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Co-variation between the intensity of behavioural manipulation and parasite development time in an acanthocephalan–amphipod system

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parasite development time;
phototaxis;
trade-offs.

Abstract

Pomphorhynchus laevis, a fish acanthocephalan parasite, manipulates the behaviour of its gammarid intermediate host to increase its trophic transmission to the definitive host. However, the intensity of behavioural manipulation is variable between individual gammarids and between parasite populations. To elucidate causes of this variability, we compared the level of phototaxis alteration induced by different parasite sibships from one population, using experimental infections of *Gammarus pulex* by *P. laevis*. We used a naive gammarid population, and we carried out our experiments in two steps, during spring and winter. Moreover, we also investigated co-variation between phototaxis (at different stages of infection, 'young' and 'old cystacanth stage') and two other fitness-related traits, infectivity and development time. Three main parameters could explain the parasite intra-population variation in behavioural manipulation. The genetic variation, suggested by the differences between parasite families, was lower than the variation owing to an (unidentified) environmental factor. Moreover, a correlation was found between development rate and the intensity of behavioural change, the fastest growing parasites being unable to induce rapid phototaxis reversal. This suggests that parasites cannot optimize at the same time these two important parameters of their fitness, and this could explain a part of the variation observed in the wild.

Introduction

In host–parasite interactions, intraspecific variation within and among populations in terms of standard infection parameters (prevalence, intensity and abundance) and components of transmission (virulence or fecundity of parasites, resistance or susceptibility of host) has been repeatedly demonstrated over the last decade (e.g. Kaltz & Shykoff J, 2002; and see Poulin, 2006 for an overview). Genotypes of both hosts and parasites have been demonstrated to be involved in the variation and evolution of these traits (e.g. Lively, 1989; Kraaijeveld *et al.*, 1998; Carius *et al.*, 2001; de Roode *et al.*, 2007). Most of these studies have dealt with microparasites characterized by direct life cycles, but some studies have revealed the same phenomena in macroparasites with complex life cycles (Davies *et al.*, 2001; Incani *et al.*, 2001; Hammerschmidt & Kurtz, 2005; Cornet *et al.*, 2009).

Many complex-life cycle parasites have developed the ability to induce specific changes in their intermediate host phenotype (e.g. behaviour, appearance) that promote the trophic transmission of their infective stage to the definitive host (Lafferty, 1999; Moore, 2002; Thomas *et al.*, 2005). Although behavioural manipulation induced by parasites is now a widely documented phenomenon, particularly in larval helminths (e.g. Bethel & Holmes, 1973; Poulin *et al.*, 1992; Thomas & Poulin, 1998), little is known about the factors responsible for the intraspecific variation often described in these systems. Indeed,

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behavioural manipulations have often been studied using naturally infected animals without thorough knowledge of genotypes of either hosts or parasites or a control of the environmental factors during the infection. The analysis of this variation is nonetheless essential to understand the evolution of parasite-induced phenotypic changes (Thomas *et al.*, 2005; Webster, 2005). For example, the detection of genetic variation in parasites for inducing behavioural manipulation would be a first step indicating a potential for coevolutionary processes on this trait (Carius *et al.*, 2001).

