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Evidence of parasite-mediated disruptive selection on genetic diversity in a wild fish population

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Abstract

Identifying the processes maintaining genetic variability in wild populations is a major concern in conservation and evolutionary biology. Parasite-mediated selection may strongly affect genetic variability in wild populations. The inbreeding depression theory predicts that directional selection imposed by parasites should act against the most inbred hosts, thus favouring genetic diversity in wild populations. We have tested this prediction by evaluating the strength and shape of the relationship between the load of a harmful fin-feeder ectoparasite (*Tracheliastes polycolpus*) and the genome-wide genetic diversity (i.e. heterozygosity measured at a set of 15 microsatellites) of its fish host, the rostrum dace (*Leuciscus leuciscus*). Contrary to expectation, we found a nonlinear relationship between host genetic diversity and ectoparasite load, with hosts that were either homozygous or heterozygous harbouring significantly fewer parasites than hosts with an intermediate level of heterozygosity. This relationship suggests that parasites could increase the variance of global heterozygosity in this host population through disruptive selection on genetic diversity. Moreover, when genetic diversity was measured at each locus separately, we found two very strong positive associations between host genetic diversity and the ectoparasite load. This latter result has three main implications: (i) genome-wide effect cannot alone explain the nonlinear relationship between global heterozygosity and ectoparasite load, (ii) negative non-additive allelic interactions (i.e. underdominance) may be a mechanism for resisting ectoparasite infection, and (iii) ectoparasites may favour homozygosity at some loci in this host population.

Keywords: additive effects, ectoparasite, heterozygosity–fitness correlations, host–parasite interaction, host resistance, non-additive effects, underdominance

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Introduction

A clear evaluation of the relative role of the evolutionary processes shaping the genetic variability of wild populations is a primary goal in evolutionary and conservation biology (Kirschner & Gerhart 1998; Keller & Waller 2002; Frankham 2005; Whitlock 2008). Natural selection is one of the main processes by which genetic diversity might be maintained in wild population (Fisher 1930; Whitlock 2008). Parasites are arguably an important constituent of biological systems (Poulin & Morand 2000; Kuris *et al*. 2008), and it has been proposed that any selection they impose may considerably affect the genetic diversity of their host populations (Hamilton 1982; Poulin *et al*. 2000).

In the case of the loci involved in immune resistance (e.g. genes of the major histocompatibility complex, MHC), either non-additive genetic effects leading to balancing selection (i.e. overdominance of heterozygous) or additive genetic effects leading to frequency-dependent selection (i.e. selection for rare alleles, Clarke & Partridge 1988; Combes 2001) shape the genetic diversity of host species (Penn *et al*. 2002; Bernatchez & Landry 2003; Wegner *et al*. 2003). Nonetheless, as proposed by the inbreeding depression theory, parasites can also have an effect at the genome-wide level (Poulin *et al*. 2000; Keller & Waller 2002; Spielman *et al*. 2004). In this case, parasites would impose directional selection against the most inbred hosts. Inbred hosts are indeed

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more prone to express deleterious recessive alleles, have lower probabilities to carry adaptive alleles for resisting infection and have fewer chances to be heterozygous at genes under balancing selection (Coltman *et al*. 1999; Keller & Waller 2002). Since inbreeding is thought to intimately correlate with genome-wide heterozygosity, such a parasite-mediated directional selection should increase the whole genetic diversity of a host population.

Multilocus heterozygosity (MLH) measured at a set of neutral loci (e.g. microsatellites) is often used as a surrogate for genome-wide genetic diversity [i.e. global heterozygosity–fitness correlations (HFCs), global HFCs; Houle 1994; David 1997; Hansson & Westerberg 2002] to infer association between parasite load and host genetic diversity (Hansson & Westerberg 2002; Coltman & Slate 2003; Balloux *et al*. 2004). Several studies have validated the inbreeding depression hypothesis by detecting significant negative associations between parasite load and MLH (e.g. Coltman *et al*. 1999; Acevedo-Whitehouse *et al*. 2003; MacDougall-Shackleton *et al*. 2005; Ortego *et al*. 2007a), while some others have failed to do so (e.g. Côté *et al*. 2005; Ortego *et al*. 2007b). These failures might have biological causes (e.g. low pathogenic effects of the studied pathogens, Ortego *et al*. 2007b), but it is also possible that the set of microsatellites used to infer the relationship did not reflect perfectly the genome-wide genetic diversity. Indeed, theoretical and empirical studies have shown that MLH (when measured at a relatively small set of markers) was a poor estimator of the individual's inbreeding coefficient (Balloux *et al*. 2004; Markert *et al*. 2004; Bensch *et al*. 2006). Moreover, Väli *et al*. (2008) recently demonstrated, in four wild mammal species, that microsatellite marker heterozygosity was uncorrelated with the genome-wide genetic diversity at the individual level. These observations have led to the hypothesis that global HFCs might be better explained by physical associations, through linkage disequilibrium, between a single or few neutral markers and effective genes experiencing balancing selection (i.e. the local-effect hypothesis, David 1998; Hansson & Westerberg 2002) rather than by a genome-wide effect (i.e. the general-effect hypothesis, David 1998; Hansson & Westerberg 2002). In this case, even if there is no (or weak) variation in the inbreeding coefficient in a population, random segregation of chromosomes at meiosis can cause variation in the genome-wide level of homozygosity (Hansson & Westerberg 2002; Hansson *et al*. 2004). A way to test the local-effect hypothesis is to assess the shape and significance of the correlations between each locus and fitness traits (i.e. single-locus HFCs, Balloux *et al*. 2004; Lieutenant-Gosselin & Bernatchez 2006). According to the relative occurrence and strength of positive and negative single-locus HFCs in the data set, it has been demonstrated that positive, null and even nonlinear global HFCs can arise without invoking inbreeding depression (Lieutenant-Gosselin & Bernatchez 2006; Blanchet *et al*. 2009a).

