Marker-Based Inferences About Epistasis for Genes Influencing Inbreeding Depression

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ABSTRACT

We describe a multilocus, marker-based regression method for inferring interactions between genes controlling inbreeding depression in self-fertile organisms. It is based upon selfing a parent heterozygous for several unlinked codominant markers, then analyzing the fitness of progeny marker genotypes. If loci causing inbreeding depression are linked to marker loci, then viability selection is manifested by distorted segregation of markers, and fecundity selection by dependence of the fecundity character upon the marker genotype. To characterize this selection, fitness is regressed on the proportion of loci homozygous for markers linked to deleterious alleles, and epistasis is detected by nonlinearity of the regression. Alternatively, fitness can be regressed on the proportion of heterozygous loci. Other modes of selection can be incorporated with a bivariate regression involving both homozygote and heterozygote marker genotypes. The advantage of this marker-based approach is that "purging" is minimized and specific chromosomal segments are identified; its disadvantage lies in low statistical power when linkage is not strong and/or the linkage phase between marker and selected loci is uncertain. Using this method, in the wildflower Mimulus guttatus, we found predominant multiplicative gene interaction determining fecundity and some negative synergistic (nonmultiplicative) interaction for viability.

Understanding the genetic basis of inbreeding depression (i.e., the reduced survival and reproduction of inbred progeny) is important for addressing many issues in evolutionary and conservation biology, including the evolution of plant mating systems (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Uyenoyama et al. 1993; Hedrick 1994). Previous studies have indicated that inbreeding depression is caused by many deleterious genes and that the expression of these genes can be influenced by gene interaction within a locus (dominance) and among loci (epistasis) (Crow 1952, 1993; Jinks 1983; Sprague 1983; Lande and Schemske 1985; Sved and Wilton 1989; Charlesworth et al. 1990; Fu and Ritland 1994a; Johnston and Schoen 1995). Recent theoretical work (e.g., Kondrashov 1982; Charlesworth et al. 1991) has emphasized the importance of epistasis of deleterious genes, as shown that selection can favor the maintenance of sexual reproduction and the evolution of increased recombination when these genes interact such that their harmful effects are not independent and increase in a synergistic manner. Empirical studies, particularly those in Drosophila (Dobzhansky et al. 1963; Spassky et al. 1965; Mukai 1969; Temin et al. 1969; Seager and Ayala 1982; Seager et al. 1982; Clark 1987) suggest that synergism is fairly weak, and generally, evidence for synergistic gene interaction on fitness remains scarce (Crow 1970; Barker 1981; Falconer 1981; Willis 1993).

To infer interactions between loci determining inbreeding depression, two experimental approaches are frequently applied. The first is to compare the fitness of individuals differing for inbreeding coefficients $F$ (i.e., proportional average genomic homozygosity). If interactions among loci are multiplicative, the relationship between the logarithm of fitness and $F$ is expected to be linear; deviations from linearity indicate gene interaction. However, to obtain individuals of differing $F$, enforced inbreeding for one or more generations is required, during which natural selection may "purge" deleterious genes (Lynch 1988; Willis 1993) and change the actual relationship between fitness and $F$. The second approach is to compare the fitness of individuals differing in the number of chromosomes made entirely homozygous by one generation of mating (e.g., Mukai 1969). While this approach allows inferences of a more detailed picture of fitness interactions (Simmons and Crow 1977), it is limited to those experimental organisms in which such chromosome manipulations are possible, such as Drosophila.

Ideally, epistasis should be inferred via direct measurement of fitness of all multilocus genotypes (e.g., in the two locus case, the fitnesses of $AABB$, $AABb$, . . . $aabb$). However, actual identification of individual fitness loci is difficult (Wright 1977; Mather and Jinks 1982), although genomic maps may allow eventual identification (e.g., see Tanksley 1993). Also, total fitness is not easily
be estimated (Lewontin 1974) and one is often restricted to fitness components such as fecundity and viability. In addition, significant fitness interactions of higher order (i.e., greater than three) may go undetected statistically (Hastings 1986; Clark 1987).

