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LOCAL HETEROZYGOSITY-FITNESS CORRELATIONS WITH GLOBAL POSITIVE EFFECTS ON FITNESS IN THREESPINE STICKLEBACK

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Abstract.—The complex interactions between genetic diversity and evolution have important implications in many biological areas including conservation, speciation, and mate choice. A common way to study these interactions is to look at heterozygosity-fitness correlations (HFCs). Until recently, HFCs based on noncoding markers were believed to result primarily from global inbreeding effects. However, accumulating theoretical and empirical evidence shows that HFCs may often result from genes being linked to the markers used (local effect). Moreover, local effect HFCs could differ from global inbreeding effects in their direction and occurrence. Consequently, the investigation of the structure and consequences of local HFCs is emerging as a new important goal in evolutionary biology. In this study of a wild threespine stickleback (*Gasterosteus aculeatus*) population, we first tested the presence of significant positive or negative local effects of heterozygosity at 30 microsatellites loci on five fitness components: survival, mating success, territoriality, length, and body condition. Then, we evaluated the direction and shape of total impact of local HFCs, and estimated the magnitude of the impacts on fitness using regression coefficients and selection differentials. We found that multilocus heterozygosity was not a reliable estimator of individual inbreeding coefficient, which supported the relevance of single-locus based analyses. Highly significant and temporally stable local HFCs were observed. These were mainly positive, but negative effects of heterozygosity were also found. Strong and opposite effects of heterozygosity are probably present in many populations, but may be blurred in HFC analyses looking for global effects only. In this population, both negative and positive HFCs are apparently driving mate preference by females, which is likely to contribute to the maintenance of both additive and nonadditive genetic variance.

Key words.—Fitness, *Gasterosteus aculeatus*, heterozygosity, inbreeding depression, linkage disequilibrium, mate choice, microsatellites.

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The interaction between genetic diversity and fitness is a key element in evolutionary biology, and particularly for studies related to the evolution of mate choice, dispersal, host-pathogen interactions, as well as speciation (Charlesworth and Charlesworth 1987; Thornhill 1993; Lynch et al. 1995; Hedrick and Kalinowski 2000; Keller and Waller 2002). The importance of understanding such processes for conservation biology is also well acknowledged (DeSalle and Amato 2004). Elucidating the structure and dynamics of genetic diversity-fitness interactions may allow the evaluation of the relative importance of local adaptation at the genetic level versus evolutionary potential of populations in a conservation context (Vergeer et al. 2004). This issue is also intimately related to testing the alternate hypotheses of good genes versus complementary genes in studies of mate choice (Mays and Hill 2004; Neff and Pitcher 2005).

A common way of studying the dynamics of genetic diversity-fitness interactions has been to investigate inbreeding depression and its possible evolutionary consequences (e.g., Charlesworth and Charlesworth 1987; Thornill 1993; Coltman and Slate 2003). The level of inbreeding has traditionally been determined by pedigree analysis, but is increasingly being documented by means of quantifying heterozygosity using either allozymes or noncoding markers such as microsatellites (Slate et al. 2004). For noncoding markers, it has generally been assumed that mean heterozygosity reflects a global level of heterozygosity, which in turn should correlate with individual inbreeding level (e.g., Coltman et al. 1998, 1999; Coulson et al. 1998, 1999; Saccheri et al. 1998; Marshall and Spalton 2000; Slate et al. 2000; Höglund et al. 2002; LeBas 2002; Foerster et al. 2003; Grant et al. 2003).

This interpretation was radically challenged by three recent studies that revealed a weak association between heterozygosity, even when measured over many markers, and inbreeding coefficient (Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004; reviewed in Pemberton 2004). According to Slate et al. (2004), the relationship between heterozygosity and inbreeding is expected to be strong only in the case of unusually high variance in individuals' inbreeding coefficients. Similarly, using stochastic individual-based simulations, Balloux et al. (2004) found a high correlation between heterozygosity and inbreeding only in highly substructured populations or populations with mating systems favoring frequent incestuous pairings. Yet, it is unlikely that the numerous empirical studies in which heterozygosity-fitness correlations (HFCs) were detected all represent such extreme cases (Pemberton 2004). These observations suggest that HFC may be better explained by relationships at the level of individual markers (direct effects) or genes closely linked to them (local effects) than by global inbreeding depression (Pemberton 2004).

Thus, the difference between direct, local, and global inbreeding effects stands in what is characterized by genetic markers. In global inbreeding effects, marker heterozygosity is expected to reflect genome wide heterozygosity, which should correlate with the individual inbreeding level. In direct effects, marker heterozygosity represents only its own heterozygosity, whereas in local effects it represents linked loci heterozygosity. Direct effects are unlikely in the case of noncoding markers (Hansson and Westerberg 2002). Therefore,

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the most likely alternative hypothesis to global inbreeding effects is that of local effects, which should primarily result from a genetic association, through linkage disequilibrium, between effective genes and one or more of the markers used (Hansson and Westerberg 2002).

Arguably, approaching HFC from a local rather than global inbreeding perspective reorients several basic questions and goals of HFC studies. First, because the importance of local HFCs is not resolved (Pemberton 2004), the proportion and number of loci presenting local HFCs, and the importance of their effects on fitness still have to be investigated. Several studies recently provided evidence for HFCs associated with local effects (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005; Tiira et al. 2006). However, most of those studies included relatively small numbers of loci with unknown chromosomal positioning, which limits the interpretation of the results obtained (Pemberton 2004). A second important aspect of local effects concerns the possibility of observing both negative and positive HFCs in a single population. Thus, under the inbreeding hypothesis, heterozygosity at one locus is expected to represent the global inbreeding level (Hansson and Westerberg 2002). As inbreeding generally affects fitness negatively (Charlesworth and Charlesworth 1987; Thornill 1993), heterozygosity at every locus is expected to correlate positively with fitness. In the case of local effects, however, the effect of heterozygosity on fitness components at each particular locus is independent (David 1997), and could be positive, negative, or null, depending on the functional importance and allelic interactions (e.g., additivity versus dominance or overdominance) at linked genes. This issue can be approached by (1) first testing whether heterozygosity at each locus has more often a positive or negative effect on fitness, and (2) then assessing the global relationship over all local HFCs. These are two important analyses as the relative importance of both types of effects could dictate the direction of evolution in terms of the maintenance of genetic diversity under the effect of either directional or stabilizing selection.

