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Evidence for host variation in parasite tolerance in a wild fish population

S. Blanchet · O. Rey · G. Loot

Abstract Hosts can protect themselves against parasites by actively reducing parasites burden (i.e. resistance) or by limiting the damages caused by parasites (i.e. tolerance). Disentangling between tolerance and resistance is important for predicting the evolutionary outcomes of host-parasite interaction. Dace (*Leuciscus leuciscus*) are often parasitized by the ectoparasite *Tracheliastes polycolpus* which feeds on (and destroys) fins, reducing thus the host's condition. We tested the hypothesis that genetically-based variation in ecto-parasite tolerance exists in a wild dace population. We found that moderately heterozygous dace, which are less resistant than highly heterozygous or homozygous dace, tolerated better the effect imposed by *T. polycolpus* for a given parasite burden. However, tolerance also varied upon environmental conditions, suggesting that genetic and environmentally-based variation exists for both resistance and tolerance in this natural host-parasite system. Moreover, a negative genetic correlation may exist between tolerance and resistance, and hence several evolutionary outcomes are possible in this interacting system.

Keywords Resistance · Inbreeding · Heterozygosity-fitness correlations · Ectoparasite · Co-evolution · Arm race · Genetic correlation · Virulence · Pathogenic effects · Environmental effects · Rivers · Pathogens · Microsatellites

S. Blanchet (✉)
Station Expérimentale du CNRS à Moulis, U.S.R 2936, 09100 Moulis, France
e-mail: blanchet@cict.fr

S. Blanchet · G. Loot
Laboratoire Evolution et Diversité Biologique, U.M.R 5174, C.N.R.S, University Paul Sabatier,
118 route de Narbonne, 31062 Toulouse cedex 4, France

O. Rey
Centre de Biologie et de Gestion des Populations, Campus international de Baillarguet, CS 00016,
34988 Montferrier-sur-Lez cedex, France

Introduction

Hosts have evolved two types of defence mechanisms against parasites and pathogens. The first mechanism, resistance, actively reduces the parasite burden before or after infection has occurred. The second mechanism, tolerance, limits the damage caused by a given parasite burden (Koskela et al. 2002; Kover and Schaal 2002; Rausher 2001). The fitness of parasitized hosts may depend upon these two mechanisms. Although this possibility has been explicitly recognized by plant biologists in response to both grazing and infectious diseases (Fineblum and Rausher 1995; Restif and Koella 2004; Simms and Triplett 1994), very few studies have yet considered this possibility in animals. Indeed, both theoretical and empirical studies on animal-parasite interactions usually consider that host fitness only results from their ability to resist parasites (Antonovics and Thrall 1994; Boots and Bowers 1999).

Distinguishing between resistance and tolerance is however, critical when considering the ecological and evolutionary consequences of parasites and pathogens. From a host perspective, both mechanisms will enhance the fitness of the host and could hence be selected for. However, from a parasite perspective, the fitness consequences of these mechanisms are strikingly different. For instance, resistance diminishes parasite fitness and hence affects its evolution. However, tolerance will have a nearly neutral effect on parasite fitness if tolerance does not affect parasite optimal virulence, or can, on the contrary, have unpredictable effects if tolerance modifies parasite optimal virulence (Boots 2008; Miller et al. 2006; Roy and Kirchner 2000). Several theoretical studies have demonstrated that these fundamental difference are critical to predict the outcome of co-evolutionary processes between hosts and parasites (Rausher 2001; Råberg et al. 2009).

