Effect of parasite-induced behavioral alterations on juvenile development

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Like many trophically transmitted parasites, the trematode *Microphallus papillorobustus* alters the behavior of its intermediate host, the crustacean gammarid *Gammarus insensibilis*, in a way that favors its vulnerability to definitive hosts (aquatic birds). Parasitized females still produce eggs, but because juvenile development occurs inside the female marsupial brood pouch, young gammarids are subject to the same risk of predation as their mothers until they exit the marsupium. We explored the idea that developing juveniles can adjust their developmental schedule in a state-dependent manner according to the parasitic status of the mother. We predicted that juveniles from parasitized females would accelerate their development, or exit the marsupium at an earlier stage, to avoid predation by birds. Contrary to our expectations, we observed the opposite, that is, juveniles from parasitized females exited the marsupial brood pouch significantly later than those from uninfected mothers. We discuss these results in relation to current ideas on host manipulation by parasites in ecosystems. *Key words:* amphipod, developmental schedule, manipulative parasite, maternal effect, predation, trematode. *[Behav Ecol 20:1020–1025 (2009)]*

The role of parasites in the evolution of host life-history traits is a question that has attracted considerable interest in evolutionary ecology (Møller 1997). By definition, parasites are costly to their hosts because they exploit resources that could otherwise be channeled into host growth, maintenance, or reproduction (Price 1980). Direct costs resulting from this exploitation are a first cause of between-individual or between-population variations in life-history traits such as fecundity, growth, or survival (for a review, see Møller 1997; Thomas, Guégan, et al. 2000; Sorensen and Minchella 2001).

Alternatively, changes in host life-history traits after infection can also be adaptive responses to parasitism (Minchella 1985; Hurd 2001). Hosts that are unable to resist infection by other means (e.g., immunological resistance or inducible defenses) are favored by selection if they partly compensate for the parasite-induced losses by adjusting their life-history traits. This prediction is now supported by several theoretical and empirical examples (Minchella 1985; Hochberg et al. 1992; Forbes 1993; Michalakis and Hochberg 1994; Møller 1997; Sorensen and Minchella 2001). For instance, parasitized hosts can adaptively alter their reproductive effort before dying or being castrated, by either enhancing immediate fecundity (Minchella and Loverde 1981) or reducing age at maturity

(Lafferty 1993; Michalakis and Hochberg 1994; Sorci et al. 1996; Agnew et al. 1999; Fredensborg and Poulin 2006). Parasitized hosts also have the potential to adjust life-history traits such as dispersal (Sorci et al. 1994; Heeb et al. 1999; Lion et al. 2006), growth schedule (Sousa 1983; Minchella 1985), and sexual behavior (Polak and Starmer 1998; Adamo 1999).

Beyond selection for responses which alleviate the direct impact of parasites on infected hosts, there is the case of adaptive transgenerational phenotypic plasticity, in which parents provide their offspring with phenotypes to cope with, resist to, and/or avoid infections (see Sorci and Clobert 1995; Rolff 1999). For instance, parental infection has been found to enhance offspring immunity in both vertebrates (e.g., Hanson 1998) and invertebrates. Moret (2006) showed that parental challenge in the mealworm beetle enhanced offspring immunity through the inducible production of antimicrobial peptides in the hemolymph. In addition to parental influences, offspring themselves can, in theory, perceive cues correlated with parasitism and/or its consequences and adjust their own developmental strategies accordingly (Poulin and Thomas 2008). Adaptive responses by the progeny in parasitized individuals can then be the product of natural selection acting on the parent as well as on the descendant genomes.

