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Effects of genetic similarity on the life-history strategy of co-infecting trematodes: are parasites capable of intrahost kin recognition?

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Paracorophium excavatum.

Abstract

For conspecific parasites sharing the same host, kin recognition can be advantageous when the fitness of one individual depends on what another does; yet, evidence of kin recognition among parasites remains limited. Some trematodes, like *Coitocaecum parvum*, have plastic life cycles including two alternative life-history strategies. The parasite can wait for its intermediate host to be eaten by a fish definitive host, thus completing the classical three-host life cycle, or mature precociously and produce eggs while still inside its intermediate host as a facultative shortcut. Two different amphipod species are used as intermediate hosts by *C. parvum*, one small and highly mobile and the other larger, sedentary, and burrow dwelling. Amphipods often harbour two or more *C. parvum* individuals, all capable of using one or the other developmental strategy, thus creating potential conflicts or cooperation opportunities over transmission routes. This model was used to test the kin recognition hypothesis according to which cooperation between two conspecific individuals relies on the individuals' ability to evaluate their degree of genetic similarity. First, data showed that levels of intrahost genetic similarity between co-infecting *C. parvum* individuals differed between host species. Second, genetic similarity between parasites sharing the same host was strongly linked to their likelihood of adopting identical developmental strategies. Two nonexclusive hypotheses that could explain this pattern are discussed: kin recognition and cooperation between genetically similar parasites and/or matching genotypes involving parasite genotype–host compatibility filters.

Introduction

Cooperative or altruistic interactions between conspecifics are costly to implement but costs can be offset if cooperation increases individual and/or inclusive fitness of all interacting partners (Foster *et al.*, 2006 and references therein). If performing an altruistic act has a direct reproductive cost, the additional reproduction achieved by the recipient of the altruistic act can offset the cost to the donor of the act if the two individuals share many genes (Hamilton, 1964; West *et al.*, 2007). Therefore, and according to kin selection theory, for actors of altruistic behaviours, potential fitness benefits depend on genetic relatedness among the actors (Foster *et al.*, 2006 and references therein). Cooperation levels, that is, the frequency of altruistic acts between two individuals, should increase with the genetic relatedness between them, suggesting that selection should favour kin recognition abilities alongside cooperative behaviours.

Kin recognition is the ability for individuals to evaluate their genetic relatedness with conspecifics and is a key factor in numerous biological processes ranging from mating strategies (e.g. inbreeding avoidance, Gerlach & Lysiak, 2006) to altruistic behaviours like

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communal breeding in birds where nonbreeding individuals help raise genetically related offspring (Brown, 1987). Kin recognition has also been documented in simpler organisms. For example, some protists use polymorphic plasma membrane proteins for kin recognition (Benabentos *et al.*, 2009). Among parasites, *Plasmodium* spp. can use kin recognition to evaluate intrahost genetic diversity before adopting a reproductive strategy (Reece *et al.*, 2008). Parasitoid wasps (*Goniozus legneri*) use kin recognition to minimize competition for laying sites/hosts with close relatives (Lizé *et al.*, 2012). Also, cestodes can recognize and preferentially mate with kin when given a choice between kin and nonkin partners (Schjørring & Jäger, 2007).