In this current study, we examined the relationships between the intensity of a harmful fin-feeder ectoparasite (the copepod *Tracheliastes polycolpus*) and the microsatellite marker heterozygosity of its threatened fish host, the rostrum dace (*Leuciscus leuciscus*), to determine if parasites can influence the genome-wide genetic variability of their host population. This has been achieved by considering both global and single-locus HFCs (Lieutenant-Gosselin & Bernatchez 2006). Ectoparasites are widespread in fish; they can be highly pathogenic and hence provoke major mortality outbreaks in both wild and farmed fish populations (Chai *et al*. 2005; Costello 2006; Wagner *et al*. 2008). The worldwide development of industrial aquaculture has led to a global awareness of the necessity to understand the genetic causes and consequences of ectoparasite infection in wild fish populations (Krkošek *et al*. 2007). Rostrum dace is a threatened cyprinid fish species living in cold-water streams or rivers in Western Europe and are often infected by *T. polycolpus*. Only adult females are parasitic and they attach to fins where they feed on the epithelial cells and mucus. The grazing activity of *T. polycolpus* damages the fins, resulting in local lesions and their partial or total destruction [see Fig. 1 and Loot *et al*. (2004)]. These direct pathogenic effects reduce the fitness of their hosts by enhancing local bacterial inflammation (see Fig. 1b), reducing feeding success and decreasing the growth rate of infected hosts (Blanchet *et al*. 2009b).

According to the inbreeding depression hypothesis, we expected to detect a significant negative relationship between host heterozygosity and ectoparasite load, indicating that parasites might maintain a high genetic diversity in their host population through directional selection for heterozygous individuals (Coltman *et al*. 1999). Contrary to expectation, we demonstrated that, at the MLH level, large ectoparasite loads were significantly associated to hosts with an intermediate level of heterozygosity. These results provide new insight into the way that parasites could mediate selection on genetic diversity in host populations. Furthermore, by analysing single-locus HFCs, we detected strong positive associations between host genetic diversity and ectoparasite load, explaining the nonlinear global HFC. By adopting this approach, researchers in ecology and evolution will find a valuable method to understand the genetic mechanisms by which hosts might resist pathogen infections.

Materials and methods

Sampling strategy

We sampled dace by electrofishing in the river Viaur, a 169-km long river situated in southwestern France. The river Viaur is highly fragmented with up to one mill weir every 3 km (Grenouillet *et al*. 2008). We have strong evidence that

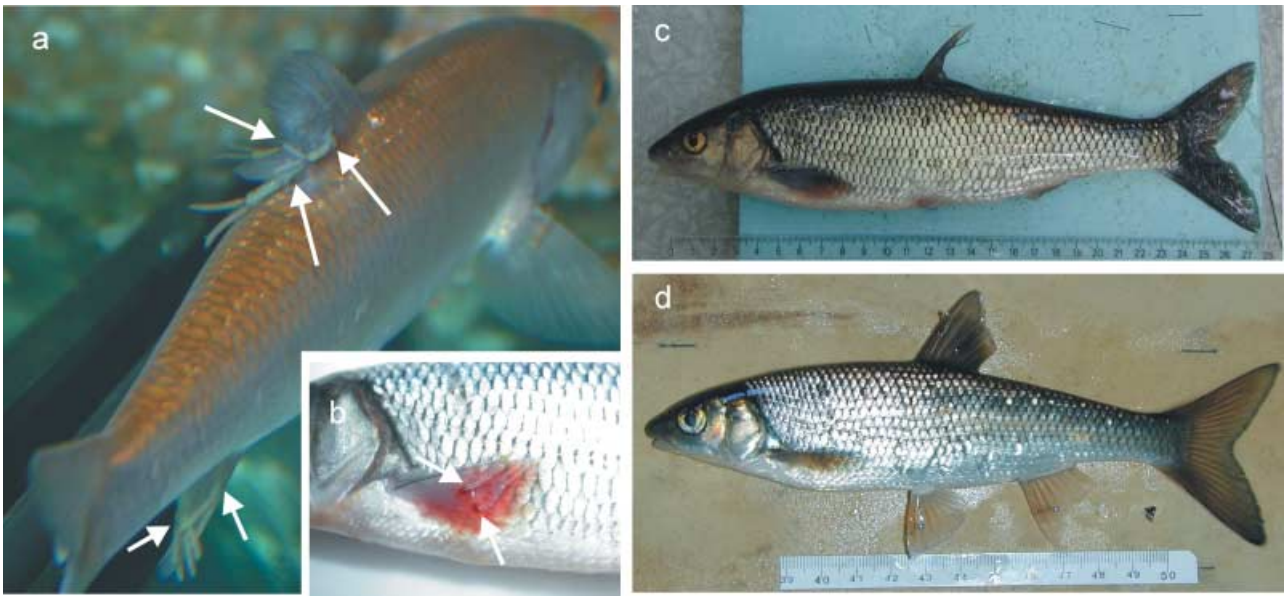

Fig. 1 Pictures showing (a) the localization of the ectoparasite *Tracheliastes polycolpus* on the fins of its host, the rostrum dace (*Leuciscus leuciscus*); (b) the secondary bacterial inflammation occurring during infection; (c) a heavily parasitized dace with the dorsal, pelvic and anal fins partially or totally destroyed and more than 20 *T. polycolpus* on the caudal fin; (d) a non-infected dace for comparison.

the fragmentation has affected the genetic structure of several fish species (including rostrum dace), and in particular, has reduced effective population size and decreased gene flow among sub-populations, which should favour inbreeding (Rey, Blanchet and Loot, unpublished). Sampling was carried-out from 15 to 25 of June in two consecutive years (2006 and 2007). Dace were sampled at several sampling sites (eight sites in 2006 and seven of these sites in 2007) to cover the whole geographic distribution of dace in this river (see Grenouillet *et al*. 2008 for details on sampling sites). We sampled 10–20 dace per site (16.8 individual/site on average, see Table S1, Supporting Information) according to the abundance of dace at each site (N_{2006} = 145; N_{2007} = 105). Note that because the same sites were sampled in two consecutive years, it is possible that a same individual was sampled in both years, thus creating pseudo-replication. Based on individual genotypes (see the section *Genetic analyses*) and using the software GeneCap (Wilberg & Dreher 2004), we indeed detected that three dace were sampled in both years. These three fish were therefore deleted from the 2007 data set to avoid pseudo-replication.