Gammarus insensibilis, Stock 1966 (Amphipoda), is one of the most common invertebrate species in the salt marsh ecosystems of southern France (Brun 1971). *G. insensibilis* from southern France lagoons is frequently parasitized by the trematode *Microphallus papillorobustus*, Rankin 1940 (Microphallidae; Helluy 1981, Thomas, Renaud, Derothe, et al. 1995). This parasite has a complex life cycle including

snails from the genus *Hydrobia* as first intermediate hosts, *G. insensibilis* as second intermediate host, and various aquatic birds as definitive hosts (Rebecq 1964). *M. papillorobustus* is a manipulative parasite for gammarids: infective larvae, called cercariae, migrate into the amphipod's brain, encyst in the cerebroid ganglia, and then induce strong behavioral alterations in the host (i.e., positive phototaxis, negative geotaxis, and an aberrant evasive behavior). Parasitized gammarids are typically found near the surface water (Ponton, Biron, Joly, et al. 2005), a behavior that renders them more susceptible to predation by small wading birds (definitive hosts of the parasite; Helluy 1981, 1984; Thomas, Renaud, Derothe, et al. 1995). Life-history theory suggests that optimal timing for juveniles to exit the maternal marsupium should be based on the optimal balance between maximizing growth and minimizing mortality. Because juveniles are exposed to the same predation risk as their mothers during all the developmental period, we predicted that those developing inside parasitized females should exit the brood pouch earlier, thus avoiding avian predation, by either accelerating their development or exiting at an earlier developmental stage. An alternative hypothesis that is nonadaptive for the host is that juvenile development is prolonged because the parasite somehow disrupts the reproductive capacity of the mother: It has been shown that *M. papillorobustus* imposes important costs on host reproduction (Thomas, Renaud, Derothe, et al. 1995). We conducted an experiment under controlled conditions in which we disentangled the influences of parasite and microhabitat on the responses displayed by juveniles.

MATERIALS AND METHODS

Collection and maintenance of specimens

A large sample of pairs of *G. insensibilis* (ca. 200) in precopula mate guarding was randomly collected during April 2004 in the Thau lagoon (southern France, 43°25′N, 3°35′E) following Thomas, Renaud, Derothe, et al. (1995). The reproductive biology of *G. insensibilis* (described in Helluy 1981) is similar to that of the majority of *Gammarus* species (Sutcliffe 1992). Males select females close to their moults and guard them until fertilization of eggs is possible. After insemination, the male generally guards the female for a few hours before abandoning her. Fertilized eggs then develop in the female's brood pouch.

Pairs with infected males were identified in the field through their aberrant occurrence at the water surface. Assuming that assortative pairing based on infection status predominated in the field (see Thomas, Renaud, Derothe, et al. 1995; Thomas, Renaud, and Cézilly 1996), we expected that pairs captured at the surface of the lagoon would comprise both infected males and infected females and, on the other hand, that pairs captured at the bottom would consist of uninfected males and uninfected females. In the laboratory (Station Méditerranéenne de l'Environnement Littoral, Sète), pairs were kept isolated in small plastic cups (2 cm diameter, 5 cm height) in large tanks (diameter 1.5 m, 1 m depth) with a continuous flow system with aerated water from the Thau lagoon (18 °C, 38°/°°), until mating occurred and the females moulted. The top and bottom of the cups were closed with a plankton net so that tank water could circulate freely through the cups. After insemination, the male of each pair was sacrificed by exposure to −80 °C for a few seconds, and its head was dissected in order to confirm parasitic status. The metacercariae of *M. papillorobustus* are permanent ovoid cysts (270 × 350 μm; Rebecq 1964) located within the amphipod brain (Helluy 1981). Females from the bottom were kept only if their partners were uninfected, whereas females from the surface were kept only if their partners were infected.

Experimental design

Parasitized gammarids are typically found near the surface in open water, whereas uninfected ones are found in the benthic zone, hidden under algae. Thus, at least 2 environmental parameters differ for infected and uninfected individuals: light and depth. We assessed, both separately and jointly, the effects of depth, lighting, and mothers' parasitic status on the developmental schedule of juveniles. For this, we placed presumed infected and uninfected fertilized females in 4 different treatments: 1) light surface (control for infected females), 2) light bottom, 3) dark surface, and 4) dark bottom (control for uninfected females; Figure 1). To manipulate the level of exposure to light, we used transparent tubes and dark opaque tubes, painted black; half of each kind of tube was placed in the surface and at the bottom (1m depth) of a large tank filled with water from the Thau lagoon. The experiment started with 20 replicates for each of the treatments. Therefore, after insemination, 20 females presumed parasitized and 20 presumed uninfected were placed in each of the 4 different treatments described above. The experiment took place in a room exposed to the natural photoperiod. The cups were examined and cleaned daily and provided each time with an excess of fish food (Tetra AniMin). The experiments finished when the females moulted again, which corresponds to one episode of reproduction. During the first 3 days of the experiment, dead females were replaced. At the end of the experiment, all females were preserved in 70% ethanol (EtOH) (v/v), measured in length (from head to tip of telson), and dissected in order to verify their parasitic status.