Conspecific helminth parasites, when co-infecting a common host, may thus be capable of kin recognition. Trematodes have asexual multiplication phases producing numerous clonal larvae, ranging from hundreds to several thousands, depending on the species. If genetically close, or even identical, individuals end up sharing the same host, then it should be advantageous for these parasites to be capable of kin recognition and cooperative interactions. *Coitocaecum parvum* is a trematode parasite infecting fish as definitive hosts after the parasite's juvenile stages inside an amphipod intermediate host are ingested by the fish. Adult *C. parvum* colonize the digestive tract of the fish host where they sexually reproduce. Eggs are released with host faeces and hatch into free-swimming larvae. However, *C. parvum* may facultatively adopt progenesis (i.e. early maturation into a hermaphroditic adult and reproduction) while still inside the intermediate amphipod host and omit the fish definitive host in response to different environmental factors (Holton, 1984a,b; Lagrue & Poulin, 2007, 2008a). For instance, under experimental conditions, if amphipod hosts are exposed to chemical cues from fish, the parasites they harbour are much less likely to adopt progenesis, compared with those in amphipods not exposed to fish cues (Lagrue & Poulin, 2007). The perceived absence of fish definitive hosts thus promotes the adoption of progenesis within the intermediate host. In such a case, eggs are released in the environment upon the intermediate host's death and hatch when in contact with the water. The free-swimming larvae hatched from eggs must then find and enter the first intermediate host, the snail *Potamopyrgus antipodarum*, where they asexually multiply to eventually produce genetically identical parasite larvae (cercariae). Cercariae emerge from snails at low rates, typically 1–20 cercariae per infected snail per day (C. Lagrue, pers. obs.), although these rates can be maintained for over a year until snail death. The cercariae of *C. parvum* have no tail and move by crawling; after leaving the snail, they must infect an amphipod second intermediate host where they encyst as metacercariae. The parasite then adopts one of the two alternative life strategies mentioned above; either wait for a fish definitive host to consume the infected amphipod or undergo progenesis while still inside the amphipod intermediate host and produce viable eggs by selfing (Poulin & Cribb, 2002; Lefebvre & Poulin, 2005). Selfing is the only option for progenetic worms encased individually within cysts, whereas outcrossing appears widespread among adult worms in the digestive tract of fish hosts, as mating pairs are frequently observed (C. Lagrue, pers. observation). *Coitocaecum parvum* can use two different amphipod species as second intermediate hosts. *Paracalliope fluviatilis* and *Paracorophium excavatum* occur in sympatry but are ecologically different; *P. fluviatilis* is free swimming and mobile, whereas *P. excavatum* is purely sedentary, burrowing in the sediment. These interspecific differences may affect the likelihood of amphipods to harbour genetically similar parasites. The sedentary *P. excavatum* may be more likely to acquire closely related *C. parvum* individuals given its own and the parasite's limited dispersal abilities.

Available evidence indicates that progenesis is neither genetically determined nor heritable. Its frequency does not differ among clones under standardized experimental conditions (Lagrue *et al.*, 2009), and it also does not differ between offspring of progenetic parents and offspring of 'normal' adult worms living in fish definitive hosts (Lagrue & Poulin, 2009). Adoption of progenesis by metacercariae is influenced by numerous external factors in addition to definitive host presence in the environment. For example, because progenetic metacercariae grow much larger than nonprogenetic ones and thus require more resources, intrahost competition with other parasite species and amphipod host size also influence whether or not progenesis is adopted (Lagrue & Poulin, 2008a; Ruiz Daniels *et al.*, 2012). The developmental plasticity offered by progenesis could also allow parasites to adjust life strategies according to specific contexts, including genetic relatedness between individual parasites sharing the same host. Up to 40% of infected amphipod hosts harbour two metacercariae (most amphipods harbour a single metacercaria, very few harbour more than three, with a maximum observed of six; Lagrue & Poulin, 2008a) meaning that over 60% of *C. parvum* metacercariae share their intermediate host with a conspecific (Lagrue & Poulin, 2008b). Co-infecting *C. parvum* individuals can either adopt the same (normal three-host life cycle or progenesis) or two different developmental strategies.

There is thus an obvious situation of conflict when two *C. parvum* metacercariae have to share the same amphipod host but adopt different life-history strategies (Lagrue *et al.*, 2009). Parasites adopting the classical three-host cycle need their amphipod host to be eaten by a fish as soon as possible; in contrast, individuals adopting progenesis benefit from keeping the amphipod host alive as long as possible to maximize their egg output, meanwhile using most, if not all, of the space and resources available in the host to grow and produce

eggs. Therefore, in double infections, there is a conflict of interests between the two life-history strategies that is potentially very costly for one of them (Dezfuli *et al.*, 2001). Requirements from the intermediate host are very different: long life for egg production for progenetic individuals vs. ingestion by a definitive host for metacercariae adopting the three-host cycle. Alternatively, co-infecting *C. parvum* individuals could adopt the same strategy to reduce potential conflicts. If kin recognition is possible between *C. parvum* individuals, genetic similarity between two parasites sharing a host may influence their likelihood of adopting the same developmental strategies (Gardner *et al.*, 2011; Marshall, 2011), thus reducing competition between closely related parasites sharing the same host/resources (Lizé *et al.*, 2012).