The general objective of this study was to investigate the occurrence and magnitude of local and global effect HFC in a wild population of threespine stickleback (*Gasterosteus aculeatus*) from the St. Lawrence River estuary, Quebec, Canada. The threespine stickleback represents a remarkable model for studying HFC in natural conditions. First, many microsatellite loci have been described and positioned on a QTL linkage map (Peichel et al. 2001). Second, stickleback populations have apparently evolved under different selective pressures, both in terms of directionality and intensity, such that the species comprises highly specialized, as well as more generalist populations (Bell and Forster 1994; Braithwaite and Odling-Smee 1999). The species therefore offers the potential to evaluate the nature of HFC under diverse population histories and selective regimes. Third, the threespine stickleback is also characterized by complex behavioral interactions, including female mate choice and male-male competition, as well as paternal care (Wootton 1976; Bell and Forster 1994; Bell 1995). Therefore, mate choice may lead to both direct and indirect benefits to females. In addition, the cost of female choice is likely to be relatively low as males nest in high density and display intensively (Blais et al. 2004). Multiple mating cues, the occurrence of both direct and indirect benefits and low costs in female mate choice are all likely to increase the possibility of complex mate choice evolution, such as mating based on genetic compatibility (Jennions and Petrie 1997). Moreover, intense male-male competition could contribute to inflated fitness differences between more or less heterozygous individuals, as reported for other types of stresses (e.g., Danzmann et al. 1988; Borsa et al. 1992; Audo and Diehl 1995).

In this context, our specific objectives were: (1) to test for the occurrence of significant positive or negative local effects of heterozygosity at 30 microsatellites loci evenly distributed over the genome, on five fitness components; (2) to evaluate the direction and shape of the total impact of local HFCs, relative to global HFC effects; (3) to estimate the magnitude of the impact on fitness by means of both regression coefficients and selection differentials. Finally, we discuss possible evolutionary consequences of local HFCs.

Materials and Methods

Study Site and Field Sampling

We studied a natural population of threespine stickleback located at the Isle-Verte Ecological Reserve on the south shore of the St. Lawrence River middle estuary (Quebec, Canada). Sticklebacks of this population live one to two years (De Fraipont et al. 1993) and most males complete only one breeding cycle per year (Lachance and FitzGerald 1992). They reproduce in coastal tide pools reached during spring high tides (Blais et al. 2004).

Fish were collected in May after the egg-collecting phase of the first breeding cycle (detailed in Blais et al. 2004). Virtually all males and the majority of females from five interconnected tide pools were captured. A total of 605 adult fish were captured including 325 (54%) females and 280 males. In this study, only males were considered. Approximately half the males were guarding a nest (139) and 35% of the nests had eggs (Blais et al. 2004). In this population, nonterritorial sneakers achieve virtually null reproductive success. Thus, nonterritorial males, which represented nearly 50% of all males in the pools, fertilised only 3% of the eggs (Blais et al. 2004). A total of 208 males were available for genotyping, including most territorial ($n = 135$) and 73 randomly selected nonterritorial ones. Sixty percent of these males were 1+ and 40% were 2+ age classes (Blais et al. 2004).

Fitness Components

Five male fitness related traits were measured: survival, standard length, body condition, territoriality status (having a nest or not), and mating success (detailed in Blais et al. 2004). The two cohorts were analyzed separately except for survival (see below). Standard length at a given age was used as a surrogate for growth. Blais et al. (2004) previously found a highly significant effect of this trait on the probability of being a territorial male for 1+ males. Body condition was defined as the residual of the cohort linear regression predicting body weight from standard length using JMP IN version 3.2.6 (SAS Institute 1999). Territoriality relates to male-

TABLE 1. Descriptive statistics and PCR annealing temperatures for the 30 loci genotyped. Linkage groups, and QTL are those defined by Peichel et al. 2001; A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, average inbreeding coefficient of individuals relative to hypothetical subpopulations according to Robertson and Hill 1984. Loci with significant single-locus HFC for at least one fitness component are bold.

Locus	Linkage group	QTL	A	H_o	H_e	F_{IS}	Annealing T (°C)
Stn1	I	—	16	0.67	0.85	0.185*	60
Stn9	I	First dorsal spine	13	0.73	0.84	0.075*	56
Stn14	I	—	21	0.78	0.90	0.073*	56
Stn16	II	—	5	0.50	0.48	0.029	60
Stn23	II	—	16	0.76	0.82	0.074*	56
Stn26	II	First dorsal spine	13	0.79	0.82	0.054*	60
Stn30	III	—	18	0.77	0.79	0.058*	60
Stn31	III	—	14	0.84	0.88	0.041	60
Stn34	III	—	16	0.72	0.83	0.097*	60
Stn38	IV	—	2	0.21	0.22	0.025	60
Stn61	VI	—	18	0.75	0.86	0.101*	62
Stn65	VI	—	30	0.75	0.94	0.173*	52
Stn67	VI	—	27	0.83	0.89	0.012	60
Stn69	VII	—	13	0.78	0.84	0.118*	60
Stn70	VII	—	7	0.71	0.73	0.074	60
Stn78	VII	—	27	0.76	0.91	0.115*	60
Stn82	VII	—	17	0.73	0.73	0.049	60
Stn83	VIII	—	10	0.68	0.70	0.029	56
Stn93	VIII	—	7	0.52	0.53	−0.006	62
Stn96	VIII	Second dorsal spine	19	0.75	0.83	0.062	62
Stn98	VIII	—	16	0.72	0.88	0.100*	56
Stn107	IX	—	10	0.77	0.66	−0.051*	60
Stn130	XI	Second dorsal spine	14	0.85	0.83	0.023	52
Stn131	XI	Number of gill rakers	18	0.69	0.78	0.098*	52
Stn134	XII	—	4	0.36	0.50	0.310*	56
Stn147	XII	—	11	0.62	0.70	0.052	60
Stn152	XIII	Number of lateral plates	27	0.75	0.95	0.158*	52
Stn158	XIII	—	6	0.46	0.45	0.001	62
Stn177	XV	—	12	0.75	0.76	0.044	62
Stn209	XXVI	Number of lateral plates	2	0.20	0.22	0.090	62

* $P \leq 0.05$ after Bonferroni correction.

male competition and was defined as the possession (or lack thereof) of a nest, and was determined by visual observations of the males' behavior in the field (detailed in Blais et al. 2004). Mating success of territorial males ($n = 77$ males 1+, $n = 58$ males 2+) was defined as successful (egg clutch(es) present in their nest) or unsuccessful (no eggs). Finally, to assess the possibility of differential survival as a function of genetic diversity, we tested the null hypothesis of no impact of heterozygosity value on the likelihood of being 1+ or 2+ years old, assuming equality of heterozygosity at the outset of each cohort. Hereafter, estimated differential survival is simply called ''survival.'' These five fitness components were largely independent from each other; relationships between them were weak ($r \leq 0.17$) except for standard length and territoriality in 1+ males ($r = 0.45$).