Alteration of intermediate host behaviour is particularly well studied in acanthocephalan parasites (Kennedy, 2006). Among them, *Pomphorhynchus laevis* is a fish acanthocephalan parasite widely distributed in Europe, which uses several crustacean amphipod species as intermediate hosts. *P. laevis* is known to modify several behaviours in its gammarid intermediate host, *Gammarus pulex*. Indeed, infected gammarids become less photophobic (Bauer *et al.*, 2000; Perrot-Minnot, 2004; Franceschi *et al.*, 2008), are more attracted by predator odours (Kaldonski *et al.*, 2007) and are more often found in the river current than uninfected individuals (Lagrue *et al.*, 2007). These modifications increase their vulnerability to predation (Lagrue *et al.*, 2007). Levels of behavioural manipulation are variable between individual gammarids collected from the field; some infected animals showing, for example, a complete phototaxis reversal, whereas others are almost not manipulated (Tain *et al.*, 2006). Part of this variation can be explained by the age of infection, as the older the parasite larva is, the more it manipulates its *G. pulex* host (Franceschi *et al.*, 2008). Manipulation is also variable between parasite populations, but no local adaptation pattern was found for this trait (Franceschi *et al.*, 2010). On average, there was very little variation between host populations in their ability to resist manipulation, and the intensity of behavioural manipulation therefore appears to be controlled by the parasites more than by the hosts (Franceschi *et al.*, 2010). Within-population variation in behavioural manipulation was not investigated by Franceschi *et al.* (2010), because experimental infections were carried out with a mix of several parasite clutches.

The first aim of this study was to compare the variability in behavioural alterations induced by different families of parasites using experimental infections of *G. pulex* by *P. laevis*. One of the main difficulties in studying the effects of genotype in a gammarid–acanthocephalan interaction is that we are not yet able to control parasite and amphipod reproduction in the laboratory. Investigation into the manipulation ability of controlled lines is therefore impossible. Comparison of genetic variation in behavioural manipulation can nevertheless be undertaken using different parasite clutches of full sibs. Indeed, after insemination, the male *P. laevis* produces a copulatory cap that seals the female gonopore, preventing subsequent inseminations by other males (Crompton & Nickol, 1985; Dezfuli *et al.*, 1999). Moreover, taking only one female parasite per definitive fish host will also insure that the different clutches cannot be sired by the same male. The second aim of the study was to investigate potential trade-offs between the intensity of behavioural alterations and two other parasite fitness-related traits, infectivity and rapidity of maturity. Prior to being able to manipulate their host's behaviour, parasites have to optimize their ability to infect the intermediate host. On the other hand, they also have to optimize their growth to reach the infective stage for the next host before the intermediate host dies. Behavioural manipulation, growth and fighting host defences are probably costly for the parasite (Poulin, 1994), and we can expect some trade-offs between these different traits (Stearns, 1992), which could also explain part of the variation in behavioural manipulation.

Materials and methods

Origin of hosts and parasites

Gammarus pulex individuals were collected in May and October 2007 in a small tributary of the Suzon River (N 47° 24′12.6″; E 4° 52′58.2″), located in Burgundy, eastern France. In surveys over 10 years, *P. laevis* parasite has never been found in this population (L. Bollache, unpublished data), which can therefore be considered naive for the parasite (see Franceschi *et al.*, 2008, 2010). In the laboratory, gammarids were maintained in well-aerated aquaria of 37 × 55 × 10 cm, containing dechlorinated, UV-treated tap water at 15 ± 1 °C and elm leaves for food, under a 12:12 h light/dark cycle. Only males were used for experimental infections.

Parasite eggs were taken from naturally parasitized chubs (*Leuciscus cephalus*) sampled by electrofishing in the Ouche River (N 47° 17′52.91″; E 5° 02′27.28″), a tributary of the Saône River naturally infected by *P. laevis* (e.g. Perrot-Minnot, 2004). Fish were anaesthetized, killed and dissected within 24 h after collection. Adult parasites were immediately collected from the fish intestines, and eggs were obtained by dissecting female worms. Eggs were placed in 400 μL of water (each clutch in a separate tube), and parasite tissues were preserved in 300 μL of alcohol for species molecular identification.

Parasite molecular identification

In Burgundy, gammarids and fish may be infected by two closely related species of acanthocephalan parasites, *P. laevis* and *Pomphorhynchus tereticollis*. These two species cannot be reliably distinguished based on morphology, and thus a molecular method based on differences in the length of the ITS1 sequence (first internal transcribed spacer of the rDNA) was used for parasite identification (see details in Franceschi *et al.*, 2008).