For each female, we recorded the intermoult duration (in days). Intermoult duration is the maximum period of time in which juveniles can develop. The total number of emerged viable juveniles was counted daily and preserved in 70% EtOH (v/v). We defined as the peak day the day on which the majority of juveniles (more than 50%) was released. The time between the female's last moult and the peak was an estimator of the length of the juveniles' development. We randomly selected one juvenile among those that emerged on the peak day as representative of the brood to which it belonged. In order to determine developmental stage, these representative juveniles were measured in length (from head to tip of the third metasomal segment), and the number of articles of both antennae was counted under a stereomicroscope (Helluy 1981). The
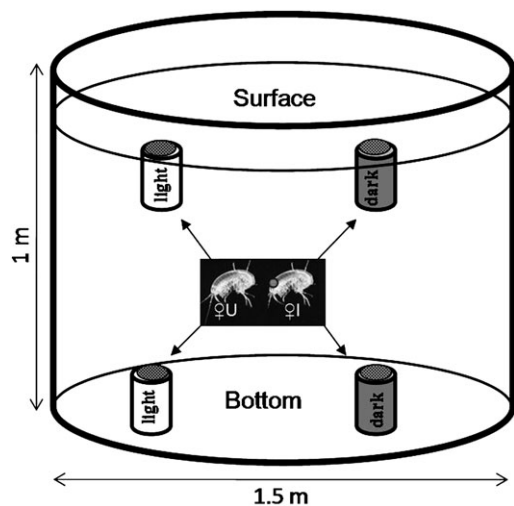

Figure 1
Experimental procedure (♀U: uninfected gammarid females, ♀I: infected gammarid females).

length of the juveniles was measured on digital standardized pictures (Olympus Camedia C-5060 wide zoom, 5.1 megapixels, magnification ×4 on a stereomicroscope Olympus SZ61, ×45) using Image J software. To verify that measurement error, due to the focusing of the numeric camera, was small enough to detect size differences between juveniles from different females, body length of 10 juveniles from the marsupium of 1 infected female and 10 juveniles from the marsupium of 2 uninfected ones were measured twice on each photograph. The estimated percentage of error obtained was 18.66% following the methodology described by Bailey and Byrnes (1990).

Data analysis

We used 1-way analyses of variance (ANOVAs) to study the influence of infection status and parasitic load on female body length. To identify factors acting on the number of juveniles and on female intermoult duration, we used 2 linear models. Explanatory terms considered in these models were maternal infection status, maternal size, depth, and light. We also considered second-order interactions. More precisely, full models were constructed as follow:

Number of juveniles (or intermoult duration) ~ status
+ maternal size + depth + light + status : depth
+ status : light + depth : light.

The variable "status" refers to the maternal infection status. The symbol ":" means that the 2 terms surrounding the symbol are considered as well as their interaction.

For each linear model performed, stepwise selection procedures were used. At every step, the normality of residuals was evaluated by the Shapiro test, and the homogeneity of variance and independence in residuals were checked using the Fligner test (Conover et al. 1981) and the Durbin and Watson (1950) test, respectively. In cases of assumption violability, due to a few outliers, the corresponding data were excluded (maximum 2 of 99 individuals). Note that including outliers in the analysis did not lead to different conclusions.

Concerning juveniles, the length of their development and their size have been studied. The variability on the number of days for the development of juveniles was too low to be directly studied in a regression model. Therefore, a dummy variable was used to study juvenile development duration. This variable indicated, for each individual, whether or not the length of

juvenile development was greater than the average value in our populations. Then, this variable was used as the response variable of a generalized linear model (GLM) with a logit link and binomial error distribution. This model family allows to take into account the binary structure of the response variable. Explanatory terms considered in this model were maternal infection status, maternal size, maternal intermoult duration, depth, and light. We also considered second-order interactions.