Genetic Analyses

A total of 30 microsatellites were chosen among 209 loci identified by Peichel et al. (2001) to cover half of the 26 identified linkage groups (Table 1). Seven of those loci are linked to QTL associated either with the number of lateral plates, length of first or second dorsal spine, or gill raker numbers (Peichel et al. 2001). Lateral plates and dorsal spines play a role in defense against bird and fish predators, whereas gill raker numbers are important in prey selectivity (Bell and Foster 1994).

Polymerase chain reactions (PCRs) were carried out on individual loci in a 11-μL reaction containing: 2 μL (25–50 ng) of total genomic DNA, 1.2 μL of reaction buffer (10.9 mM Tris-HCL, ph 9.0, 54.5 mM KCl, 1.6 mM $MgCl_2$, 0,1% Triton X-100), 56.8 μM of dGTP, dCTP and dATP, 28.4 μM of dTTP, 0.5 ρmol of forward and reverse primers, 1.7 μM of dUTP TAMRA (Molecular Probe) fluorescent incorporation labelling and 1 unit of *Taq* DNA polymerase. Polymerase chain reactions were conducted in a Perkin-Elmer 9600 thermocycler (vers. 2.01) with an initial denaturation step of 5 min at 94°C followed by 30 cycles of 45 sec at 94°C, 1 min at appropriate annealing temperature (Table 1) and 1 min at 72°C, ending with a 10 min of elongation phase at 72°C. Polymerase chain reaction products were electrophorized on 8% denaturating polyacrylamide gels, allelic bands were visualized on a FMBIO II scanner (Hitachi), and scored using the GENESCAN-500 size standard (Applied Biosystems, Inc., Foster City, CA).

Single-Locus HFC Analyses

Expected and observed heterozygosity were calculated using GENETIX, version 4.01 (Belkhir et al. 1996–2002), and departure from Hardy-Weinberg equilibrium (HWE) and F_{is} estimates were calculated using GENEPOP version 3.1d (Raymond and Rousset 1995). The relationship between departure from HWE and presence of significant HFC was test-

ed by a χ^2 test and the effect of allele number and expected heterozygosity on departure from HWE was tested using logistic regressions in JMP IN software (SAS Institute 1999).

Prior to analyzing single-locus HFCs, we first tested for evidence of global inbreeding effect. Under the assumption that heterozygosity reflects the individual inbreeding level, theory predicts that heterozygosity of loci within an individual should be correlated (Balloux et al. 2004; Pemberton 2004; Slate et al. 2004). This can be verified by randomly subdividing the sample of loci into two groups and asking whether the mean multilocus heterozygosity (MLH) of the first group of loci is correlated with the MLH of the second group of loci within individuals (Balloux et al. 2004). The MLH-MLH correlation coefficient is then interpreted as an indication of the magnitude of the association between MLH and inbreeding coefficient (Balloux et al. 2004). We randomly subdivided loci into two groups of 15, recalculated individual MLH for both groups, and measured the correlation between those measures with a linear regression. This was repeated 10,000 times using MAPLE release 7 (Waterloo Maple, Inc., Waterloo, Ontario, Canada) to obtain the mean and standard deviation of the correlation coefficient.

The occurrence of positive or negative effects of single-locus heterozygosity on fitness components was first investigated by χ^2 tests in the case of binary components (survival, territoriality, mating success) and two-tailed *t*-tests for continuous traits (standard length, body condition) using JMP IN software (SAS Institute 1999). Correction for multiple tests was applied by calculating the number of false-positive associations expected at $\alpha = 0.05$ for 30 independent tests (one per locus). For each component and each age class, 1.5 false-positive associations were expected, or 13.5 in total (nine components-age classes $\times$ 1.5). Thus, the presence of real associations was tested by a χ^2 comparing the observed number of significant associations ($P \leq 0.05$) with the expected number of false positive (13.5) using SAS release 8.02 (SAS Institute 1999–2001).

Second, the predominance of negative versus positive single-locus HFCs was investigated by a cumulative binomial exact probability test on the direction of the associations (using SAS release 8.02, SAS Institute 1991–2001). For example, suppose that 20 of the 30 loci revealed a positive association between heterozygosity and a given fitness component, and 10 revealed a negative association. The test calculated the probability of obtaining $\geq$20 positive relationships on 30 trials assuming equal probabilities for one result or another (50%), and independently of the absolute level of significance of single-locus HFCs. Also, in order to investigate the stability of the response among fitness components and age cohorts (temporal stability), we performed a categorical model analysis using the CATMOD procedure in SAS release 8.02 (SAS Institute 1999–2001). This procedure determines the importance of each independent variable (fitness component and age cohort) in explaining the variance observed in the response variable (direction of the effect of heterozygosity; positive or negative).

Third, we also tested whether the combination of loci falling into one of the two categories (positive or negative single-locus HFC) had a significant impact on each fitness component. This was achieved using the Fisher method combining probabilities of independent tests (Sokal and Rohlf 1995), as employed by Bierne et al. (2000) in an analogous context.

Finally, we determined whether the distribution of single-locus HFCs was random among loci by performing a χ^2 comparing the distribution of the observed associations versus the expected uniform distribution for all components and both age classes using SAS release 8.02 (SAS Institute 1999–2001).

