

Heterozygote advantage and the maintenance of polymorphism for multilocus traits

Alexandre Ding*, Jérôme Goudet

Department of Ecology and Evolution, Biology Building, University of Lausanne, 1015 Lausanne, Switzerland

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Abstract

Explaining how polymorphism is maintained in the face of selection remains a puzzle since selection tends to erode genetic variation. Provided an infinitely large unsubdivided population and no frequency-dependance of selective values, heterozygote advantage is the text book explanation for the maintenance of polymorphism when selection acts at a diallelic locus. Here, we investigate whether this remains true when selection acts at multiple diallelic loci. We use five different definitions of heterozygote advantage that largely cover this concept for multiple loci. Using extensive numerical simulations, we found no clear associations between the presence of any of the five definitions of heterozygote advantage and the maintenance of polymorphism at all loci. The strength of the association decreases as the number of loci increases or as recombination decreases. We conclude that heterozygote advantage cannot be a general mechanism for the maintenance of genetic polymorphism at multiple loci. These findings suggest that a correlation between the number of heterozygote loci and fitness is not warranted on theoretical ground.

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1. Introduction

A puzzling problem in population genetics revolves around the question of the maintenance of genetic polymorphism. Empirical evidences show that natural selection in the wild is widespread for many traits (Endler, 1986; Kingsolver et al., 2001). And in many experiments, sustained response to artificial selection attests the presence of genetic polymorphism (Hill and Caballero, 1992). Since selection tends to erode genetic polymorphism, there is clearly a paradox between widespread selection and large amounts of genetic polymorphism. Mutation and balancing selection are two major factors invoked for the resolution of this paradox (Barton and Keightley, 2002). It is well established that mutation plays a role in generating genetic polymorphism under selection (e.g. Zhang and

Hill, 2002). Here, we focus on one factor that maintains (rather than create) genetic polymorphism: the advantage of heterozygote in an infinitely large, unsubdivided, population.

Theoretical studies diverge as to whether heterozygote advantage is the main reason for the maintenance of genetic polymorphism. Some showed that heterozygote advantage can maintain polymorphism. When selection acts at one diallelic locus, heterozygote advantage is necessary and sufficient to maintain genetic polymorphism (e.g. Hedrick, 1999). Ginzburg (1979) showed that, when selection acts at one multiallelic locus, the arithmetic mean fitness of heterozygotes (weighted or not by allelic frequencies) must be higher than the (weighted respectively unweighted) arithmetic mean fitness of homozygotes in order to maintain genetic polymorphism. Similarly, when stabilizing selection acts at multiple loci with purely additive effects, Zhivotovsky and Feldman (1992) showed that genetic polymorphism can be maintained if heterozygote genotypes have a higher stability of development than homozygote

*Corresponding author. Fax: +41 21 692 42 65.

E-mail addresses: alexandre.ding@unil.ch (A. Ding), jerome.goudet@unil.ch (J. Goudet).

genotypes (i.e. a form of heterozygote advantage). And when selection acts at two diallelic loci, Turelli and Ginzburg (1983) and Gimelfarb (2000) showed, using numerical simulations, that the probability of maintaining polymorphism is high when there is “heterozygote advantage”. While these studies confirmed that heterozygote advantage can maintain polymorphism, others did not. Lewontin et al. (1978) showed numerically that the advantage of the heterozygote cannot maintain a large amount of polymorphism when selection acts at one multiallelic locus (but see Spencer and Marks, 1992). Other relevant studies for these associations between heterozygote advantage and polymorphism are Hastings (1981, 1982). Using numerical examples for selection acting at two diallelic loci, he showed that genotypic polymorphism can be maintained even when the two loci show an heterozygote disadvantage. And when selection acts at multiple loci, Gimelfarb (1998) came to equivocal results depending on how heterozygote advantage is measured (see below). However, this study was incomplete according to heterozygote advantage for traits coded by multiple loci (did not include all potential definitions for heterozygote advantage for traits coded by multiple loci). Therefore, it remains unclear whether heterozygote advantage is a general mechanism for the maintenance of polymorphism.

Our purpose here is to test whether heterozygote advantage is a general mechanism for maintaining polymorphism when selection acts at two, three, or four diallelic loci. To this end, we quantified the association between allelic polymorphism and heterozygote advantage for randomly generated fitness matrices. We use five different definitions of heterozygote advantage for traits coded by multiple loci. These definitions and their extensions to multiple loci are based on notions proposed by Lewontin et al. (1978), Turelli and Ginzburg (1983), and Karlin (1990). These notions and their extensions are fully described below in the method section. To find whether all alleles at all loci are maintained under selection, we use the classical model of genetic selection for multilocus systems (see Bürger, 2000). We predict that matrices maintaining full allelic polymorphism should show some form of heterozygote advantage, while heterozygote advantage should be absent in those matrices not maintaining allelic polymorphism. In general these predictions are not fulfilled. We therefore conclude that heterozygote advantage is not a general mechanism for the maintenance of allelic polymorphism at multiple loci.

2. Method

2.1. Population

Our model consists of a diploid monoecious population of infinite size, where mating occurs at random.

Generations are discrete and non-overlapping. Mutation is absent.

2.2. Fitness matrices

We consider traits coded by two, three, and four diallelic loci. A matrix of fitness defines fitness values of all possible genotypes (i, j) in the population. It is a square and symmetric matrix where rows and columns represent the different gametes. Each cell of the matrix, w_{ij} , gives the fitness value of the genotype (i, j) . We consider that there are no imprinting effects (i.e. $w_{ij} = w_{ji}$) and no cis-trans effects (e.g. $w(AB/ab) = w(Ab/aB)$).