With these qualifications, we present and evaluate a third approach to detect synergism between loci, a regression approach based upon molecular markers. It is based upon the principle that selection at loci controlling inbreeding depression will cause “apparent” selection at linked neutral marker loci in progeny (Charlesworth 1991) and, by observing this apparent selection at linked markers, inferences can be made about the true nature of selection (Hedrick and Muona 1990). The experimental design involves selfing a multiple marker heterozygote and analyzing the marker segregation of selfed progeny using regression. Divergences from linearity of regression indicate the average nature of synergism between individual loci. The advantage of this approach is that purging is minimized and specific chromosomal segments are identified. The main disadvantage lies in low statistical power when linkage is not strong and/or the linkage phase between marker and selected loci is not immediately apparent in the data. The problems and prospects of this procedure are illustrated by a study involving the common monkeyflower, *Mimulus guttatus* (Scrophulariaceae).

**Inference of Gene Interactions Using Multiple Marker Loci**

When neutral marker loci are genetically associated with “selected loci” (loci directly controlling inbreeding depression), neutral marker loci show apparent selection (Sax 1929; Charlesworth 1991). We have previously shown (Fu and Ritland 1994a) that, through an experiment that involves selfing a marker heterozygote and genotyping her progeny, we can make inferences about selected loci. This inference is possible because in such a selfed progeny array, genotypes at marker loci among her progeny are not immediately apparent in the data. The problems and prospects of this procedure are illustrated by a study involving the common monkeyflower, *Mimulus guttatus* (Scrophulariaceae).

Univariate regression for recessive viability selection: We first consider the case of a completely recessive deleterious allele \( h = 0 \). In this case, the frequencies of marker genotypes after selection in progeny produced by selfing are, after Equation 1a,

\[
\begin{align*}
    f_A &= \frac{[3 - s(2r - r^2)]}{\bar{f}}, \\
    f_a &= \frac{[1 - s(1 - r)]}{\bar{f}},
\end{align*}
\]

where \( \bar{f} = 4 - s(1 + 2h) \) is the mean fitness and \( r \) is the recombination fraction between marker and selected loci. If the above fitnesses denote fecundity selection, then the mean fecundities, conditioned upon the marker locus genotypes, are as follows:

\[
\begin{align*}
    x_{AA} &= \frac{[1 - s[r^2 + 2hr(1 - r)]]}{\bar{f}}, \\
    x_{Aa} &= \frac{2[1 - s[r(1 - r) + h(1 - 2r(1 - r))]]}{\bar{f}}, \\
    x_{aa} &= \frac{[1 - s(1 - r)^2 + 2hr(1 - r)]}{\bar{f}},
\end{align*}
\]

where \( \bar{f} = 1 - s(1 + 2h) \) for the unlinked case. When \( r < 1/2 \), the relative frequency of \( aa \) is less than \( 1/4 \) (i.e., \( r = 1/2 \), the unlinked case). For brevity, here we term the homozygote with reduced apparent fitness due to linkage (genotype \( aa \)) the “apparent deleterious homozygous” genotype, or “ADH.”

Multivariate interactions: Consider \( n \) unlinked marker loci. Next to the \( k \)th marker, there resides a selected loci with selection coefficient \( s_k \) and linkage \( r_k \) with the marker. The mother is a double heterozygote for all \( n \) pairs of marker-selected loci. Now, in her selfed progeny, segregation can result in any number of these \( n \) loci being homozygous for marker associated with the deleterious allele (e.g., ADH genotypes). Consider a single
observation, where i of n loci are ADH marker genotypes. For notational convenience, let the first i of these n loci be ADHs and the remaining n – i loci be the alternative marker genotypes. If the fitness is determined multiplicatively across selected loci, the expected frequency \( f_{i,n} \) of a selfed progeny with i ADH marker loci is

