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VARIATION BETWEEN POPULATIONS AND LOCAL ADAPTATION IN ACANTHOCEPHALAN-INDUCED PARASITE MANIPULATION

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Many trophically transmitted parasites manipulate their intermediate host phenotype, resulting in higher transmission to the final host. However, it is not known if manipulation is a fixed adaptation of the parasite or a dynamic process upon which selection still acts. In particular, local adaptation has never been tested in manipulating parasites. In this study, using experimental infections between six populations of the acanthocephalan parasite *Pomphorhynchus laevis* and its amphipod host *Gammarus pulex*, we investigated whether a manipulative parasite may be locally adapted to its host. We compared adaptation patterns for infectivity and manipulative ability. We first found a negative effect of all parasite infections on host survival. Both parasite and host origins influenced infection success. We found a tendency for higher infectivity in sympatric versus allopatric combinations, but detailed analyses revealed significant differences for two populations only. Conversely, no pattern of local adaptation was found for behavioral manipulation, but manipulation ability varied among parasite origins. This suggests that parasites may adapt their investment in behavioral manipulation according to some of their host's characteristics. In addition, all naturally infected host populations were less sensitive to parasite manipulation compared to a naive host population, suggesting that hosts may evolve a general resistance to manipulation.

KEY WORDS: **Behavioral manipulation, experimental infections, *Gammarus pulex*, host-parasite coevolution, local adaptation, *Pomphorhynchus laevis*.**

Many parasites with complex life cycles have developed the ability to manipulate the behavior of their intermediate hosts. Such behavioral alterations increase the parasites' fitness by improving their trophic transmission to their final host, where they can reproduce (Lafferty 1999; Moore 2002; Thomas et al. 2005; Lagrue et al. 2007; Perrot-Minnot et al. 2007). The evolution of behavioral manipulation is a subject of debate. Many models have been developed to explain the emergence and selection of manipulation (e.g., Lefevre et al. 2008; Parker et al. 2009). Poulin (1994) proposed that an "optimal manipulation effort" evolved in parasites, with an intensity depending on the balance between the costs that manipulation imposes on the parasite and a number of key factors, including parasite prevalence, fecundity, and host and parasite survival. This suggests that this trait is under the sole control of the parasite, without any influence of the host. However, as noted by Poulin (1994) and others (e.g., Cézilly and Perrot-Minnot 2005; Lefevre et al. 2008), it is not clear whether manipulation is a fixed adaptation in parasites, or a dynamic process upon which selection still acts, notably through coevolution (see also Parker et al. 2009). Manipulative parasites may have detrimental effects on host fitness (Bollache et al. 2002; Duclos et al. 2006), and behavioral manipulation itself can be seen as a form of virulence

(it increases host mortality by predation). It is therefore possible that manipulative parasites are subject to an ongoing antagonistic coevolution (reciprocal evolution of host defense and parasite counter-defense), given enough genetic variation in both partners (Thompson 1998). The genetic polymorphism of host resistance to parasites is widely distributed in animals and plants, as well as the genetic polymorphism of parasites' ability to infect and/or exploit their hosts (e.g., Carius et al. 2001; Thrall et al. 2002; Greischar and Koskella 2007). This leads to an arms race between hosts and parasites and, when populations are not panmictic, this may lead to patterns of local adaptation (Ebert 1994; Kawecki and Ebert 2004). Local adaptation occurs when the mean fitness of a population is higher in its own habitat than in a foreign one. Host–parasite systems are particularly rewarding models for the study of local adaptation (reviews in Morand et al. 1996; Kaltz and Shykoff 1998; Kawecki and Ebert 2004; Greischar and Koskella 2007). A number of experiments have found higher mean parasite performance in sympatric combinations than in allopatric ones (e.g., Ebert 1994; Lively and Dybdahl 2000; see Greischar and Koskella 2007 for a synthesis), but others detected no local adaptation in parasites or even found the reverse pattern (e.g., Dufva 1996; Kaltz et al. 1999). In fact, migration rate (or gene flow), which introduces genetic variability in populations upon which selection can act, is one of the most important parameters influencing local adaptation (Gandon et al. 1996; Gandon and Michalakis 2002). A recent meta-analysis confirmed this general prediction: parasites are more likely to be locally adapted when they migrate more than their hosts (Greischar and Koskella 2007). Most of the numerous local adaptation studies on host–parasite systems have measured adaptation as the success of infection (Greischar and Koskella 2007), but no study has ever been conducted on the parasite's ability to manipulate its host. Concomitantly, the variation among populations in parasitic manipulation remains overlooked (Thomas et al. 2005).

Behavioral manipulation is particularly well documented in larval helminths (e.g., Poulin et al. 1992; Bakker et al. 1997; Thomas and Poulin 1998), and especially in acanthocephalans (Bethel and Holmes 1973; Crompton and Nickol 1985; Kennedy 2006; Lagrue et al. 2007). Among these, *Pomphorhynchus laevis* infects fish intestine when adult, and uses crustacean amphipods as intermediate hosts (Kennedy 2006). In the amphipod *Gammarus pulex*, this acanthocephalan induces numerous behavioral alterations, such as reversal in phototaxis behavior, change in drift behavior and reversal in anti-predator behavior (Bauer et al. 2000; Kaldonski et al. 2007; Lagrue et al. 2007; Franceschi et al. 2008). Specifically, infected gammarids become less photophobic than uninfected ones and are thus more often exposed to light instead of being hidden in dark areas (Bauer et al. 2000; Franceschi et al. 2008). These modifications lead to a higher presence in open water, where they face higher predation risk by their final hosts, compared to uninfected gammarids (Lagrue et al. 2007). As a consequence, the trophic transmission to final host is significantly improved (Lagrue et al. 2007). The generation times of the two protagonists are equivalent. It takes the gammarid about 6 months from birth to reproduction and the parasite a minimum of 4 months from birth to sexual reproduction in its final host (Kennedy 2006 and pers. obs.). However, their dispersal potentials are different: gammarids have a low dispersal rate (Elliott 2003) and populations are genetically structured according to river watersheds on small scales (Meyran et al. 1997), whereas *P. laevis* have a higher dispersal potential because of three possibilities of dispersal. The eggs, released in the water, can disperse with the river current; the larval stages can move with the intermediate host they infect; finally, adult parasites are carried by fish. In particular, the common final host of *P. laevis* in Western Europe, the chub *Leuciscus cephalus*, is characterized by high dispersal ability (Hänfling and Brandl 1998; Larno et al. 2005). We therefore predict that, if there is a coevolutionary process at work between *P. laevis* and *G. pulex*, parasites should be locally adapted to their hosts (Gandon et al. 1996; Greischar and Koskella 2007).