We generated 4000 fitness matrices for each of the three traits (respectively coded by two, three, and four loci). The value of each w_{ij} (within the constraints given above) is a random integer number between 1 and 100 obtained from a uniform distribution. We use a uniform distribution since the a priori distribution of fitness values in the wild is unknown.

2.3. Heterozygote advantage

When fitness is coded by one locus with two alleles, there is heterozygote advantage if the fitness of the heterozygote is higher than the fitnesses of both homozygotes. This notion of heterozygote advantage needs to be extended when traits are genetically complex. A trait coded by multiple loci having multiple alleles has more than one heterozygote state at a given locus and can have a mixture of heterozygote and homozygote loci. This complicates the definition of heterozygote advantage.

In this section we extend the definitions of the heterozygote advantage for traits coded by (i) one diallelic locus (e.g. Hedrick, 1999) and (ii) one multiallelic locus (Lewontin et al., 1978; Turelli and Ginzburg, 1983; Karlin, 1990) to traits coded by multiple diallelic loci. This results in five extended definitions of heterozygote advantage that are reported under points (a)–(e) below and summarized in Table 1. Importantly, these definitions are based on raw fitness values (i.e. fitness values not weighted by allelic frequencies, see Section 4), since our goal is to determine which fitness matrices allow the maintenance of polymorphism independently of the population state.

When fitness is coded by more than one locus, the locus ℓ has an *average heterozygote advantage* if the average fitness for the heterozygote at this locus, $\bar{w}(\ell_1, \ell_2)$, is higher than average fitnesses of homozygotes at this same locus ℓ , $\bar{w}(\ell_1, \ell_1)$ and $\bar{w}(\ell_2, \ell_2)$. The average fitness value $\bar{w}(\ell_i, \ell_j)$ is calculated over all genotypes having alleles i and j at the locus ℓ . And when all loci are considered:

- (a) A matrix of fitness coded by ℓ loci has an *average heterozygote advantage* (abbreviated *aha*) if average heterozygote advantage is verified for all loci (Table 1).

Table 1

Definitions of heterozygote advantage and their abbreviation (abr.) for fitness coded by ℓ diallelic loci with their references (ref.)

Definitions of Heterozygote advantage	Abr.	Definitions	Ref.
(a) Average heterozygote advantage	<i>aha</i>	$\bar{w}(z_1, z_1), \bar{w}(z_2, z_2) < \bar{w}(z_1, z_2) \forall z \in \{1, \dots, \ell\};$ $\bar{w}(z_i, z_j)$ is an average over all genotypes having alleles i and j at the locus z .	
(b) Ordered matrix of fitness	<i>omf</i>	$\bar{w}_\ell > \bar{w}_{\ell-1} > \dots > \bar{w}_0;$ \bar{w}_k is an average over all genotypes that have k heterozygous loci, $k \in \{0, 1, \dots, \ell\}$.	1,2
(c) Local heterozygote advantage	<i>lha</i>	$w(i, j) > [w(i, i) + w(j, j)]/2 \forall (i \neq j) \in \{1, \dots, g\};$ $w(i, j)$ is the fitness value of the genotype (i, j) and g is the number of gametes.	1
(d) Invariable heterozygote fitness	<i>ihf</i>	$w(i, j) < w(i, m) + w(j, n)$, $w(i, j)$ as defined under (c) above.	1
(e) Simple overdominance	<i>so</i>	all gametes are maintained at equilibrium when recombination is absent.	3

Ref.: (1) Lewontin et al., 1978, (2) Turelli and Ginzburg, 1983, (3) Karlin, 1990.

When fitness is coded by one locus with n alleles, intuitively, there should be heterozygote advantage if the fitnesses of all heterozygotes are higher than the fitnesses of all homozygotes. However Mandel (1959) showed that this is neither a sufficient, nor a necessary condition for maintaining all alleles at equilibrium. He derived the necessary and sufficient conditions for the maintenance of multiallelic polymorphism. From these conditions Lewontin et al. (1978) derived two relations between the fitnesses of heterozygotes and homozygotes and one relation between heterozygotes that are all necessary for maintaining allelic polymorphism. These definitions can be extended to traits coded by multiple loci. They are treated here as three different definitions even if all are necessary for maintaining polymorphism at a single multiallelic locus. We define a matrix of fitness coded by ℓ loci as having heterozygote advantage if:

- (b) the average fitnesses \bar{w}_k 's, defined as the average fitness of all genotypes having k heterozygous loci ($k = \{0, 1, \dots, \ell\}$), are ordered according to the number of heterozygous loci (abbreviated *omf* for *ordered matrix of fitness*, Table 1), this is the definition of heterozygote advantage used by Gimelfarb (1998);
- (c) there is an overall *local heterozygote advantage* (abbreviated *lha*), if each genotype made of two different gametes (i, j) has a higher fitness than the average fitness of the two corresponding homozygous genotypes (i, i) and (j, j) (Table 1);
- (d) each heterozygote (i, j) has a lower fitness than the sum of fitness of any two others heterozygous genotypes (i, m) and (j, n) , this definition is called *invariable heterozygote fitness* and is abbreviated *ihf* (Table 1).

Definitions given under (b)–(d) form three necessary conditions for maintaining all alleles when selection acts at one multiallelic locus (Lewontin et al., 1978). Since these three definitions are all necessary, they may be seen as a unit that predicts if polymorphism is maintained under selection. However, below we treat them independently to disentangle the effect of each of these definitions.

The last definition of heterozygote advantage is derived from Karlin (1990):

- (e) a matrix of fitness is *simply overdominant* (abbreviated *so*) if it maintains all gametes at equilibrium when recombination is absent (Table 1).