The full model was constructed as follow:

Length of juvenile development ~ intermoult + status
+ maternal size + depth + light + intermoult : status
+ status : maternal size + status : depth
+ status : light + depth : light.

In GLM with a binomial error distribution, testing covariate effects involve a chi-square-distributed statistic (Crawley 2007). Assumptions concerning the error distribution were checked by estimating dispersion parameters in GLM; no significant overdispersion was detected. The size of juveniles was studied by using a linear model as follows:

juvenile size ~ (depth + light + mothersize) × status
+ light : depth.

All statistical analyses were performed using the software R (R Development Core Team 2008).

RESULTS

Females' biological characteristics

Among the 99 females analyzed (see Table 1), 41 were uninfected and 58 were infected. The mean body size of females was not significantly different among the 8 different categories of the experimental design (ANOVA, $F_{7,91} = 1.20$, $P = 0.31$) nor was the parasitic load of infected females (ANOVA, $F_{3,56} = 0.13$, $P = 0.72$). As expected, the linear model shows that the number of juveniles increased with maternal size ($F_{1,94} = 226$, $P < 0.0001$) and that uninfected females produced more juveniles than infected females ($F_{1,94} = 16.3$, $P = 0.0001$). In this minimal model, the parasitic status and the maternal size together explained 72% of variance in the number of juveniles produced. The light,

Table 1

Biological characteristics of *Gammarus insensibilis* females infected or not infected by *Microphallus papillorobustus* in relation to experimental treatment

	Bottom				Surface			
	Infected		Uninfected		Infected		Uninfected	
	Light $N = 18$	Dark $N = 15$	Light $N = 11$	Dark $N = 11$	Light $N = 11$	Dark $N = 14$	Light $N = 9$	Dark $N = 10$
Female size (mm)	14.24 ± 0.4	13.17 ± 0.4	12.76 ± 0.5	13.46 ± 0.5	12.94 ± 0.5	13.31 ± 0.4	13.76 ± 0.5	13.55 ± 0.5
Parasite intensity	3.0 ± 0.5	4.13 ± 0.6			3.18 ± 0.7	4.28 ± 0.6		
Number of juveniles	37 ± 4.3	24.8 ± 4.7	26.09 ± 5.5	34.72 ± 5.5	23.18 ± 5.5	17.14 ± 4.9	38.67 ± 6.1	33.90 ± 5.8
Maternal intermoult duration (days)	11.39 ± 0.28	11.27 ± 0.31	9.55 ± 0.36	10 ± 0.36	10.72 ± 0.36	11.14 ± 0.32	10.44 ± 0.40	10.10 ± 0.38
Juvenile size (mm)	1.33 ± 0.01	1.32 ± 0.02	1.30 ± 0.02	1.31 ± 0.02	1.28 ± 0.02	1.32 ± 0.02	1.32 ± 0.02	1.30 ± 0.02
Development time (days)	9.05 ± 0.15	9.46 ± 0.17	9 ± 0.2	8.91 ± 0.2	9.36 ± 0.2	9.5 ± 0.17	9.11 ± 0.22	9.2 ± 0.21

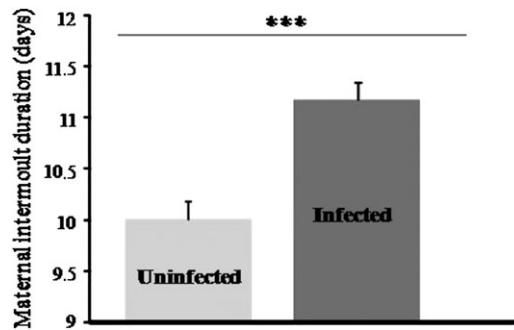Values are given as mean ± standard error.

Figure 2
Females intermoult duration in relation to parasitism status (±standard error).

depth, and second-order interactions considered had no effect on the number of juveniles (for all covariates, $P > 0.05$). Moreover, maternal intermoult duration increased with maternal size ($F_{1,94} = 20.4$, $P < 0.0001$), and infected mothers had longer intermoult duration than uninfected ones ($F_{1,94} = 41.0$, $P < 0.0001$). This latter result is also found when comparing directly the maternal intermoult duration between infected and uninfected females (Wilcoxon rank sum test, $W = 500$, $P < 0.0001$, Figure 2). In this minimal model, the parasitic status and the maternal size together explained 39% of variance of the maternal intermoult duration. Again, the light, depth, and second-order interactions considered had no effect on the intermoult duration (for all covariates, $P > 0.05$).