Only *P. laevis* parasites were found in the present samples.

Infection procedure

Because of laboratory constraints, experimental infections were carried out on two occasions, in May and October 2007, following the same protocol, detailed below. In the laboratory, the gammarids were acclimated for 4 weeks prior to the infection experiments, by groups of 250 individuals, and fed with elm leaves.

Parasite eggs from each female were examined under a Nikon microscope E600 (20 ×, Nikon, Champigny sur Marne, France) to evaluate their number and maturity (mature eggs contain a developed larval stage called acanthor, see Crompton & Nickol, 1985). We used nine different parasite families of similar maturity in the spring 2007 experiment and 10 in the winter 2007. After dilution with water, suitable egg exposure doses were obtained. We considered each clutch as a 'parasite sibship', because all eggs are assumed to come from one female and only one male, thus making them full sibs.

Prior to infection, gammarids were deprived of food for 24 h. The infection was then carried out as described in Franceschi *et al.* (2008, 2010). Two gammarids were placed in a dish of 6 cm diameter and filled with water at 15 ± 1 °C, and the egg suspension at a concentration of 100 parasite eggs per gammarid (this dose provided an acceptable ratio of infection success versus multiple infection rate, see Franceschi *et al.*, 2008) was deposited on a 1-cm^2 dry elm leaf placed in the dish. The gammarids were allowed to feed on it for 48 h, whereas uninfected leaves were provided to control groups. For each treatment (one treatment corresponding to the exposure to one parasite sibship), 108 male gammarids were used.

At the end of the exposure period, the gammarids were rinsed and placed in 0.5-L aquaria by groups of eighteen individuals. Thus, each treatment was carried out in six replicates of 18 individuals randomly assigned to each aquarium, themselves randomly distributed on shelves in a single room. They were then maintained in standard conditions (they all received the same oxygenated water at 15 ± 1 °C, automatically replaced six times during the day, and were under a 12:12 h light/dark cycle).

Survival was checked every week and, from the sixth week, all gammarids were inspected once a week under a binocular microscope to detect the presence of parasites (see Franceschi *et al.*, 2008). As soon as an acanthella parasite larval stage (not infective for the definitive host) was detected through the cuticle, the gammarid was isolated in a 0.2-L plastic dish filled with water at 15 ± 1 °C to follow the parasite development individually. At the same time, uninfected individuals from the control treatment were also isolated. Parasite development was then followed once a week, and the date on which the parasites reached the cystacanth stage (infective for the definitive host) was noted. The prevalence (number of infected hosts/total number of surviving gammarids) was calculated after the last acanthella detection occurred, i.e. 90 days post-exposure in the May 2007 experiment and 66 days post-exposure in the October 2007 experiment (because of a more rapid development of parasites, see Results).

Behavioural measurements

The phototaxis of each individual was measured the day after the parasite reached the cystacanth stage (called 'young cystacanth' stage hereafter) and then 2 weeks later (called 'old cystacanth' stage hereafter). Phototaxis of uninfected control animals was also measured at the same time. The reaction to light of isolated individuals was measured as described in Franceschi *et al.* (2007). A single gammarid was introduced into a horizontal tube filled with well-aerated water, comprising a dark zone and a light zone of equal size. After a 5-min period of acclimatization, the position of the gammarid was recorded each 30 s for 5 min. At each observation, a score of 0 was given if the individual was located in the dark area and a score of 1 was given if it resided in the lighted area. At the end of each trial, summed scores ranged from 0 (always in the dark, strongly photophobic) to 10 (always in the light, strongly photophilic).

The number of parasites per infected host was verified at the end of the experiment by dissecting all animals.