\[
f_{i,n} = \left( \frac{n}{i} \right) \left( \frac{1}{4} \right)^i \left( \frac{3}{4} \right)^{n-i} \prod_{k=0}^{i} (1 - s_k (1 - r_k)^2) \]

\[
\times \prod_{k=i+1}^{n} (1 - s_k (2r_k - r_k^2)/3) \approx \left( \frac{n}{i} \right) \left( \frac{1}{4} \right)^i \left( \frac{3}{4} \right)^{n-i} \exp \left( - \sum_{k=0}^{i} s_k (1 - r_k)^2 \right) - \sum_{k=i+1}^{n} s_k (2r_k - r_k^2)/3 / \bar{f}, \]  

where \( \bar{f} = \prod (4 - s_k) \). Among selfed progeny of an n-locus heterozygote, there are \( \binom{n}{i} \) ways that i of n loci can be heterozygous. Averaging over all such combinations, and if s and r are independent across loci, it follows that

\[
E[f_{i,n}] \approx \left( \frac{n}{i} \right) \left( \frac{1}{4} \right)^i \left( \frac{3}{4} \right)^{n-i} \exp \left( \frac{1}{3} \bar{r} (1 - 2r + r^2 + \sigma_r^2) - \frac{1}{3} (n - i) \bar{r} (2\bar{r} - \bar{r}^2 - \sigma_r^2) / \bar{f}, \right.  
\]

where \( E \) denotes the expectation across all such combinations, and bars denote means among loci across all combinations (note that for any single progeny, the mean \( s \) for \( k = 0 \), \( i \) does not equal the mean \( s \) for \( k = i + 1, n \)). After dividing the above expectation by the binomial proportions expected under no selection, and taking the natural log, one obtains a regression equation linear in \( i \)

\[
\ln \left( E[f_{i,n}] / \left( \frac{n}{i} \right) \left( \frac{1}{4} \right)^i \left( \frac{3}{4} \right)^{n-i} \right) \approx a + bi,  
\]

wherein

\[
a = -\kappa \bar{r}c / 4,  
\]

\[
b = -\bar{s}c,  
\]

and \( c = \left( \frac{1}{3} - 2\bar{r} \right) \left( 3 - 2\bar{r} \right) + 4\sigma_r^2 / 3. \)

Thus, under multiplicative selection, a regression of \( \ln ( \text{fitness} ) \) on the proportion of ADHs is approximately linearly negative regardless of the distribution of linkage and selective values among loci, as long as their values are not correlated. Selected loci contribute to the slope roughly in proportion to the squared recombination rate with the marker, and variation of \( r \) increases the slope. Also, because the expectation is defined across all combinations of loci, this equation properly applies to data from a large number of offspring, sufficiently large such that any single category of i homoygous genotypes contains most, if not all, groupings of \( i \) from \( n \).

Synergistic epistasis: We assert that deviations from multiplicative selection cause the regression described by Equation 2d to be nonlinear in \( i \), and hence that synergism can be detected by a significant estimate of a nonlinear regression coefficient. A specific synergistic model will illustrate the basic characteristics to expect in this nonlinear relationship. Let us assume the recessive fitness (1 – \( s_0 \)) (1 – \( s_1 \)) (1 – \( s_2 \)) for any pair of selected loci \( kl \). Higher-order interactions are assumed absent, but all \( n(n-1) \) pairwise interactions enter among \( n \) loci. After a similar derivation as above, we find the expected slope of the regression of \( \ln ( \text{fitness} ) \) on \( i \) to be (to order \( \sigma^2 \))

\[
b = (\text{above linear term}) - 2i^2 c.  
\]

Note that only more closely linked loci contribute substantially to the nonlinear term (in proportion to \( (1 - 2r)^2 (1 - 3r)^2 \)), limiting the statistical power to detect nonlinearity.