Among these five definitions of heterozygote advantage, *average heterozygote advantage* and *ordered matrix of fitness* are the two definitions closest to the definition when fitness is coded by one diallelic locus. Definition of *simple overdominance* uses the tautology between heterozygote advantage and the maintained polymorphism at one locus (see Karlin, 1990 for details). This last definition has never been used empirically.

For each matrix of fitness, we recorded the occurrence of each of the five definitions of heterozygote advantage (defined under points (a)–(e) above). Note that these five definitions are not independent. A matrix of fitness showing *lha* can also show *so* and *aha* for example. These definitions should be regarded, therefore, as different ways of characterizing a “general” heterozygote advantage. The next step consists in finding if these same fitness matrices maintain polymorphism under selection.

2.4. The model

We use the classical model of genetic selection for traits coded by multiple loci (first developed by Lewontin and Kojima, 1960 for two diallelic loci) as a mean to find if genetic polymorphism is maintained by a given matrix of fitness. Bürger (2000) gives a full and general description of this model that we briefly summarize here. Selection is constant through generations and acts on viability differences. Let $x_i(t)$ be the frequency of gamete i at generation t ($\sum_i x_i(t) = 1$), and w_{ij} be the fitness of the genotype constituted by gametes i and j . The frequency of gamete i in generation $t + 1$ is given by

$$x_i(t + 1) = \frac{1}{\bar{w}(t)} \sum_{j,k} w_{j,k} x_j(t) x_k(t) R(j, k \rightarrow i), \quad (1)$$

where $\bar{w}(t)$ is the average fitness of the population at generation t given by

$$\bar{w}(t) = \sum_{i,j} w_{i,j} x_i(t) x_j(t) \quad (2)$$

and $R(j, k \rightarrow i)$ is the probability that genotype (j, k) produces gamete i . This probability is a function of the recombination rate (r). The recombination rate between two adjacent loci is independent of the loci considered (i.e., all loci are equidistant) and of the occurrence of recombination at other loci. It remains constant through generations. We use numerical simulations to seek the equilibrium properties of this system.

2.5. Numerical simulations

This method of numerical simulation has been developed by Karlin and Carmelli (1975). It is commonly used for the study of the model given by Eq. (1) (e.g. Lewontin et al., 1978; Turelli and Ginzburg, 1983; Gimelfarb, 1998). Parameters of the model given by Eq. (1) are (1) the matrix of fitness and (2) the recombination rate (r). We use 4000 randomly generated matrices for traits coded by two, three, and four loci (as defined above) and 4 different recombination rates (0.00, 0.01, 0.05, and 0.50). We used each of the four recombination rates for each of the 4000 matrices of fitness. When loci are linked ($r = 0.00$), a trait coded by ℓ loci is equivalent to a trait coded by one locus having 2^ℓ alleles (e.g. Roughgarden, 1979). Recombination rates of 0.01 and 0.05 were chosen because previous studies (Karlin and Carmelli, 1975; Gimelfarb, 1998) showed that it is around these values of r that changes in equilibrium properties might occur. Finally the maximal recombination rate ($r = 0.50$) represents a system in which the loci segregate independently.

We assume that initially all gametes are present according to the product of the frequency of their constituting alleles (i.e. gametic phase equilibrium). These starting conditions constitute a regular grid covering the space of gametic frequencies. Frequencies of the alleles take the following values:

For two loci: $\ell_1 = \{0.01, 0.09, 0.19, 0.29, 0.39, 0.49, 0.59, 0.69, 0.79, 0.89, 0.99\}$;

For three loci:

$\ell_1 = \{0.01, 0.09, 0.24, 0.39, 0.54, 0.69, 0.84, 0.99\}$;

For four loci: $\ell_1 = \{0.01, 0.35, 0.70, 0.99\}$;

And $\ell_2 = 1 - \ell_1$.

The number of starting conditions used is 121 for fitness coded by two loci (11^2), 512 for the three loci (8^3) and 256 for the four loci (4^4). There is a lower number of starting allelic frequencies for four loci compared to two loci (and for three compared to two), in order to maintain a reasonable number of starting gametic frequencies.

Changes of gametic frequencies (Eq. (1)) for each matrix of fitness were followed until an equilibrium was

reached. Equilibrium was reached when differences in frequency for all gametes between two consecutive generations are less than 10^{-12} , i.e. $|x_i(t+1) - x_i(t)| \leq 10^{-12} \forall i$. Simulations that did not reach equilibrium after 100,000 generations were discarded from the analysis. A matrix of fitness was discarded if no equilibrium was attained for any of the starting conditions. The total number of discarded matrices is negligible (21 over 4000 matrices in the worst case). Two equilibria are considered equal if the absolute differences between frequencies of all gametes are less than 10^{-3} (a higher stringency does not alter the results).

We checked that the implementation of the model was correct by using fitness matrices with known solutions for two loci (e.g. Roughgarden, 1979) and numerical results for three and four loci. Another check was the congruence of our results with those of Karlin and Carmelli (1975), Lewontin et al. (1978), Turelli and Ginzburg (1983), and Gimelfarb (1998), despite our use of an evenly spaced larger number of starting conditions.

2.6. Classes of polymorphism

Once equilibria for a given matrix of fitness were reached, each matrix was classified according to the maximal number of loci it maintains polymorphic. A locus is considered polymorphic if the rarest allele has a frequency higher than 0.01. For simplicity and clarity, we define two classes of polymorphism:

- (i) *Fully polymorphic* (abbreviated *f*): all of the ℓ loci are maintained polymorphic at equilibrium,
- (ii) *Non-fully polymorphic* (abbreviated \bar{f}): at most $\ell - 1$ (out of ℓ) loci are maintained polymorphic at equilibrium (this includes the case where none of the loci remained polymorphic).