Data analyses

For each parasite sibship, we used the mean values for each six replicates to estimate the infection success (proportion of infected gammarids), the multi-infection rate (proportion of infected gammarids with more than one parasite) and the parasite development time (the time lapse between the day of exposure and the day when the parasite reached the 'young cystacanth' stage). For phototaxis scores, we used the median score per replicate. Because the data issued from the average of these six values met homoscedasticity conditions, the analyses were performed using general linear models. For the analysis of the infection success and multi-infection rate, models include the following factors: exposure date (spring 2007/winter 2007) and a random 'parasite sibship' factor nested within the 'exposure date' factor. For the analysis of development time and phototaxis scores at both 'young cystacanth' and 'old cystacanth' stages, the additional 'multi-infection rate' factor was included in the models.

Changes in the phototaxis between the two stages of parasite development were analysed using paired *t*-tests.

To analyse the relationship between phototaxis scores, infection success and development time, we used Spearman correlations. Two types of correlations were investigated. The first took into account the average values per sibship, which could be seen as a genetic correlation in a

broad sense, including additive, nonadditive and interaction genetic covariance plus maternal covariance (Roff, 1997; Hammerschmidt & Kurtz, 2005); the second took into account values from replicates. Because several correlations were performed for each type of correlation, a Bonferroni adjustment was applied to avoid type I error.

All tests were performed using JMP 6.0 Software (SAS Institute Inc., Cary, NC, USA) and were two-tailed. P values < 0.05 were considered significant.

Results

Infection success and development time

The average prevalence ranged from 2.5 to 84% for the first experiment and from 55 to 84% for the second one (Fig. 1a). The infection success and multi-infections were not significantly different according to exposure date but differed according to parasite families (Table 1). Contrastingly, development time to the 'young cystacanth' stage was influenced by the date of exposure (with an overall median development time of 12.3 weeks in the spring 2007 experiment and 8.7 weeks in the winter 2007 one) but not by parasite family (Table 1, Fig. 1b). In the winter 2007 experiment, parasites were also more synchronized in their development than in the spring 2007 (O'Brien test for equal variances: $F_{1,105} = 5.37$, $P = 0.02$). Multi-infections did not affect development time (Table 1).

Phototaxis scores

Preliminary to the analysis of variation between parasite sibship, we verified that parasitism induced a change in phototaxis, by comparing the infected versus uninfected control gammarids. Because animals of the two control series did not differed in their phototaxis score, either at young cystacanth stage (ANOVA: $F_{1,9} = 0.20$, $P = 0.66$) or at old cystacanth stage ($F_{1,9} = 0.14$, $P = 0.71$), they were grouped for further analyses. At 'young cystacanth' stage, the phototaxis score was higher than that in controls in the spring experiment, but not in the winter experiment (ANOVA: $F_{2,115} = 35.56$, $P < 0.0001$; Fig. 2). At 'old cystacanth' stage, the scores were higher in the two infection series compared to controls ($F_{2,109} = 18.97$, $P < 0.0001$; Fig. 2). The phototaxis scores of infected individuals of the two exposure experiments significantly increased between young and old cystacanth stages (paired t-test: $t_{41} = 3.13$, $P = 0.003$ for the spring experiment; $t_{58} = 13.03$, $P < 0.0001$ for the winter experiment). This contrasts with the uninfected control animals, where the phototaxis scores remained low and stable between the two measures ($t_{10} = -0.39$, $P = 0.71$)(Fig. 2).

We then compared phototaxis scores between parasite families at the 'young cystacanth' stage (Fig. 1c). The