If one performed a quadratic regression and obtained estimates of linear (\( b_1 \)) and quadratic (\( b_2 \)) coefficients, one could then estimate \( 1/\sigma = b_1/\sigma b_2 \), as suggested by CHARLESWORTH et al. 1991 (p. 178), to measure the relative degree of synergism, assuming linkage (\( c \) in Equation 2d) is known. However, due to the limited degrees of freedom in the data, estimates of linkage are impossible (one could resort to flanking markers; see Fu and RITLAND 1994b); thus our inferences are limited to detecting the presence or absence of synergism, and if present, the sign of interaction (positive or negative).

Figure 1 graphically illustrates the regressions expected under three cases of viability selection: (1) no selection, (2) multiplicative selection with \( s = 0.2 \) and (3) synergistic selection with \( s = 0.2 \) and \( t = 0.04 \). Figure 1A shows the binomial distributions expected under each scenario. The ratio of the observed to neutral proportions is given in Figure 1B. With log transformation of this ratio, a negative slope for the regression occurs under multiplicative selection, and deviation from linearity occurs only under synergistic selection (Figure 1C).

Alternatives to unknown linkage phase: The inference procedure described so far (i.e., Equation 2d) assumes a known “phase” of linkage between marker and selected alleles. With a large number of progeny, linkage phase is fairly obvious (a marker homozygote showing reduced fitness almost surely represents the marker allele linked to the deleterious allele). However, with few progeny, the homozygote not subject to selection may show less apparent fitness merely because of sampling error. If one performs regression on the proportion of such misclassified marker homozygotes, one may spuriously infer selection, due to accumulated bias over loci.
while at \( r = 0.2 \), the slope is 67\%, and at \( r = 0.4 \) the slope is 86\%.

Another approach is to regard the data as “incomplete” and use a likelihood weighted regression. In this approach, at each locus, each of the alternative homozygotes has an equal prior probability of \( \frac{1}{2} \) of being the ADH. Over \( n \) loci, there are \( 2^n \) unique “orderings” of ADHs (e.g., at each of \( n \) loci, each of the two alternative homozygote genotypes is considered as deleterious), each with probability \( \frac{1}{2^n} \). The likelihood of the \( k \)th ordering (cf. Edwards 1992) given the data, is

\[
l_k = \prod_{i=0}^{n} \left( \frac{f_{i,A\mid k}}{\sum_{j=0}^{n} f_{i,\bullet\mid j}^n} \right) N_{i,k},
\]

where \( N_{i,k} \) is frequency of individuals homozygous for \( i \) ADH loci in the \( k \)th ordering, and \( f_{i,\bullet\mid k} = \left( \frac{1}{2} \right)^i \left( \frac{1}{2} \right)^{n-i} \exp(a_k + b_k) \) wherein \( a_k \) and \( b_k \) are the regression parameters estimated using Equation 2d. The final estimate is then obtained as a weighted average over all orderings,

\[
b = \sum b_k \left( \sum l_k \right).
\]

Although the number of orderings increases greatly with many loci (e.g., there are 32768 orderings for 15 loci), the speed of present day computers makes this weighting approach practical for the number of loci typically employed by isozyme studies.

**Univariate regression for recessive fecundity selection**:

Fecundity selection at a linked locus is manifested via a dependence in the mean of the fecundity trait on the marker genotype, as shown above. This differs from the viability selection expectations by the omission of the binomial term and a slightly different mean fitness. For the case of a completely recessive deleterious allele, the apparent fecundity of marker genotypes (Equation 1b) simplifies to

\[
x_{i,a} = \left[ 1 - s(2r - r^2)/3 \right]/\bar{\pi},
x_{i,m} = \left[ 1 - s(1 - r)^2 \right]/\bar{\pi},
\]