Assessing tolerance is a challenging task since its measurement readily depends upon the definition that is used (Roy and Kirchner 2000). For instance, in the laboratory, Råberg et al. (2007) studied host tolerance across a range of parasite burden to demonstrate—for the first time—that genetic variation exists for tolerance in an animal model, namely rodent malaria in lab mice. They further provided evidence that a negative genetic correlation exists between resistance and tolerance, such that a highly tolerant individual is necessarily weakly resistant (and vice versa). This empirical demonstration was rapidly followed by a genetic screen in the fruitfly *Drosophila melanogaster* which showed -by studying tolerance at a single parasite burden- that variation in both tolerance and resistance can originate from a single mutation (Ayres and Schneider 2008). Although indisputably elegant and innovative, these two studies, as well as most others suggesting a variation in animal tolerance to parasite (Allen et al. 1997; Corby-Harris et al. 2007; Ma et al. 1998; Williams et al. 2005), suffer one main limitation for their results to be generalized. Indeed, they have been performed in the laboratory on selected strains, where genetic and phenotypic variability is likely low and where parasite burden are often higher than what they can be in the wild. In addition, for natural populations, the effect of environmental variation on hosts might be strong enough to override or to interact with potential genetic specificities.

In a wild dace population (*Leuciscus leuciscus*), we have previously demonstrated genetically-based variation for resistance to a harmful ectoparasite, the copepod *Tracheliastes polycolpus* (Blanchet et al. 2009a). Only females of this species are parasitic, attaching to fins after having been fecundated by dwarf free-living males. During this parasitic phase (which spans between 1 and 2 months), females feed on the epithelial cells and mucus, incurring local lesions and the partial or total destruction of fins (see Fig. 1 in

Blanchet et al. 2009a). Following this, females lay eggs in the water column and die. There is no apparent infective seasonality, with dace being parasitized all year long. The overall pathogenic effect has several fitness consequences on dace, such as reducing feeding and growth rates (Blanchet et al. 2009b), and increasing the rate of mortality for young individuals (Blanchet et al. 2009a). However, dace that are either highly homozygous or heterozygous have lower parasite burdens (i.e. a higher resistance) than moderately heterozygous individuals (Blanchet et al. 2009a). In addition, dace can tolerate the parasite by limiting the level of fin degradation for a given number of parasites (e.g. through investment in cell regeneration). Therefore, the level of fin degradation is a reliable measure of "fitness" that can be used to compare tolerance between genetic sub-groups (Råberg et al. 2009 for a review).

In this paper, we tested the hypothesis that genetically-based variation in ectoparasite tolerance exist in this wild population of dace. Because resistance and tolerance have been shown to be negatively correlated with each other (Råberg et al. 2007), we predict that the genetic sub-groups found previously to be weakly resistant (i.e. the moderately heterozygous fish, Blanchet et al. 2009a) will exhibit high tolerance to this ectoparasite. This means that for a given parasite burden, the level of fin degradation will be lower in this genetic sub-group compared to the highly homozygous or heterozygous groups. This hypothesis was tested using the framework proposed in Råberg et al. (2007, 2009) and inspired from plant literature where tolerance is usually defined as the slope of host fitness against infection intensity (Simms and Triplett 1994).

Materials and methods

Sampling strategy

Adult and juvenile dace were sampled by electric-fishing at eight sampling sites in the river Viaur (South-Western France) in June 2006 ($n = 145$ dace) and 2007 ($n = 105$ dace). These sites cover the whole geographic distribution of dace in this river and show important levels of environmental variation (see Table 1 for details on the physical and chemical characteristics of these sites). For instance, difference in mean annual water temperature between the most upstream and downstream sites are greater than 4°C (Table 1). Upon capture, each fish was anesthetized, measured to the nearest mm, examined for parasite burden by counting the total number of parasites on fins and body surface, and scored for the level of fin degradation. To evaluate fin degradation, we scored 0, 1, 2, 3 or 4 points if a fin was 0, 25, 50, 75 or 100% eroded by the parasites, i.e. a score of 2 points means that fifty percent of the area of the fin was eaten by the parasites. The scores attributed to each fin were summed over all the fins to obtain a single total score (i.e. count data) of fin degradation per individual fish. The same observer (G.L.) scored the fin degradation for all dace. Parasite prevalence was 90.3% and 95.3% in 2006 and 2007, respectively. Parasite burden was 12.01 ± 4.67 (mean $\pm$ SD) in 2006 and 12.08 ± 2.42 (mean $\pm$ SD) in 2007. Score of fin degradation was 1.55 ± 2.48 (mean $\pm$ SD) in 2006 and 1.03 ± 1.48 (mean $\pm$ SD) in 2007. Finally, five scales and a small cut from one pelvic fin were used to determine individual age and genotype, respectively. After recovering from the anaesthesia, all fish were returned to their original sampling sites.