In this context, our study aimed at testing (i) whether genetic similarity among co-infecting *C. parvum* metacercariae varied between amphipod host species (i.e. second intermediate hosts) and (ii) whether co-infecting *C. parvum* metacercariae adopting the same life-history strategy in the same individual host were more genetically similar to each other than pairs of metacercariae drawn at random from the entire population, which would be consistent with kin recognition.

Materials and methods

Field sampling and dissection

Paracalliope fluviatilis and *Paracorophium excavatum* amphipods were sampled in 2008 and 2012, respectively, in Lake Waihola, South Island, New Zealand. They were collected by dragging a hand-held net along the bottom of the lake, along a short stretch (< 20 m) of the lakeshore. Amphipods were dissected under a dissecting microscope to assess infection status, and only amphipods harbouring two *C. parvum* metacercariae were used in analyses. *Coitocaecum parvum* metacercariae were identified as 'no egg' (i.e. non-egg producing individuals) or 'eggs' (i.e. progenetic, egg producing individuals); the two types of metacercariae also differ markedly with respect to size and internal organ development (Holton, 1984a,b; Lagrue & Poulin, 2007). They were then preserved individually in 1.5 mL Eppendorf tubes filled with 100% ethanol before genetic identification.

DNA extraction, PCR and genotyping

DNA was extracted from each individual metacercaria by digestion in 100 mM Tris–HCI, pH 8.0/10 mM EDTA/100 mM NaCI/0.1% SDS/50 mM dithiothreitol/proteinase K (0.5 g mL^{-1}) for 4 h at 37 °C. DNA was then purified with phenol/chloroform-isoamyl [1/2 : 1/2 (vol/vol)] and chloroform-isoamyl. DNA was concentrated through precipitation by adding 1/10 volume of potassium acetate and 1 volume of isopropanol and resting at −20 °C overnight. DNA was finally rinsed in 70% ethanol, dissolved in 50 μL of sterile water and stored at −20 °C.

Nine microsatellite markers available in GenBank were pooled in two multiplexes (Table 1) and used to genotype *C. parvum* metacercariae (Lagrue *et al.*, 2007, 2009). Forward PCR primers were 5′ fluorescein labelled, and PCRs were carried out in 10 μL reaction volumes using the QIAGEN Multiplex PCR kit (Qiagen Inc, Düsseldorf, Germany) with 5 μL of QIAGEN Multiplex PCR master mix, 1 μL of primer mix (2 μM of each primer from multiplex 1 or 2), 1 μL of extracted DNA (diluted by 1/20) and RNase-free water. Amplification was conducted according to the PCR programme for amplification of microsatellite loci defined for QIAGEN Multiplex PCR kit: 15 min at 95 °C (initial activation step), followed by 30 cycles consisting of 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 60 s. The last extension step was at 60 °C for 30 min.

A small volume (0.7 μL) of each PCR product was mixed with 0.25 μL of red-dye-labelled GeneScan size standard ROX 500 (Applied Biosystems Inc, Foster City, CA, USA) and 9.25 μL of deionized formamide. After denaturation, the mixture was electrophoresed using an automatic sequencer (Applied Bio System 3130 Avant). Allele sizes were then determined using the GENEMAPPER v3.7 software (Applied Biosystems). The statistical software FSTAT (FSTAT® version 2.9.3; Goudet, 1995) was then used to calculate number of alleles (An) and test for genetic linkage disequilibrium between microsatellite markers.

Genetic dissimilarity coefficient

The genetic dissimilarity coefficient A of Rousset was used to determine genetic dissimilarity between *C. parvum* metacercariae (Rousset, 1996). This coefficient does not require Hardy–Weinberg equilibrium and can be used in clonal populations. High coefficient values indicate high genetic dissimilarities between individuals and vice versa. Genetic dissimilarity coefficients were calculated using the SPAGEDI software (Spatial Pattern Analysis of Genetic Diversity® version 1.2, Université Libre de Bruxelles, Brussels, Belgium).