2.7. Analysis

The measure of interest is the strength of the association between polymorphism maintained at equilibrium and each of the five definitions of heterozygote advantage. To quantify this association, we used proportions of fitness matrices which maintain polymorphism and satisfy each of the five definitions of heterozygote advantage.

For fully (*f*) and for non-fully (\bar{f}) polymorphic fitness matrices, we estimated the proportions of matrices having each of the five definitions of heterozygote advantage. This calculation is done for the four rates of recombination and for matrices of fitness coded by two, three, and four loci. For instance, the proportion of fully polymorphic fitness matrices showing an average heterozygote advantage, abbreviated $P_{ahal/f}$, is estimated

as the number of matrices showing *aha* among the fully polymorphic fitness matrices. A proportion $P_{x|y}$ is zero when no matrices of a given class (y) of polymorphism have the considered definition (x) of heterozygote advantage (for a given trait and recombination rate). It takes value one when all matrices have the considered definition (x) of heterozygote advantage. Note that a condition x is necessary if $P_{x|f} = 1$ and is sufficient if, in addition to $P_{x|f} = 1$, $P_{x|\bar{f}} = 0$.

Having defined these proportions, two predictions can be made. First, if heterozygote advantage maintains genetic polymorphism, fully polymorphic matrices should have proportions close to one, while non-fully polymorphic matrices should have proportions close to zero (i.e. $P_{x|f} \approx 1$ and $P_{x|\bar{f}} \approx 0$). For each trait and recombination rate, data can be arranged in a two-by-two contingency table (one entry is the presence/absence of the definition of heterozygote advantage, the other entry is the classes of polymorphism). The significance of observed differences is tested using Fisher's exact tests.

A second prediction is that if heterozygote advantage is a general mechanism for the maintenance of genetic polymorphism, the proportions of fully polymorphic matrices showing one of the definitions of heterozygote advantage should be the same for two, three, and four loci (i.e. $P_{x|f}(\ell = 2) \approx P_{x|f}(\ell = 3) \approx P_{x|f}(\ell = 4)$).

3. Results

Out of the five different definitions of heterozygote advantage (summarized in Table 1), *local heterozygote advantage* and *simple overdominance* are never found for the 4000 random fitness matrices for traits coded by three and four loci. Of the three remaining definitions, the proportions of fully polymorphic fitness matrices showing *average heterozygote advantage* ($P_{aha|f}$) and *ordered matrix of fitness* ($P_{omf|f}$) decrease as the number of loci increases, contrary to our second prediction. The proportion of matrices that have invariable heterozygote fitness increases with the number of loci for fully ($P_{ihf|f}$) and non-fully polymorphic ($P_{ihf|\bar{f}}$) matrices. Again, this does not correspond to our second prediction. These results are reported in more details in the next sections, after a description of the proportions of fully polymorphic matrices.

3.1. Classes of polymorphism

Fig. 1 gives the proportions of fully polymorphic matrices, i.e. matrices that maintain all alleles for all coding loci, for the four rates of recombination (the proportion of non-fully polymorphic matrices is one minus the proportion of fully polymorphic matrices).

For traits coded by two, three, and four loci, the highest proportion of fully polymorphic fitness matrices are found when loci are totally linked ($r = 0.00$, Fig. 1).

These proportions decrease as the rate of recombination increases for the three traits.

When loci are loosely linked ($r \geq 0.05$), the proportion of fully polymorphic fitness matrices diminishes as the number of loci increases. On the contrary, when loci are tightly linked ($r \leq 0.01$), the proportion of fully polymorphic matrices increase as the number of loci increases. There is clearly a strong interaction between recombination rate and the number of loci on the maintenance of polymorphism.

3.2. Average heterozygote advantage

Fig. 2A gives the proportions of fully (above the x -axis) and non-fully (below the x -axis) polymorphic matrices that have an *average heterozygote advantage* (*aha*, as defined under a in Table 1). The highest $P_{aha|f}$ is about 0.8 ($\ell = 2, r = 0.50$). Therefore, at least 20% of the matrices that maintain polymorphism do not show average heterozygote advantage. In addition, at least 1% of the non-fully polymorphic matrices have an average heterozygote advantage ($\ell = 3, r = 0.00$). This condition is therefore neither necessary, nor sufficient.

Differences between $P_{aha|\bar{f}}$ and $P_{aha|f}$ are strongly affected by the number of loci (Fig. 2A). When traits are coded by two and three loci, $P_{aha|f}$ are significantly higher than $P_{aha|\bar{f}}$ (all $p < 0.001$). Unexpectedly, for fitness matrices coded by four loci, the situation is reversed: $P_{aha|\bar{f}}$ are significantly higher than $P_{aha|f}$ (all $p < 0.001$, except for $r = 0.50, p = 0.51$).

$P_{aha|f}$ decreases as the number of loci increases and as the rate of recombination decreases (Fig. 2A). The lowest proportions of fully polymorphic *aha* matrices are for traits coded by four loci.