The aim of this work was thus to investigate whether behavioral manipulation is variable between parasite and host populations, and if a manipulative parasite may show local adaptation to its host. We conducted a common garden experiment with reciprocal cross-infections between hosts and parasites from six natural populations. Because studies of local adaptation can sometimes lead to contrasting results according to the fitness trait under investigation (Refardt and Ebert 2007), we quantified two different measures of parasite fitness, infectivity, and manipulative ability, and we also measured host survival rate. We finally compared these results to those obtained, with the same six parasite populations, on a host population where no coevolution occurred with *P. laevis*.

Materials and Methods

ORIGIN OF HOSTS AND PARASITES

Gammarus pulex and their *P. laevis* parasites were collected in March 2008 in six locations from three different watersheds located in eastern France (Fig. 1). These locations were chosen, after a preliminary census, because they harbored the final host (the chub *L. cephalus*), the intermediate hosts, and the parasites. For the Loire watershed, we chose the Arconce river, a tributary of the Loire river (Site L1; 46°28′20.18″N; 4°19′48.45″E) and the Mesvrin river, a tributary of the Arroux river (L2; 46°51′51,14″N; 4°15′53.94″E). For the Meuse watershed, we chose the Madon river, a tributary of the Moselle river (M1; 48°14′51,46″N; 6°09′04,28″'E) and the Vair river, a tributary of the Meuse river (M2; 48°11′47.23″N; 5°54′10.12″E). Finally, for the Saône watershed, we chose two tributaries of the Saône

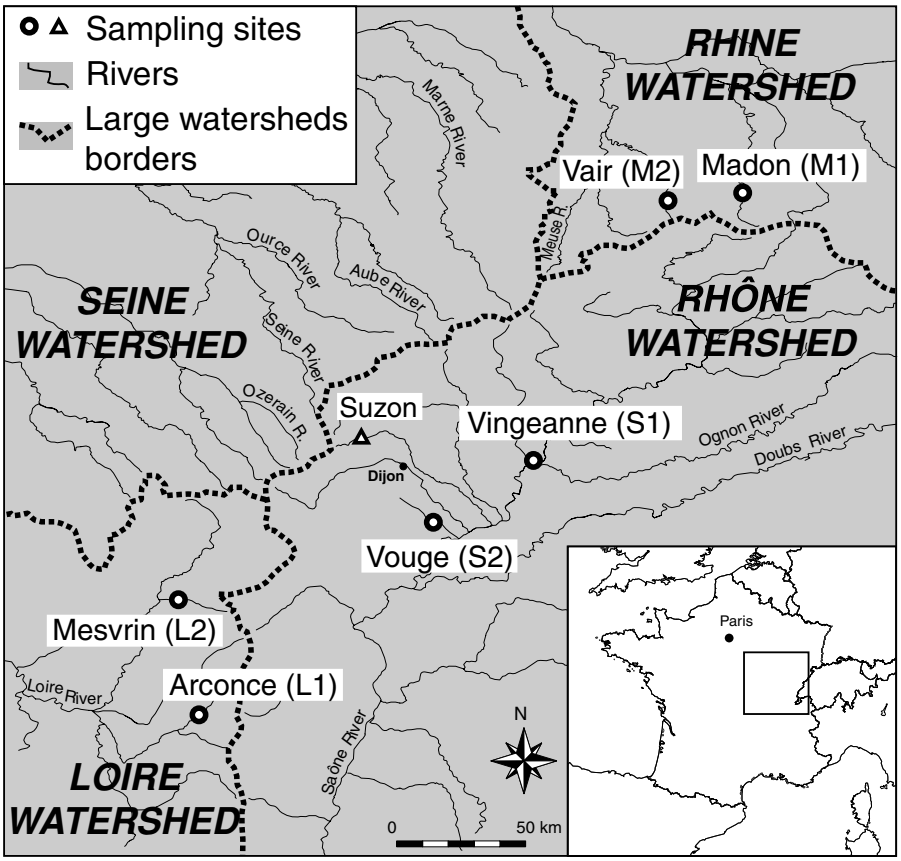

Figure 1. **Localization of the different studied populations in Burgundy, Eastern France.**

river, the Vingeanne river (S1; 47°20′52.17″N; 5°27′04.20″E) and the Vouge river (S2; 47°08′03.65″N; 5°10′45.61″E). We also collected gammarids at another site, Val-Suzon, in a small tributary of the Suzon River (47°24′12,6″N; 4°52′58,2″E) in the Saône watershed. This population is totally isolated from the others due to regular summer drying out of a part of the river, and no *P. laevis* have been found here for at least 10 years. Given that *G. pulex* in Val-Suzon are sensitive to experimental infection and to behavioral manipulation by *P. laevis* (Franceschi et al. 2008), this population represents a naive control population.

In the laboratory, uninfected gammarids were acclimated by groups of 100 individuals for two weeks prior to infection experiments, 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. To standardize the recipient hosts for experimental infections, only adult reproductive males (i.e., taken paired with a female) were used. Because of differential predation on infected individuals (Lagrue et al. 2007), a field-recorded prevalence never reflects the "real" parasite prevalence in the intermediate host. Therefore, the prevalence of *P. laevis*-infection in the six naturally infected populations was estimated in the laboratory after 60 days, in a subsample of individuals unexposed to experimental infection and kept in standard conditions without any predation pressure (Table 1).

Parasite eggs were taken from naturally parasitized chubs (the final host of *P. laevis*) sampled by electrofishing in the rivers described above, except at Val-Suzon where no *P. laevis* occurs.

Table 1. **Estimated prevalence of *P. laevis* in gammarids and proportion of *P. laevis* in fish for the six naturally infected populations investigated (see text for details).**

Population	Prevalence in *G. pulex* (%[1]; Ng)	Proportion in fish (%; Nf; Np)
L1: Arconce	0.00 (1.19); 251	100.0; 3; 10
L2: Mesvrin	1.80 (0.00–4.31); 110	30.0; 3; 10
M1: Madon	8.40 (4.45–12.30); 191	95.2; 7; 21
M2: Vair	0.00 (1.09); 273	92.8; 5; 14
S1: Vingeanne	0.00 (0.73); 406	88.5; 4; 26
S2: Vouge	0.87 (0.00–2.59); 114	100.0; 7; 37

Ng, Nf, and Np are sample sizes of *G. pulex*, fish, and *P. laevis* investigated, respectively.

[1]The confidence interval at 95% is provided in parenthesis. For populations in which no parasite was observed, the CI was replaced by the maximal likely frequency of the infection at $P = 0.05$, calculated according to Post and Millest (1991).