According to developmental strategies of metacercariae sharing the same host, pairs of co-infecting *C. parvum* individuals were classified as 'no egg' (i.e. the two parasites sharing a host are normal, non-egg producing individuals), 'eggs' (i.e. the two parasites sharing a host are progenetic, egg producing individuals) or 'mixed' pairs (i.e. one normal, non-egg producing and one progenetic, egg producing individual). Genetic dissimilarity coefficients between co-infecting *C. parvum* individuals were used to calculate mean intrahost genetic dissimilarity (IHD) coefficients between co-infecting individuals in each amphipod host species. Mean genetic dissimilarity coefficients between individuals sharing a

Table 1 Microsatellite markers developed for *Coitocaecum parvum* (see Lagrue *et al.*, 2007, 2009) and used here. bp: number of base pairs, Accession no: GenBank Accession Number, A_n: overall number of alleles found in *C. parvum* at a given locus in each amphipod host.

						A_n	
Locus	Repeat motif	Primer sequences (5′–3′)	Range (bp)	Accession no	Multiplex no	*P. fluviatilis*	*P. excavatum*
Cpa-3	(AG)8	F: GTTTAGTCAGGCACTGTAGC R: GTAAGATACCTTGGACCGATG	68–78	DQ789050	1	5	10
Cpa-4	(GT)9(AT)4	F: CAAGACAAACTGAGGACGC R: ATAAGAGCATTGGGAGGGG	93–102	DQ789051	2	3	8
Cpa-8	(CT)7(CT)20(GT)8	F: CATCGTGCTTGAGATATACTACG R: GTGAGTCGGGCTGGTGAAG	113–144	DQ789052	1	17	22
Cpa-12	(GA)13	F: CATTTTCCAATTCTAACGAGTG R: CCCTATTCCTTTTGACCTCTC	131–170	DQ789053	1	13	15
Cpa-19	(CT)20	F: GAAACCAGATTGCGTATCC R: GTATCAAGTTTACCGTTACAGAAC	75–116	DQ789055	1	14	23
Cpa-26	(AC)7	F: GATTACGCAACTCATTCCAG R: CATCAACGTTTATGTTCC	110–123	EF088680	2	7	12
Cpa-28	(GT)10	F: CCATTTGACATTGAATTGCG R: CATCGTATGAGGGTGAAATACC	57– 75	EF088681	2	3	8
Cpa-29	(AC)10	F: GCTTGGAATGAGTGATAACAC R: GTTCCCTATGGTAAGTCAGC	56–72	EF088682	1	2	10
Cpa-48	(GT)16	F: GAAATGAAATATGGGTATCGTTGTG R: CGTTCGCCATCGACATACAC	65–87	EU203673	2	13	16

host and all other parasites in the population were also calculated to obtain total population dissimilarity (TPD) coefficients in each amphipod host species.

Statistical analyses

First, we tested whether *C. parvum* metacercariae sharing the same amphipod host were genetically more similar to each other than they were with individuals in the rest of the parasite population. If so, IHD should be lower than TPD. Intrahost dissimilarity and total population dissimilarity were thus compared within each host amphipod species separately. The data set did not follow a normal distribution; thus, the nonparametric paired Wilcoxon test was performed.

Secondly, genetic dissimilarity coefficients between *C. parvum* metacercariae sharing the same host (i.e. intra host dissimilarity; IHD) and overall allele numbers (A_n) were compared between the two amphipod host species. The IHD/A_n ratio was used to weight the genetic dissimilarity between parasites sharing the same host by the number of alleles found in each host species. This ratio was calculated for the two amphipod host species and compared with the nonparametric unpaired Mann–Whitney *U*-test.

All statistical analyses were performed using GRAPH PAD in Stat (Version 3.10 Copyright® 1992–2009 by GraphPad Software, Inc., La Jolla, CA, USA).