Estimating the Total Impact of Local HFCs

To infer the possible evolutionary consequences of the observed pattern of local HFCs, we explored the shape and strength of relationships between each fitness component and global heterozygosity resulting from the cumulative effect of local associations. Global heterozygosity was estimated by multilocus heterozygosity (MLH) that is the proportion of heterozygote loci over all loci for each individual. The regressions of fitness traits on MLH were then examined graphically to detect any nonlinear relationships, as performed by Amos et al. (2001) (for an example of nonlinear HFC, see also Neff 2004). The significance of the best-fit model was tested using JMP IN software (SAS Institute 1999). The strength of these ''global'' impacts was also evaluated by calculating selection differentials. For both continuous components (standard length and body condition), we used the method of Lande and Arnold (1983) whereby the selection differential may be inferred from the slope of the regression between statistically standardized MLH and the relative fitness component. In the case of fitness components with a binary response (survival, mating success and territoriality), we calculated standardized selection differentials according to Janzen and Stern (1998), which is an adaptation of the Lande and Arnold (1983) method for using a logistic regression. We performed this analysis separately for both groups of loci showing evidence for either positive or negative HFC. Note that in the alternative view that detected single-locus HFCs may nevertheless be explained by inbreeding (see Discussion), the above analyses may be considered a novel approach for characterizing global effect HFCs.

RESULTS

Descriptive Genetic Parameters and MLH-Inbreeding Correlation

The mean number of alleles per locus was 14.3, and ranged from two to 30 (Table 1). H_o were on average slightly below H_e ($H_o = 0.67 \pm 0.17$; $H_e = 0.74 \pm 0.19$), and 16 loci revealed significant departure from HWE, which resulted in a mean F_{is} of 0.075 ± 0.068 (Table 1). Departure from HWE was associated with allelic richness at a given locus (logistic regression, $\chi^2 = 6.241$, $P = 0.013$, df = 1, $n = 30$, $r^2 = 0.15$), as well as to single-locus H_e (logistic regression, $\chi^2 = 8.211$, $P = 0.004$, df = 1, $n = 30$, $r^2 = 0.20$). However, the departure from HWE had no significant effect on the probability of loci to show significant HFC ($\chi^2 = 0.002$, $P = 0.961$, df = 1), with almost identical results if positive and negative HFCs were considered separately. Allelic richness had no significant effect on the probability of loci to show significant HFC, whether positive and negative rela-

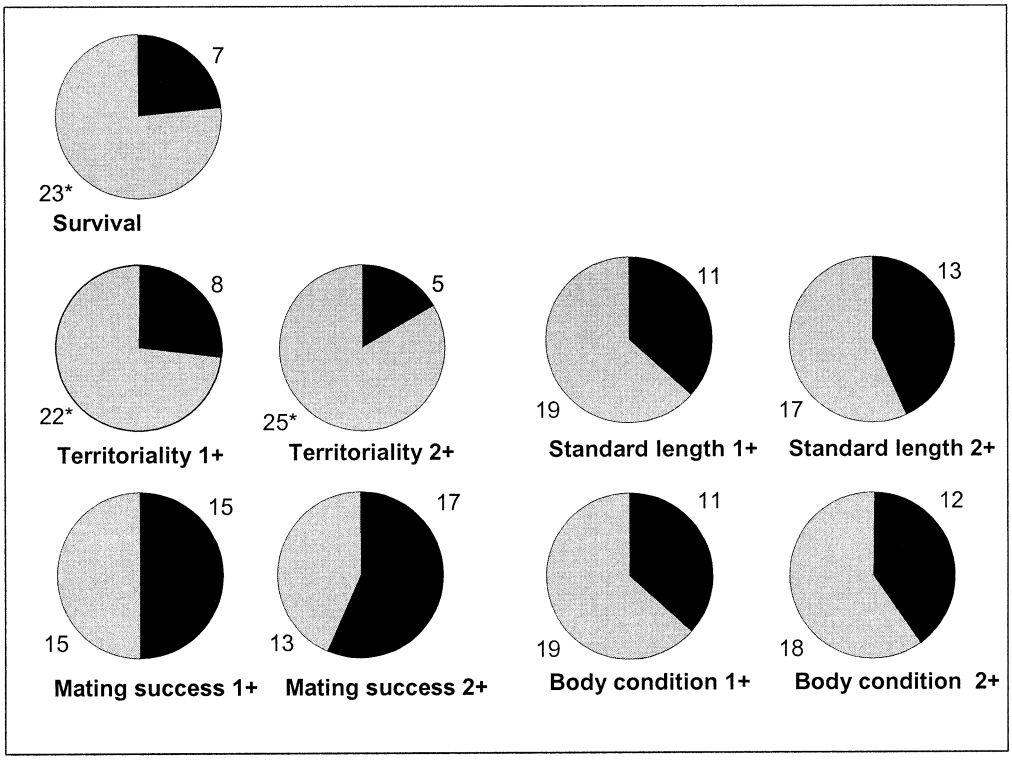

FIG. 1. Numbers of positive and negative single-locus HFCs for each fitness component and cohort. Grey and black shading represent positive and negative HFCs, respectively. Significant differences in proportions, as estimated by cumulative binomial probability tests ($P \leq 0.05$), are indicated by an asterisk.

tionships were considered together or separately ($P > 0.60$). The correlation coefficient between MLH computed with the randomly created subsets of 15 loci was weak (mean $r^2 = 5.5\% \pm 1.9\%$, 10,000 permutations). This indicated that MLH may not be a reliable estimator of individual inbreeding coefficient, and confirmed the relevance of subsequent single-locus based analyses.

Single-Locus HFCs

Occurrence of positive and negative single-locus HFCs

Between zero and six significant single-locus HFCs (mean $= 2.78 \pm 1.56$) were observed depending on the fitness component and the age class analyzed. In total, 25 of the 270 single-locus HFCs were significant, which represented a highly significant difference from the expected false discovery rate of 13.5 ($\chi^2 = 10.312$, $P < 0.001$, df = 1). Eighteen of the 25 significant HFCs were positive, and seven were negative. Considering all single locus HFCs independently of their level of significance, positive relationships represented 63% of all associations (171/270), a highly significant difference from random expectation (cumulative binomial probability test, $P < 0.0001$, trials = 270). When considering fitness components separately, a significant predominance of positive HFCs was observed for survival and territoriality at both ages (Fig. 1). Positive single-locus HFCs were predominantly observed, albeit nonsignificantly, for standard length and body condition as well. In contrast, similar (for 1+ fish) or larger numbers (for 2+ fish) of negative HFCs were observed for male mating success (Fig. 1). Furthermore, the categorical model analysis indicated that proportions of positive versus negative single-locus HFCs significantly varied among fitness components, and were temporally stable between cohorts (Table 2).