Table 1 Environmental description of the eight sites sampled for dace (*Leuciscus leuciscus*) and the ectoparasite *Tracheliastes polycolpus*

Sampling site	Distance from the source (km)	Slope (%)	Water velocity (m s^{-1})	Mean water temperature (°C)	Dissolved ammonia (mg l^{-1})	Dissolved Nitrates (µg l^{-1})	Phosphate (mg l^{-1})
Site 1	47.60	0.49	20.40	10.38	0.04	2.06	0.04
Site 2	53.00	0.65	18.31	10.92	0.06	6.43	0.06
Site 3	68.00	0.69	26.79	11.20	0.05	6.76	0.09
Site 4	71.00	0.44	23.31	11.51	0.04	6.74	0.09
Site 5	76.60	0.46	25.79	11.55	0.04	6.69	0.10
Site 6	95.00	0.47	41.73	11.67	0.04	8.05	0.13
Site 7	101.00	0.29	53.20	11.75	0.04	8.63	0.09
Site 8	130.20	0.20	42.00	14.58	0.04	8.04	0.06

The value is the mean of two sampling years, 2006 and 2006. Distance from the source as well as slope coefficient were recorded from a geo-references map. Water velocity was measured each year using a wadding rod and a specific recorder. Water temperature was continuously recorded over the 2 years using automatic data loggers, chemical parameters (dissolved ammonia, nitrates and phosphate) were sampled once a year and analysed in the laboratory

Laboratory analyses

Age estimation

Age was evaluated by counting the annual growth rings observed in the scales Francis (1990). Individual age was observed to vary between 1 and 11 years old. Because of this, ages were then pooled into six categories, (cat. 1 = age 1–3; cat. 2 = age 4; cat. 3 = age 5; cat. 4 = age 6; cat. 5 = age 7–8; and cat. 6 ≥ age 9). These categories were necessary because some ages (i.e. very young or very old individuals) were underrepresented in the dataset, potentially leading to statistical incoherence, particularly when testing for the interactions between predictors.

Genetic analyses

After DNA extraction, individual genotypes were obtained at 15 polymorphic microsatellite loci (see Blanchet et al. 2009a). No linkage disequilibrium or null alleles were detected in this set of microsatellites (see Blanchet et al. 2009a for further details). For each individual, we then calculated an index of multilocus heterozygosity (the internal relatedness, Amos et al. 2001). Internal Relatedness (IR) is a widely used measure of heterozygosity which estimates the relatedness of an individual's parents based on the extent of shared alleles relative to random expectations (Amos et al. 2001). IR often outperforms other heterozygosity indices when predicting individual fitness (Chapman et al. 2009). In our dataset, IR ranged from −0.208 to 0.550 (mean ± SD, 0.051 ± 0.133). Individuals with low IR values are the most heterozygous of the population. We have previously shown that individuals with moderate levels of IR harboured significantly more parasites (i.e. are less resistant) than highly heterozygous or homozygous individuals (Blanchet et al. 2009a). To test for genetically-based variation in tolerance, we arbitrarily divided our data in three categories according to individual IR (moderately heterozygous, −0.011 < IR < 0.082; extremely heterozygous IR < −0.011; extremely homozygous individuals, IR > 0.082). The arbitrary

splitting point for each category was chosen so that the three IR categories were represented by the same number of individuals. Splitting IR in categories has the advantage of improving visualizing and testing for slope differences that might exist between the different genetic categories (Råberg et al. 2007). However, transforming a continuous variable such as IR in categories might appear statistically illogical at first glance. Hence, to avoid misinterpretation due to the categorization of IR values, we performed the statistical tests using IR either as a continuous or a categorical variable (see below).