Results

A total of 202 *P. fluviatilis* and 51 *P. excavatum* infected with two *C. parvum* individuals were found. In *P. fluviatilis*, 49 amphipod hosts contained two progenetic metacercariae ('eggs' pairs), 68 contained two normal metacercariae ('no egg' pairs) and 85 contained one progenetic individual and one normal individual ('mixed' pairs). In *P. excavatum*, 13 amphipods contained an 'egg's' *C. parvum* pair, 23 contained a 'no egg' pair and 15 contained a 'mixed' pair.

In 'mixed' pairs (one normal and one progenetic co-infecting individuals), there was no clear difference between intrahost genetic dissimilarity (IHD) and total population dissimilarity (TPD) in the case of the amphipod *P. fluviatilis* (paired Wilcoxon test, $n = 85$, $P = 0.0651$; Fig. 1), and no difference at all in the amphipod *P. excavatum* (paired Wilcoxon test, $n = 15$, $P = 0.30$; Fig. 2). Co-infecting *C. parvum* individuals were as dissimilar from each other as they were from the rest of the population. In 'no egg' pairs, IHD was significantly lower than TPD in both *P. fluviatilis* (paired Wilcoxon test, $n = 68$, $P < 0.0001$; Fig. 1) and *P. excavatum* (paired Wilcoxon test, $n = 23$, $P = 0.0010$; Fig. 2). Similarly, *C. parvum* individuals in 'eggs' pairs were significantly less dissimilar from each other than from metacercariae in the rest of the population, in *P. fluviatilis* (paired Wilcoxon test, $n = 49$, $P = 0.0008$; Fig. 1) and *P. excavatum* (paired Wilcoxon test, $n = 13$, $P = 0.0046$; Fig. 2).

The intrahost genetic dissimilarity (IHD) coefficient between co-infecting *C. parvum* was significantly higher in *P. fluviatilis* than *P. excavatum* regardless of parasite life-history strategies (unpaired Mann–Whitney *U*-test, $P < 0.0001$). The numbers of alleles detected among parasites were significantly different between host species (Table 1); an average of 8.56 (± 1.90) alleles per locus were found in *C. parvum*

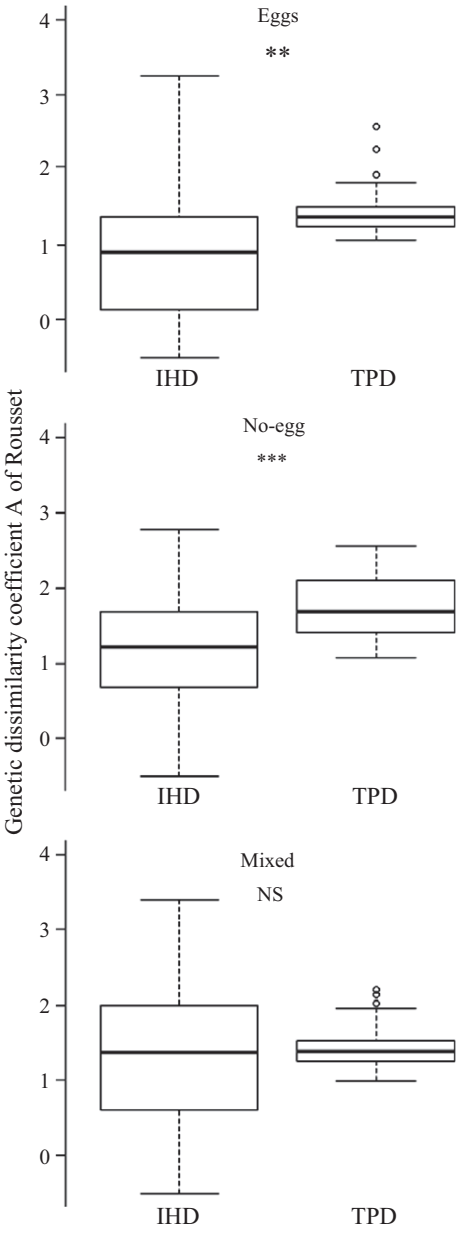

Fig. 1 Intrahost (IHD) and total population (TPD) genetic dissimilarity (boxplots represent the median, minimum, maximum, Q1 (25th percentile), Q3 (75th percentile) and outliers) in co-infecting *Coitocaecum parvum* metacercariae found in *Paracalliope fluviatilis* amphipods for each pair category ('eggs', 'no egg' and 'mixed' pairs). $^{**}P < 0.005$, $^{***}P < 0.0001$, NS: $P > 0.05$.