TABLE 2. Categorical model analysis of the stability of the direction of effects, positive or negative, of heterozygosity (response variable) in relation to fitness components and age cohorts (independent variables). Complete saturated model in (a), main effects model in (b).

	df	χ^2	P
(a)			
Intercept	1	13.54	<0.001
Cohort	1	0.07	0.785
Fitness component	3	12.23	0.007
Cohort × F. component	3	1.43	0.698
Likelihood ratio	0	—	—
(b)			
Intercept	1	13.54	<0.001
Cohort	1	0.07	0.785
Fitness component	4	12.23	0.007
Likelihood ratio	4	1.43	0.698

Overall significance of single-locus HFCs

Fisher's method for assessing the overall significance of multiple tests was applied separately for loci showing either positive or negative effects (Table 3). This allowed the testing of the probability to observe this combination of either positive or negative single-locus HFCs. The overall effect of loci showing a positive association between heterozygosity and

TABLE 3. Fisher's method for combining probabilities from the single-locus tests, both for positive and negative HFCs. Loci with equal values for either positive or negative effects are omitted. The number of single-locus tests for combined probability is given in parentheses. Values of MLH-fitness correlation coefficient are presented only for significant ($P \leq 0.05$) or nearly significant ($P \leq 0.10$) tests. Significant probabilities ($P \leq 0.05$) are in bold.

		Positive associations		Negative associations	
		P (df)	r^2	P (df)	r^2
Survival		**0.0393** (21)	6.3%	0.8162 (5)	—
Territoriality	1+	0.0731 (19)	8.0%	0.4279 (7)	—
	2+	**0.0030** (18)	16.0%	0.2062 (4)	—
Mating success	1+	0.1803 (15)	14.8%	**0.0172** (12)	11.1%
	2+	**0.0036** (11)	18.6%	**0.0002** (13)	22.0%
Standard length	1+	0.0659 (19)	11.7%	0.8494 (11)	—
	2+	**0.0358** (17)	13.3%	0.6585 (13)	—
Body condition	1+	**0.0370** (19)	7.8%	0.5882 (11)	—
	2+	0.4945 (18)	—	0.2559 (12)	—

fitness was significant for all fitness components in at least one age class (Table 3). Moreover, the overall effect of loci showing a negative association was highly significant for mating success for both age classes (Table 3). No overall significant effect was observed between loci with a negative association and any other trait.

Nonrandom HFCs distribution

The distribution of the significant single-locus HFCs among loci for the different fitness traits was also investigated to test if some loci were particularly involved in creating HFCs. The observed distribution of significant single-locus HFCs tended to be non-random ($\chi^2 = 40.000$, $P = 0.069$, df = 29). Thus, three loci (*Stn34*, *Stn78*, *Stn83*) were associated with three different fitness components, and six other loci (*Stn9*, *Stn31*, *Stn107*, *Stn130*, *Stn147*, *Stn177*) revealed two significant associations, the remaining four being associated with a single component (*Stn14*, *Stn23*, *Stn26*, *Stn96*,). These 13 loci were distributed over nine linkage groups and included loci associated with the length of both the first (*Stn9*, *Stn26*), and second dorsal spines (*Stn96*, *Stn130*) (Table 1).

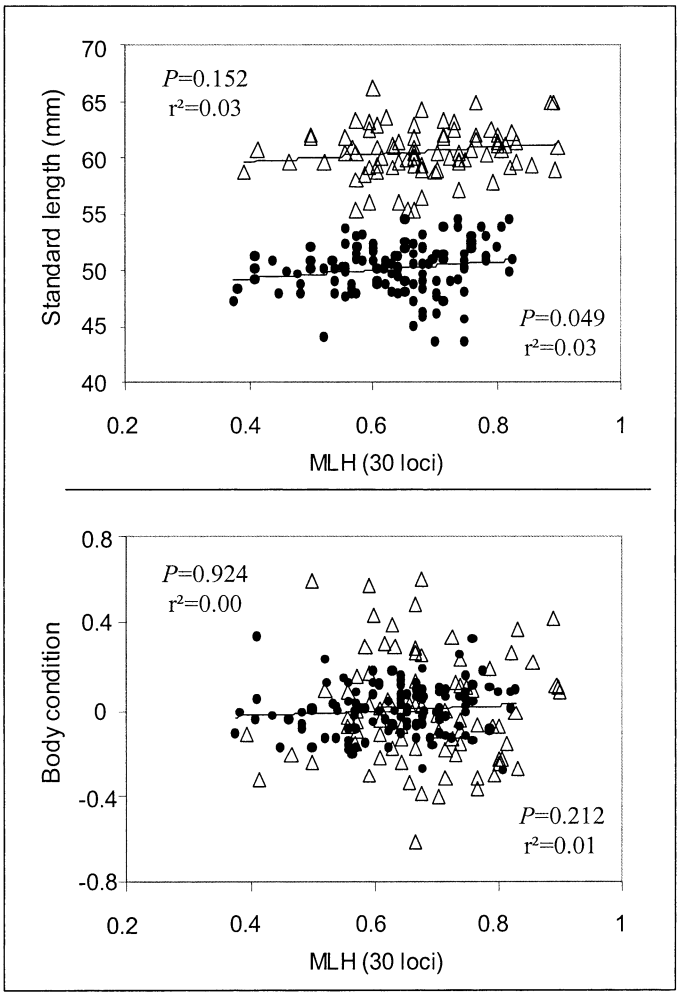

FIG. 2. Relationships between MLH calculated from 30 loci and, from top to bottom, standard length and body condition for each age class. Males of the 2+ cohort are represented by open triangles, while 1+ males are represented by solid circles. P-values and regression coefficients (r^2) of the linear regressions are indicated in the bottom-right corners for the relationships obtained with 1+ males, and upper-left corners for relationships with 2+ individuals. The slope of the linear relationship is presented given a significant correlation or trend, as suggested by the data.