Fish were anesthetized, killed and dissected within 24 h after collection. Adult female parasites were immediately collected from the fish intestines and eggs were obtained by dissecting the worms. Eggs were placed in 400 μL of water and parasite tissues were preserved in 300 μL of alcohol for species molecular identification.

PARASITE MOLECULAR IDENTIFICATION

Chubs may be infected by two closely related species of acanthocephalan parasites, *P. laevis* and *Pomphorhynchus tereticollis*, and these two species cannot be reliably distinguished based on morphology. A molecular method was thus used for parasite identification prior experimental infections (see details in Franceschi et al. 2008). Several parasites from different chubs were examined. *Pomphorhynchus laevis* was the dominant parasite in the final host, except in the Mesvrin river. Prevalence was generally weak in *G. pulex*, but variable between populations (Likelihood-ratio $\chi^2 = 58.18$, $P < 0.0001$) (Table 1).

INFECTION PROCEDURE

Gammarids from the seven populations (Arconce, Mesvrin, Madon, Vair, Vingeanne, Vouge and Val-Suzon) were infected by parasites from the six naturally infected populations (Arconce, Mesvrin, Madon, Vair, Vingeanne and Vouge). We therefore obtained a full-crossed design between the six naturally infected populations, plus a naive control for each parasite origin.

Parasite eggs from each female were examined under a Nikon microscope (20×) to evaluate their maturity (mature eggs, containing a larval stage called acanthor, are usually mixed with immature eggs in the female genital tract, see Crompton and Nickol 1985). Eggs were counted in five viewing areas, to select clutches having approximately the same proportion of mature eggs (70–80%). Six clutches of *P. laevis*, from three different fish, were then selected for each population, except for the Mesvrin river, where only three parasites from three different fish were suitable. The clutches from a single site were mixed, and the number of mature eggs in each suspension was then estimated by averaging the counts made under a microscope in 10 samples of 1 μL. Suitable exposure doses (100 eggs per gammarid, Franceschi et al. 2008) were then obtained after dilution with water.

Prior to infection, gammarids were deprived of food for 24 h. The infection was then carried out as described in Franceschi et al. (2008). Two gammarids were placed in a dish of 6 cm diameter, filled with water at 15 ± 1°C, and the egg suspension was deposited on a 1 cm^2 dry elm leaf placed in the dish. Uninfected leaves were provided to control groups. The gammarids were allowed to feed on the leaves for 48 h. For each treatment, 108 gammarid males were used. At the end of the exposure, the gammarids were rinsed, placed in aquaria of 0.5 L, and maintained in standard conditions (water at 15 ± 1°C, 12:12 h light:dark cycle). Eighteen individuals in the same treatment group (exposed to eggs from the same parasite population) were randomly assigned to each aquarium. There were therefore six replicates (aquaria) for each treatment.

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 (parasites are visible through host cuticle; see Franceschi et al. 2008). As soon as a parasite was detected, the gammarid was isolated in a plastic dish of 0.20 L filled with water at 15 ± 1°C. At the same time, uninfected individuals from control treatments were also isolated. The prevalence (number of infected hosts/total number of surviving gammarids) was calculated 90 days postexposure. The intensity of infection (number of parasites per infected host) was estimated on the same date and measured at the end of the experiment by dissecting all animals.

BEHAVIORAL MEASUREMENTS

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, featuring a dark zone and a light zone of equal size. After a 5-min period of acclimatization, the position of the gammarid was recorded every 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 (gammarid always in the dark, strongly photophobic) to 10 (always exposed to light, strongly photophilic). Because hosts exhibit more photophilic behavior as parasites mature (Franceschi et al. 2008), phototaxis was measured twice for each individual during parasite development: the day after at least one parasite reached the cystacanth stage ('young cystacanth stage'; the cystacanth is the acanthocephalan larval stage infective for the final host), and two weeks after this first measurement (old cystacanth stage).

DATA ANALYSES

Except where specified, tests were performed using JMP 6.0 Software (SAS Institute Inc.) and were two-tailed. P values < 0.05 were considered significant.

Survival was analyzed during the growth phase of the parasite, between the day of exposure and the visual detection of the parasite (Franceschi et al. 2008). Survival was therefore assessed for 70 days. The analysis was performed using Cox proportional hazard models with random effects. We used the function "coxme," from the package "kinship" (Therneau 2007) implemented in the R software (R development Core Team 2008) to fit mixed-effects Cox models. We tested, in the six naturally infected populations, the effects of host and parasite origins and their interactions on host survival, with aquaria (replicates) as a nested

random factor. In these exposed groups, the analysis includes the survival of both infected individuals and exposed-uninfected individuals (because at these stages the infection cannot be seen). Unexposed hosts from the six populations were therefore included in the model as a control. Any effect of the exposure on survival (direct effect of the parasite or indirect effect, e.g., resistance to the infection) can then be seen by comparing this control group to the different exposed groups. Comparisons of nested models were undertaken using a stepwise procedure based on the likelihood ratio test (Collett 1994). Local adaptation for virulence was not tested because we had no a priori hypothesis for the optimal value of virulence (in terms of parasite fitness) in this system.

The infection success of the different parasite populations on the different host populations was analyzed by means of generalized linear models using binomial distribution and a logit link function (logistic regression). The following factors were included in a first model: "host watershed," "parasite watershed," the interaction between these two factors, a "host population" factor nested within the "host watershed" factor, a "parasite population" factor nested within the "parasite watershed" factor, and their interactions. Interactions between hierarchical levels (e.g., host watershed $*$ parasite population or parasite watershed $*$ host population) are difficult to interpret biologically because we have no idea of the population structure within watersheds. Because they were nonsignificant ($P = 0.27$ and $P = 0.94$, respectively), they were excluded from the presented model. Watershed effects were tested over the nested terms, that is, over the population variation within watershed, by calculating pseudo-F tests from the mean deviances. We then ran a second model only with host and parasite population as fixed factors, and their interactions (see Results). We then conducted contrast analyses subsequent to the logistic regressions. Following Thrall et al. (2002) we first compared sympatric combinations to allopatric combinations (i.e., cells of the diagonal of the host $\times$ parasite matrix against off diagonal cells). Moreover, as Kawecki and Ebert (2004) highlighted, there are two alternative ways of examining parasite local adaptation. On one hand, the "home versus away" criterion compares the performance of a given parasite origin on sympatric (home) or allopatric (away) hosts. On the other hand, the "local versus foreign" criterion examines the performance of sympatric (local) or allopatric (foreign) parasites on a given host origin. We therefore analyzed these two criteria by running contrasts of sympatric versus allopatric combinations for each line and each columns of the host $\times$ parasite matrix.