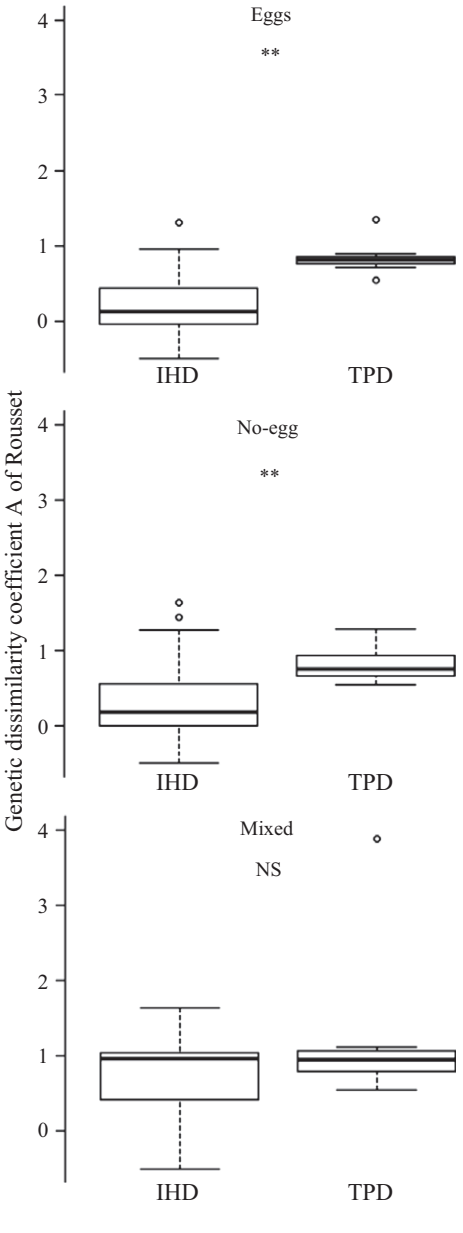

Fig. 2 Intrahost (IHD) and total population (TPD) genetic dissimilarity (boxplots represent the median, minimum, maximum, Q1 (25th percentile), Q3 (75th percentile) and outliers) in co-infecting *Coitocaecum parvum* metacercariae found in *Paracorophium excavatum* amphipods for each pair category ('eggs', 'no egg' and 'mixed' pairs). $^{**}P < 0.005$, NS: $P > 0.05$.

metacercariae infecting *P. fluviatilis* compared with 13.78 (± 1.89) in *P. excavatum* (Student's *t*-test, $P < 0.0001$). Finally, the IHD/A_n ratio was significantly higher in *P. fluviatilis* than *P. excavatum* regardless of parasite life-history strategies (unpaired Mann–Whitney *U*-test, $P = 0.0049$; Fig. 3).

Discussion

Our data showed that, regardless of host species, *C. parvum* metacercariae sharing the same amphipod host and adopting the same strategy were genetically more similar (i.e. less dissimilar) to each other than they

were to the rest of the parasite population. In contrast, two parasites sharing the same host that adopted different developmental strategies (i.e. one was progenetic and had produced eggs; the other had not) were as genetically dissimilar from each other as they were from the rest of the parasite population. Admittedly, this last pattern was only clear-cut for one of the two amphipod species; nevertheless, it still differs from the situation in which the two parasites within a host have adopted the same strategy. Three hypotheses can explain this pattern: (i) a genetic determinism of *C. parvum* life-history strategy, (ii) kin recognition between co-infecting parasites and subsequent adjustment of life-history strategy, and/or (iii) host–parasite matching genotypes involving host immunity.