Global Impact, and Single-Locus HFCs

To infer the possible evolutionary consequences of the observed pattern of local HFCs, and also for the sake of comparison with previous studies that focussed on global effects only, we explored the shape and strength of relationships between each fitness component and global heterozygosity resulting from the cumulative effect of local associations. Multilocus heterozygosity over all 30 loci ranged from 0.38 to 0.90, with an average of 0.66 ± 0.11; MLH explained between 3% and 9% (mean = 5%) of the variance in fitness components, depending upon the trait for which a significant association was observed. A significant positive relationship was observed between MLH calculated over all 30 loci and survival (logistic regression, $\chi^2 = 9.405$, $P = 0.002$, df = 1, $n = 208$, $r^2 = 0.034$), territoriality in both age groups (logistic regressions, 1+: $\chi^2 = 4.982$, $P = 0.026$, df = 1, $n = 125$, $r^2 = 0.030$; 2+: $\chi^2 = 9.461$, $P = 0.002$, df = 1, $n = 83$, $r^2 = 0.093$) and standard length for 1+ fish (F-ratio = 3.972, $P = 0.049$, df = 1, $n = 125$, $r^2 = 0.031$), whereas similar, nonsignificant trends were observed for 2+ males in standard length and for 1+ males in the case of body condition (Fig. 2). No apparent relationship with MLH was detected with mating success in either age classes, or with body condition in 2+ males (Figs. 2 and 3). In all cases where a relationship was observed between MLH at the 30 loci and a fitness trait, the best fit was obtained by positive linear relationship (Figs. 2, 3).

Selection differentials

Selection differentials for each fitness-related trait were calculated for the effects of MLH calculated over all 30 loci, but also for loci with either positive or negative HFC rela-

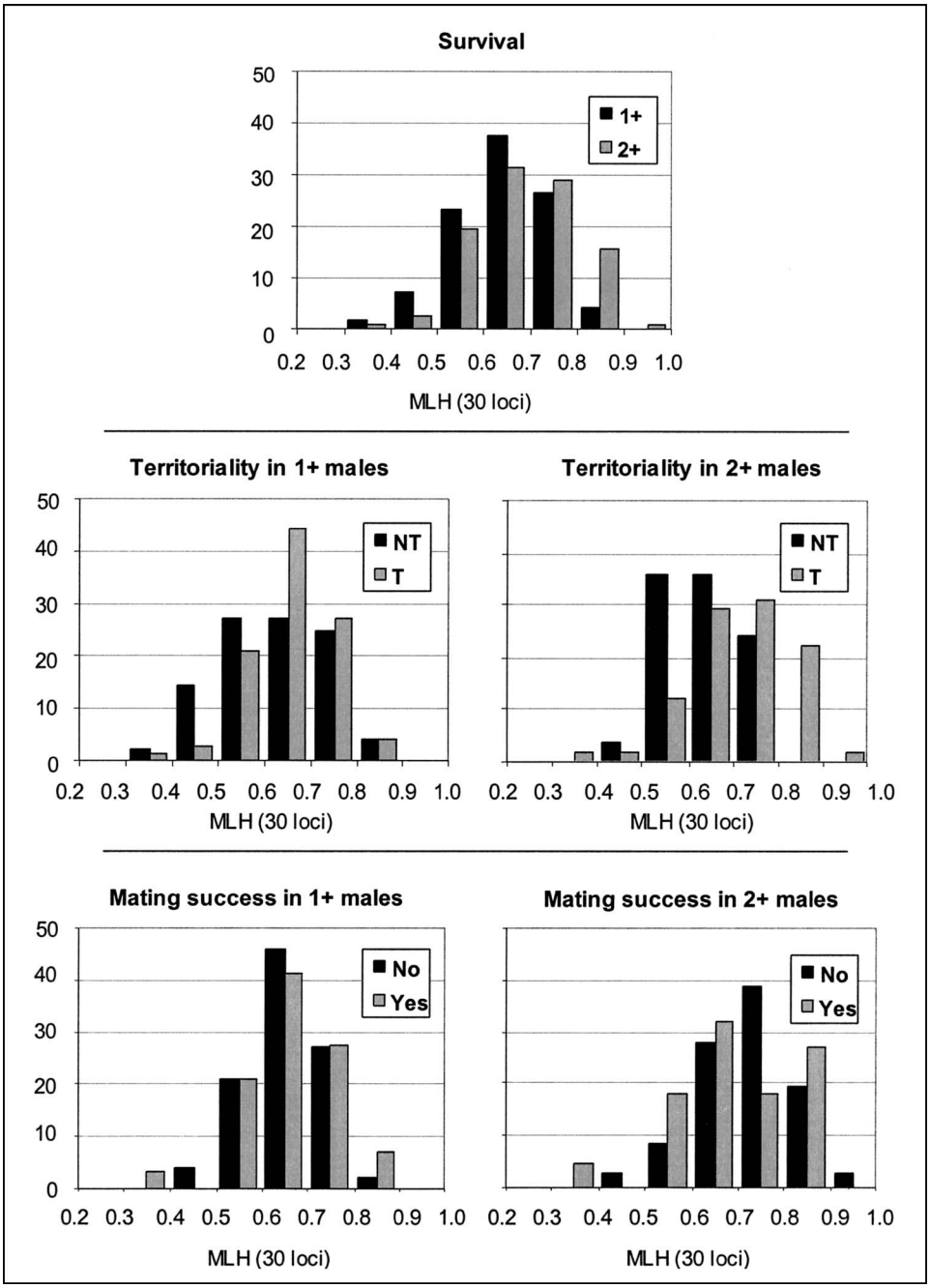

FIG. 3. Relationships between MLH calculated from 30 loci and, from top to bottom, survival, territoriality, and mating success for males of both ages. Multilocus heterozygosity (30 loci) is divided into distinct intervals to facilitate visualization of the relationships. The y-axis presents the percentage of males in each interval for each category. NT and T refer to territorial and nonterritorial males, respectively. Regarding mating success, the categories refer to nesting males with (Yes) or without (No) eggs in their nests.

tionships (Table 4) considered separately. This was done to allow evaluation of the potential strength of selection acting upon specifically implicated loci. Selection differentials (S) calculated with MLH (30 loci) were all positive except for mating success in 2+ fish (−0.12 SE 0.17). The average S value was 0.06, with the highest value being observed for survival (0.26 SE 0.09) and 2+ territoriality (0.21 SE 0.08). Selection differentials calculated with loci showing positive HFC were globally higher with an average effect of 0.24 and ranged from 0.01 SE 0.00 (2+ length) to 0.64 SE 0.20 (2+ mating success) (Table 4); S acting upon length and body condition were consistently lower, possibly because of the small variance in these traits (data not shown). Finally, S calculated from MLH restricted to negative single-locus HFCs for mating success were −0.46 SE 0.15 in 1+ males, and −0.59 SE 0.17 in 2+ males (Table 4).