After capture, the dace were anaesthetized, weighed (to the nearest 0.1 g) and the total body length was measured (to the nearest millimetre). On average, dace were 200.73 ± 10.51 mm in 2006 and 201.05 ± 19.12 mm in 2007 (mean ± SD). *Tracheliastes polycolpus* attached to the fins were counted for each parasitized individual. Parasite prevalence was 90.3% and 95.3% in 2006 and 2007, respectively. Parasite load (as the number of parasites per individual) was 12.01 ± 4.67 (mean ± SD) in 2006 and 12.08 ± 2.42 (mean ± SD) in 2007 (see Table S1 for more details). Finally, we gently removed a minimum of five scales to determine age and a small surface tissue sample of one pelvic fin (*c*. 5 mm^2) per fish for genetic analyses. All fish were returned alive to their original sampling sites.

Laboratory analyses

Age estimation. Age was evaluated by counting the annual growth zones that form the scales (these zones are called 'annuli' and are formed during winter, when the growth is low; Francis 1990). The dace sampled ranged from age 1 to 11.

Genetic analyses. Total DNA was extracted from pelvic fin tissue as described in Aljanabi & Martinez (1997). Individual genotypes were obtained at 15 microsatellite loci (Table 1) isolated and developed on closely related cyprinid species (see Table 1). Loci were co-amplified using the QIAGEN Multiplex PCR Kit. Polymerase chain reactions (PCR) were carried out in a 10-μL final volume containing 5–20 ng of genomic DNA, 5 μL of 2× QIAGEN Multiplex PCR Master Mix, and locus-specific optimized combination of primers (detailed recipes are available upon request). PCR amplifications were performed in a Mastercycler PCR machine (Eppendorf) under the following conditions: 15 min at 95 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C and finally followed by a 60-min final elongation step at 72 °C. Amplified fragments were then

Table 1 Description of the 15 microsatellites used in this study. Each sampling year (2006 and 2007) is showed separately. Asterisks (***) indicate significant F_{IS} after Bonferroni corrections; H_E, expected heterozygosity; H_O, observed heterozygosity

	Allelic richness		H_E		H_O		F_{IS} (W & C)		
	2006	2007	2006	2007	2006	2007	2006	2007	Reference
LC290	7	4	0.615	0.632	0.633	0.673	0.028	–0.063	Vyskocilova *et al*. 2007
Lid8	14	9	0.784	0.799	0.821	0.804	–0.044	–0.001	Barinova *et al*. 2004
CypG24	25	21	0.865	0.836	0.896	0.803	–0.032	0.044	Baerwald & May 2004
CypG30	14	14	0.844	0.845	0.841	0.894	0.007	–0.053	Baerwald & May 2004
LceC1	13	11	0.871	0.813	0.847	0.826	0.03	–0.012	Larno *et al*. 2005
Lid2	10	8	0.702	0.667	0.731	0.689	–0.037	–0.029	Barinova *et al*. 2004
CypG03	18	21	0.762	0.783	0.713	0.805	0.068	–0.024	Baerwald & May 2004
Lid11	10	9	0.339	0.318	0.345	0.297	–0.012	0.071	Barinova *et al*. 2004
LC27	16	16	0.861	0.867	0.854	0.875	0.012	–0.005	Vyskocilova *et al*. 2007
Z21908	10	12	0.838	0.832	0.786	0.854	0.066	–0.022	Shimoda *et al*. 1999
Ca12	13	13	0.753	0.776	0.669	0.757	0.116	0.03	Dimsoski *et al*. 2000
Rru4	7	7	0.567	0.572	0.542	0.543	0.049	0.054	Barinova *et al*. 2004
MFW1	6	7	0.741	0.735	0.75	0.686	–0.008	0.071	Crooijmans *et al*. 1997
Lco5	9	9	0.759	0.753	0.793	0.679	–0.041	0.103***	Turner *et al*. 2004
Rhca20	5	6	0.377	0.383	0.379	0.335	–0.001	0.145***	Girard & Angers 2006

separated on an ABI PRISM 3730 automated capillary sequencer (Applied Biosystems). Allelic sizes were then scored using GeneMapper version 4.0 (Applied Biosystems).

Statistical analyses

Descriptive genetic analyses. Locus-by-locus heterozygosity levels (observed and expected) and F_{IS} estimates were calculated using Genetix version 4.05.2 (Belkhir *et al*. 2004). Departure from Hardy–Weinberg equilibrium (i.e. deficit in heterozygotes) was tested for each locus and each year independently using GenePop version 3.4 (Raymond & Rousset 1995). Significance tests of linkage disequilibrium between all pairs of loci (both years pooled) were performed in the program FSTAT version 2.9.3.2 (Goudet 1995). Global heterozygosity at the 15 loci was calculated using the internal relatedness (IR, Amos *et al*. 2001). This measure attempts to estimate the relatedness of an individual's parents using the extent of allele sharing relative to random expectations (Amos *et al*. 2001). IR was calculated based on pooled gene frequencies across all sampling sites and years. If there is variation in the inbreeding coefficient and/or random segregation of chromosomes in the population, IR values will show variation and will be approximately normally distributed around zero for offspring born to random 'unrelated' parents. Individuals with low IR values can be considered as the most heterozygous of the population.