(i) If *C. parvum* metacercariae sharing the same host have a similar genotype, adoption of the same developmental strategy may be due to a genetic determinism of progenesis. However, as stated in the introduction, progenesis in *C. parvum* is not genetically predetermined: its frequency does not differ among clones in standardized experimental infections (Lagrue *et al.*, 2009). Progenesis in this species is a facultative and nonheritable strategy (Lagrue & Poulin, 2009), adopted by the parasite mainly as a response to environmental variables. For example, definitive host density can influence *C. parvum* developmental strategy, with a low density of definitive hosts promoting the adoption of progenesis (Lagrue & Poulin, 2007; Lagrue *et al.*, 2009). Competition between parasites for host resources also limits the number of individuals that can achieve progenesis in a given host (Ruiz Daniels *et al.*, 2012).

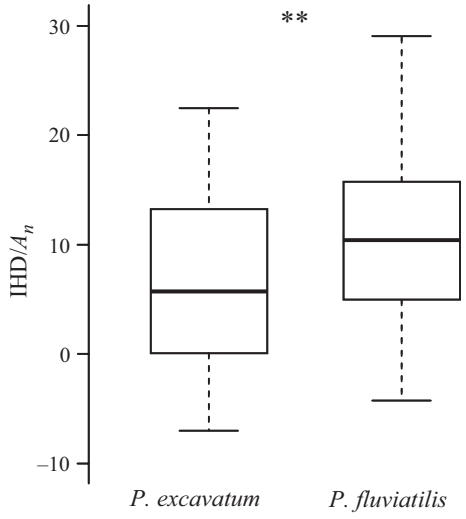

Fig. 3 Intrahost genetic dissimilarity (IHD) weighted by the overall number of alleles (A_n) between co-infecting parasites (boxplots represent the median, minimum, maximum, Q1 (25th percentile), Q3 (75th percentile) and outliers) in *Paracalliope fluviatilis* and *Paracorophium excavatum* (**P = 0.0049).

(ii) Data showed that co-infecting parasites that are genetically more similar tend to adopt the same strategy, indicating that they may be capable of kin recognition, that is, evaluating genetic relatedness with their co-infecting conspecific and responding accordingly. When parasites sharing the same host are genetically close, adopting a cooperative strategy may have a selective advantage (Lizé *et al.*, 2012). Either they increase their chance of passing their common genetic heritage through cross-fertilization in the final host by adopting the normal, 'no egg' strategy, or both parasites adopt progenesis, thus maximizing the number of offspring with common genetic inheritance when their shared amphipod host dies. When co-infecting *C. parvum* individuals are genetically dissimilar, each one should try to maximize its own reproductive output with no regard for its co-infecting conspecific. Competition over host resources and transmission pathways should thus reduce the likelihood of both parasites adopting the same strategy (López-Villavicencio *et al.*, 2011).

(iii) When infecting the same host, co-infecting parasites face similar challenges and compatibility filters from the host (Kuris *et al.*, 2007), potentially inducing a phenomenon of matching genotypes (immune compatibility between parasite and host genotypes). This is equivalent to a system of 'key' and 'lock' where a parasite can infect a host only if it has the right 'key' (i.e. genotype; Théron & Coustau, 2005). Two parasites found sharing the same host may therefore be genetically closer to each other than they are to other individuals of the parasite population because they needed to carry a similar genetic 'key' to infect that particular host in the first place. In our study, parasites sharing the same host were indeed closer genetically. However, this mechanism of matching genotypes cannot be separated from that of kin recognition using our data; both involve a higher genetic similarity between parasites sharing the same host.

Additional mechanisms could affect whether metacercariae sharing the same amphipod host and adopting the same strategy are genetically more similar to each other than to the rest of the population. For instance, the two parasites inside an amphipod may have infected the host at different times. If the two infections are almost simultaneous, the likelihood that the two cercariae are clones is much higher than if the infections are separated by several days, due to the mobility of the source (snail) and target (amphipod) hosts. In the case of simultaneous infections, external conditions such as the presence of fish cues would be the same for both parasites, promoting the adoption of the same strategy independently of genetic similarity. If the infections are separated by a long time interval, the parasites are less likely to be genetically similar and would possibly develop under different external conditions. There is, however, no way of distinguishing between timing effects and genetic

effects using field samples. Additionally, selective removal of amphipods by fish predation based on what types of metacercariae they carry could potentially bias our results. Many trophically transmitted helminths manipulate the phenotype of their intermediate host to enhance their transmission success to the definitive host (Poulin, 2010). However, laboratory tests have shown that there are no behavioural differences between uninfected amphipods and those harbouring either progenetic or nonprogenetic metacercariae (Poulin, 2001).