Statistical analyses

First, we tested if categorizing individuals according to their IR values reproduced correctly the genetically-based differences in resistance reported elsewhere (Blanchet et al. 2009a). To do so, we fitted a generalized linear model (GLM), where the parasite burden was the dependent variable and the sampling sites (with eight levels), age categories (with six levels) and IR categories (with three levels) were the predictors.

We then tested for genetically-based variation in tolerance in this dace population using the statistical framework described in Råberg et al. (2007, 2009). In statistical terms, tolerance can be defined as the slope of the relationship between parasite burden and host fitness (Zar 1999). Slope differences are assessed using ANCOVA-like analyses. Because degradation is a direct consequence of parasite burden, we used this measure as a surrogate of fitness. Variation in tolerance can also have an environmental (i.e. sampling sites) or developmental (i.e. age) basis (Råberg et al. 2009). To deal with this, we built a full model in which level of fins degradation was the dependent continuous variable, and parasite burden (continuous), sampling year, sampling site, age category and IR category were the categorical predictors. All two-way interactions between each categorical predictor and parasite burden were initially fitted to test for environmentally-based, developmentally-based and/or genetically-based variation in tolerance (a significant interaction indicates slope differences between categories). We also included the quadratic term of parasite burden to test for a non-linear relationship between parasite burden and fin degradation. Indeed, we can expect that the effect of parasite burden increases exponentially-like rather than linearly. Two-way interactions between the quadratic term and each categorical predictor were also fitted (Råberg et al. 2009). Parasite burden was log $(x + 1)$ transformed. Because categorizing an initially continuous variable can inflate type I errors, we also tested if similar results were obtained when IR was used as a continuous, rather than a categorical predictor. Because of the complexity of the full model, we used sequential log-likelihood ratio tests to simplify the model by removing non-significant terms (Crawley 2007). Such method also allows testing the influence of one variable independently of other confounding variables (Crawley 2007). Only the final simplified model is presented here. For all models we assumed a quasi-Poisson error term distribution to deal with over-dispersion of the dependent variable.

Results

As expected, the *a priori* genetic categories we used here provided similar patterns as those reported in Blanchet et al. (2009a). That is, moderately heterozygous hosts were less resistant since they harboured significantly more parasites than extremely heterozygous and homozygous hosts (GLM, $F_{2,\ 238} = 6.602$, $P = 0.002$, see Fig. 1).

Concerning variation in tolerance, none of the interactions involving the age of the hosts (i.e. age*parasite burden and age*[parasite burden]^2) and the sampling year (i.e. sampling
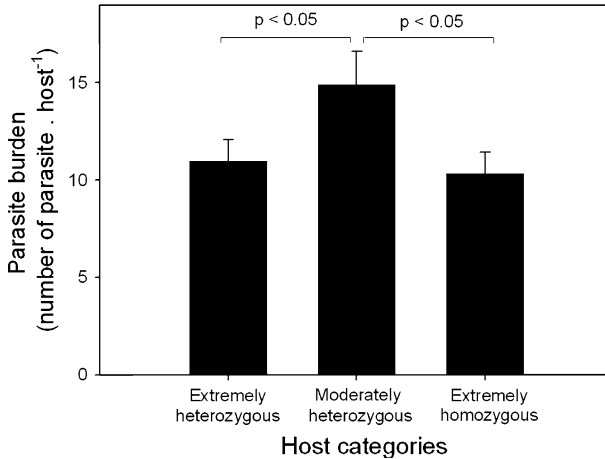
Fig. 1 *Barplot* showing the average parasite burden (mean + SE) of three genetic categories of dace (*Leuciscus leuciscus*). Significant pairwise comparisons are indicated ($P < 0.05$)