Juveniles' biological characteristics

Three variables significantly affected the length of juvenile development: the infection status (GLM, $\chi^2 = 4.18$, degree of freedom [df] = 1, $P = 0.041$), the intermoult duration (GLM, $\chi^2 = 10.6$, df = 1, $P = 0.001$), and the depth (GLM, $\chi^2 = 4.93$, df = 1, $P = 0.026$). More precisely, being infected, living at the surface and being the offspring of a female with a long intermoult duration, increased the probability that juvenile development was longer than average. According to raw data, offspring's average development time is increased by approximately 7 h when mothers are infected (mean development time is 9.33 or 9.05 days for infected or uninfected mothers, respectively). Although this average difference is small, 34% of the offspring from infected females left the brood pouch after the median development duration compared with 10% of offspring from uninfected females (Fisher's Exact test, df = 1, $P = 0.004$). Thus, 83% of offspring that left the brood pouch after the median development duration came from infected mothers. Accordingly, GLM predictions show that offspring from infected females have around 3.48 times more chance of leaving the brood pouch after 9 days than offspring from uninfected females, irrespective of depth and maternal intermoult duration.

Maternal intermoult duration has a positive effect on the development duration of offspring. According to raw data, maternal intermoult duration for offspring that left the brood pouch after the median development duration is, on average, 1 day longer than intermoult duration for offspring that left before the median development duration (mean intermoult duration is 11.46 days compared with 10.44 days after or before median development time, respectively; Wilcoxon rank sum test: $W = 433$, $P < 0.0001$). In addition, GLM predictions show that increasing the maternal intermoult duration by 1 day

leads to an increase in the odds of leaving the brood pouch after 9 days of 1.7 times.

Depth has a negative effect on the development duration of offspring. According to raw data, 33% of offspring growing at the surface left the brood pouch after 9 days, whereas 16% of offspring growing at the bottom did (Fisher's Exact test, df = 1, $P = 0.06$). Thus, 62.5% of offspring that left the brood pouch after the median development duration are growing at the surface. Accordingly, GLM predictions show that offspring growing at the surface have around 3.52 times more chance to leave the brood pouch after 9 days than offspring growing at the bottom.

None of the variables considered significantly affect the size of juveniles (for all covariates, $F < 3.2$, $P > 0.08$). In addition, development stage of the juveniles at the exit of the marsupial brood pouch was the same for infected and uninfected females: All juveniles showed 7 articles in their antennae.

DISCUSSION

A major challenge for life-history theory is to explain and predict the phenotypic variation in ages and sizes at transitions between life stages (Roff 1980; Stearns 1992; Berrigan and Koella 1994). Our study of *G. insensibilis* suggests that both maternal environment and parasitism by the manipulative trematode *M. papillorobustus* can have an effect on offspring life-history traits. However, contrary to our prediction, young gammarids did not reduce the time spent in the pouch of their infected mothers, either by accelerated development or premature release. Conversely, our results suggest that the timing of exit from the marsupial brood pouch is delayed for parasitized females and for mothers exposed to the surface environment.

Although statistically significant, the effects of both depth and infection status on the developmental period of juveniles seem small. To assess their biological significance, further work investigating the effect of developmental period on offspring fitness in the natural environment is required. Several explanations, adaptive or not, can potentially explain why juveniles from parasitized females remain in the marsupium longer. One of the simplest explanations is that predation rates may be too low for faster development to be adaptive. The predation risk for an amphipod is minute even after behavioral modifications induced by parasitism (Thomas, Renaud, Rousset, et al. 1995). The cost of leaving the brood pouch early may therefore be much higher than the gain from leaving early. However, data on the temporal dynamics of predation are not available, and thus, we cannot verify this explanation.