The intensity of infection was analyzed in the same way as infection success, with a generalized linear model using a log link function.

Variation in phototaxis scores was analyzed using nonparametric tests, as data did not meet either normality or homoscedasticity conditions, even after transformation attempts. We analyzed the effect of the intensity of infection, this trait being treated as a categorical variable. Because small sample sizes were obtained for intensities >2 parasites per host, three categories were established, following Franceschi et al. (2008): one parasite per host, two parasites per host, and more than two parasites per host. As for infection success, we then analyzed the main factor effects (origin of hosts and parasites) on behavioral manipulation, and finally the phototaxis scores of hosts exposed to "home versus away" parasites, and of parasites infecting "local versus foreign" hosts.

Results

HOST SURVIVAL

The interaction was nonsignificant ($P = 0.11$) and was removed from the Cox model comparing the effects of host and parasite populations (with aquaria as a nested random factor). Host survival was affected by both host and parasite populations (whole model: Likelihood-Ratio $\chi^2_{12} = 559.0$, $P < 0.0001$). There was huge variation in survival between host populations (L-R $\chi^2_5 = 109.52$, $P < 0.0001$, Fig. 2B), with higher mortality in L2 and S2 populations and the best survival in the M1 population. Parasite origins also differently affected host survival (L-R $\chi^2_6 = 23.87$, $P = 0.0006$, Fig. 2A). Using unexposed animals as references in the analysis, parasites from all origins meant a decrease in host survival. The least virulent parasites were those in the M2 population, increasing mortality by 14.3% compared to the control and the most virulent were those in S2 (39.8% increase in mortality). Removing the control unexposed individuals from the analysis confirmed that parasites from different populations have different virulence (L-R $\chi^2_5 = 11.70$, $P = 0.039$). The statistics were qualitatively similar when host populations resistant to the infection (see below) were removed from the analyses (not showed).

INFECTION SUCCESS

Global infection success in the six naturally infected populations was influenced by host origin and by parasite origin, both at the population level only (Table 2, Fig. 3). A maximum of 38.17% infection was found in hosts from the M1 population, whereas hosts from two populations, L2 and S1, remained totally uninfected (Fig. 3A). Parasites from the M2 and S2 populations showed a high infective capacity, around 18%, whereas parasites from the L2 and S1 populations were poorly infective (yielding 3.21% and 4.47% infection rates, respectively) (Fig. 3B). Interactions between host and parasite origins were not significant, either at watershed or population levels (Table 2). The fact that two host populations, from two different watersheds, were "resistant" to infection by parasites of any origin probably dragged the results toward a statistical homogeneity. We therefore carried out analyses excluding these two host populations. This resulted

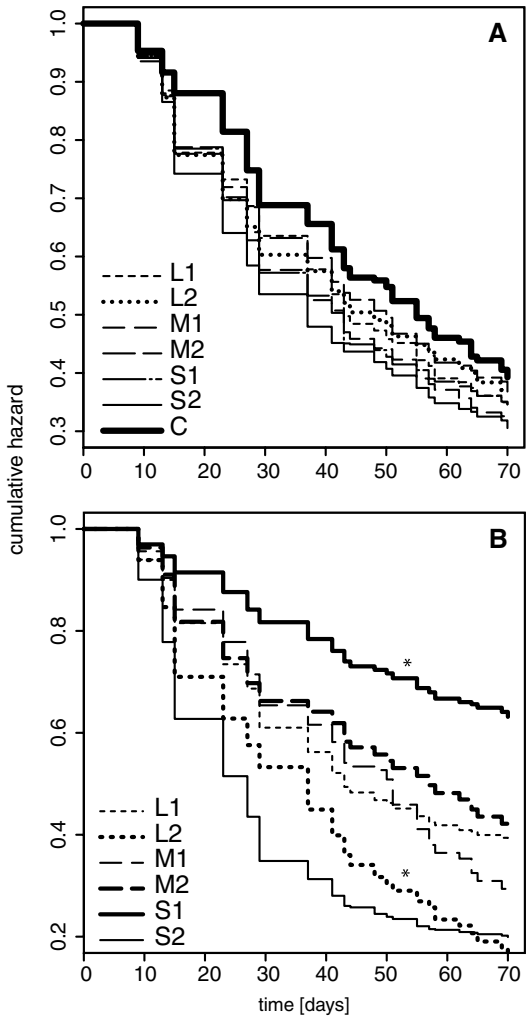

Figure 2. **Survival of gammarids according to infection status by the different parasite origins (A) and host populations (B). The y-axis represents the Kaplan–Meier estimates of the cumulative mortality proportion. These lifetime distributions were obtained by pooling all the aquaria (replicates) under the same treatment (same origin for host or parasite). C are control, unexposed gammarids. The two curves labeled with asterisks denote host populations that were resistant to infections. See Figure 1 for population labeling.**

in an unbalanced design between populations and watersheds. Because under this restriction watersheds and host populations were confounded for L and S origins, we limited our analyses to the population level. Results concerning the significance of the main factors were similar to results found in the full model, but this second analysis revealed significant interactions between host and parasite origins (Table 3). The "sympatric versus allopatric" contrast revealed higher infection rates in sympatric combinations (Table 3, the overall infection success in sympatric and allopatric combinations were 0.27 and 0.14, respectively). However, the "local versus foreign" contrasts showed that only hosts from the M2 population were significantly more susceptible to infection

Table 2. **Generalized linear model analyzing the infectivity of the different parasite populations on the different host populations (whole model: Likelihood Ratio $\chi^2_{23} = 234.09$, $P < 0.0001$).**

Source	df	L.R. χ^2 and F	P
Host watershed	2	χ^2=92.86	
	2, 3	F=2.42	0.24
Parasite watershed	2	χ^2=1.38	
	2, 3	F=0.08	0.93
Host watershed × parasite watershed	4	χ^2=4.41	0.35
Host population [host watershed]	3	χ^2=57.58	<0.0001
Parasite population [parasite watershed]	3	χ^2=25.47	<0.0001
Host population × parasite population [host watershed, parasite watershed]	9	χ^2=11.31	0.25

by their sympatric parasites, whereas differences were not significant in the other populations (near significance at S2 location, Table 3, Fig. 3C). The "home versus away" contrasts revealed that sympatric infections were significantly more successful in the M1 and the M2 populations (Table 3, Fig. 3D).