year*parasite burden and sampling year *[parasite burden]² were significant, indicating that there was no variation in tolerance among age categories and among years (results not shown). However, the final selected model included three significant interactions terms; IR categories*[parasite burden]², IR categories*parasite burden and sampling sites*parasite burden (see Table 2a). The two interactions involving IR categories indicated that the slopes of the non-linear relationship between parasite burden and fin degradation varied significantly among the genetic categories (Table 2a, Fig. 2a). This result suggests a genetically-based variation for tolerance in this fish population. Extremely heterozygous and homozygous fish had similar low parasite tolerance relative to the moderately heterozygous fish (Fig. 2a). This was particularly obvious in highly parasitized fish, because under high parasite burdens, the level of fin degradation was lower in moderately heterozygous fish than in extremely heterozygous and homozygous fish (Fig. 2a). The interaction involving sampling sites (Table 2a) further suggests that environmental variation also influences the level of tolerance variation in this population. As shown in Fig 2b, we detected striking differences among sampling sites in the relationship between parasite burden and fin degradation. For example in two sites (sites 1 and 2), fish had a lower tolerance to this ectoparasite (Fig. 2b). Similar results were obtained using IR as a continuous, rather than a categorical predictor (see Table 2b). The only notable difference was found for the interaction term between sampling sites and the quadratic term for parasite burden. This term was significant when IR was considered as continuous (Table 2b) while it was not when IR was included as a categorical factor. Despite this slight difference, we still found significant interactions between IR, parasite burden and its quadratic term, as well as between sampling sites and parasite burden (Table 2b).

Discussion

Hosts can use two different mechanisms to defend against pathogens and parasites: resistance and tolerance (Råberg et al. 2009). Typically, studies focus on understanding genetic variation for resistance. Here, we use a novel method to unravel genetically-based

Table 2 Output of generalized linear models aiming at testing the effect of several predictors on the level of fin degradation of dace (*Leuciscus leuciscus*) induced by the ectoparasite *Tracheliastes polycolpus*

	df	*F* values	*P* values
(a) IR as a categorical factor			
Null			
Sampling sites	7,238	11.11	**<0.001**
Age	5,236	2.76	**0.019**
Parasite burden	1,232	3.43	0.065
[Parasite burden]2	1,232	0.66	0.419
IR categories	2,233	2.30	0.102
Parasite burden * IR categories	2,224	3.43	**0.033**
[Parasite burden]^2 * IR categories	2,224	4.78	**0.009**
Parasite burden * Sampling sites	7,229	2.28	**0.019**
(b) IR as a continuous factor			
Null			
Sampling sites	7,239	10.81	**<0.001**
Age	5,237	2.75	**0.019**
Parasite burden	1,233	4.25	**0.04**
[Parasite burden]2	1,233	1.26	0.262
IR	1,233	1.13	0.299
Parasite burden * IR	1,224	5.55	**0.019**
[Parasite burden]^2 * IR	1,224	5.31	**0.022**
Parasite burden * Sampling sites	7,223	3.26	**0.003**
[Parasite burden]^2 * Sampling sites	7,223	3.07	**0.004**

(a) The measure of multilocus heterozygosity (IR) was split in categories. (b) The measure of multilocus heterozygosity (IR) was included in the model as a continuous factor. Only the final models are showed. These models allow testing for genetic and environmental variation in parasite tolerance in this dace population. Bold *P*-values are significant at the threshold $\alpha = 0.05$

variation for tolerance in a wild fish host population. This is, to our knowledge, one of the first evidence that genetic variation for tolerance exists in natural hosts' population (Råberg et al. 2009).