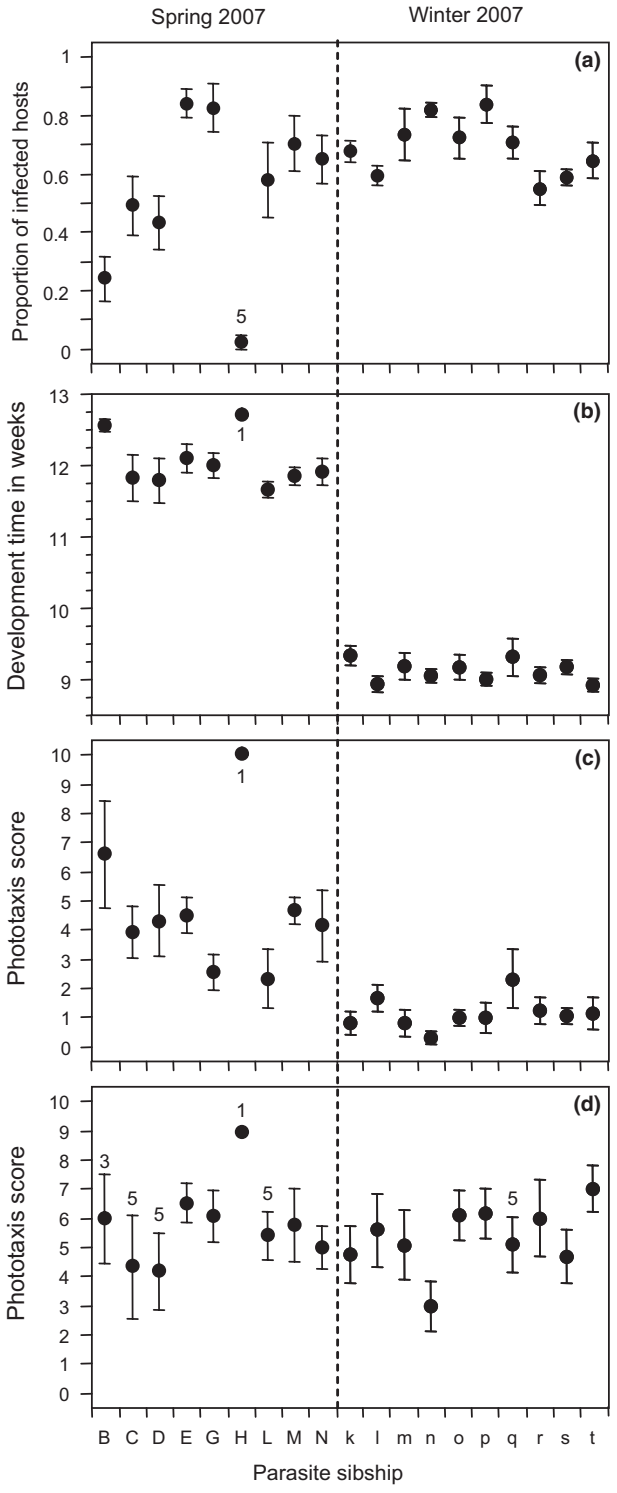

Fig. 1 Proportion of infected gammarids (a), development time of parasites (b), phototaxis scores of infected gammarids at 'young cystacanth stage' (c) and 'old cystacanth stage' (d), according to the different parasite sibships and according to the two experiments (spring and winter). All data are mean ± SEM of the six experimental replicates, except when the number of replicates is specified.

Table 1 Variation in the proportion of infected hosts, proportion of multiple infections, development time and phototaxis scores (at young cystacanth stage (y) and old cystacanth stage (o)), measured in *Gammarus pulex* infected by *Pomphorhynchus laevis*, as a function of the exposure date (spring vs. winter) and parasite sibship nested as a random factor within the exposure date. Multi-infection was also included as a source of variation in the three last models.

Dependant variable	Source of variation	$F_{(d.f.)}$	P
Proportion of infected hosts	Global model	$7.82_{(18, 94)}$	< 0.0001
	Exposure date	$3.14_{(1, 17.01)}$	0.094
	Sibship (exposure date)	$7.12_{(17, 94)}$	< 0.0001
Proportion of multi-infection	Global model	$2.46_{(18, 89)}$	0.003
	Exposure date	$1.10_{(1, 20.60)}$	0.306
	Sibship (exposure date)	$2.25_{(17, 89)}$	0.007
Development time	Global model	$65.16_{(19, 85)}$	< 0.0001
	Exposure date	$697.86_{(1, 21.95)}$	< 0.0001
	Sibship (exposure date)	$1.60_{(17, 85)}$	0.081
	Multi-infection	$0.89_{(1, 85)}$	0.347
Phototaxis score (y)	Global model	$5.12_{(19, 86)}$	< 0.0001
	Exposure date	$44.80_{(1, 21.29)}$	< 0.0001
	Sibship (exposure date)	$1.83_{(17, 86)}$	0.037
	Multi-infection	$0.20_{(1, 86)}$	0.653
Phototaxis score (o)	Global model	$1.16_{(19, 80)}$	0.317

