THE FITNESS CONSEQUENCES OF MULTIPLE-LOCUS HETEROZYGOSITY UNDER THE MULTIPLICATIVE OVERDOMINANCE AND INBREEDING DEPRESSION MODELS

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Abstract.—There is a growing body of literature suggesting that the fitness of an individual increases with the observed number of heterozygous loci. Broad theoretical considerations indicate that under various sorts of balancing selection, this is what one should generally expect in a population of multiple-locus genotypes. To date, however, it has not been possible to distinguish between two potential explanations of the phenomenon. The first explanation is that the loci examined are themselves responsible for the fitness differences observed (or, equivalently, are very closely linked to those that do). The genetic variation in question is thought to be maintained in polymorphic equilibrium by some form of balancing selection. The second explanation assumes that the observed loci are themselves selectively irrelevant but that their heterozygosity reflects that of the total genome. Genomic heterozygosity is thought to be predictive of fitness, being an obverse measure of generalized inbreeding depression. We provide a formal derivation of an explicit relationship between fitness and multiple-locus genotype for a simple form of the first explanation, the multiplicative overdominance model. The inbreeding depression model is a degenerate special case of this more general formulation. A formal estimation and testing framework is constructed that should facilitate evaluation of the two models with empiric data on heterozygosity and fitness.

The accumulating allozyme data from natural populations have established convincingly that genetic variation is widespread in a great many organisms, but we still have no compelling evidence to suggest how much of that variation is maintained by natural selection. Quite a bit of effort has been expended on the search for meaningful selective differences among alleles (or genotypes) at particular loci, with at least modest success (e.g., Tsakas and Krimbas, 1970; Vigue and Johnson, 1973; Cavener and Clegg, 1981*a*, 1981*b*; DiMichele and Powers, 1982; Hilbish et al., 1982; Oakeshott et al., 1982; Burton and Feldman, 1983; Watt, 1983; Watt et al., 1983), but the vast majority of segregating loci in most of the species surveyed so far have never been examined in enough detail to characterize selective factors in satisfactory detail. Indeed, the effort required to "prove" the selective relevance of even a single locus in even a single species is so prohibitive that we can never hope to do so for more than a small fraction of the locus/species combinations of interest. The careful effort to evaluate particular cases has been rewarding in a variety of ways, and is to be encouraged, but exhaustive enumeration is neither an attractive nor a feasible route to wide generalization about the maintenance of observed genetic variability; some other approach is needed.

Single-locus analysis of the relation between genetic variation and fitness has not been very rewarding, and a number of workers have been led to consider the impact of multiple-locus genotypes on fitness. In particular, several authors have attempted to correlate fitness or some surrogate measure with the number of heterozygous loci in an individual. Some workers find an increase in fitness with increasing heterozygosity (Schall and Levin, 1976; Singh and Zouros, 1978; Bottini et al., 1979; Zouros et al., 1980; Mitton et al., 1981; Koehn and Shumway, 1982; Pierce and Mitton, 1982; Ledig et al., 1983; Leary et al., 1984), but others do not (e.g., Mukai et al., 1974; Gaines et al., 1978; Knowles and Grant, 1981; Mitton et al., 1981). Turelli and Ginzburg (1983) have theoretically demonstrated that if allozyme polymorphisms are maintained by any of several forms of balancing selection, then we should observe a general increase in fitness with an increase in the number of heterozygous loci. Whether the variation in

question is actually maintained by balancing selection remains to be seen, of course, but the observations are generally in agreement with predictions based on a broad concept of balancing selection.

Alternatively, it has been suggested that measured heterozygosity may merely reflect overall genomic heterozygosity and that highly homozygous genotypes suffer from inbreeding depression (Ledig et al., 1983). Mitton and Pierce (1980) and Chakraborty (1981) have shown, however, that heterozygosity from a small sample of loci is a poor indicator of total genomic heterozygosity for an individual, suggesting that there should be little or no association if inbreeding depression is the root cause of the phenomenon. On the other hand, inbreeding depression can be a powerful force (cf. Franklin, 1970), so even a small correlation between observed and genomic heterozygosity might be sufficient to account for the loose association observed between heterozygosity and fitness.

It is difficult to choose between these alternative explanations, because our current assessment procedures are almost hopelessly crude; they are not designed to distinguish between the two selective hypotheses. In current practice, no attention is paid to which loci are heterozygous, and most of the potentially useful information is suppressed in the interest of convenient summarization. The objective of this paper is to develop a predictive model for the relationship between fitness and the particular multiple-locus genetic state of a single individual. The model employed is the simplest I could use, the multiplicative overdominance model for unlinked loci. I present an evaluation procedure: (1) that is very easily implemented, (2) that provides a test of the null hypothesis of no selective differences among observed genotypes, (3) that distinguishes between genomic inbreeding and specific-locus effects, (4) that permits a determination of the marginal effect of each locus, and (5) that facilitates an assessment of the multiplicative overdominance assumption.

I begin with a consideration of the pattern of fitness to be expected from a set of two-allele loci, then describe the pattern to be expected of multiple-allele loci, and then

present an assessment procedure for evaluating the hypothesis. I argue that the inbreeding depression hypothesis is a degenerate special case of the more general formulation. In a later paper (Bush et al., unpubl.), the predictions of the two models are compared for a series of population samples from pitch pine (*Pinus rigida* Mill.).