We demonstrate that heterozygosity at supposedly neutral loci was not only a good genetic predictor of ectoparasite resistance (see Blanchet et al. 2009a), but also of ecto-parasite tolerance. This "genetic" effect was statistically independent of other confounding factors such as other environmental effects. Although quantifying genetically-based tol-erance is important for predicting the evolutionary outcomes of host-parasite interaction (Best et al. 2008), another important feature of the resistance-tolerance dynamics is to understand the genetic relationship between resistance and tolerance. We found that moderately heterozygous hosts that were less resistant than extremely heterozygous or homozygous hosts (Blanchet et al. 2009a) were better to tolerate the ectoparasite, and vice versa. As found elsewhere (Råberg et al. 2007), this suggests that a genetic correlation might exist between resistance and tolerance in this fish population. Further detailed studies are however, needed to confirm such a genetic correlation since our data cannot be used to make inferences about the genetic architecture of resistance and tolerance. Par-ticularly, in the near future we hope to use pedigree information to estimate levels of additive genetic variance for both resistance and tolerance (e.g. Theriault et al. 2007).
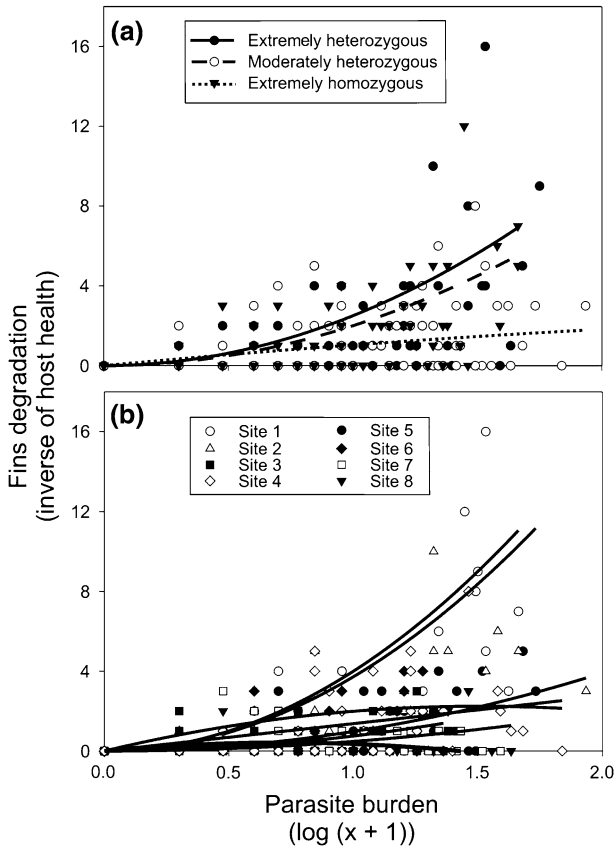
Fig. 2 Relationships between parasite burden [log $(x + 1)$ transformed] and the level of fin degradation of dace (*Leuciscus leuciscus*) according to **a** three genetic categories of dace and **b** the eight sampling sites. This figure illustrates that both genetic and environmental variation for parasite tolerance exist in this dace population

Tolerance is usually measured using fitness traits such as growth rate (Råberg et al. 2009). However, a major weakness in using growth rate in the wild is that this complex trait is under the control of several internal and external factors that are not necessary related to parasite infection. Moreover, it is hard in natural condition to control for co-infection, which can strikingly blur measures of tolerance when non-specific traits such as growth rate are used (Råberg et al. 2009). Causalities are in addition difficult to ascertain for such complex traits (Blanchet et al. 2009d). Indeed, does parasite burden affect growth rate? Or does growth rate affect parasite burden? This sort of chicken-egg dilemma is often difficult to resolve and can strikingly complicate interpretations within the resistance-tolerance framework (Blanchet et al. 2009b, d). Here, we take advantage that fins degra-dation is a "simple" trait that is weakly affected by external factors other than this ectoparasite. In other words, our host-parasite system constitutes a nice model for quan-tifying variation for tolerance in the wild. A disadvantage of our model is however, that, as a non-model species, it is still very hard to speculate for mechanisms responsible for the variation in host tolerance. Genome-wide heterozygosity (as measured by neutral markers,