3.2.1. Ordered fitness matrices

Results for *ordered fitness matrices* (Fig. 2B, *omf* as defined under b in Table 1) are very similar to those obtained for the average heterozygote advantage. The highest $P_{omf|f}$ equals 0.675 ($\ell = 2, r = 0.50$) and all other

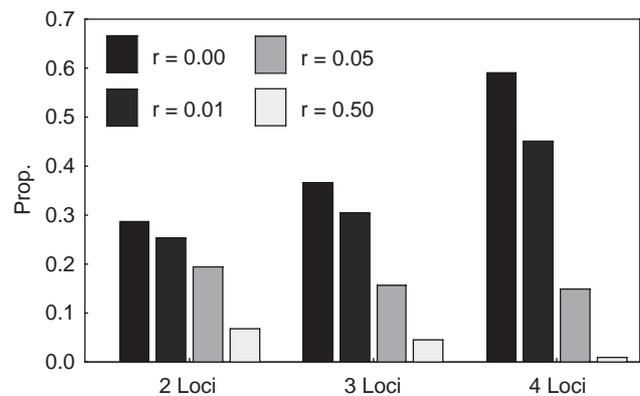


Fig. 1. Proportions of fully polymorphic matrices for the traits coded by two, three, and four loci and the four rates of recombination.

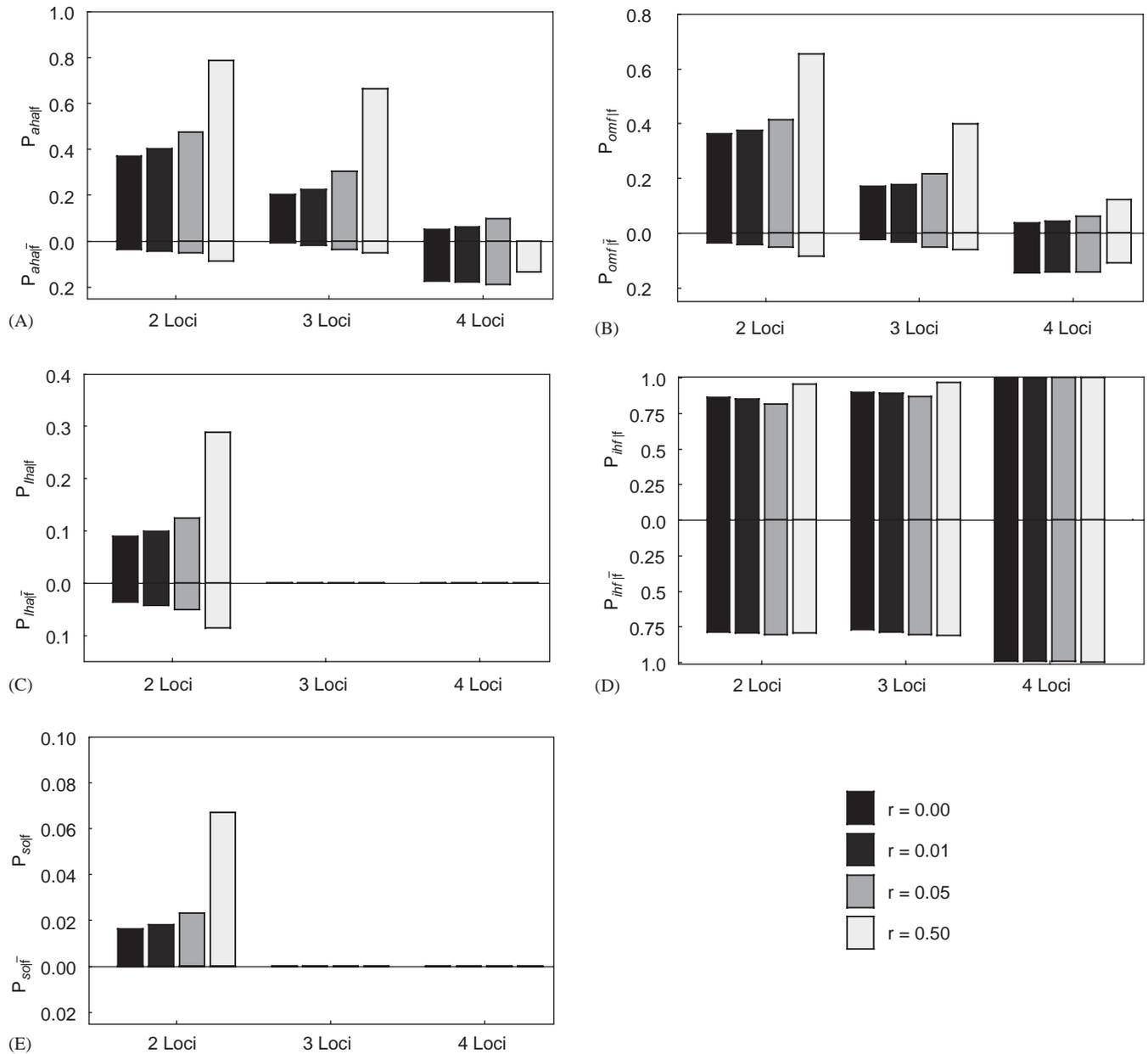


Fig. 2. Proportions among fully polymorphic matrices (above the x-axes, f) and among non-fully polymorphic matrices (below the x-axes, \bar{f}) that (A) have an average heterozygote advantage (aha), (B) are ordered (omf), (C) have a local heterozygote advantage (lha), (D) have an invariable heterozygote fitness (ihf), and (E) are simply overdominant (so) for fitness coded by two, three, and four loci. The abbreviation on the y-axis should be read as follow: $P_{aha|f}$, for example, is the proportion of fitness matrices having an average heterozygote advantage among the non-fully polymorphic fitness matrices (see Section 2.7; Analysis for a complete description of the proportions reported on the y-axes).

$P_{omf|f}$ are lower than 50%. The lowest $P_{omf|\bar{f}}$ equals 0.025 and increases as the number of loci increases.

Differences between fully and non-fully polymorphic fitness matrices change as the number of loci increases (Fig. 2B). $P_{omf|f}$ are higher than $P_{omf|\bar{f}}$ when $\ell = 2$ or 3. This is reversed for matrices coded by four loci. Observed differences between fully and non-fully polymorphic ordered fitness matrices are all highly significant (all $p < 0.001$).