A Set of Two-Allele Loci

Consider a diploid population with K segregating loci, unlinked and with two alleles each. I assume that the two alleles at each locus are codominant, as is usual with allozyme electromorphs; denote them as A_1 and A_2, B_1 and B_2, and their allele frequencies as P_A and Q_A, P_B and Q_B, etc. I presume that the population has already attained multiple-locus Hardy-Weinberg equilibrium.

I envisage a broad sort of balancing selection that maintains the genetic variation in equilibrium. The marginal fitness values for any one locus are functions of genotypic frequencies and of the fitness values of the multiple-locus genotypes. I postulate that at equilibrium the net balance of forces is such that the marginal fitness array at each locus shows overdominance. Karlin and Liberman (1979a, 1979b) have shown that for either additive or multiplicative fitness across a set of "loosely linked" loci, stable polymorphic equilibria will exhibit marginal overdominance. More general fitness schemes leading to multiple-locus stable polymorphism are not guaranteed to yield marginal overdominance at equilibrium (Lewontin and Kojima, 1960; Ewens and Thomson, 1977; Hastings, 1981, 1982), but Turelli and Ginzburg (1983) have shown that marginal overdominance is the most frequent consequence of multiple-locus polymorphism. For purposes of exposition, then, let the A-locus be represented as shown in the panel below.

Genotype	A_1A_1	A_1A_2	A_2A_2
Frequency	$P_A{}^2$	$2P_AQ_A$	$Q_A{}^2$
Fitness (W_A)	e^{-S_A}	1	e^{-T_A}

The values of S_A and T_A are assumed to be small and positive, compatible with the notion that marginal selective differentials are small. It will be convenient to use the fact

that $(1 - S_A) \approx \exp\{-S_A\}$ and $(1 - T_A) \approx \exp\{-T_A\}$ for small S_A and T_A, and I shall switch back and forth between these notational forms whenever convenient.

I have assumed Hardy-Weinberg frequencies for expository purposes, and this warrants some comment. Not all types of heterotic selection lead to Hardy-Weinberg proportions at equilibrium, but where selective differentials are small, departures from Hardy-Weinberg proportions will be small as well. Moreover, there is little evidence in natural populations for large departures from Hardy-Weinberg proportions that could be attributed to large selective differentials, although the difficulties of detecting departures from Hardy-Weinberg proportions are well known (e.g., Lewontin and Cockerham, 1959; Ward and Sing, 1970; Chakraborty and Rao, 1972; Haber, 1980). Given the observations to date, the assumption of Hardy-Weinberg proportions at equilibrium is a reasonable first approximation.

Using the fact that $(1 - S_A) \approx \exp\{-S_A\}$ and $(1 - T_A) \approx \exp\{-T_A\}$ for small S_A and T_A, standard theory (e.g., Li, 1978) yields the following equilibrium frequencies for the A-locus:

$$P_A = \frac{T_A}{S_A + T_A} \text{ and } Q_A = \frac{S_A}{S_A + T_A}. \quad (1)$$

The more fit of the two homozygotes is the more common. The central idea in what follows is that some homozygotes are more fit than others. Rather than using the number of homozygous loci as a criterion of fitness, I construct testable hypotheses about the differences of homozygote fitness values from the observed allele frequencies. To do that, I construct a measure of the "adaptive distance" between any homozygote and the (heterozygous) optimum genotype. The least fit homozygote should be furthest from this optimum. The scaling of adaptive distance is also helpful in determining the relative fitness impact of selection at different loci. Consider the quantity (X_A) as a candidate measure of adaptive distance for A-locus genotypes.

Genotype	A_1A_1	A_1A_2	A_2A_2
Fitness (W_A)	e^{-S_A}	1	e^{-T_A}
Distance (X_A)	P_A^{-1}	0	Q_A^{-1}

The fitness values (W_A) above are marginal genotypic averages, of course, and we really desire values for individuals. For the jth individual, the natural logarithm of fitness (denoted Y_A) and adaptive distance (X_A) are simply related:

$$Y_A(11_j) = \log[W_A(11_j)]$$
$$= -S_A + \epsilon_j$$
$$= -\alpha \cdot X_A(11) + \epsilon_j$$
$$= [-\alpha/P_A] + \epsilon_j \quad (2a)$$

$$Y_A(12_j) = \log[W_A(12_j)]$$
$$= -\alpha \cdot X_A(12) + \epsilon_j$$
$$= \epsilon_j \quad (2b)$$

$$Y_A(22_j) = \log[W_A(22_j)]$$
$$= -T_A + \epsilon_j$$
$$= -\alpha \cdot X_A(22) + \epsilon_j$$
$$= [-\alpha/Q_A] + \epsilon_j, \quad (2c)$$

where the error terms (ϵ_j) reflect the fact that individuals have fitness (or surrogate) values departing from the genotypic averages, due to differences in genotype at other loci, due to random environmental influences, measurement error, and so on. These error terms (deviations from genotypic expectations) are assumed to be independent and identically distributed, with mean $(\bar{\epsilon}) = 0$ and variance (σ_ϵ^2). The slope coefficient (α) takes the value:

$$\alpha = \frac{S_A T_A}{S_A + T_A}. \quad (3)$$

The term α is traditionally referred to as the "segregational genetic load" for the A-locus (Morton et al., 1956). Whether one views genetic load as the cost of heterosis or as a measure of lost opportunity resulting from the impossibility of maintaining a pure population of the optimal (heterozygous) genotype, it is a measure of the magnitude of differential selection operating on genotypes at the locus. The relationship between log(fitness) and adaptive distance is linear, and the slope of the line is the intensity of selection for the locus. For scaling the asymmetry between $X_A(11)$ and $X_A(22)$, α is of no concern, since:

$$\frac{[-\alpha/P_A]}{[-\alpha/Q_A]} = \frac{S_A}{T_A}. \quad (4)$$

	A_1A_1	A_1A_2	A_2A_2	
B_1B_1	$e^{-S_A-S_B}$	e^{-S_B}	$e^{-T_A-S_B}$	$\bar{W}_A \cdot e^{-S_B}$
B_1B_2	e^{-S_A}	1	e^{-T_A}	$\bar{W}_A$
B_2B_2	$e^{-S_A-T_B}$	e^{-T_B}	$e^{-T_A-T_B}$	$\bar{W}_A \cdot e^{-T_B}$
	$\bar{W}_B \cdot e^{-S_A}$	$\bar{W}_B$	$\bar{W}_B \cdot e^{-T_A}$	$\bar{W}_A \cdot \bar{W}_B$

MARGINAL FITNESS

$$A_1A_1 : W_{11} = \frac{\bar{W}_B \cdot e^{-S_A}}{\bar{W}_B} = e^{-S_A}$$

$$A_2A_2 : W_{22} = \frac{\bar{W}_B \cdot e^{-T_A}}{\bar{W}_B} = e^{-T_A} \qquad W_{12} = \frac{\bar{W}_B}{\bar{W}_B} = 1$$

$$B_1B_1 : W_{11} = \frac{\bar{W}_A \cdot e^{-S_B}}{\bar{W}_A} = e^{-S_B}$$

$$B_2B_2 : W_{22} = \frac{\bar{W}_A \cdot e^{-T_B}}{\bar{W}_A} = e^{-T_B} \qquad W_{12} = \frac{\bar{W}_A}{\bar{W}_A} = 1$$

FIG. 1. Two-locus fitness values for the multiplicative overdominance model, with marginal relative fitness values for each locus.

Now, consider a second locus, also marginally overdominant. Genotypic frequencies are given (at least approximately) by two-locus Hardy-Weinberg expectations at equilibrium. Having assumed multiplicative fitness across a set of unlinked loci, there should be no gametic disequilibria between the A and B loci at equilibrium (Karlin and Liberman, 1979a, 1979b). The adaptive distances of B_1B_1, B_1B_2, and B_2B_2 are $X_B(11) = P_B^{-1}$, $X_B(12) = 0$, and $X_B(22) = Q_B^{-1}$, respectively. In keeping with the multiplicative overdominance model, the two-locus fitness array is as shown in Figure 1. Each double homozygote (corner) has lower fitness than either flanking single-locus homozygote, and each single-locus homozygote has lower fitness than the two-locus heterozygote (center). Fitness decreases when additional loci are homozygous, but the decreases are not identical for all genotypes with the same level of homozygosity, a fact I make use of below.

Since the fitness values are assumed to be multiplicative across loci, and since there are no disequilibria, the natural logarithm of fitness can be represented by (cf. Appendix):

$$Y_j = \log(W_j) = -\alpha \cdot X_A - \beta \cdot X_B + \epsilon_j, \quad (5)$$

where the slope coefficients are:

$$\alpha = \frac{S_A T_A}{S_A + T_A} \quad \text{and} \quad \beta = \frac{S_B T_B}{S_B + T_B}, \quad (6)$$

the respective intensities of selection for the two loci. Allele frequencies are sufficient to gauge the asymmetry of adaptive distance within a locus, but the respective intensities are needed to weight the loci. Note, for example, that both the parameter sets $\{S = 0.01, T = 0.02\}$ and $\{S = 0.05, T = 0.10\}$ imply $\{P = 2/3, Q = 1/3\}$, but they represent rather different intensities of selection. The allele frequencies are observable, but it is necessary to estimate the slopes. There are nine genotypes, each with a different adaptive distance. To see what this means, consider the following example; let $S_A = 0.02$, $T_A = 0.04$, $S_B = 0.015$, and $T_B = 0.045$. The equilibrium allele frequencies are $P_A = 2/3$, $Q_A = 1/3$, $P_B = 3/4$, and $Q_B = 1/4$, and the slopes (intensities) are $\alpha = 0.0133$ and $\beta = 0.0113$.

A plot of the logarithm of fitness against two-locus adaptive distances is presented in Figure 2a, where the X-values for the A and B loci are weighted by α and β, respectively. The optimal genotype ($A_1A_2B_1B_2$) is at one end of the scale and the worst ($A_2A_2B_2B_2$) is at the other end, but there is no strict order of fitness with the number of heterozygous loci. For example, $A_1A_1B_1B_1$ has higher fitness than either $A_1A_2B_2B_2$ or $A_2A_2B_1B_2$. An analogue of the more usual treatment is presented in Figure 2b, where log(fitness) is plotted against the number of homozygous loci. The usual monotonicity is evident, but this presentation is less informative than that of Figure 2a. Moreover, since the points of the graph are averages for two-locus genotypes, there is individual-to-individual variation around the regression line, more variation in Figure 2b than in Figure 2a.