Global HFC analyses. We tested the shape and strength of the relationship between individual parasite load and IR using generalized linear models (GLMs). We computed two GLMs with individual parasite load as the response variable. The first model included only genetic terms as continuous factors; namely IR and its quadratic term (IR^2) [to test for nonlinear relationships (Neff 2004)]. The second model included nongenetic terms (i.e. sampling sites, sampling year, dace age and body length) as well as the genetic terms (IR and IR^2) as predictors. In this model, IR and IR^2 were included after the other factors to test its independent explanatory power on the parasite load (Crawley 2007). All two-term interactions including the terms of interest (IR and IR^2) were fitted into the second model to test for the consistency of the relationship between genetic diversity and parasite load across sampling sites, sampling years, dace age and body length. A quasi-Poisson error term was assumed in these models (Crawley 2007). For comparison, we computed models with three other indices of heterozygosity. The first was measured as the proportion of heterozygous loci (i.e. MLH *sensu stricto*) and was highly correlated to IR ($r = –0.97$, $P < 0.001$). The second index was a measure of homozygosity that accounted for the presence of rare alleles (i.e. homozygosity by locus, HL, Aparicio *et al*. 2006). In our data set, IR and HL were highly correlated to each other ($r = 0.95$, $P < 0.001$). Finally, the third index taking into account the mutational dynamic of microsatellites, was supposed to better represent the entire continuum the degree of inbreeding in the population (i.e. mean d^2, Coltman & Slate 2003) and was less correlated to IR ($r = 0.17$, $P = 0.005$).

Considering individual prevalence rather than parasite load as an index of parasitism can also provide interesting insight into the relationships between host genetic diversity and risk of parasitism (Ortego *et al*. 2007a). However,

prevalence was very high in the studied population (i.e. only 18 individuals were not parasitized out of the 250 individuals sampled, see Table S1) and computing such an analysis inherently led to a very low statistical power. Such an analysis was therefore computed only using IR and IR^2 as predictors, and the results are provided for comparison only. A binomial error term was assumed.

General or local effect hypothesis? Under the assumption that heterozygosity reflects the individual's inbreeding level, theory predicts that the heterozygosity of loci within an individual should be correlated (Balloux *et al*. 2004; Markert *et al*. 2004). This is particularly true for highly inbred populations (Balloux *et al*. 2004), which may be the case in the river Viaur given the high level of fragmentation (Grenouillet *et al*. 2008 and see above). As proposed by Balloux *et al*. (2004), we first tested for evidence of a global inbreeding effect by (i) randomly subdividing our loci into two groups, (ii) calculating individual heterozygosity (as the proportion of the loci at which an individual was heterozygous) for the two groups, and (iii) calculating the correlation between those measures (this procedure was repeated 5000 times). The heterozygosity–heterozygosity correlation coefficient is interpreted as an indication of the magnitude of the association between heterozygosity and the inbreeding coefficient (Balloux *et al*. 2004).

Second, we investigated whether any of the 15 loci showed significant associations with parasite load (i.e. single-locus HFCs). For this, we re-analysed our data, testing each marker independently by fitting GLMs with the individual parasite load as the response variable. Each initial model included sampling sites, sampling year, dace age and body length as co-factors as well as each locus (as a categorical factor) and IR calculated by excluding the marker being considered as two genetic terms (Acevedo-Whitehouse *et al*. 2006). All two-term interactions were also included. Significant *P* values were corrected for multiple comparisons using a Bonferroni procedure. Nonsignificant terms (but the two genetic terms) were excluded from the final models. From the final models, we extracted the sign of the slopes (even if not significant) of the relationships between each individual locus and the parasite load (a negative slope indicates that heterozygous individuals harboured on average more parasites than homozygotes).

Finally, for significant single-locus HFCs, we tested for associations between alleles of the specific loci and parasite load under a 'good allele' model and 'compatible allele' model (Von Hardenberg *et al*. 2007). A good allele model assumes that a given allele increases fitness independently from another allele (including its homologue, Neff & Pitcher 2005). A compatible allele model assumes that an allele increases fitness when in a specific combination with another allele (including its homologue, Neff & Pitcher 2005). In the case of the good allele model, for each allele, we scored 0 if the allele was absent and 1 if present. For the compatible allele model, we scored 0 if an allele was present, 1 if one copy of the allele was present and 2 if two copies were present (Von Hardenberg *et al*. 2007). We then fitted GLMs with parasite load as the dependent variable and the genetic terms instead of IR (once for the good allele and once for the compatible allele model). The significance association was tested comparing the fit of a model with the genetic terms to one with no genetic terms (i.e. the null model).

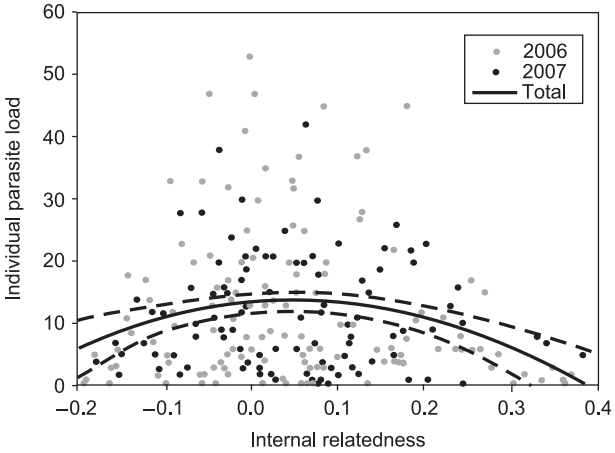

Fig. 2 Patterns of global HFCs linking genetic diversity and parasite load in rostrum dace (*Leuciscus leuciscus*). Relationships between the internal relatedness (a measure of genetic diversity) and individual parasite load harboured by dace sampled in 2006 (grey dots) and in 2007 (black dots). The black line is the relationship for both sampling years when pooled and dotted lines are the 95% confident intervals for such a relationship.