$P_{omf|f}$ decreases with the number of loci (Fig. 2B). The highest proportions on average are for fitness matrices

coded by two loci, while the lowest proportions are for fitness matrices coded by four loci.

We also note that all ordered matrices of fitness coded by four loci also show aha (independently of the maintained polymorphism). The reverse is not true.

3.3. Local heterozygote advantage

The local heterozygote advantage (lha , as defined under c in Table 1) is strongly influenced by the number of loci since none of the 4000 fitness matrices coded by

three and four loci show this definition of heterozygote advantage (Fig. 2C). Local heterozygote advantage is found only for fitness coded by two loci. In order to have a better idea of the proportion of matrices coded by three and four loci showing *local heterozygote advantage*, we generate 100,000 matrices coded by three loci and 100,000 matrices coded by four loci. Two matrices coded by three loci and none coded by four loci have a *local heterozygote advantage* among these 100,000 matrices. This definition of heterozygote advantage is therefore extremely rare as soon as more than two loci are involved.

For two loci, fully polymorphic matrices have a statistically significant higher *lha* proportion than non-fully polymorphic matrices (all $p < 0.001$). This difference decreases as the rate of recombination decreases. But overall, $P_{lha|f}$ are low (lower than 0.3, Fig. 2C), and $P_{lha|\bar{f}}$ never goes to zero (the lowest equals 0.037).

3.4. Invariable heterozygote fitness

The proportion of matrices that have *invariable heterozygote fitness* (*ihf*, as defined under d in Table 1) increases with the number of loci (Fig. 2D). This increase is found for $P_{ihf|f}$ as well as for $P_{ihf|\bar{f}}$. Nearly all fitness matrices show invariable heterozygote fitness when they are coded by four loci (be they fully or non-fully polymorphic).

There is a statistically significant difference between fully and non-fully polymorphic *ihf* proportions for fitness matrices coded by two and three loci (all $p < 0.001$). Fully polymorphic matrices have higher *ihf* proportions than non-fully polymorphic matrices (Fig. 2D). But for both traits, $P_{ihf|f}$ are smaller than 1 and $P_{ihf|\bar{f}}$ are higher than zero. Finally, there are no significant differences between fully and non-fully polymorphic *ihf* proportions for matrices coded by four loci ($p = 0.73$ for $r = 0.00$, $p = 0.60$ for $r = 0.01$, $p = 0.29$ for $r = 0.05$, and $p = 0.28$ for $r = 0.50$).

3.5. Simple overdominance

There is a strong effect of the number of loci on *simple overdominance* (*so*, as defined under e in Table 1), as none of the 4000 fitness matrices coded by three or four loci are simply overdominant (Fig. 2E).

When fitness is coded by two loci, no non-fully polymorphic matrices are simply overdominant (by definition when loci are linked). This indicates that simple overdominance is a sufficient condition for the maintenance of polymorphism when fitnesses are coded by two loci. Importantly, while sufficient, *so* is not necessary since $P_{so|f}$ are very low (smaller than 0.07, Fig. 2E). All *so* matrices of fitness also show all other definitions of heterozygote advantage (except for two matrices not showing *omf*).

4. Discussion

This study investigates the role of heterozygote advantage in the maintenance of allelic polymorphism when selection acts on traits encoded by two, three or four loci. Our first prediction was that the proportions of matrices showing heterozygote advantage among the fully polymorphic matrices should be close to one ($P_{x|f} \approx 1$), and close to zero among non-fully polymorphic fitness matrices ($P_{x|\bar{f}} \approx 0$). None of the five definitions of heterozygote advantage summarized in Table 1 met this prediction (Fig. 2). In addition, the association between heterozygote advantage and polymorphism class was strongly dependent on the number of loci involved and on the recombination rate. Therefore, these results do not meet our second prediction ($P_{x|f}(\ell = 2) \approx P_{x|f}(\ell = 3) \approx P_{x|f}(\ell = 4)$). Last combining any or all of these five definitions of heterozygote advantage led to the same conclusions.

The two definitions of heterozygote advantage that are the closest to the definition of heterozygote advantage for one diallelic locus (*aha* and *omf*) yield results in the expected direction only for traits coded by two and to a lesser extent three unlinked loci. Heterozygote advantage, therefore, does not seem to be a mechanism that maintains polymorphism for traits coded by more than three loci. This might explain why some studies found that heterozygote advantage maintains polymorphism and others did not. Studies showing that heterozygote advantage may maintain polymorphism had either traits coded by one or two loci (Ginzburg, 1979; Turelli and Ginzburg, 1983; Gimelfarb, 2000) or they considered traits coded by loci with only pure additive effects (Zhivotovsky and Feldman, 1992). On the other hand, Hastings (1981, 1982) showed that, even for traits coded by two loci, heterozygote disadvantage could allow the maintenance of polymorphism. We show that heterozygote advantage as defined here is not a plausible explanation for the maintenance of polymorphism under selection when more than two loci are involved.

4.1. Limits of the model

The number of fully polymorphic fitness matrices is low when loci are unlinked. Among the 4000 fitness matrices, there are 268 fully polymorphic matrices for two loci, 183 for three loci, and 41 for four loci. Among these 41 fully polymorphic matrices not all matrices fulfilled the definitions of heterozygote advantage. Since $P_{x|f}$ is based on a small number of matrices, this may bias our conclusions for unlinked loci. For a trait coded by one locus, Spencer and Marks (1992) showed that if mutations are added to the model used here (Eq. (1)), many alleles (up to 38) may be maintained. Such a “constructionist” approach might also increase the

number of fully polymorphic matrices for traits coded by more than one locus. But, Spencer and Marks (1992) obtained fitness matrices that all showed heterozygote advantage ($\bar{w}_{ij} > \bar{w}_{ii}$), i.e. they arrived at the same conclusions as Lewontin et al. (1978) and Ginzburg (1979) regarding the maintenance of allelic polymorphism. Our method is identical to the one used by Lewontin et al. (1978). Therefore, while this “constructionist” approach might allow increasing the proportion of fully polymorphic matrices, it is not obvious that it will change our conclusions.