We then compared the infection success of the different parasite populations, between the naive host population and the naturally infected ones (Fig. 3B,E). A two-way logistic regression (whole model: Likelihood-ratio $\chi^2_{11} = 252.14$, $P < 0.0001$) revealed an effect of host population type (naive vs. others L-R $\chi^2_1 = 30.79$, $P < 0.0001$), the naive population being more susceptible to infection (overall prevalence: 43.08%) than naturally infected ones (overall prevalence: 9.98%). It also confirmed the previously observed effect of parasite population (L-R $\chi^2_5 = 107.78$, $P < 0.0001$), with M2 parasites being the most infectious and L2 and S1 being the least infectious (Fig. 2). In addition, the interaction between host and parasite populations was significant (L-R $\chi^2_5 = 15.85$, $P < 0.007$). Indeed, although the general pattern of infection was similar, L1 parasites performed relatively better in infecting the naive population, whereas parasites from L2 and S1 populations performed relatively better in naturally infected populations (Fig. 3B,E).

The number of parasites per infected host varied between 1 and 8, with an average of 2.0 ± 0.09 parasites per host. Most gammarids (70.0%) were infected by one or two parasites. These values were slightly higher than those found in nature, where the mean intensity is around 1.5 (Outreman et al. 2002). In the analysis of naturally infected populations (uninfected host populations L2 and S1 being excluded), parasite intensity was not influenced by host origin, parasite origin or their interaction (GLM, whole

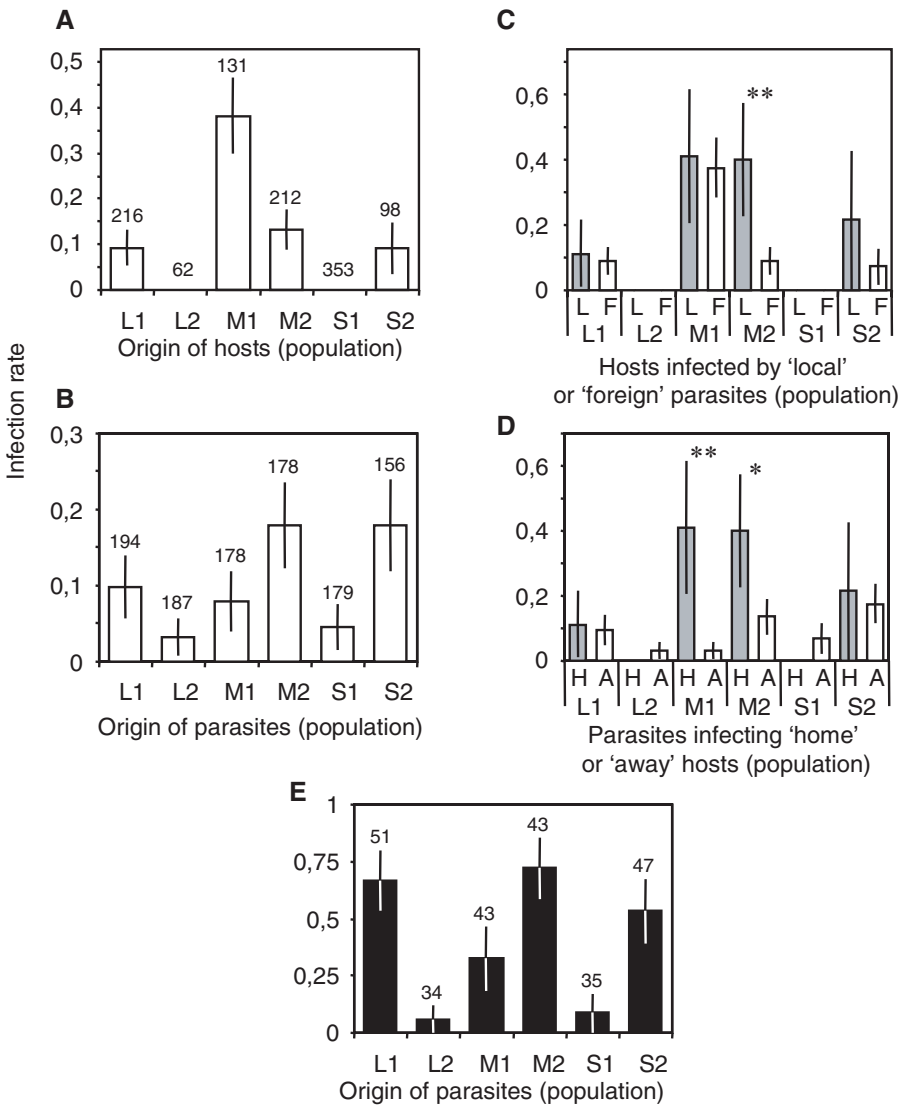

Figure 3. Infection rates of *P. laevis* parasites in *G. pulex* hosts. (A) infection rates according to the different naturally infected host populations (all parasite sources taken together). (B) infection rates according to the different parasite populations (all naturally infected host populations taken together). (C) and (D) show "local versus foreign" and "home versus away" comparisons for infectivity, respectively. E: infection rates according to the different parasite populations, in hosts from the naive population. Error bars are confidence intervals at 95%, calculated assuming a binomial distribution of the variance. Numbers above the bars are sample sizes. Stars highlight significant differences in contrasts comparison, see Table 3 for details and other comparisons. (*$P < 0.05$, **$P < 0.01$).

model: L-R $\chi^2_{23} = 29.09$, $P = 0.18$). There was no significant difference in parasite intensities between naturally infected hosts and naive hosts from Val-Suzon (GLM: L-R $\chi^2_1 = 1.61$, $P = 0.20$).

BEHAVIORAL MANIPULATION

Host populations L2 and S1, in which no host became infected, were of course not included in the analysis of phototaxis scores. Therefore, as previously mentioned, we did not analyze the watershed level.

Parasite intensity had no effect on phototaxis scores, either globally or when analyses were conducted separately for each population (Kruskall–Wallis, all $P > 0.07$). All individuals were thus analyzed together. Phototaxis scores at the "young cystacanth" stage were not significantly different between host (Kruskal–Wallis: $\chi^2_3 = 2.50$, $P = 0.47$) or between parasite populations (Kruskal–Wallis $\chi^2_5 = 9.53$, $P = 0.09$). Globally, the median phototaxis score at this "young cystacanth" stage was close to 0 in all combinations, and did not significantly differ from scores in uninfected controls (Table 4). "Local versus foreign" and "home versus away" comparisons gave similar results (Wilcoxon tests, all $P > 0.07$). Moreover, the various parasite populations did not differ in the modifications they induced in the naive host population at this stage (Kruskal–Wallis: $\chi^2_5 =$

Table 3. Generalized linear model and contrasts analyzing the infectivity of the different parasite populations on the different host populations, without L2 and S1 host populations but taking into account L2 and S1 parasite infections (whole model Likelihood Ratio. $\chi^2_{23} = 122.43$, $P < 0.0001$).