The extension to multiple loci, each with two alleles, is obvious. Given the same sort of adaptive distance measures, the predictive model becomes:

$$Y_j = \log(W_j)$$
$$= -\alpha \cdot X_A - \beta \cdot X_B - \ldots$$
$$- \kappa \cdot X_K + \epsilon_j, \qquad (7)$$

where the slope coefficients are the respective intensities of selection for the various loci. The model encompassed by Equation (7) is richer in predictive detail than the standard treatment; with K loci, the usual treatment allows for only $(K + 1)$ heterozygosity classes. With the same K loci, there are almost always more than $(K + 1)$ different genotypes in the adaptive distance model, sometimes substantially more.

A Multiple-Allele Locus

The mathematical theory for the selective maintenance of multiple-alleles in polymorphic equilibrium is considerably more complicated than is that for a pair of alleles. The conditions that must be satisfied are very demanding, and simple heterosis is neither necessary nor sufficient (e.g., Mandel, 1970; Lewontin et al., 1978; Li, 1978). However, provided that a state of balanced equilibrium is assumed to exist, there are certain conditions that will be satisfied. Consider the three-allele C-locus, which can be represented as follows.

Genotype	C_1C_1	C_1C_2	C_1C_3	C_2C_2	C_2C_3	C_3C_3
Frequency	P_C^2	$2P_CQ_C$	$2P_CR_C$	Q_C^2	$2Q_CR_C$	R_C^2
Fitness	W_{11}	W_{12}	W_{13}	W_{22}	W_{23}	W_{33}

Now, pool C_2 and C_3 into $C_{1'}$, reducing the locus to a pair of allelic classes (C_1 and $C_{1'}$).

Genotype	C_1C_1	$C_1C_{1'}$	$C_{1'}C_{1'}$
Frequency	P_C^2	$2P_C(1 - P_C)$	$(1 - P_C)^2$
Fitness	W_{11}	$W_{11'}$	$W_{1'1'}$

The fitnesses of the synthetic genotypes ($W_{11'}$ and $W_{1'1'}$) are:

$$W_{11'} = \frac{(2P_CQ_CW_{12} + 2P_CR_CW_{13})}{(2P_CQ_C + 2P_CR_C)}$$
$$= \frac{(Q_CW_{12} + R_CW_{13})}{(1 - P_C)} \qquad (8a)$$

$$W_{1'1'} = \frac{(Q_C^2W_{22} + 2Q_CR_CW_{23} + R_C^2W_{33})}{(1 - P_C)^2}. \qquad (8b)$$
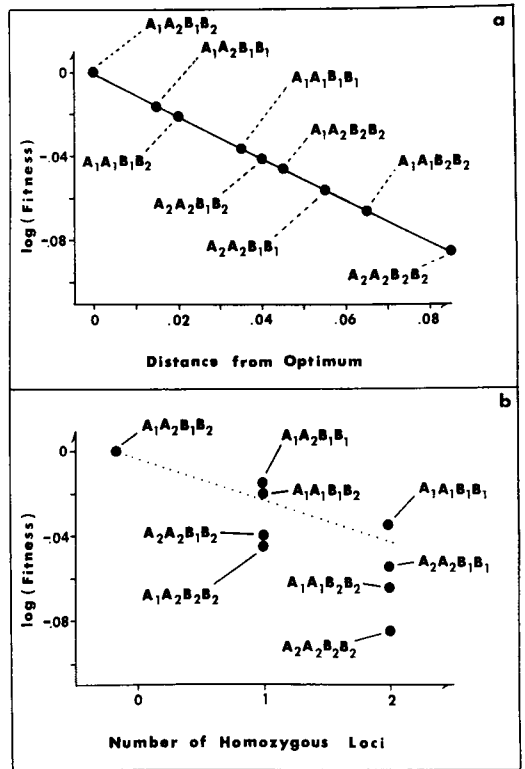
Fig. 2. An example of the relationship between log(fitness) and genotype for two loci: a) according to the adaptive distance formulation, and b) reflected in the number of homozygous loci.

Alternatively, one might pool C_1 and C_3 into $C_{2'}$, reducing the locus to a different pair of allelic classes, or even C_1 and C_2 into $C_{3'}$, reducing the locus to a third two-allele form. For each of these alternative lumpings, it is straightforward to define synthetic fitness measures along the lines indicated by Equations (8a) and (8b). A necessary condition for (consequence of) a stable polymorphic equilibrium is that:

$$W_{11}, \ W_{1'1'} < W_{11'}$$

and

$$W_{22}, \ W_{2'2'} < W_{22'}$$

and

$$W_{33}, \ W_{3'3'} < W_{33'} \qquad (9)$$

To treat a three-allele locus, it is only necessary to pool two of the alleles into an "other alleles" class, and then use the two-allele treatment, merely adding the locus to the
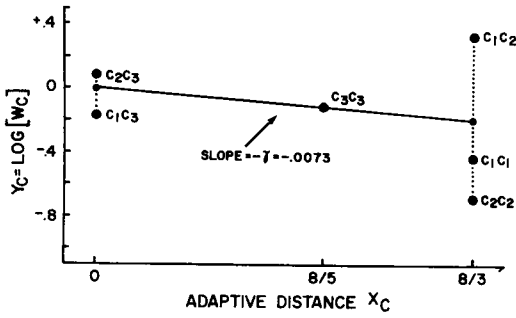
FIG. 3. An example of the relationship between log(fitness) and genotype for a three-allele locus. The line represents the model specification, which is an exact representation if C_1C_1, C_1C_2, and C_2C_2 are pooled into $C_3C_{3'}$, and if C_1C_3 and $C_2C_{3'}$ are pooled into $C_3C_{3'}$. The actual log(fitnesses) of all six genotypes are also plotted.

array in (7). The only question is which of the three available pooling strategies to use. The best strategy is to use the most frequent allele as C_1, lumping the other two into $C_{1'}$. The major reason for this is that evaluation of the model will be most effective if the rarer of the two homozygotes is present in the sample in nontrivial frequencies. Pooling of the rarer alleles should generally ensure this.

To illustrate the three-allele analysis further, I envision a three-allele C-locus with a frequency/fitness specification slightly modified from an example by Li (1978 p. 44).

Genotype	C_1C_1	C_1C_2	C_1C_3	C_2C_2	C_2C_3	C_3C_3
Frequency	$\dfrac{1}{64}$	$\dfrac{4}{64}$	$\dfrac{10}{64}$	$\dfrac{4}{64}$	$\dfrac{20}{64}$	$\dfrac{25}{64}$
Fitness	0.925	1.000	0.950	0.900	0.975	0.955

The genotypic frequencies are determined by the fitness values and are computed as in Li (1978):

$$\tilde{P}_C = 0.125$$
$$\tilde{Q}_C = 0.250$$
$$\tilde{R}_C = 0.625. \qquad (10)$$

These equilibrium frequencies and fitness values imply frequencies and fitness values for the synthetic genotypes ($C_1C_{1'}$ and $C_{1'}C_{1'}$), ($C_2C_{2'}$ and $C_{2'}C_{2'}$), ($C_3C_{3'}$ and $C_{3'}C_{3'}$), and these latter satisfy, as they must, the conditions required for a stable polymorphism:

$$W_{11} = 0.9250, \quad W_{1'1'} = 0.9587$$
$$< W_{11'} = 0.9643 \qquad (11a)$$
$$W_{22} = 0.9000, \quad W_{2'2'} = 0.9527$$
$$< W_{22'} = 0.9792 \qquad (11b)$$
$$W_{33} = 0.9550, \quad W_{3'3'} = 0.9472$$
$$< W_{33'} = 0.9667. \qquad (11c)$$

Since C_3 is the most frequent allele, lump C_1 and C_2 into $C_{3'}$, and construct the following (X, Y)-specification for the C-locus.

Genotype	C_1C_1	C_1C_2	C_1C_3	C_2C_2	C_2C_3	C_3C_3
$\hat{Y}_C$	−0.0195	−0.0195	0	−0.0195	0	−0.0117
X_C	2.6667	2.6667	0	2.6667	0	1.6000
Log(W_C)	−0.0426	+0.0328	−0.0168	−0.0690	+0.0083	−0.0117

The log(W_C) values are scaled relative to the average fitness of C_1C_3 and C_2C_3 (0.9667). A plot of log(W_C) against X_C is presented in Figure 3. The theoretical line ($\hat{Y}_C$) is that predicted by the model (with C_1 and C_2 pooled); the slope is $-\gamma = -0.0073$. The six logarithmically transformed fitness values are plotted against their respective adaptive distance values. The weighted averages of the fitness values of the genotypes having the same X_C-values fit the line exactly, but individual genotypes do not. This is an unavoidable consequence of pooling the least common alleles. There is always some loss of information with pooling, but what information remains is valid.

The extension of this treatment to multiple-allele loci is straightforward. Stable polymorphic equilibrium for multiple alleles implies overdominance for all of the synthetic pairs:

$$W_{ii}, \ W_{i'i'} < W_{ii'} \quad \text{for all alleles } (i). \ (12)$$

All but the most common allele can be lumped into a single "other alleles" class, and the locus can be treated in two-allele form.

An Estimation and Testing Framework

The estimation of the regression coefficients (the intensities of selection) is a relatively straightforward matter that I shall develop below. Before beginning that dis-

cussion, however, some assurance is needed that the adaptive distances (X-values) are measured without error or at least with small error, relative to the error in the Y-values. The X-values are simply assigned from the allele frequencies of the population, but these frequencies are estimated with some error. Given large samples, the allele frequencies (and hence the X-values) will be estimated with minimal error, and it might be well in general to estimate those frequencies from a different (and hopefully larger) sample than is used to assess the relationship between heterozygosity and fitness. The real uncertainty in all of this work is the measurement of fitness (or some credible surrogate) for an individual. That task is formidable under the best of circumstances, and one can anticipate measurement and estimation errors at least an order of magnitude larger than the errors in the adaptive distances. Both problems will be discussed in more detail in a later paper (Bush et al., unpubl.). I assume here that the requisite measures of fitness are in hand.