4.2. Allelic versus gametic polymorphism

We focused on allelic polymorphism because it is the measure of polymorphism used in experimental studies. When all alleles are maintained at equilibrium, not all gametes are necessarily maintained for traits coded by more than one locus (of course the reverse is true). For instance, for four diallelic loci, maintaining two out of the 16 possible gametes (*ABCD* and *abcd*) is sufficient to maintain all alleles. Thus conditions for the maintenance of allelic polymorphism at equilibrium are less stringent than conditions for the maintenance of gametic polymorphism. This is illustrated by the results on *ordered matrix of fitness (omf, Table 1)*, *local heterozygote advantage (lha, Table 1)*, and *invariable heterozygote fitness (ihf, Table 1)*. When loci are completely linked ($r = 0.00$), a trait coded by multiple loci is equivalent to a trait coded by one multiallelic locus (e.g. Roughgarden, 1979). Lewontin et al. (1978) showed that *omf*, *lha*, and *ihf* are necessary conditions for the maintenance of all alleles when selection acts at one locus. Therefore, for linked loci, proportions of fully polymorphic *omf*, *lha*, and *ihf* matrices should be equal to one. We found proportions lower than one (Fig. 2B–D). This difference reflects the difference between conditions for the maintenance of allelic and gametic polymorphism. Indeed, Lewontin et al. (1978) derived these three conditions (*omf*, *lha*, and *ihf*) for maintaining all *gametes* (i.e. all alleles at a single locus) at equilibrium. We focused on fitness matrices that maintain all *alleles* at equilibrium. This explain why we found low fully polymorphic proportions of *omf*, *lha*, and *ihf* even with $r = 0.00$. Note that, when all gametes were maintained at equilibrium in our numerical iterations, the three conditions (*omf*, *lha*, and *ihf*) were always met.

4.3. Unweighted versus weighted definitions

Heterozygote advantage could be defined using either raw fitness values (unweighted) or raw fitness values multiplied by gametic frequencies (weighted). Difference between unweighted and weighted definitions of heterozygote advantage is illustrated by the results we found on the *average heterozygote advantage (aha, Table 1)*

compared to previous studies (Turelli and Ginzburg, 1983; Gimelfarb, 1998). The definition of *average heterozygote advantage* we used differs from the *induced heterozygote advantage* used by Turelli and Ginzburg (1983) and Gimelfarb (1998). Like heterozygote advantage at one diallelic locus ($w_{11}, w_{22} < w_{12}$), *average heterozygote advantage* is unweighted by gamete frequency (as already mentioned in the method section). It takes into account raw values of fitness only. The *induced heterozygote advantage* uses fitness values that are weighted by gametic frequencies once the population has reached a polymorphic equilibrium (*induced heterozygote advantage* at one diallelic locus would thus be given by $p^2 w_{11}, q^2 w_{22} < 2pq w_{12}$). So *induced heterozygote advantage* reflects conditions that are function of the population state since fitness values are multiplied by gametic frequencies present in the population. *Average heterozygote advantage* does not depend on population state. Additionally, *induced heterozygote advantage* is defined only for polymorphic equilibria, *average heterozygote advantage* is always defined.

For fully polymorphic matrices, Turelli and Ginzburg (1983) and Gimelfarb (1998) found high proportions (> 0.99) of *induced heterozygote advantage* for traits coded by two to five diallelic loci. We found low *aha* proportions that decrease as the number of loci increases or as the loci become partially or totally linked. This difference between proportions (high *induced heterozygote advantage* and low *average heterozygote advantage*) clearly indicates that *induced heterozygote advantage* is not a characteristic of the fitness matrices per se, but a characteristic of the population state and the matrix of fitness.

We argue that the unweighted definition is more appropriate than the weighted one because it is closer to the measures taken by experimentalists. Often fitness values of different genotypes are assessed via competition between different genotypes (e.g. Ebert et al., 2002; Sanjuan et al., 2004). The classical setup for such experiments is to introduce two different genotypes in equal frequencies, thus assuming no frequency dependence. At a theoretical level, the effort of understanding what maintains genetic polymorphism in a population should de facto be centered around unweighted measures, unless we seek solution around an equilibrium. Note also that the original definition of heterozygote advantage for one locus with two alleles is unweighted.

4.4. Should individual fitness increase with heterozygosity?

This question has been raised by Turelli and Ginzburg (1983) within the framework of the neutralist selectionist debate. If genetic polymorphism is neutral, it should have no effect on the reproductive success of its carrier. Turelli and Ginzburg (1983) found that around 75% of

the fully polymorphic fitness matrices coded by two unlinked loci were ordered (i.e. *omf* as defined in Table 1). We found a similar proportion of fully polymorphic ordered fitness matrices coded by two unlinked loci (Fig. 2B). However P_{omf} decreases drastically as the recombination rate decreases or as the number of loci increases, a finding corroborated by Karlin and Carmelli (1975) and Gimelfarb (1998). Hereafter, we emphasize the relevance of this finding for the ongoing debate on heterozygosity—fitness correlation (see e.g. David, 1998).