Results

Descriptive genetic analyses

Only two loci (*Lco5* and *Rhca20*, see Table 1) showed a significant heterozygous deficit (after Bonferroni correction). These deficits were not observed consecutively over the two sampling years (Table 1) and did not impact on the outcomes of the global HFC analyses. Over the 105 possibilities, no pair of loci displayed significant linkage disequilibrium (the adjusted significant threshold was $\alpha = 0.0005$, see details in Table S2, Supporting Information).

Global HFCs

We found a highly significant curvilinear association (percentage of explained deviance, 7.35%; GLM, $P < 0.001$, Table 2) between parasite load and IR; with both heterozygous and homozygous dace harbouring, on average, fewer parasites (see Fig. 2 for a graphical representation

Table 2 Results of generalized linear models aimed at evaluating (a) the effect of the internal relatedness (IR) on the individual parasite load and (b) the effect of IR after accounting for other confounding factors. The quadratic term for IR enables nonlinearity in the relationship to be tested. Bold *P* values are significant at the 0.05 level

	Degree of freedom	Deviance	Residual deviance	*P* value
(a) Simple model				
Null			2640.24	
Internal relatedness	1, 249	26.34	2613.90	0.12
[Internal relatedness]^2	1, 247	167.74	2446.17	**< 0.001**
(b) Full Model				
Null			2640.24	
Host age	1, 248	675.6	1964.64	**< 0.001**
Sampling site	7, 241	193.1	1771.54	**< 0.001**
Sampling year	1, 240	0.48	1771.06	0.79
Host body size	1, 239	7.02	1764.03	0.30
Internal relatedness	1, 238	13.22	1750.81	0.16
[Internal relatedness]^2	1, 237	68.96	1681.85	**< 0.001**
Host body size*Internal relatedness	1, 236	0.47	1681.38	0.79
Host body size*[Internal relatedness]^2	1, 235	2.42	1678.96	0.55
Host age*Internal relatedness	1, 234	0.01	1678.95	0.97
Host age*[Internal relatedness]^2	1, 233	13.6	1665.35	0.15
Sampling site*Internal relatedness	7, 226	56.77	1608.58	0.28
Sampling site*[Internal relatedness]^2	7, 219	56.95	1551.63	0.28
Sampling year*Internal relatedness	1, 218	1.09	1550.54	0.68
Sampling year*[Internal relatedness]^2	1, 217	14.28	1536.26	0.14

without considering other nongenetic factors). It is worth noting that, even if the percentage of deviance explained by IR (and IR^2) decreases in a more complex model (see Table 2), this nonlinear trend was still significant after considering all other confounding factors, and was highly consistent across sampling sites, sampling years and dace age (Table 2).

Both HL and MLH provided similar but weaker trends than IR did, while mean d^2 did not give significant trends (see Table S3, Supporting Information for further details). As found elsewhere, IR, HL and MLH are often highly correlated (Aparicio *et al*. 2006), and IR outperforms mean d2 in most cases (Coltman & Slate 2003). Simulations have suggested that HL outperforms IR for populations with high expected heterozygosity values (i.e. > 0.6, Aparicio *et al*. 2006). However, differences were less clear when a relatively small number of loci (i.e. 10) were screened (Aparicio *et al*. 2006). This could explain why our empirical data do not follow results obtained from simulations.

To gain insights into the mechanisms underlying this nonlinear relationships (see the Discussion), we explored if average age of dace differed significantly between moderately diverse and extremely homozygous/extremely heterozygous individuals. When we divided our data in three equal categories according to the individual IR (moderately heterozygous, –0.032 < IR < 0.071; extremely heterozygous –0.032 < IR; extremely homozygous individuals, IR > 0.071) we found a significant difference in average age between categories (Kruskal–Wallis test, $\chi^2 = 6.14$, d.f. = 2, $P = 0.04$, results not shown). Particularly, fish being moderately heterozygous were, on average, older than fish from the two other categories (results not shown). Moreover, the proportion of young fish (< 5 years old) was significantly lower in the moderately heterozygous group than in the two other groups ('moderately heterozygous' vs. 'extremely heterozygous', $\chi^2 = 4.04$, d.f. = 1, $P = 0.044$; 'moderately heterozygous' vs. the 'extremely homozygous', $\chi^2 = 7.54$, d.f. = 1, $P = 0.006$; 'extremely heterozygous' vs. the 'extremely homozygous', $\chi^2 = 0.41$, d.f. = 1, $P = 0.521$, results not shown), while the proportion of older fish (> 5 years old) did not differ significantly between categories ('moderately diverse' vs. 'extremely heterozygous', $\chi^2 = 2.72$, d.f. = 1, $P = 0.098$; 'moderately diverse' and the 'extremely homozygous', $\chi^2 = 3.23$, d.f. = 1, $P = 0.072$; 'extremely heterozygous' vs. the 'extremely homozygous', $\chi^2 = 0.08$, d.f. = 1, $P = 0.753$, results not shown).

As expected, we found no significant relationship between individual prevalence and heterozygosity [GLM with a binomial error term, P(IR) = 0.273; P(IR^2) = 0.18]. This means that the few individuals that were not parasitized (n = 18) did not have a significantly different heterozygosity value than parasitized hosts (n = 232).

General or local effect hypothesis?

