.* 1997; Cézilly *et al.* 2000). These results are based on amphipods infected in the field, and ideally experimentally-infected individuals should be used to assess the impact of parasites on host behaviour. Also, it is possible that *C. parvum* metacercariae affect host behaviours other than those examined here, such as their responses to a disturbance. Nevertheless, normal metacercariae of other trematode species show clear manipulation of similar behaviours in their intermediate hosts in ways that benefit the parasite (Helluy, 1984; McCurdy *et al.* 2000); the fact that none was observed here is striking. The very high densities of amphipods and the presence of at least 1 other predatory fish species unsuitable as definitive hosts (perch, *Perca fluviatilis*), however, may greatly reduce the efficiency of host manipulation in the present system. The odds are stacked against any given metacercaria making it to a suitable fish host, and alternative transmission strategies can be favoured.

The contrast in virulence between normal and progenetic metacercariae is very revealing. In other host-parasite systems, the normal, non-progenetic metacercariae of trematodes can harm and even kill their amphipod intermediate host (Thomas *et al.* 1995; Jensen, Jensen & Mouritsen, 1998; but see Meissner & Schaarschmidt, 2000). In the present

system, the normal fully-developed metacercariae of *C. parvum* were more likely to occur in amphipods that died quickly than in hosts that lasted until the end of the experiment, independent of amphipod body size. This suggests that they have a negative effect on amphipod survival. In contrast, no such effect was detected for progenetic metacercariae. This is surprising, because one would expect that progenetic metacercariae divert more energy from their amphipod host for the production of eggs, and that they may benefit from killing their host to release those eggs. However, the reduced virulence displayed by progenetic metacercariae instead allows the precocious worm to accumulate as many eggs as it can before the death of the host. Indeed, progenetic metacercariae in amphipods that died during the experiment had produced close to the maximum number of eggs observed by Macfarlane (1939), whereas those found in hosts that survived until the end of the experiment had released less than half that number of eggs. These will be the only eggs produced by the progenetic metacercariae, and premature host death would only interrupt egg production before the maximum number is reached. If progenetic metacercariae kill their host only when egg production is terminated, they may do so when hosts are close to the end of their natural life-span, and thus their actual virulence is low. In contrast to the progenetic trematode *Aphalloides coelomicola*, which has evolved higher virulence to kill its long-lived host as a means of achieving egg dispersal (Pampoulie *et al.* 1999, 2000), progenetic *C. parvum* show reduced virulence to maximize egg output and rely to some extent on the short life-span of their host for egg release. Variability in trematode virulence has led to divergent life-history strategies in other species (Davies, Webster & Woolhouse, 2001), and provides further evidence of how flexible the evolution of virulence really is (Ebert & Herre, 1996; Frank, 1996; Poulin, 1998).

Progenesis has evolved independently on several occasions in the Trematoda (Grabda-Kazubska, 1976; Carney & Brooks, 1991; Combes, 1995). In some taxa it is only facultative, in others it is the only developmental pattern observed. Extreme truncation of the trematode life-cycle, from 3 hosts to a single one, has also been documented: in such cases, the entire life-cycle takes place in the snail host (Mohandas, 1975; Barker & Cribb, 1993; Barger & Esch, 2000). There must be one or more selective pressure, acting in several different trematode populations to account for the frequent independent abbreviations of the ancestral life-cycle during the phylogenetic history of trematodes. Holton (1984*b*) remarked that progenesis appears to be relatively common among the few trematode species known from New Zealand freshwater systems. He suggested that the unpredictability, including floods and droughts, which characterizes many New Zealand

freshwater habitats could make definitive hosts periodically unavailable for the completion of the parasites' life-cycles, and that progenesis could serve as insurance. The study system in the present investigation is relatively stable over time. In this case, it may be the extremely low probability of any given amphipod being ingested by a fish that has promoted the evolution of progenesis in *C. parvum* as a key strategy capable of getting metacercariae out of an impasse. In addition to the low probability of reaching the definitive host, establishment success in this host may be quite low. Common bullies, *Gobiomorphus breviceps*, from the study site show a 100% prevalence of infection by adult *C. parvum* but only a low average intensity of about 5 worms per fish (F. Wilhelm, personal communication). Density-dependent establishment success in the intestine of the fish host may further reduce the likelihood of a metacercaria reaching adulthood. It would be interesting, if comparative data were available, to contrast intermediate host life-span and transmission rates to (and establishment success within) the definitive host, between the few trematode species using progenesis and those relying solely on the ancestral, 3-host life-cycle.

Why, then, is progenesis not more frequent among members of that species? If this alternative strategy has not spread through the population, it must be that its net fitness benefits are no greater than those resulting from the normal life-cycle. A closer examination of the pros and cons of the two strategies provides some answers. On the one hand, progenesis almost guarantees the production and release of at least a modest number of eggs (median in this study = 173 eggs per worm from hosts that died during the experiment). On the other hand, the normal developmental route appears to be a high risk, high profit option: it offers a slim chance of a longer, more prolific adult life in the gut of a fish. The likelihood of producing eggs and the average number of eggs associated with each strategy may result in similar reproductive success and fitness payoffs for both normal and progenetic metacercariae. However, offspring quality may play a role as well as offspring quantity. Because they are enclosed alone within a cyst, progenetic metacercariae can only self-fertilize; the genetic heterogeneity of their offspring will be reduced, possibly leaving them at a disadvantage (Font, 1980). Under these conditions, by-passing the definitive host and the opportunity for cross-fertilization may remain a minority strategy. Studies of the viability of offspring of both normal adult and progenetic worms would shed light on this issue.

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