If the matrix of fitness is ordered, the correlation between the number of heterozygote loci of a genotype and its fitness is strong and positive. If the matrix of fitness is not ordered, the correlation must be weaker than when the matrix is ordered, because the average fitness of genotypes having fewer heterozygote loci is higher than the average fitness of genotypes having more heterozygote loci. Using this rationale, Turelli and Ginzburg (1983) concluded that, when polymorphism is maintained, one should find a strong positive correlation between the number of heterozygous loci and individual fitness (since 75% of the fully polymorphic matrices are ordered). This conclusion is often used in studies investigating the relation between heterozygosity and fitness. But our results indicate that, for fully polymorphic matrices, the correlation between the number of heterozygote loci and fitness becomes weaker as the number of loci increases (since P_{omf} decreases as the number of loci increases). We therefore posit that a positive association between fitness and the number of heterozygote loci is not warranted on theoretical grounds.

5. Conclusion

We show that heterozygote advantage is not a major mechanism for the maintenance of allelic polymorphism, at least for the randomly generated fitness matrices we use. We acknowledge that our conclusions might come from our fitness values being generated at random whereas real fitness values are likely not. However, since the genetic architecture of fitness related traits is far from being elucidated, our approach seems to be the only appropriate for the time being. With these restrictions in mind, our results imply that heterozygote advantage is not a general mechanism for maintaining polymorphism because proportions of fully polymorphic matrices having a heterozygote advantage decrease as the number of loci increases or as the recombination rate decreases. In quantitative genetic models, heterozygote advantage translates into a non-zero dominance deviation. Dominance deviation is therefore neither necessary nor sufficient to maintain polymorphism at multiple loci. The next step would be

to consider a new set of allelic interactions, those between alleles at different loci. As exemplified by many others (e.g. Hastings, 1982; Gimelfarb, 1989 or Wolf et al., 2000), here too, epistasis seems to be the essence.

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References

- Barton, N.H., Keightley, P.D., 2002. Understanding quantitative genetic variation. *Nature Rev. Genet.* 3, 11–21.
- Bürger, R., 2000. *The Mathematical Theory of Selection, Recombination, and Mutation*. Wiley, Chichester.
- David, P., 1998. Heterozygosity—fitness correlations: new perspectives on old problems. *Heredity* 80, 531–537.
- Ebert, D., Haag, C., Kirkpatrick, M., Riek, M., Hottinger, J.W., Pajunen, V.I., 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295, 485–488.
- Ender, J.A., 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
- Gimelfarb, A., 1989. Genetic variation for a quantitative character maintained under stabilizing selection without mutations: Epistasis. *Genetics* 123, 217–227.
- Gimelfarb, A., 1998. Stable equilibria in multilocus systems: a statistical investigation. *Theor. Popul. Biol.* 54, 133–145.
- Gimelfarb, A., 2000. Balance of fitnesses maintaining polymorphisms in two-locus genetic systems. *Theor. Popul. Biol.* 58, 187–195.
- Ginzburg, L.R., 1979. Why are heterozygotes often superior in fitness? *Theor. Popul. Biol.* 15, 264–267.
- Hastings, A., 1981. Marginal underdominance at a stable equilibrium. *Proc. Natl. Acad. Sci. USA* 78, 6558–6559.
- Hastings, A., 1982. Unexpected behavior in the two locus genetic systems: an analysis of marginal underdominance at a stable equilibrium. *Genetics* 102, 129–138.
- Hedrick, P.W., 1999. *Genetics of Populations*, second ed. Jones and Bartlett Publisher.
- Hill, W.G., Caballero, A., 1992. Artificial selection experiments. *Annu. Rev. Ecol. Syst.* 23, 287–310.
- Karlin, S., 1990. Levels of multiallelic overdominance fitness, heterozygote excess and heterozygote deficiency. *Theor. Popul. Biol.* 37, 129–149.
- Karlin, S., Carmelli, D., 1975. Numerical studies on two-loci selection models with general viabilities. *Theor. Popul. Biol.* 7, 399–421.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., et al., 2001. The strength of phenotypic selection in natural population. *Am. Nat.* 157, 245–261.
- Lewontin, R.C., Kojima, K.I., 1960. The evolutionary dynamics of complex polymorphisms. *Evolution* 14, 458–472.
- Lewontin, R.C., Ginzburg, L.R., Tuljapurkar, S.D., 1978. Heterosis as an explanation for large amounts of genetic polymorphism. *Genetics* 88, 149–170.
- Mandel, S.P.H., 1959. The stability of a multiple allelic system. *Heredity* 13, 289–302.
- Roughgarden, J., 1979. *Theory of Population Genetics and Evolutionary Ecology: An Introduction*. Macmillan Publishing Company, New York.

- Sanjuan, R., Moya, A., Elena, F., 2004. The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. *Proc. Natl. Acad. Sci. USA* 101, 8396–8401.
- Spencer, H.G., Marks, R.W., 1992. The maintenance of single-locus polymorphism. IV. Models with mutation from existing alleles. *Genetics* 130, 211–221.
- Turelli, M., Ginzburg, L.R., 1983. Should individual fitness increase with heterozygosity? *Genetics* 104, 191–209.
- Wolf, J.B., Brodie III, E.D., Wade, M.J., 2000. *Epistasis and the Evolutionary Process*. Oxford University Press, Oxford.
- Zhang, X.-S., Hill, W.G., 2002. Joint effects of pleiotropic selection and stabilizing selection on the maintenance of quantitative genetic variation at mutation—selection balance. *Genetics* 162, 459–471.
- Zhivotovsky, L.A., Feldman, M.W., 1992. On models of quantitative genetic variability: A stabilizing selection-balance model. *Genetics* 130, 947–955.