Heterozygosity–heterozygosity correlation was weak within our set of microsatellites (r = –0.071 ± 0.050, mean ± SD), suggesting that IR was a poor reliable estimator of the

	Single locus			Global	
Locus	Homozygous	Heterozygous	*P* value (slope)	*P* value IR	*P* value IR^2
LC290	13.03 (1.53)	11.79 (0.85)	0.171 (+)	0.132	**0.011**
Lid8	**8.33** (**1.29**)	**13.21** (**0.91**)	**0.002** (–)**	0.324	**0.043**
CypG24	10.5 (1.48)	12.59 (0.88)	0.384 (–)	0.383	< **0.001****
CYpG30	10.34 (1.40)	12.62 (0.88)	0.982 (–)	0.188	**0.002****
LceC1	11.54 (1.64)	12.40 (0.89)	0.443 (+)	0.243	< **0.001****
Lid2	**15.72** (**1.66**)	**10.88** (**0.85**)	**0.042** (+)	0.121	**0.021**
CypGo3	12.41 (1.48)	12.19 (0.93)	0.060 (–)	0.585	**0.002****
Lid11	11.85 (0.99)	12.97 (1.31)	0.947 (+)	0.164	< **0.001****
LC27	11.81 (2.90)	12.29 (0.78)	0.605 (+)	0.187	**0.001****
Z2190	12.41 (2.10)	12.15 (0.83)	0.912 (+)	0.237	**0.014**
Ca12	12.34 (1.39)	12.14 (0.94)	0.842 (+)	0.190	**0.002****
Rru4	**9.39** (**0.95**)	**14.56** (**1.17**)	**0.001** (–)**	0.328	**0.005**
MFW1	11.98 (1.47)	12.23 (0.95)	0.871 (+)	0.290	**0.050**
Lco5	12.5 (1.36)	12.05 (0.94)	0.995 (–)	0.266	**0.012**
Rhca20	11.57 (0.97)	13.21 (1.31)	0.353 (–)	0.288	< 0.001**

Table 3 Table showing the *P* values of each of the 15 single-locus HFCs and global HFCs (all loci excluding the locus being considered, measured as IR) after accounting for sampling sites and age of the host. For the single-locus HFCs, we also reported the average value (± SE) of parasite load for both homozygous and heterozygous individuals (before accounting for sampling sites and age of the host) and the sign of the slope of each relationship (after accounting for sampling sites and age of the host). For the global HFCs, the quadratic term (IR^2) was included for testing nonlinear relationships. In both cases, bold *P*-values are significant at the 0.05 level and asterisks indicate a significant effect after Bonferroni correction

Table 4 Genetic associations between alleles of the *Rru4* and *Lid8* loci and parasite load in the rostrum dace *Leuciscus leuciscus* under two models, a 'good allele' model and a 'compatible allele' model. 'Null model' is the model without genetic terms. Bold *P* values are significant at the 0.05 level

	'Good allele' model				'Compatible allele' model			
	Residual deviance	Deviance	d.f.	*P* value	Residual deviance	Deviance	d.f.	*P* value
Rru4								
Null model	2633.00				2633.00			
Model with genetic terms	2445.54	187.46	6	**0.01**	2363.50	269.50	9	**< 0.001**
Lid8								
Null model	2512.54				2512.54			
Model with genetic terms	2224.64	287.90	14	**0.04**	2171.89	340.66	14	**< 0.001**

individual inbreeding coefficient for our study (Balloux *et al*. 2004).

In the case of the single-locus HFCs, over the 15 initial models, we detected only two significant interactions at $\alpha < 0.05$ (*Lid11*, sampling sites*heterozygosity, $P = 0.01$; *LC27*, age*heterozygosity, $P = 0.01$). After correcting for multiple tests, these interactions were not significant ($\alpha > 0.003$) and were thus deleted from the final models, together with the factor 'sampling year' and 'host body size' that were not significant in any of the models. After accounting for differences among sampling sites and individuals' age, two single-locus HFCs were significant after Bonferroni corrections (*Lid8* and *Rru4*, see Table 3). Both single-locus HFCs were negative: for each locus, heterozygous individuals harboured significantly more parasites than homozygous dace (Fig. 3a). The shape of these single-locus HFCs was consistent across age classes, sites and sampling years (GLMs, interaction terms: $P > 0.05$, results not shown). When these loci were each excluded one at a time from the calculation of IR, we found no strong influence of any loci on global HFCs (Table 3). This indicates that these two loci have significant individual effects on parasite load but have no 'disproportionate' effects (i.e. they do not explain all the deviance contained in the genetic terms, Acevedo-Whitehouse *et al*. 2006).

We tested for specific associations between alleles of *Rru4* or *Lid8* and parasite load using both good allele and compatible allele models of genetic effects. For both loci, we found that both kinds of models explained a significant part of the deviance when compared to the null model (Table 4). However, for both *Rru4* and *Lid8*, the explicative power of the compatible allele model was strikingly higher than that of the good allele model.

Because each locus (*Lid8* and *Rru4*) was not genetically linked to another (see Table S2), we tested if the combined effect of these loci on parasite load was additive. To do so, we computed a GLM including both loci as categorical fixed factors and we tested the interaction term between both loci. We found a nonsignificant interaction term between both loci ($P = 0.38$), indicating that they acted additively on the individual parasite load. In other words, heterozygotes at one out of the two loci harboured an