where \( \bar{\pi} = 1 - s/4 \) is the mean fecundity of the array of selfed progeny. Given the multiplicative effects over \( n \) selected loci, each with selection coefficient \( s_k \), the average fecundity of selfed progeny with \( i \) ADH loci is

\[
x_{i,a} = \prod_{k=0}^{i} \left( 1 - s_k(1 - r_k)^2 \right)
\times \prod_{k=i+1}^{n} \left( 1 - s_k(2r_k - r_k^2)/3 \right)/\bar{\pi}
\approx \exp \left( - \sum_{k=0}^{i} s_k(1 - r_k)^2 \right.
\left. - \sum_{k=i+1}^{n} s_k(2r_k - r_k^2)/3 \right)/\bar{\pi},
\]

\[
x_{i,m} = \prod_{k=0}^{i} \left( 1 - s_k(1 - r_k)^2 \right)/\bar{\pi},
\]
where $\bar{x} = \prod (1 - s_i/4)$. By taking the natural log of the average fecundity, one obtains the same linear relationship as with viability selection

$$\ln(E[x_{i,a}]) = a + bi,$$  \hspace{1cm} (6c)

where the regression coefficient $b$ is the same as defined in Equation 2d. Thus, by regressing the average fecundity on the proportion of marker ADHs, one again estimates a quantity proportional to the average selection coefficient, and any significant nonlinearity could be taken as evidence for a synergistic interaction.

With smaller progeny sizes, one again encounters the problem of unknown linkage phase between markers and selected loci. The same two alternatives exist as in the case of viability selection, i.e., to regress the average fecundity on the number of marker homozygotes, instead of the number of ADH loci, or to apply a likelihood weighted regression. For the latter, one can use the following likelihood function, which assumes a normal distribution,

$$l_k = \prod_{i=0}^{n} \exp \left\{ \frac{-(x_{i,n} - a_k - ib_k)^2}{2\sigma^2_k} \right\}$$  \hspace{1cm} (7)

(omitting constants), for $\sigma^2_k$ is the variance of the trait about the predicted mean and $x_{i,n}$ the observed mean trait value among individuals homozygous for $i$ putative ADH loci given the particular ordering $k$.

**Bivariate regression for partial recessive selection:** The above procedures assume completely recessive ($h = 0$) selection, but it is possible that deleterious alleles are partially recessive (see Simmons and Crow 1977 discussion). Partial recessivity, if present, may bias inferences (Fu and Ritland 1994b) and can accordingly decrease the efficiency of regressions. To include partial recessive selection, we now consider a bivariate regression.

**Bias due to partial recessivity:** Equation 1, a and b completely specifies the marker expectations in the respective cases of viability and fecundity selection. With multiplicative effects among viability loci, the total fitness is the product of these quantities over $n$ marker loci. Using the same procedure that leads to the derivation of Equation 2d, one finds that the slope of the univariate regression of ln(fitness) on the number of ADHs is

$$b = -\bar{r}[(1 - 2\bar{r})(3 - 2\bar{r}) - 2\text{h}(1 - 2\bar{r})^2]$$

$$+ 4(1 - 2\bar{h})\sigma^2_n] / 3.$$  \hspace{1cm} (8)

When $h = 0$ (i.e., recessive case), Equation 8 reduces to Equation 2d. For $h > 0$ (partial recessivity), the slope is less than that for $b$ in Equation 2d, implying that selection is less likely to be detected. For example (ignoring variation of linkage), at $r = 0.2$ and $h = 0.3$, the slope is 86% that of Equation 2d. When $h > 0.5$ (i.e., for partial dominance and underdominance), the slope may actually become positive (the condition for sign change is $h > 0.5 + 1/(1 - 2\bar{r} + 4\sigma^2_n)$. For $h < 0$ (overdominance), the slope is greater than that for $b$ in Equation 2d. Synergistic effects due to partial recessivity are greater than those described for multiplicative epistasis, as the quadratic coefficient for synergism is proportional to the squared linear coefficient (see Equation 3).