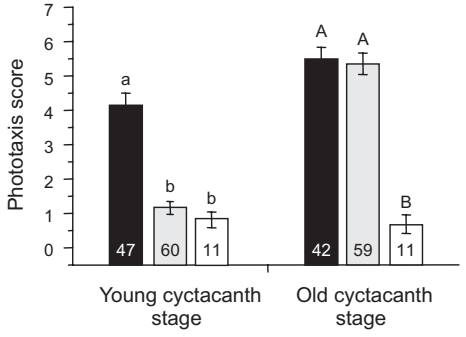

Fig. 2 Phototaxis scores (mean ± SEM) of gammarids according to their infection status by *P. laevis* parasites and according to the stage reached by the parasite in infected animals. Black bars denote the infected animals during the spring experiment, grey bars the infected animals during the winter experiment and white bars the uninfected control animals of both experiments. The number of replicates is provided in the bars.

global level of light attraction was much lower in the winter experiment than in the spring one (Table 1). Despite this large difference, the variation between parasite families in their ability to alter phototaxis of their intermediate host was significant (Table 1; Fig. 1c). This difference was attributable to a difference between sibships during the spring experiment ($F_{8,38} = 2.39$, $P = 0.034$), whereas the difference was not significant in the winter experiment ($F_{9,50} = 1.13$, $P = 0.36$). Multi-infections did not affect this phototaxis score (Table 1).

At the 'old cystacanth' stage, none of the analysed factors had a significant effect on variation in phototaxis score (Table 1; Fig. 1d).

Correlations between infection parameters and phototaxis scores

Because we found no significant variation among parasite families in either development time or phototaxis scores in winter, we only analysed data from the spring experiment. In addition, the family H was removed because only one gammarid was infected. At the young cystacanth stage, no significant correlation at the sibship level was found (Table 2, Fig. 3a). However, at the replicate level, we found a significant positive correlation between development time and phototaxis score (Table 2, Fig. 3b). Such a positive correlation was also found between development time and phototaxis score at the 'old cystacanth' stage, but it not statistically supported enough to be considered significant (Table 2). The correlations between other traits were all nonsignificant. It is worth noting that these results were quantitatively similar when the analyses were made at the level of individual gammarids (results not showed).

Discussion

Our study first revealed that phototaxis scores were generally higher in infected animals than in control ones but that attraction to light increased as parasite larvae aged. These results confirm previous ones. Indeed, previous individual repeated measures showed that the behavioural alteration (attraction by light instead of repulsion) appears in gammarids only when parasites reach the cystacanth stage and then continues to increase with parasite age (Franceschi *et al.*, 2008). In addition, the present experimental design allowed us to find that three other parameters influenced the parasite intra-population variation in behavioural alteration: parasite sibships (genetic basis), experiment replicates (environmental factor) and parasite development time (life history strategy).

Variation among parasite sibships

There were differences in terms of infection success, proportion of multi-infections and behavioural alteration among the different parasite families. These differences among parasite sibships could be owing to genetic variation. However, our experimental design did not allow us to differentiate genetic effects from maternal effects. Neither can we ignore the possibility of an 'environmental' effect of the definitive host from which female parasites were sampled (see also discussion below for the observed variation between experiments). In numerous host–parasite systems, within-population variation in infection success has been reported and linked

Table 2 Correlations between four traits linked to the infection of *G. pulex* by *P. laevis*.