TABLE 4. Selection differentials (S) and standard errors calculated using MLH estimate based on all 30 loci, those showing positive HFC (+ restricted MLH), or those showing negative HFC (− restricted MLH) in the case of mating success. Values that are not presented were all near zero. The number of loci used to estimate restricted MLH is provided in parentheses. Significant HFCs ($P \leq 0.05$) are in bold.

		S, 30 loci MLH	S, + restricted MLH (number of + loci)	S, − restricted MLH (number of − loci)
Survival		**0.26 ± 0.09**	**0.36 ± 0.09 (21)**	—
Territoriality	1+	**0.15 ± 0.07**	0.25 ± 0.07 (19)	—
	2+	**0.21 ± 0.08**	**0.26 ± 0.07 (18)**	—
Mating success	1+	0.02 ± 0.15	0.56 ± 0.17 (15)	**−0.46 ± 0.15 (12)**
	2+	−0.12 ± 0.17	**0.64 ± 0.20 (11)**	**−0.59 ± 0.17 (13)**
Standard length	1+	**0.01 ± 0.00**	0.02 ± 0.00 (19)	—
	2+	0.01 ± 0.00	**0.01 ± 0.00 (17)**	—
Body condition	1+	0.01 ± 0.01	**0.03 ± 0.01 (19)**	—
	2+	0.00 ± 0.03	0.07 ± 0.03 (18)	—
Mean		0.06	0.24	−0.53

DISCUSSION

Balloux et al. (2004), and Slate et al. (2004) have both shown the inadequacy of considering a priori HFCs as a manifestation of global inbreeding depression. As a result, their findings have reinforced the notion of heterozygosity effects on fitness at a local level, consistent with recent empirical studies finding support for local effect HFCs (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005; Tiira et al. 2006). Yet, doubts persist around the existence and importance of local effect HFCs in natural populations (Pemberton 2004). In this context, our aim was to investigate the presence, characteristics, magnitude and consequences of local heterozygosity effects in a natural population of threespine sticklebacks.

Strong Evidence of Positive and Negative Single-Locus HFCs

Significant single-locus HFCs were observed in all fitness components: survival, mating success, territoriality, standard length, and body condition. Together with the weak observed correlation of heterozygosity among loci, these results strongly suggest that HFC may arise without global inbreeding effects (Balloux et al. 2004). In the case of global effects, positive HFC is usually expected (Charlesworth and Charlesworth 1987; Thornhill 1993), with global negative HFCs being rarely reported and typically weak (Coltman and Slate 2003). It is intuitive to expect more variable interactions at the local HFCs level than at the global scale. Indeed, variation arising from the locus-specific characteristics is added to variation resulting from the population history and fitness component (also impacting at the global effects level). Thus, an intuitive hypothesis in local effect HFCs is to expect complex assortment of null, positive, and negative HFCs in a single population at a given moment, depending on the loci and the traits under study. In this study, positive single-locus HFCs were predominant in all fitness components except mating success, and showed a temporal stability between cohorts. Moreover, Fisher's test revealed significant grouped positive effects for all components. Nevertheless, grouped negative effects were strong and highly significant with respect to mating success. Moreover, heterozygosity at some of the loci had no apparent impact at all on fitness for the traits we studied. Thus, within population heterozygosity-fitness correlations were more complex for local effects than for global inbreeding effects in this study.

Possible Evolutionary Consequences

Our results indicated that more heterozygous males at some particular loci have a higher total fitness because: (1) they are generally larger; (2) they have a higher probability of establishing a territory; (3) territorial males obtain the vast majority of fertilizations (Blais et al. 2004); (4) among territorial males, heterozygous individuals (at some loci) are more likely to reproduce and finally; (5) heterozygous males are more likely to live two years, and therefore, potentially reproduce more than once (Lachance and FitzGerald 1992). Moreover, the impacts of heterozygosity on fitness were relatively strong in many cases. For instance, heterozygosity at 11 to 21 loci explained on average 13% (and up to 22%) of the variance in fitness components. Furthermore, heterozygosity at those loci was associated with high selection differentials via change in males' survival, territoriality, and mating success probabilities. For example, the selection differential from positive HFCs was 0.36 SE 0.09 for males' survival, 0.56 SE 0.17 for mating success in 1+ males and 0.64 SE 0.20 in 2+ males. Admittedly, one cannot readily compare these values with selection differentials calculated from phenotypes. Yet, it is noteworthy that these are substantially higher than the median value of 0.16 reported by Kingsolver et al. (2001) in their meta-analysis of selection on phenotypes.

Our finding of substantial heterozygosity impacts on fitness contrasts with the small effects usually reported in studies of inbreeding depression on natural animal populations, studies which have led to the general conclusion of weak impacts of heterozygosity on fitness (David 1998; Hasson and Westerberg 2002; Slate and Pemberton 2002; Coltman and Slate 2003). The difference between most previous studies and ours stems primarily from differences in analytical approaches, rather than the specificity of the focal population. Thus, we believe that focusing on single-locus analysis may be more efficient at deciphering the complexity of interactions between heterozygosity and fitness. Here, this approach allowed quantifying the differential impacts of loci with negative and

positive effects separately, which would have been hidden in analyses focusing on global effects only. Indeed, strong impacts of heterozygosity on fitness associated with local effects are not so surprising when considering the emerging picture of consistent, nonadditive genetic effects on phenotypic expression, including dominance and overdominance (e.g., Meffert et al. 2002; Sgrò and Blows 2003; Gibson et al. 2004; Syed and Chen 2005; see also Neff and Pitcher 2005).

Different evolutionary outcomes could arise from local heterozygosity effects on fitness. For instance, it is probable that, at loci showing positive HFCs, alleles will persist longer in the population as a result of balancing selection. Moreover, positive HFC could increase positive selection upon new mutations, and together, these two effects could lead to the maintenance of genetic diversity within the population (Balloux et al. 2004). Interestingly, loci showing at least one significant positive HFC tended to have slightly more alleles (Table 1), and included none of the seven loci having fewer than 10 alleles. Hansson et al. (2004) found similar results in a HFC study on great reed warblers. In their study, the strongest single-locus positive HFCs were found in the four markers (out of 19) showing the highest heterozygosity (but see Tiira et al. 2006). Positive HFCs could also promote dispersal through an increase of migrant indirect fitness because their offspring are more likely to be heterozygous compared to those of local parents. This would be analogous to theoretical expectations in the case of inbreeding depression (Thornhill 1993).