**Bivariate regression:** For simplicity, we first consider fecundity selection. For $x_{i,j,a}$ the fitness of a selfed progeny homozygous for $i$ ADH marker loci and heterozygous for $j$ marker loci ($i + j = n$), one can show that the expected slope of the bivariate partial regression of ln($x_{i,j,a}$) on $i$ is

$$b_{hoo} = -\bar{r}(1 - 2\bar{r}),$$

and on $j$ is

$$b_{hoo} = -\bar{r}[1 - 2\bar{r}]\left[\bar{r} + \bar{h}(1 - 2\bar{r})\right]$$

$$- 2(1 - 2\bar{h})\sigma^2_n] / 3.$$  \hspace{1cm} (9)

Clearly, the slope of the regression of fitness on $i$ is more negative in this bivariate case than in the univariate case (Equation 2), roughly by the factor $3/(3 - 2\bar{r})$, and is unaffected by variation of linkage and level of dominance. Interestingly, Equation 9 also shows that $b_{hoo}$ can be nonzero even when average dominance is zero, implying that a linear relationship between fitness and heterozygosity in selfed progeny cannot distinguish between dominance and overdominance (Houle 1989).

Extension of such a bivariate regression to partial recessive viability selection is straightforward. Observed frequencies are first divided by the trinomial probabilities $(\text{t}_i)(\text{t}_j)(\text{t}_{i+j})$, and the log of these transformed frequencies are then bivariately regressed on $i$ and $j$. The quantities given in Equation 9 are estimated.

Again, these formula assume the phase of association between markers and selected loci is known. If uncertain, the likelihood weighted regression approach must be used. The number of possible orderings remains the same as in the univariate regression ($2^n$), and the likelihood equation for fecundity requires a bivariate normal distribution.

**Experimental design:** The power of detecting synergism via this marker-based regression approach is generally low. To improve the precision of estimates, one can either increase the number of loci or increase the number of individuals assayed. We used Monte-Carlo simulation to investigate this issue by computing variances of estimates in the univariate quadratic regression (i.e., Equations 2d and 3) with no actual selection. Scenarios consisting of 5–30 markers and 100–500 individuals were considered in 30 combinations with 10,000 replications for each. The observed variances of estimates among replications were multiplied by the corresponding number of loci and size of progeny to deter-
were examined. Sensitive to the number of loci. Size, while the estimate of quadratic coefficient is more sensitive to only the progeny size; while the estimate of linear coefficient is sensitive to only the progeny size. These results indicate that the estimate of linear coefficient increases, while in contrast, the variance of the quadratic coefficient estimate decreases, the per-individual, per-locus variances of the linear coefficient estimate increases, while in contrast, the variance of the quadratic coefficient estimate decreases dramatically. These results indicate that the estimate of linear coefficient is sensitive to only the progeny size, while the estimate of quadratic coefficient is more sensitive to the number of loci. A progeny size of 200 or more assayed for ~10 loci is sufficient for linear regression estimates.

A STUDY OF SYNERGISM IN M. GUTTATUS

Materials and methods: To assess the multilocus, marker-based method described above and detect synergism for viability and fecundity genes, we analyzed a dataset from an experiment with M. guttatus (the common monkeyflower). The construction of the crosses, the experimental procedures (including growing conditions, phenotyping and genotyping of the progeny) and the isozyme loci used have been previously described (Fu and Ritland 1994a,c). The dataset consisted of genotypic data for nine unlinked marker loci and fecundity data for five traits for 1874 surviving F2 progeny among 31 selfed progeny arrays (~60 progeny per array). The five fecundity traits were relative growth time, number of nodes in main stem, total number of flowers, plant height, and above-ground dry weight. All data were log-transformed before analysis.