Consider a pair of two-allele loci, and recall the fitness array of Figure 1. Define a parameter vector $\theta = [\alpha, \beta]'$. It can be shown (Appendix) that the usual regression estimator $\hat{\theta}$ is unbiased:

$$E[\hat{\theta}] = E[\hat{\alpha}, \hat{\beta}]' = [\alpha, \beta]' = \theta. \quad (13)$$

The model (regression) and error (residual) sums of squares are easily shown to be functions of sample size (N), the error variance (σ_ϵ^2), and the selection intensities (Appendix):

$$E[SS(MODEL)] = 2 \cdot \sigma_\epsilon^2 + (N - 1)$$
$$\cdot (\alpha^2 + \beta^2) \quad (14a)$$
$$E[SS(ERROR)] = (N - 3) \cdot \sigma_\epsilon^2. \quad (14b)$$

Given K loci, each with two alleles, the regression treatment can be extended to models involving the K-dimensional vector $\theta = [\alpha, \beta, \ldots, \kappa]'$, merely by extending (13), (14a), and (14b) in the obvious fashion. Multi-allelic loci can also be treated in two-allele fashion, as I have indicated above, but it must be remembered that the error terms (ϵ_j) will contain variation among genotypes within a pooled class.

Since individual fitness (or surrogate) values will vary around the genotypic averages, due both to measurement error and fitness variation ascribable to unobserved loci, the observed SS(ERROR) will not be identically zero, and the regression model can be compared with observations in the usual fashion, testing the hypothesis that any subset of θ is zero, indicating a lack of selective relevance for that subset of loci. In the event that SS(ERROR) is large, it might be useful to determine whether the linear model (multiplicative overdominance) is itself adequate as a representation, since one can augment the model by adding cross-product (epistatic) terms.

One final remark on estimation and testing is in order. All of the above is predicated on the assumption that it is possible to determine relative fitness values of all K-locus genotypes, by reference to the optimal genotype, the K-locus heterozygote. In routine practice, with even a modest number of loci under examination, the K-locus heterozygote will frequently not be observed. In that event, some other genotype must be used as a fitness reference, which results in the addition of a nonzero intercept to (7). This convention represents a mere shift of scale, and has no consequences for the evaluation techniques presented here.

The Inbreeding Depression Model

I pointed out above that the observed relationship between heterozygosity and fitness could be the result of nonspecific inbreeding depression, with a more heterozygous genotype merely indicating a lower homozygosity level of the individual. The convention of counting the number of heterozygous loci is a special case of the adaptive distance formulation I have used here. A count of the number of heterozygous loci is tantamount to the assumption that all observed loci are equivalent in fitness terms. If the genetic loci under consideration are mere indicators of the genomic heterozygosity of the individual, then the details concerning which of the alternate allelic markers at any particular locus are homozygous will convey no useful information about the fitness of that multiple-locus genotype. In essence, that amounts to scoring all heterozygotes as $X = 0$ and all homozygotes as $X = 1$ and setting all regression coefficients equal. That reduces the inbreeding depression model to a degenerate special

case of the adaptive distance model, a treatment developed by Morton et al. (1956). To evaluate that model, it is only necessary to regress log(W) on the number of homozygous loci; serious lack of fit would be an indication that the inbreeding depression model is too simple. Any significant improvement in the prediction due to fitting separate slopes for the separate loci and/or different adaptive distances for different homozygotes would be evidence that the loci under examination are important in themselves, rather than being indicators of a generalized inbreeding effect.

It is appropriate to close this section with a final comment on the inbreeding depression model. Mitton and Pierce (1980) and Chakraborty (1981) have examined the correlation between the heterozygosity/homozygosity of a subset of observed loci and that of the total genome, and have shown that the correlation is generally quite small. If the loci under examination are assumed to be of no adaptive relevance, then one might argue that the fitness effects of genomic homozygosity must be large to account for the moderate size of the necessarily indirect association between observed homozygosity and fitness. It develops, however, that this whole line of argument is in need of closer scrutiny.

Mitton and Pierce, and Chakraborty as well, employed sets of independently segregating loci. There is no correlation of homozygosity across a set of independently segregating loci, and the correlation between observed and genomic homozygosity is due solely to the fact that the former is a subset of the latter. Formally, let homozygosity at the observed loci be denoted by (X) and that of all other (unobserved loci) be denoted by (X'). If genomic homozygosity is denoted by (Z), then the relation between Z and X is merely:

$$Z = \zeta \cdot X + (1 - \zeta) \cdot X', \qquad (15)$$

where ζ is the fraction of segregating loci under actual observation. The correlation between X and Z is thus:

$$r_{XZ} = \frac{\sigma_{XZ}}{\sigma_X \cdot \sigma_Z}$$

$$= \frac{\zeta \cdot \sigma_X}{\sqrt{\zeta^2 \cdot \sigma_X^2 + (1 - \zeta)^2 \cdot \sigma_{X'}^2}} \qquad (16)$$

which is nonzero because ζ is nonzero. For the inbreeding depression model, X is thought to have no direct effect on fitness, so what we really need is the indirect relationship between X and Y, through X'. The relationship between X, X', and Y is shown in path diagram form in Figure 4. If observed homozygosity (X) has no direct effect on Y, then the direct path coefficient $p_{XY} = 0$, and since $r_{XX'} = 0$ as well, there is no correlation between observed homozygosity and fitness, direct or indirect (Fig. 4a). If there is an association between X and Y, it must be due to the direct effect of X on Y, with $p_{XY} \neq 0$, as shown in Figure 4b. The basic phenomenon cannot be due to independently segregating loci that are not under observation.