By manipulating the behavior of its host and forcing it to stay in the surface of the water, *M. papillorobustus* may indirectly protect it from predation by predators other than aquatic birds (e.g., fish). This could lead to uninfected amphipods suffering a higher rate of predation than infected ones. For young gammarids inside the maternal marsupium, the optimal balance between maximizing growth and minimizing mortality would then differ between parasitized and unparasitized females but not in the way a priori predicted. A lower (net) predation risk of gammarids at the surface could explain why juveniles from parasitized females stay longer inside the maternal marsupial compared with those of unparasitized females. How then can the fact that uninfected females do not naturally prefer living at the water surface be explained? This can be due to other fitness-related variables such as for instance the food abundance or the temperature. When all ecological factors are taken into account, surface habitats could be on average better than bottom habitats for developing embryos but worse for female fitness in the long term (e.g., lower number of reproductive episodes).

The idea that manipulated gammarids are less likely than uninfected conspecifics to die from predation by nonhost predators is indirectly supported by other studies (Levri 1998; Médoc et al. 2006). This suggests that, to understand the selective landscape in which manipulative changes and its evolutionary consequences occur, it is necessary to consider the manipulated hosts inside the ecosystem. Directs costs (for instance the increase in predation rate by predators that are hosts) and indirect consequences of being infected (for instance the decrease in predation rate by nonhost predators) can act in opposite directions so that the net fitness of infected individuals might be similar to or even greater than that of uninfected ones (Michalakis et al. 1992; Thomas, Poulin, et al. 2000). However, further investigations are necessary to evaluate the true predation rate by both fish and birds in infected and uninfected *G. insensibilis*.

Nonadaptive mechanisms could account for the longer development time of juveniles in parasitized individuals. Our study confirms that *M. papillorobustus* imposes significant costs on parasitized females, influencing several aspects of their reproductive biology. For example, infected females have a longer intermoult duration when compared with uninfected females and suffer a significant reduction in fecundity (producing fewer juveniles; see also Thomas, Verneau, et al. 1996). The longer development time of juveniles in parasitized females was observed under all experimental conditions (surface/bottom, light/dark) and was not associated with faster development. Body size and developmental stage at the exiting time were similar for all studied females, whatever their parasitic status. This suggests that the progeny of parasitized gammarids requires a longer time to reach the same size and development stage than juveniles from uninfected females. Because *M. papillorobustus* directly affects host physiological conditions, we must also consider the possibility that not only the number but also the quality of eggs produced by parasitized hosts is affected. In that case, an extended development would be necessary to compensate for the poor quality of eggs. This scenario is certainly nonadaptive for the host but it can be adaptive for the parasite: The decreased female host condition could represent a reallocation of host resources to parasitic growth.

The fact that juveniles from uninfected females have a longer development time when females are placed at the surface underlines the significant influence of environmental conditions on development, but it is in accordance with both the adaptive and nonadaptive hypotheses mentioned above. Indeed, we cannot exclude the possibility that surface conditions could be perceived by juveniles as a signal of reduced predation risk (i.e., parasitized females) to which they react by changing their exit date. Alternatively, surface conditions may also be stressful for juveniles. Additional experiments are necessary to determine which variables among those characterizing the surface conditions are most relevant and how they actually operate to generate the longer developmental time observed.

Overall, our study supports the hypothesis that the behavioral changes seen in this system are a result of manipulation *sensu stricto*, that is, the direct modification of host behavior. It has been recently suggested that instead of this strategy, certain parasites may select for collaborative behaviors in their hosts by imposing additional fitness costs in response to "disobedience" (Zahavi 1979; Soler et al. 1998; Ponton, Biron, Moore, et al. 2005; Lefèvre et al. 2008). Our experimental design allowed us to keep infected females in a situation of partial (i.e., light or depth) or total (i.e., light and depth) disobedience with respect to behavior favoring the transmission of the parasite. We found that all infected females suffered a reduction in direct fitness across all experimental conditions, suggesting that the behavioral change displayed by the gammarid is more the result of true parasitic manipulation (see also Helluy and Thomas 2003) than a compromise between the host and the parasite strategies.

In conclusion, this study does not support the initial prediction that juveniles from parasitized females accelerate their development to avoid predation by birds. The opposite result found is difficult to interpret as it may illustrate either a cost of parasitism or an adaptive phenomenon.

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