Source	df	L.R. χ^2	P
Host population	3	40.79	<0.0001
Parasite population	5	31.26	<0.0001
Host population × parasite population	15	25.72	0.039
Contrasts 1			
Sympatry versus allopatry, global	1	11.89	0.0005
Contrasts 2			
Local versus Foreign, L1 hosts	1	0.82	0.36
Local versus Foreign, M1 hosts	1	0.25	0.61
Local versus Foreign, M2 hosts	1	18.75	<0.0001
Local versus Foreign, S2 hosts	1	3.56	0.059
Contrasts 3			
Home versus Away, L1 parasites	1	1.39	0.24
Home versus Away, M1 parasites	1	13.74	0.0002
Home versus Away, M2 parasites	1	3.85	0.049
Home versus Away, S2 parasites	1	0.46	0.50

3.51, $P = 0.62$). However, the median phototaxis score in the naive population was significantly higher than that of the naturally infected populations (Wilcoxon: $Z = -2.54$, $P = 0.01$; Fig. 5A).

Phototaxis scores at the "old cystacanth" stage were significantly different among parasite populations, but not among host ones (Kruskal–Wallis: $\chi^2_5 = 13.65$, $P = 0.02$; and $\chi^2_3 = 1.98$, $P = 0.62$, respectively, Fig. 4A,B). Parasites from M1, M2, and S1 populations induced less alteration in light attraction (Fig. 4B). Conversely, no significant difference was found among parasite origins in the modifications they induced in the naive host population (Kruskal–Wallis: $\chi^2_5 = 3.92$, $P = 0.56$; Fig. 4E), the result remaining similar when the two groups with extremely small sample sizes were removed from the analysis (Kruskal–Wallis: $\chi^2_3 = 3.64$, $P = 0.30$). However, on average, this naturally uninfected host population was more manipulated than the naturally infected ones (Wilcoxon: $Z = -3.67$, $P = 0.0002$; Fig. 5B).

The global comparisons between sympatric and allopatric combinations showed no significant difference in manipulation (Wilcoxon: $Z = -1.93$, $P = 0.07$). The within-population "local versus foreign" comparisons showed that only the M1 host population reacted differently to allopatric versus sympatric infections, with foreign parasites inducing more pronounced behavioral alteration than local ones (Wilcoxon: M1 population: $Z = -2.79$, $P = 0.005$; other populations: all $P > 0.56$; Fig. 4C). The "home versus away" population-by-population comparisons showed no difference between parasites infecting sympatric or allopatric hosts (Wilcoxon: all $P > 0.40$; Fig. 4D). It is worth noting that parasites from the M1 population always induced very weak phototaxis scores, explaining the absence of

Table 4. Phototaxis score comparisons in host populations, between individuals infected by all parasites origins and control individuals, at "young" and "old cystacanth stage" (Wilcoxon tests).

Host population	Cystacanth stage	Status	Phototaxis median	N	Z	P
L1	Young	infected	0.5	20	1.47	0.14
		control	0	23		
	Old	infected	3	17	4.18	<0.0001
		control	0	22		
M1	Young	infected	0	48	−1.26	0.21
		control	0	16		
	Old	infected	4	39	−3.25	0.001
		control	0	15		
M2	Young	infected	1	28	−1.78	0.08
		control	0	28		
	Old	infected	2	23	3.75	0.0002
		control	0	25		
S2	Young	infected	0	9	0.26	0.80
		control	0	23		
	Old	infected	5	9	2.66	0.008
		control	0	18		
V	Young	infected	1	109	−2.00	0.045
		control	0.5	30		
	Old	infected	7	98	−5.86	<0.0001
		control	0	29		