The rest of the genome does not, of course, consist of only those loci segregating independently of the ones under observation. In general, closely linked loci can be expected to exhibit gametic disequilibrium with the loci under observation. In that case, $r_{XX'} \neq 0$, and one might very well observe an indirect correlation (associative overdominance) between X and Y (Kojima and Lewontin, 1969), an observable set of neutral markers that appear to be overdominant because of their disequilibria with unseen but linked markers that are under selection. More generally, the fitness-homozygosity associations observed by several authors probably represent the effects of small segments of chromosome surrounding the observed markers. Since it is not possible to distinguish between the heterozygosity of the observed markers and that of the flanking loci, the term "observed loci" might just as well be understood to mean "observed segments of chromosome."

Whether the fitness-homozygosity association is due to balancing selection or to inbreeding depression, the "loci" of interest are those under observation. There is nothing to be gained by invoking independently segregating portions of the genome that are not under observation. Inbreeding depression comes in two forms, the depression caused by deleterious recessive homozygotes at loci not supporting polymorphisms and the depression caused by homozygosity for alternate alleles at loci maintained as balanced polymorphisms (Morton et al.,

1956). Given that the "loci" under examination are those that matter, there should be no real need for the (1, 0) scoring system of the homozygosity treatment, and it should generally be possible to extract more information with the adaptive distance treatment.

DISCUSSION

The adaptive distance treatment is based on the idea that marginal selective differentials are quite small; the assumption is the only one compatible with the known facts. Almost no one has been able to convincingly demonstrate large selective differentials for single loci. Moreover, the lack of large single-locus marginals makes a certain amount of theoretical sense. A marginal-fitness value includes the frequency-dependent effects of all other loci, assayed or otherwise. With even a modest number of loci under consideration, no two individuals will have the same genotype, and the genetic backgrounds over which two marginal genotypes are averaged may not include a single background genotype in common. It is entirely plausible that the average fitness values of two or more random collections of genotypes will be about the same, everything else being equal. When viewing a single locus, one is merely looking at one facet of a multi-faceted genotype. Although demonstrating large marginal selective effects for single loci is bound to be difficult, the fact that several workers have detected fitness differences between groups of individuals that differ for as few as 6–12 identifiable loci (cf. Mitton and Grant, 1984) argues that the multiple-locus genome is under strong selection.

The variety of models that one might employ to generate a stable multiple-locus polymorphic array is very large. It is not possible to elaborate the detailed fitness structure of a complicated multiple-locus system from survey data alone, of course, but that is scarcely the point. An attempt to determine whether or not multiple-locus fitness is related to heterozygosity in some systematic and theoretically plausible fashion is the only real issue at stake here. Having begun with the simplest model that might do the job, multiplicative overdominance, the linearity of the relationship between
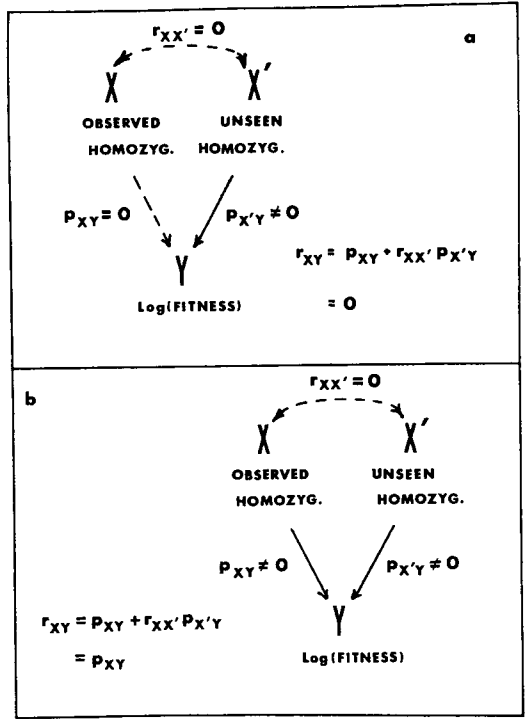

FIG. 4. Direct and indirect effects of observed homozygosity (X) on $Y = \log(W)$: a) the case where $p_{XY} = 0$ and $p_{X'Y} \neq 0$, and b) the case where $p_{XY} \neq 0$ and $p_{X'Y} \neq 0$.

adaptive distance (X) and $(Y) = \log(W)$ is the theoretical reward. My assumption that $\exp\{-S\} \approx (1 - S)$ and $\exp\{-T\} \approx (1 - T)$ is tantamount to saying that there is no real distinction between the additive overdominance and multiplicative overdominance models, and there are no changes in the basic conclusions for the additive model.