Our results also showed a difference in *C. parvum* intrahost dissimilarity (IHD) between the two amphipod host species. IHD was higher in *Paracalliope fluviatilis* than in *Paracorophium excavatum*. *Coitocaecum parvum* individuals found sharing the same *P. fluviatilis* host were thus less similar to each other than *C. parvum* sharing the same *P. excavatum* host. Three hypotheses could explain this pattern: (i) a bias in the data set, (ii) the low mobility of *C. parvum* cercariae combined with the different lifestyles of the two amphipod hosts and/or (iii) a parasite–host species-specific matching genotype effect.

(i) Differences in *C. parvum* IHD coefficients between the two host species could be related to a bias in the data set between host-specific parasite subpopulations. When the number of alleles identified in two subpopulations is different, the subpopulation presenting the highest number of alleles is likely to also have the highest IHD. Indeed, more alleles in a population induce lower probabilities for two parasites of sharing the same alleles and thus produce a higher IHD. If so, IHD should be higher in the *C. parvum* subpopulation infecting *P. excavatum* (highest number of alleles) than in parasites infecting *P. fluviatilis*. However, the opposite was observed, suggesting strongly that the difference observed in *C. parvum* IHD between the two hosts is not the result of a bias in the data set.

(ii) *Coitocaecum parvum* free-living larvae (i.e. cercariae) are short lived and do not swim (Lagrue & Poulin, 2008a). They have thus low dispersal abilities and must infect an amphipod host in close proximity to their snail host. In parallel, the two amphipod host species have contrasting mobility and dispersal abilities. *Paracalliope fluviatilis* is a demersal, free-swimming species whereas *P. excavatum* is purely benthic, sedentary and burrow dwelling (Luque *et al.*, 2010). These specific differences combined with the low dispersal ability of *C. parvum* larvae may expose *P. excavatum* hosts to parasite larvae derived from a limited, spatially close subset of snail hosts in which asexual multiplication generates thousands of clonal cercariae. Furthermore, snails within a small area of the lakeshore are more likely to have been infected by eggs derived from the same adult worms, that is, by sibling parasites. *Paracalliope fluviatilis* moves greater distances and may get infected by genetically more diverse, spatially distant *C. parvum* larvae. Parasites infecting *P. excavatum* could thus be both genetically less diverse and of more limited spatial origins than *C. parvum* individuals co-infecting *P. fluviatilis*, potentially explaining differences in IHD observed between the two host species.

(iii) Similar to the matching genotypes hypothesis described previously, parasite genotype–host species-specific compatibility filters could influence *C. parvum* allele diversity in different host species (Théron & Coustau, 2005). While both amphipod hosts may be exposed to all parasite genotypes present in the *C. parvum* population, host species-specific compatibility filters could allow different subsamples of these genotypes to infect each distinct host species. This would induce different *C. parvum* genotypes to match only one of the two host species and/or one of the host species to allow infection by a narrow subset of alleles/genotypes (Théron & Coustau, 2005).

Conclusion

Overall, our results showed that intrahost levels of genetic similarity between co-infecting parasites were influenced by the identity of the host species used. Furthermore, and regardless of host species, genetic similarity between two parasites sharing the same host influenced individual parasite life-history strategy. More genetically similar parasites tended to adopt the same developmental strategy. Two nonexclusive and indistinguishable explanations for this pattern are possible: kin recognition between co-infecting individual parasites and host–parasite matching genotypes. Kin recognition implies that parasites are capable of assessing their genetic similarity to co-infecting conspecifics whereas matching genotypes imply compatibility filters between parasites and hosts.

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