	Infection success	Parasite development time	Host phototaxis score (y)	Host phototaxis score (o)
Infection success		−0.14 (P = 0.34)	−0.22 (P = 0.14)	0.12 (P = 0.46)
Parasite development time	0.24 (P = 0.57)		**0.42 (P = 0.004)**	0.33 (P = 0.03)
Host phototaxis score (y)	−0.17 (P = 0.69)	0.57 (P = 0.14)		0.27 (P = 0.08)
Host phototaxis score (o)	0.62 (P = 0.10)	0.74 (P = 0.04)	0.24 (P = 0.57)	

Spearman r_s coefficients are given, with the associated P values within brackets. Value in bold is the one remaining significant after Bonferroni adjustment (α = 0.05).
Above diagonal: correlations between replicate averages (n = 42).
Below diagonal: correlations between sibship averages (n = 8).

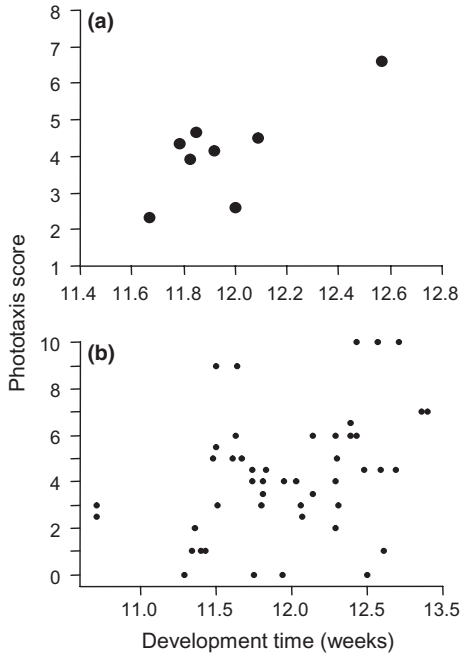

Fig. 3 Correlations between average development time and average phototaxis score at the 'young cystacanth stage', at sibship level (a) and at replicate level (b).

to differences in parasite genotypes (e.g. Carius *et al.*, 2001; Incani *et al.*, 2001; Kaltz & Shykoff J, 2002), but very little is known about behavioural manipulation. Only the results of Leung *et al.* (2010) suggest that there could be variation between trematode clones from the same natural population in their ability to reach the host tissues where they can express host manipulation. However, in this case, the presence of other parasites seems to be a stronger factor explaining parasite distribution than parasite genotype (Leung *et al.*, 2010).

The variation among parasite sibships was nevertheless significant in only one of our experiments, in the spring replicate. In the winter replicate, less variation was observed, and most *P. laevis* sibships modified host phototaxis very little at the 'young cystacanth' stage, compared to uninfected controls. This does not mean that they were intrinsically bad manipulators because, when they had grown to the 'old cystacanth' stage, their ability to alter phototaxis was the same as that of the spring parasites. Parasite development time was also much faster and less variable in the winter experiment than in the spring one. This finding strongly suggests that the winter parasites were unable to both develop rapidly and at the same time alter phototaxis when reaching the infective stage (see discussion about the possibility for a trade-off between these two traits).

Variation between experiment replicate

Environmental parameters (e.g. temperature and resource availability) can affect parasite traits such as growth or the outcome of parasite transmission by modulating the exposure of the host to their pathogens (Ebert *et al.*, 2000; Fels, 2005; Jokela *et al.*, 2005; Fels & Kaltz, 2006). A single parasite genotype can produce different phenotypes across a range of environmental conditions, and environmental variation can sometimes hide the genetic variation in parasite traits (Blanford *et al.*, 2003; Mitchell *et al.*, 2005). Variation we observed between spring and winter experiments can hardly be attributable to differences in proximate environmental conditions experienced by parasites during the infection procedure because our experiments were all conducted in the same controlled conditions. We also limited the variation owing to host condition by acclimatizing gammarids in the laboratory for 4 weeks prior to infection, both in spring and in winter experiments. Of course, despite our controlled conditions, environmental conditions (e.g. temperature) could be slightly different between the two experiments and could explain at least a part of the observed variation. However, it would be surprising that such slight (if any) differences explain 3 weeks of differences in parasite development time. However, we did not control for conditions that acanthocephalan mothers experienced before laboratory experiments. The faster development of parasites in winter may be attributable, for example, to a better food supply of parasite females in their definitive fish host and thus a higher allocation of nutrients to their eggs. In