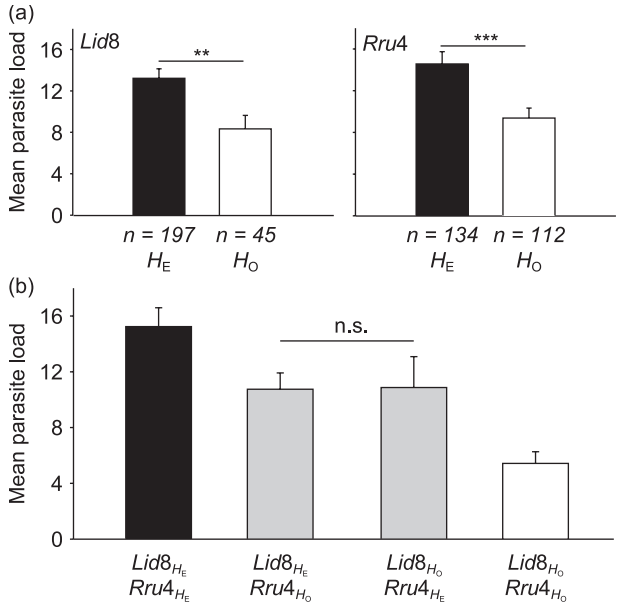

Fig. 3 Patterns of single-locus HFCs linking genetic diversity and parasite load in rostrum dace (*Leuciscus leuciscus*). (a) Mean parasite load harboured by either heterozygous (H_E: black bars) or homozygous (H_O: white bars) dace at one of the two loci (i.e. *Lid8* and *Rru4*) that displayed significant single-locus HFCs. Differences were statistically assessed using Generalized Linear Models (**$P < 0.01$; ***$P < 0.001$). (b) Mean parasite load harboured by either purely heterozygous (H_E at both loci: black bars), purely homozygous (H_O at both loci: white bars) or mixed heterozygous/homozygous (H_E or H_O at one of the loci: grey bars) dace at the two loci (i.e. *Lid8* and *Rru4*) that displayed significant single-locus HFCs. Differences between categories were assessed using contrast tests (n.s. $P > 0.05$). In all graphs, bars are means ± standard errors.

intermediate level of parasite in comparison to either purely heterozygous or homozygous individuals (see Fig. 3b).

Discussion

Contrary to most expectations (Coltman *et al*. 1999; Ortego *et al*. 2007a), we found that dace that were either highly homozygous or highly heterozygous at the multilocus level harboured on average less ectoparasites than dace of an intermediate level of heterozygosity. This nonlinear trend was solid since it was independent from other confounding factors and was highly consistent both spatially and temporally. Nonlinear HFCs have already been reported elsewhere (e.g. Wegner *et al*. 2003; Neff 2004; Ortego *et al*. 2007a; Blanchet *et al*. 2009a). However, this current study is, to our knowledge, the first to demonstrate a pattern suggesting disruptive selection acting on genetic diversity. Since the negative fitness consequences of *Tracheliastes polycolpus* on dace have been demonstrated (see Fig. 1 and Blanchet *et al*. 2009b), this result would indicate that both homozygous and heterozygous individuals might be fitter and that the variance of heterozygosity might be increased in this population. Alternatively, individuals with either low or high IR values might have a higher susceptibility to parasites, making these individuals more prone to disappear (by mortality) from the population well before sampling. In other words, the pattern we highlight here might underlie stabilizing rather than disruptive selection. For this hypothesis to be true, we should expect that the average age would differ significantly between moderately diverse and extremely homozygous/extremely heterozygous individuals. If it differed, we should further expect differences in the distribution of ages between the three categories, with some ages (particularly younger individuals) being absent from the frequency distribution of both extremely homozygous and extremely heterozygous categories. By analysing the relationships between age and heterozygosity (see the Results section), we indeed found a significant difference in average age between moderately diverse and extremely homozygous/extremely heterozygous individuals. However, we found that, for the moderately diverse category, young individuals were at a lower frequency probably because they die before sampling due to parasite infection (high parasite load and/or weak resistance to the infection). On the contrary, for extremely homozygous and heterozygous categories, all age categories could survive the infection (because of high resistance and/or low parasite load). These results led, therefore, to a very weak support for the stabilizing selection hypothesis and, on the contrary, we can reasonably suggest that dace with either low or high IR are less susceptible to ectoparasites than those with intermediate IR values and therefore have a higher fitness.

Our results suggest that the local-effect hypothesis was the most likely reason for the disruptive-like global HFC we report here. The most direct evidence being that we highlighted two highly significant single-locus HFCs which is indicative of linkage disequilibrium between these two loci and functional genes (Hansson & Westerberg 2002; Lieutenant-Gosselin & Bernatchez 2006). Linkage disequilibrium is supposed to increase as the effective population size (N_e) decreases (Balloux *et al*. 2004). We have evidence that river fragmentation has reduced N_e in this dace population (O. Rey, S. Blanchet and G. Loot, unpublished), which might have favoured a high level of linkage disequilibrium in this population. Additionally, we found no evidence that heterozygosity estimated from our set of microsatellites correctly reflected the genome-wide diversity of the hosts (Balloux *et al*. 2004). In our case, it is noteworthy that a lack of association between marker-based heterozygosity and genome-wide diversity might simply reflect the low statistical power of our analysis (i.e. heterozygosity–heterozygosity correlation) due to a low number of typed loci rather than an absence of variation in the inbreeding

coefficient (Balloux *et al*. 2004; Slate *et al*. 2004). Such a result should therefore be confirmed with a larger panel of microsatellites. This is, however, highly consistent with most empirical studies that have attempted to assess relationships between marker-based heterozygosity, inbreeding coefficient and genome-wide diversity (Balloux *et al*. 2004; Markert *et al*. 2004; Bensch *et al*. 2006; Väli *et al*. 2008). This being said, given that the two significant single-locus HFCs were negative, we should have expected to detect a negative, rather than a nonlinear, global HFC (Lieutenant-Gosselin & Bernatchez 2006). Hence, we propose that the combination of both positive and negative local HFCs (irrespectively of the *P* values, 46.6% of the single-locus HFCs were negative and 54.4% were positive, Table 3) and the presence of strong negative single-locus HFCs might translate into a trend whereby both heterozygous and homozygous individuals (at the multilocus level) are fitter. We partially confirmed this speculation since testing global HFCs, excluding the two loci showing strong single-locus HFCs (*Rru4* and *Lid8*) from the calculation of IR, led to a nonsignificant global HFC (GLM, $P > 0.06$). The possible occurrence of both positive and negative single-locus HFCs (irrespective of their statistical significance) proves the difficulty of predicting a priori the shapes of relationships between genetic diversity and fitness at the multilocus level.