Either of these simple models is only an approximation to a general fitness scheme for multiple unlinked loci, but the approximation may not be unreasonable as a first approximation. If marginal selective differentials are small at equilibrium, as seems empirically to be the case in most studies, it should be possible to approximate the log(fitness) values of particular multiple-locus genotypes by means of a Taylor expansion in the mathematical vicinity of equilibrium. In the parlance of Figure 1, the Y-value for $A_1A_1B_1B_1$ (for example) can be represented as:

$$Y(A_1A_1B_1B_1) \approx 0 - S_A - S_B$$
$$+ O(S_A + S_B)^2. \quad (17)$$

With $0 < S_A$, $S_B < 0.10$, the higher order terms should be very small. Multiplicative (additive) overdominance is surely not the true model, but within the limits of detection, it should be a reasonable first approximation. The regression strategy can be used to evaluate quadratic, cubic, or higher order (epistatic) models, of course, but with very little hope of a convincing demonstration, given small S_A and S_B.

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APPENDIX

The purpose of this appendix is to derive the expected means, sums of squares, and cross products needed for the regression analysis for a pair of unlinked, two-allele loci with multiplicative fitness values. Assume that the population is at two-locus Hardy-Weinberg equilibrium and that the allele frequencies are known without error. Using the definitions in the text, it develops that the expected value of $\bar{Y}$, the mean of the Y_j, is (since $\bar{\epsilon} = 0$):

$$E(\bar{Y}) = P_A^2 P_B^2 (-S_A - S_B) + \ldots$$
$$+ Q_A^2 Q_B^2 (-T_A - T_B) + \bar{\epsilon}$$
$$= -(\alpha + \beta), \quad (A.1)$$

since at equilibrium:

$$P_A = \frac{T_A}{S_A + T_A} \quad \text{and} \quad Q_A = \frac{S_A}{S_A + T_A} \quad (A.2a)$$

$$P_B = \frac{T_B}{S_B + T_B} \quad \text{and} \quad Q_B = \frac{S_B}{S_B + T_B}. \quad (A.2b)$$

The regression of Y on X_A and X_B requires:

$$E[SS(TOTAL)] = E[Y'Y]$$

$$= E\left[\sum_{j=1}^{N} (Y_j - \bar{Y})^2\right]$$

$$= P_A^2 P_B^2 \cdot E[-S_A - S_B - \bar{Y}]^2 + \ldots$$
$$+ Q_A^2 Q_B^2 \cdot E[-T_A - T_B - \bar{Y}]^2$$

$$+ E\left[\sum_{j=1}^{N} \epsilon_j^2\right]$$

$$= (N - 1) \cdot \sigma_\epsilon^2 + (N - 1)(\alpha^2 + \beta^2).$$

$$(A.3)$$

Similar manipulations yield:

$$\mathbf{X_A X_A} = \sum_{j=1}^{N} (X_{Aj} - \bar{X}_A)^2$$

$$= (N - 1)$$

$$= \sum_{j=1}^{N} (X_{Bj} - \bar{X}_B)^2$$

$$= \mathbf{X_B' X_B} \quad (A.4a)$$

$$\mathbf{X_A' X_B} = \sum_{j=1}^{N} (X_{Aj} - \bar{X}_A)(X_{Bj} - \bar{X}_B)$$

$$= \mathbf{X_B' X_A}$$

$$= 0, \quad (A.4b)$$

and:

$$E[\mathbf{X_A' Y}] = E\left[\sum_{j=1}^{N} (X_{Aj} - \bar{X}_A)(Y_j - \bar{Y})\right]$$

$$= (N - 1) \cdot \alpha \quad (A.5a)$$

$$E[\mathbf{X_B' Y}] = E\left[\sum_{j=1}^{N} (X_{Bj} - \bar{X}_B)(Y_j - \bar{Y})\right]$$
$$= (N - 1) \cdot \beta. \quad (A.5b)$$

The expected parameter estimates are:

$$E[\hat{\theta}] = (\mathbf{X}'\mathbf{X})^{-1} E(\mathbf{X}'\mathbf{Y})$$

$$= \begin{bmatrix} \mathbf{X_A}'\mathbf{X_A} & \mathbf{X_A}'\mathbf{X_B} \\ \mathbf{X_B}'\mathbf{X_A} & \mathbf{X_B}'\mathbf{X_B} \end{bmatrix}^{-1} \cdot E \begin{bmatrix} \mathbf{X_A}'\mathbf{Y} \\ \mathbf{X_B}'\mathbf{Y} \end{bmatrix}$$

$$= \begin{bmatrix} \alpha \\ \beta \end{bmatrix}. \qquad (A.6)$$

The expected model and error sums of squares are:

$$E[SS(MODEL)] = E[(\mathbf{Y}'\mathbf{X})(\mathbf{X}'\mathbf{X})^{-1} \cdot (\mathbf{X}'\mathbf{Y})]$$
$$= 2 \cdot \sigma_\epsilon^2 + (N - 1) \cdot (\alpha^2 + \beta^2) \quad (A.7a)$$

$$E[SS(ERROR)] = E[\mathbf{Y}'\{\mathbf{I} - \mathbf{X}(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\}\mathbf{Y}]$$
$$= (N - 3) \cdot \sigma_\epsilon^2. \qquad (A.7b)$$