Possible evolutionary consequences on mate choice

Heterozygosity-fitness correlations may also have biologically meaningful impacts in the evolution of mate choice. Mate choice by female threespine sticklebacks could have both direct (material) and indirect (genetic) benefits, because males not only provide females with sperm, but also aerate and defend eggs and fry against predators (Wootton 1976). Therefore, the observed general positive single-locus HFCs may promote the evolution of two nonexclusive types of mate choice: females could either choose a more heterozygous male to increase direct benefits (e.g., more heterozygous males have a better survival), or a compatible male (e.g., one having different alleles at effective loci) to increase offspring quality (indirect benefits). Finally, HFCs could also contribute to the evolution of polyandry, because multiple mating has been shown to result in higher genetic diversity and progeny survival (e.g.; Garant et al. 2005). However, polyandry is apparently a limited phenomenon in this population (Whoriskey et al. 1986; Bell and Forster 1995; Blais et al. 2004).

On the other hand, both important positive and negative single-locus HFCs affected males' mating success in this study. Single-loci showing positive and those showing negative effects could either influence the same trait or different traits used by females in mate choice. Given that heterozygosity had a positive effect for other fitness components, it is plausible that negative and positive HFCs may affect different traits. Traits affected by either negative or positive single-locus HFCs could be integrated through multiple cues in female mate choice, whereby females choose both heterozygous males at random loci (good fathers; direct benefit) and for indirect benefits by choosing individuals homozygous at particular good genes. This hypothesis, however, remains to be rigorously tested, as alternative explanations are likely. Finally, genetic compatibility in terms of dissimilarity could represent another cue that could be integrated in a hierarchical scheme for choosing mates, as demonstrated empirically by Roberts and Gosling (2003) and reviewed in Mays and Hill (2004).

Recently, Neff and Pitcher (2005) proposed to relate ''good genes'' with additive genetic variation in fitness and ''compatibility genes'' with nonadditive genetic variation in fitness. In their view, genetic quality becomes the sum of additive and nonadditive quality (Neff and Pitcher 2005). Thus, mate choice may (1) oscillate between a good genes mating system and compatibility genes mating system, (2) rely on both good genes and compatibility genes in the same female simultaneously, or (3) rely on both good genes and compatibility genes in different females with negative frequency dependent selection on the two female types (Neff and Pitcher 2005). Such a model for mate choice is very appealing, because it allows the maintenance of both additive and nonadditive genetic variance and thus helps to elucidate an important evolutionary paradox (Neff and Pitcher 2005; see for example Roff and Mousseau 1987). Although not considered by Neff and Pitcher (2005), this model could possibly be expanded to consider mate choice for direct benefits. For instance, here we found that females chose males that were more heterozygous at several loci. This behavior is likely to provide females with better fathers for providing care to their offspring, and also to favor maintenance of both additive and nonadditive variance. Indeed, even if females choose consistently for a nonadditive male quality, this quality is not heritable per se, and thus will not respond to selection by a decrease in variability.

Generality of the Findings

In addition to this study, several recent papers provided evidence for the importance of local associative effects in explaining HFC in different species and contexts (Bean et al. 2004; Hansson et al. 2004; Markert et al. 2004; Spielman et al. 2004; Syed and Chen 2005; Tiira et al. 2006). It thus appears that single-locus HFCs may be more common than previously assumed. In this study, however, the local characteristics of the Isle-Verte's stickleback population may have increased the prevalence of positive single-locus HFCs. For example, this population is located within the transition between fresh and salt water, which might present a particularly heterogeneous environment prone to heterozygote advantage. Also, its effective population size is likely larger than small isolated populations, and therefore less likely to be purged of recessive deleterious alleles (Lande and Schemske 1985). Moreover, intense male-male competition in this population (Blais et al. 2004) could contribute to inflated fitness differences between more or less heterozygous individuals. Yet, such life-history characteristics are by no means exclusive to this stickleback. Admittedly, loci ''that count'' may have being over represented in this study as we use seven loci for which a QTL association has been shown (Ta-

ble 1). However, QTL associated loci did not exhibit more frequently significant HFCs (results not shown).

Alternative explanations

In the previous sections, we argued that the strong consistent single-locus HFCs found in this study, together with the relatively weak MLH-MLH correlation, indicate that HFC could arise without the presence of inbreeding depression. However, it may be argued that evidence for strong single-locus HFCs is not sufficient in itself to rule out a global effect explanation. Moreover, correlation of heterozygosity among loci was weak but still enough to lead MLH-inbreeding correlation r^2 of about 0.3 (according to Balloux et al. 2004 simulations). It was also among the strongest MLH-inbreeding relationships report by Slate et al. (2004). Thus, single-locus HFCs may have alternatively been driven by a global inbreeding effect, such that nonrandom influence of particular loci could reflect their particular ''linkage'' to inbreeding level. However, this view shifts the debate to the origin and causes of the variation in heterozygosity among individuals. Does variation in heterozygosity arise from different inbreeding level or from a mix of chance and selection, or even a mix of the three? We propose that the latter is more likely. As point out by Balloux et al. (2004), there is surely a spectrum of explanations for HFCs in nature, from pure inbreeding effect (large numbers of recessive genes with small effects detected by identity disequilibrium) to chance linkage between a marker and few genes of large effects (local linkage disequilibrium). Either way, at some point, global effects must have a local cause: a given effective gene. In this view then, our results show at the very least that single-locus analysis is more efficient than a global approach to decipher heterozygosity-fitness correlations.

To conclude, we propose that future research will be more fruitful if the debate on local versus global effects is shifted to questions bringing more light on the evolutionary significance of HFCs, such as: (1) the identification of the effective genes underlying HFCs; (2) the evaluation of the mechanisms creating HFCs, namely dominance, overdominance, and genotype-by-environment interactions; (3) the determination of the relative importance of additive and nonadditive aspects of genetic quality and their interactions, and; (4) the evaluation of the impacts of different evolutionary histories and environmental factors on the expression of HFC.

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