but see Balloux et al. 2004) is thought to favour the expression of deleterious alleles that could directly or indirectly affect parasite burden (Coltman et al. 1999; Ortego et al. 2007). However, we previously found evidence that our set of loci was a weak surrogate of genome-wide heterozygosity (Blanchet et al. 2009a). Another possibility would be that a few number of loci are linked to effective genes (through linkage disequilibrium for instance, Balloux et al. 2004), and hence drive the relationships we observe. For instance, we can speculate that some loci could be linked to genes related to tissue repair and turnover or to the excretion of substances limiting ectoparasite grazing activity. Irrespectively of the mechanisms behind them, the relationships between tolerance, resistance and heterozygosity were non-linear. Non-linear relationships between heterozygosity and fitness traits have already been observed elsewhere (Neff 2004, Ortego et al. 2007, Blanchet et al. 2009c). A plausible explanation for such pattern might be that both positive and negative effects specific to individual loci may interact to shape individual resistance and the tolerance (Lieutenant-Gosselin and Bernatchez 2006, Blanchet et al. 2009c).

We also demonstrated an environmentally-based variation for tolerance in this host population. Although the specific environmental factors responsible for this variation are unknown, we have evidence that water temperature may play an important role in the host-parasite dynamics of this system (Blanchet and Loot, unpublished data). Water temperature is an important factor controlling the physiology, abundance and distribution of freshwater fish (Reyjol et al. 2001). It is for instance possible that non-optimal water temperature might be stressful for fish and hence favour harmful effects of parasites (Jokela et al. 2005). Obviously, the two sites that displayed the lowest tolerance levels (i.e. sites 1 and 2, see Fig. 2b) are also the sites were water temperature is the coldest (Table 1). Ecological implications of environmentally-based variation in parasite tolerance are important because they identify the environmental conditions that make populations more susceptible to parasites. Obviously, in one of the two sites that displayed a low tolerance level (site 1, Fig. 2b), dace density has severely declined since the last 3 years (Blanchet and Loot, unpublished data).

Disentangling between tolerance and resistance as two mechanisms for protecting against parasites has several evolutionary implications (Best et al. 2008). Particularly, if resistance is the trait under selection, host-parasite interaction will engage in an arm race, which does not necessarily hold if tolerance is the trait under selection (Best et al. 2008; Boots 2008; Read et al. 2008). In the wild fish population studied here, it is still not clear which strategy (to resist or to tolerate) is the most efficient to defend against this parasite. Blanchet et al. (2009b) have shown that parasite burden, but not fins degradation, decreases the host growth rate. It would mean that resistance should be under selection in this population, while tolerance would be rather neutral for the host fitness. Nevertheless, in highly parasitized populations such as the fish population studied here, it has been argued that tolerance would be the trait directly under selection (Restif and Koella 2004). Thus, it is possible that fish with high parasite burden had a lower growth rate not because of the effect of parasite burden *per se*, but because mechanisms limiting the grazing activity of ectoparasites are costly.

We here accomplish a first step by demonstrating that genetically-based variation exist for both resistance and tolerance in a natural host-parasite systems, and hence that several evolutionary outcomes are possible in these populations. From an evolutionary perspective, the next important step will be to understand which of these two traits is under selection in the host population. It would also be important to consider characteristics of the parasite such as virulence, because interaction between host genotype and parasite genotype are likely to occur (Ebert 1994; Lively and Dybdahl 2000). Decomposing the causal elements

of host fitness into host resistance, host tolerance and parasite virulence is a major challenge for future researches.

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