addition to external variation, we cannot exclude a seasonality effect in the physiology of both gammarids and acanthocephalans. Various traits are known to be seasonal in invertebrates, including reproduction. Seasonal changes in the prevalence of various acanthocephalans, including *P. laevis*, have been recently demonstrated in the field (Kennedy, 2006) and could be attributable to variation in infectivity, as suggested by our results. In any case, to clearly understand the factor responsible for this variation, further experiments are now needed. It nevertheless remains true that an as yet unidentified environmental condition was so important for determining the behavioural phenotype of parasitized hosts that it overrode among-sibship variation, leading to phenotypic plasticity being the main source of variation on this trait, probably via a trade-off with development time discussed below.

Co-variation with development time

In the life cycles of parasites infecting different successive hosts, there are several phases of transmission, and selection acting to improve one phase may limit the success of other phases (Gandon, 2004). In *P. laevis*, the first transmission occurs from the fish to the gammarid, the amphipods ingesting parasites eggs released by the final host, whereas the second occurs from the gammarid to the fish, when the crustacean is predated. The efficiency of these two transmission events can be inferred from the intermediate host, the first transmission being measurable by infection success, and the second by the intensity of behavioural manipulation. Obviously, the question of a trade-off between these two parameters arises. However, we found no significant relationship between infection success and behavioural manipulation, which did not support the existence of such a trade-off. By contrast, we found, in the spring experiment, a positive correlation between parasite development time and the intensity of behavioural manipulation at the 'young cystacanth' stage (at the individual level). This comes in addition to the inter-season co-variation observed in these traits and discussed earlier. Rapidly growing parasites were thus less able to manipulate the behaviour of their intermediate host, when they reached the stage that is infective for the next host. This strongly suggests a trade-off between these two traits. However, because all parasite sibships reached the same higher level of manipulation 2 weeks later, at the 'old cystacanth' stage, the correlation appears to only occur between the rapidity of development and the rapidity with which behavioural manipulation is induced (and not the intensity of manipulation that can be intrinsically reached). Hammerschmidt *et al.* (2009) recently showed that there is an optimal moment for manipulation during parasite development in the tapeworm *Schistocephalus solidus*. Our results suggest that the ability of a parasite to manipulate at this optimal moment could be constrained by the rapidity of development. Benesh (2010) found genetic variation in growth of *S. solidus*, but no correlation with behavioural changes. In this study, however, no predation-risk behaviour emerged in the copepod host after parasite maturity, perhaps owing to a non-natural host–parasite combination, therefore limiting the strength of this observation. The correlation we found in *P. laevis* involves two parameters that should optimize the time spent by the parasite in its intermediate host. By growing rapidly, a parasite limits the time during which it is not infective for the next host and therefore optimizes its transmission potential in case of predation by the final host. By inducing rapid behavioural manipulation, a parasite optimizes its transmission after reaching the infective stage. Our results suggest that these two traits cannot be optimized at the same time and may be plastically variable according to environmental conditions. It is interesting to consider that cyprinid fish foraging and feeding activity is often lower in winter than in spring (e.g. Penttinen & Holopainen, 1992; Lucas & Batley, 1996). Variation in investment in growth versus in rapidity of behavioural manipulation could therefore optimize the parasites' exploitation of their intermediate host.

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