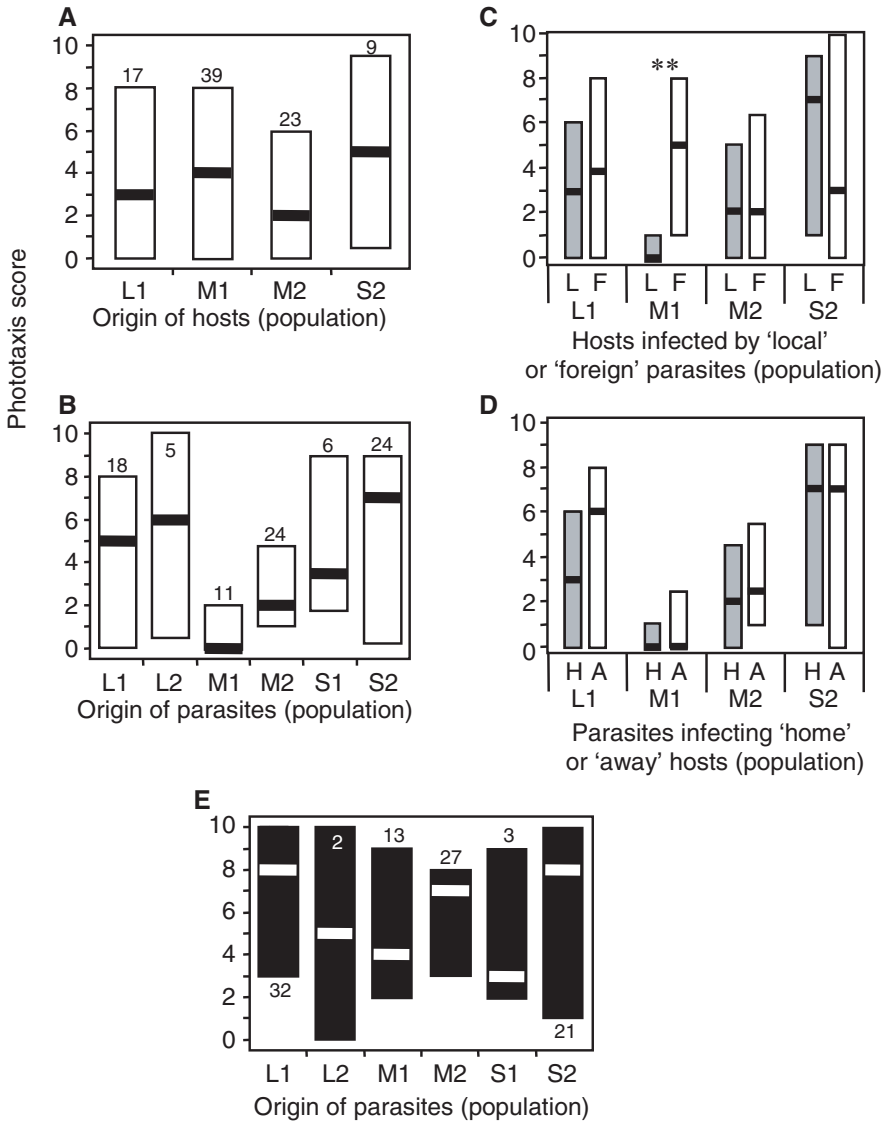

Figure 4. **Phototaxis scores in *G. pulex* hosts infected by *P. laevis* parasites, at "old" cyctacanth stage. (A) Phototaxis scores according to the different naturally infected host populations (all parasite sources taken together). (B) Phototaxis scores according to the different parasite populations (all naturally infected host populations taken together). (C) and (D) show "local versus foreign" and "home versus away" comparisons for Phototaxis scores, respectively. E: Phototaxis scores according to the different parasite populations, in hosts from the naive population. Boxes are interquartile ranges and thick lines are medians. Numbers above or below the bars are sample sizes. Stars highlight significant differences in contrast comparisons, see text for details and other comparisons. (**$P < 0.01$).**

manipulation in the sympatric combination in the "local versus foreign" analysis.

Comparison between infected and control hosts in the four naturally infected host populations showed that these gammarids were manipulated at the "old cystacanth" stage only (Table 4). On the contrary, gammarids from the naive population were manipulated at both "young" and "old cystacanth" stages, although more noticeably at the "old" stage (Table 4). Comparisons of the different parasite capacities to induce manipulation (i.e., comparisons between control hosts and hosts infected by parasites from each population) revealed more variation (Table 5). Parasites from the M1 location never manipulated their hosts at either of the two cystacanth ages considered here. Gammarids infected by the other parasite populations displayed significantly higher phototaxis scores at "old cystacanth" stages. Parasites from L1, L2, and S1 did not induce manipulation at the "young cystacanth" stage whereas the M2 and S2 parasites did (Table 5).

Discussion

This experiment showed that parasites affect negatively host survival, and that the intensity of two parasite fitness-related

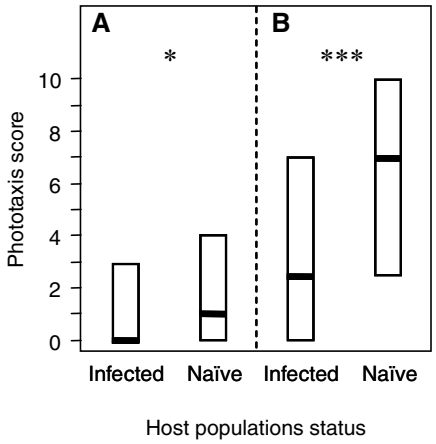

Figure 5. Phototaxis scores in *G. pulex* hosts infected by *P. laevis* parasites, at "young cystacanth stage" (A) and "old cystacanth stage" (B), in hosts from naturally infected populations (L1, M1, M2, S2) and hosts from the naive population. Boxes are interquartile ranges and thick lines are medians.

traits—infection rate and manipulation of host behavior—are variable according to the geographic origins of both *G. pulex* hosts and *P. laevis* parasites. Infection success showed a complex pattern of variation, with suggestion of adaptation of parasites to their local hosts in some populations, whereas no such pattern was found for behavioral manipulation.

HOST SURVIVAL

We found a significant negative effect of parasite infection on host survival, with a variation between parasite origins. Because we measured survival during the main growth phase of parasite development, this suggests that the virulence could be due to the parasites' high energetic demand. This is consistent with results obtained on another acanthocephalan-amphipod system (Duclos et al. 2006). The different gammarid populations also differed in their survival ability in laboratory conditions. These differences cannot be explained by differences in gammarid ages because the experiment was conducted on reproductive adult males for all populations. Mortality variation could be due to differences in environmental factors experienced by the gammarids before their quarantine in the laboratory, inducing different levels of stress in laboratory conditions.

INFECTION SUCCESS

Major variations were found between populations in the sensitivity of hosts to parasite infection. This is consistent with the among-population variation observed in *G. pulex* in their

Table 5. Comparisons of phototaxis scores induced by the parasite populations, between infected and control hosts (all host origins being grouped), at "young" and "old cystacanth stage" (Wilcoxon tests).

Parasite population	Cystacanth stage	Status	Phototaxis median	*N*	*Z*	*P*
L1	Young	infected	0	19	1.25	0.21
		control	0	90		
	Old	infected	5	18	5.11	<0.0001
		control	0	80		
L2	Young	infected	0.5	6	1.12	0.26
		control	0	90		
	Old	infected	6	5	3.43	0.0006
		control	0	80		
M1	Young	infected	0	13	−0.59	0.55
		control	0	90		
	Old	infected	0	11	0.65	0.52
		control	0	80		
M2	Young	infected	1	31	2.85	0.004
		control	0	90		
	Old	infected	2	24	5.65	<0.0001
		control	0	80		
S1	Young	infected	0	8	−1.05	0.29
		control	0	90		
	Old	infected	3.5	6	4.70	<0.0001
		control	0	80		
S2	Young	infected	1	28	2.54	0.01
		control	0	90		
	Old	infected	7	24	5.91	<0.0001
		control	0	80		

investment in immune defenses (Cornet et al. 2009). The variation could also be due to host ability to avoid infected food, but we ensured that gammarids ate the entire leaves. However, because we cannot control how many parasite eggs were ingested by each gammarid, we consider resistance in its broader sense, including behavioral and immune resistance.

Parasite origin also significantly affects infection success. One would expect to find the most infectious parasites in populations in which hosts are the most resistant, because of a reciprocal evolution of host resistance and parasite infectivity (e.g., Buckling and Rainey 2002). However, parasites from the resistant host populations were actually the least infectious, among all hosts investigated. An explanation for this finding would necessitate a better understanding of *P. laevis*'s local ecology. For example, *P. laevis* can infect other gammarid species, and particularly *Gammarus roeseli* that can live in sympatry with *G. pulex* (e.g., Moret et al. 2007). The presence of such other potential hosts can vary between populations, and if the usual intermediate host becomes too resistant to infection, it could be advantageous for the parasite to shift toward another host. Further studies should thus investigate infection success by *P. laevis* in *G. pulex* according to the presence of other intermediate host species.