Beyond elucidating mechanisms underlying global HFC, each of these two negative HFCs indicate a fitness advantage for homozygous hosts at these two specific loci and hence provide new insights into the genetic mechanisms used by hosts to resist parasite infection. It has been argued that negative HFCs could arise through outbreeding depression, as a mechanism for disrupting co-adapted gene complexes (LeBas 2002; Neff 2004). However here, since the reported negative HFCs are local, this latter mechanism seems unlikely to explain such an unexpected result. Rather, at the individual locus level, negative HFCs probably reflect negative non-additive allelic interaction as a mechanism for resisting infections. In contrast to the associative overdominance hypothesis (David 1998; Hansson & Westerberg 2002), this mechanism suggests an underdominance of heterozygotes leading to a weaker fitness of heterozygous individuals (Pitcher & Neff 2006). Alternatively, such negative HFCs could arise through good allele effect. In that case, the 'best' allele at each locus is also the most common in the population. Thus, most homozygous individuals carry two copies of the best allele and heterozygous only one, and a single copy of this allele is sufficient for increasing fitness (Neff & Pitcher 2005). However, as we showed by accounting for allele identity, these negative HFCs are best explained under a model of compatible allele underlying a non-additive interaction between alleles. We can therefore reasonably interpret these two negative HFCs as a result of underdominance of heterozygotes. Underdominance was rarely considered in natural populations, but two studies have recently demonstrated strong selection against heterozygotes at two very different non-neutral loci (i.e. MHC in salmonid fish, Pitcher & Neff 2006; *Ectodysplasin* locus coding for lateral plate number in the stickleback *Gasterosteus aculeatus*, Barrett *et al*. 2008). In our case, because frequency-dependent selection is often invocated in host–parasite interactions (Combes 2001), we can imagine that some specific alleles procure a selective advantage to the host, and that protein synthesis is lower for individuals that carry only one copy of these specific alleles (Hedgecock *et al*. 2007). Future investigations should focus on the temporal evolution of allele's frequencies at these specific loci and on pattern of genes expression in this fish species. Whatever the underlying mechanisms, our results suggest that underdominance might be more widespread than previously thought and might play an important role in shaping the genetic diversity of wild populations (Pitcher & Neff 2006; Barrett *et al*. 2008). Very interestingly, we further demonstrated that the combined effects of both loci on parasite load was additive because purely heterozygous individuals at these two loci harboured three times more parasites than purely homozygous ones. This latter result adds weight to our understanding of the transmission of genetic variation for parasite resistance since we have shown that the combined effect of loci implied in parasite resistance seems to be additive rather than epistasic (Råberg *et al*. 2007). Overall, this result opens up interesting perspectives for studying the pattern of frequency-dependent selection that often occurred in host–parasite interaction (i.e. co-evolutionary arm races, Combes 2001).

The mechanisms linking the host genotype and the level of parasite infection are still unclear. We can hypothesize that specific host genotypes have better immunological capabilities for resisting parasites. An alternative but non-exclusive hypothesis would be that individuals that are either heterozygous or homozygous are in a better condition and hence less susceptible to parasite infection. In the latter case, we should expect a relationship between IR and the condition of the dace. Using length and weight data, we evaluated the body condition using the Fulton's index (Neff 2004). We found no relationship between this index and IR (GLM, after taking into account dace age, sampling year, and sampling sites, IR, $F(1, 174) = 0.38$, $P = 0.53$; IR^2, $F(1, 174) = 0.45$, $P = 0.50$). Moreover, we demonstrated in a previous study (Blanchet *et al*. 2009b) that growth rate (that can be viewed as another measure of condition) before infection occurred does not influence the future level of parasite load, while the level of parasite load at sampling negatively influences growth during infection. These two observations hence provide more support for the immunological hypothesis. However, given the paucity of knowledge on this specific host–parasite interaction, these two hypotheses remain to be tested with the appropriate tools. We hope to do so in the near future.

To conclude, our findings underline the intimate interactions between the host genome and parasites. We have shown that these interactions occurred at the single-locus level but can rise up to the multilocus level. More importantly, our results strongly contrast with the generally accepted view that heterozygous individuals are fitter (Coltman & Slate 2003 but see LeBas 2002; Neff 2004). Indeed, as many other ectoparasites in fish (Krkošek *et al*. 2007), *T. polycolpus* incur significant fitness costs to their hosts, notably by decreasing their growth rate (Blanchet *et al*. 2009b). Since underdominance can reduce population polymorphisms through directional selection against heterozygous individuals (Pitcher & Neff 2006), we can thus expect that monomorphism will be favoured for certain loci closely linked to parasite resistance. However, in our population, this hypothesis might be challenged and a high variability around global heterozygosity could be preserved through the disruptive-like HFC observed at the multiloci level. These two opposite selective pressures imposed by *T. polycolpus* at different levels of its host's genome might well play a crucial role for shaping genetic diversity in this fish population. Our results therefore open new perspectives for future researches on the genetic interaction between hosts and their parasites and on the processes that maintain genetic variability in natural populations.

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This study is a part of an ongoing project that aims at determining how habitat fragmentation and climate change can affect the ecological and evolutionary outcome of host–parasite interactions. Simon Blanchet is a postdoctoral research fellow at the Paul Sabatier University who is interested in the environmental and genetic causes of phenotypic variability and its consequences on the functioning and the evolution of freshwater ecosystems. Olivier Rey and Pauline Berthier are PhD students interested in the use of molecular tools for replying questions on population genetic structure (O.R.) and host–parasite interaction (P.B.). Sovan Lek is a Professor at the Paul Sabatier University who conducts research on statistical modelling and fish ecology. Geraldine Loot is an Assistant Professor at the University Paul Sabatier interested in the evolutionary ecology of host–parasite interactions and in the conservation of freshwater communities.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Descriptive table of prevalence and mean parasite load according to sampling sites and sampling years

Table S2 *P* values for linkage disequilibrium for each pair of loci. The adjusted significance threshold is $\alpha = 0.0005$

Table S3 Results of GLMs aiming at evaluating (A) the effect of the genetic diversity (measured either as homozygosity by locus, multilocus heterozygosity or mean d^2) on the individual parasite load and (B) the effect of genetic diversity after accounting for others confounding factors

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