Pomphorhynchus laevis infectivity was higher when infecting their sympatric *G. pulex* hosts in populations of the Meuse watershed. Because we are not able to breed both hosts and parasites in the laboratory, we are not able to distinguish clearly if the local adaptation pattern had a genetic basis, if it is due to a maternal effect or if it is due to differences in the quality of the environment of origin (Hochberg and Van Baalen 1998; Kaltz and Shykoff 1998; Kawecki and Ebert 2004; Lopez-Pascua and Buckling 2008). Maternal effects were limited by our experimental design, but differences in the environment quality among the six host and parasite populations are likely. The variation in host survival under laboratory conditions indeed suggests different adaptations of gammarids to their local natural environment (e.g., differences in water quality and/or ecosystem productivity). However, there was no clear link between host survival and either host sensitivity or parasite infectivity, which suggests no link between local adaptation pattern and the environment quality. On the other hand, our results are consistent with a pattern of genetically based local adaptation in parasite infectivity. The adaptation pattern fits with the prediction that the higher migration rate of the parasite, relative to its intermediate host, should lead to the local adaptation of the parasite (see Introduction). In addition, the virulence imposed by *P. laevis* is consistent with the host's interest in resisting infection, a prerequisite to an arms race.

However, because local adaptation was not found in all the populations investigated, the *P. laevis–G. pulex* system resembles a geographic mosaic pattern of coevolution. Several hypotheses, that remain to be tested, could explain this pattern. There could be a variation between rivers in the dispersal potential of both hosts and parasites. Different positions in the coevolutionary cycles may explain such a mosaic as well. Such a desynchronization could be favored by variation among rivers in the selective pressures imposed by parasites on their hosts. For example, as noted above, it is likely that there are variations in the *P. laevis* host assemblages. If other intermediate hosts take an important part in the transmission of *P. laevis*, the strength of a reciprocal selection involving *G. pulex* could be lowered, generating coevolutionary cold spots (e.g., Thompson and Cunningham 2002). These phenomena are not exclusive and could act in synergy to produce the observed pattern.

Our study also revealed that gammarids from the unexposed population were more susceptible to *P. laevis* infection than those from naturally infected populations. Even though this result is based on one naive population only, Hasu et al. (2009) found a similar pattern in another crustacean-acanthocephalan interaction. This suggests that the driving of host resistance by *P. laevis* is not an isolated case in acanthocephalans.

BEHAVIORAL MANIPULATION

We found a variation in the intensity of manipulation according to parasite origin, with some populations inducing a strong manipulation whereas others failed to do so. However, because the intensity of manipulation increases with the duration of the infection (Franceschi et al. 2008; this study), the fact that M1 parasites did not manipulate their hosts 15 days after they reached the cystacanth stage does not necessarily mean that they will never be able to induce any behavioral alteration later in their maturation. Indeed, M1 parasites induced a significant manipulation in the naive hosts (Val-Suzon population). It is probably less extreme to consider that the behavioral manipulation is more rapid in some parasites than in others.

We found much less variation in behavioral manipulation according to host population. Hosts from the different naturally infected populations all had a similar level of behavioral sensitivity to parasites. Nevertheless, the hosts from the naive population were much more manipulated. This suggests that hosts that do not coevolve with *P. laevis* have a higher sensitivity to manipulation, whereas those that are naturally infected evolve a resistance to manipulation, which is consistent with the results on infectivity.

Only the M1 population shows a pattern of local adaptation according to the "local versus foreign" criterion (Fig. 4C). However, parasites from the M1 population were actually unable to manipulate any hosts, and this seeming resistance of hosts to their sympatric parasites is only due to the poor intrinsic manipulative ability of the parasites. Therefore, no pattern of local adaptation was found for behavioral manipulation, contrasting with that

observed for infectivity. The ability to efficiently manipulate several host (geno) types may be a fixed trait in a given parasite population. However, because this trait was variable among parasite populations, it does not necessarily mean that behavioral manipulation is not subject to ongoing selection. Several speculative explanations can be formulated to explain this pattern. First, the selective pressure imposed by the increased predation rate resulting from manipulation could be low enough to prevent host resistance on this trait. Such a hypothesis is supported by the overall low prevalence of *P. laevis* in the field (Lagrue et al. 2007; this study). Second, complexity and constraints inherent in the target of behavioral manipulation (i.e., host behavior) could prevent a rapid arms race. Behaviors are often the result of complex processes, with epistatic effects but also major genes with pleiotropic effects on different behaviors (Anholt and Mackay 2004). Behavioral manipulation often involves reversal of taxis (attraction to predator odors [Perrot-Minnot et al. 2007; Vyas et al. 2007], geotaxis [Ponton et al. 2006], phototaxis in the case of *P. laevis*, see this study). Such inversions are the result of changes in neuronal activities in the host brain or CNS (Helluy and Thomas 2003; Tain et al. 2007) but others physiological pathways are modified by manipulative parasites (e.g., Ponton et al. 2006). Therefore, if resistance to manipulation implies a mutation in a pleiotropic gene, also disrupting some central behaviors (reproductive or foraging behaviors for example), the host resistance could be too costly to be selected. Our results nevertheless suggest that hosts from a naive population are more sensitive to manipulation than hosts from naturally infected ones. This may mean that resisting or regulating parasite manipulation would be possible up to a certain threshold, not allowing for a rapid response to parasite counter-adaptation.

The main selective pressure explaining the variation between parasite populations in their ability to induce behavioral manipulation remains to be detected. Manipulation may induce costs to parasites (Mouritsen and Poulin 2003; Tompkins et al. 2004; Poulin et al. 2005) and may thus be counter-selected if there is another way to improve parasite transmission. The presence of other intermediate host species, and a local specialization of *P. laevis* on these hosts, could potentially explain why parasites inefficiently manipulate the behavior of *G. pulex*. However, the other main potential host in eastern France, *G. roeseli*, while infected by *P. laevis*, is known to be unaffected by manipulation on a large geographic scale (Moret et al. 2007). Our experiment also revealed the intriguing result that parasites unable to efficiently manipulate *G. pulex* came from the population in which the prevalence was the highest, not because parasites were the most infectious there, but because hosts were the most sensitive to the infection (M1 population). This stands in contradiction with one of the predictions formulated by Poulin (1994), suggesting that the optimal investment in manipulation should increase with parasite prevalence. However, Poulin (1994) postulated that parasites are under pressure to evolve toward higher levels of manipulation only when prevalence is near fixation, which was far from being the case in our study. In addition, we can also consider that a higher prevalence leads to a higher probability of passive transmission of individual parasites. In that case, under the hypothesis that manipulation is costly, the selective pressure to manipulate the host is very low (Poulin 1994). Therefore, in the M1 population, parasites would not need to invest in manipulation to improve their transmission rate, as hosts are already highly susceptible to infection. Although anecdotal, this observation deserves attention as a perspective for future research.

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