The data was analyzed using three approaches described above (of six regression models): linear and quadratic regressions of ln (fitness) on (1) the number of ADH markers, (2) the number of heterozygotes, and (3) bivariate linear and quadratic regressions of ln (fitness) where the two dependent variables were the number of ADH markers and the number of heterozygote markers. For approaches 1 and 3, a likelihood weighted regression was used because progeny size per cross in this dataset was relatively small (~60). This involved, for each cross, maximizing the likelihood function (by grid search) for each regression coefficient in each ordering of 2^"^{\text{ combinations of ADHs and weighting the regression coefficient estimate over all combinations by their likelihoods (see the above section for details). Second, bootstrap estimates (Efron 1982) of standard errors for these regression coefficients were made in 1000 resampled datasets for each cross. Finally, a grand mean regression coefficient (across the 31 arrays) and its standard error (or minimum variance) were calculated (the regression for each array was weighted by the inverse of the bootstrap variance; variances were heterogeneous among arrays due to differences in the number of heterozygous loci per parent). In the case of approach 2, the linkage phase does not need to be considered, so the likelihood weighted regression is not needed and the procedure is simpler. Significance of estimates was determined by a t-test based upon the observed bootstrap distribution. This procedure was repeated for viability component and each of five fecundity traits. To assist this analysis, a PASCAL computer program was written and is available from the authors upon request.

Results: Tables 1 and 2 give the results for viability and fecundity, respectively. Each table lists six regression models, corresponding to multiplicative recessive on ADHs, synergistic recessive on ADHs, multiplicative recessive on heterozygotes, synergistic recessive on heterozygotes, multiplicative partial recessive, and synergistic partial recessive. Negative synergism was strongly evident for viability selection (Table 1). Both univariate and bivariate quadratic regressions on ADHs revealed highly significant ($P < 0.001$) quadratic coefficients ($b_{11} = -0.0555$ and $-0.0736$, respectively). Also, two quadratic regressions on heterozygotes displayed significant ($P < 0.05$) quadratic coefficients ($b_{22} = -0.0351$ and $-0.0358$, respectively). Clearly, the power to detect synergism increased when the partial recessive model was used instead of the complete recessive model (as shown in t-values, 9.814 and 7.642 for $b_{11}$, respectively). Also, the likelihood weighted regression gave higher power to detect synergism than the regressions on heterozygotes (for
Inference of Epistasis

### TABLE 1

Results of six regressions of viability component \( (P^*) \) on the number of marker homozygotes \( (i) \) showing lower fitness values (ADHs) and/or number of marker heterozygotes \( (j) \)

<table>
<thead>
<tr>
<th>Regression model/coefficient</th>
<th>Estimate</th>
<th>Standard error</th>
<th>( t )-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplicative recessive on ADHs [ \ln(P^*) = b_i ]</td>
<td>0.0221</td>
<td>0.0141</td>
<td>1.564</td>
</tr>
<tr>
<td>Synergistic recessive on ADHs [ \ln(P^*) = b_i + b_1 ]</td>
<td>0.1750</td>
<td>0.0224</td>
<td>7.795***</td>
</tr>
<tr>
<td>Multiplicative recessive on heterozygotes [ \ln(P^*) = b_j ]</td>
<td>-0.0555</td>
<td>0.0073</td>
<td>7.642***</td>
</tr>
<tr>
<td>Synergistic recessive on heterozygotes [ \ln(P^*) = b_j + b_2 ]</td>
<td>-0.0571</td>
<td>0.0725</td>
<td>0.787</td>
</tr>
<tr>
<td>Multiplicative partial recessive [ \ln(P^*) = b_i + b_j ]</td>
<td>-0.0090</td>
<td>0.0044</td>
<td>2.062*</td>
</tr>
<tr>
<td>Synergistic partial recessive [ \ln(P^*) = b_i + b_j + b_2 ]</td>
<td>-0.0358</td>
<td>0.0153</td>
<td>2.272*</td>
</tr>
</tbody>
</table>

\( a^*0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001. \)

example, \( t = 9.814 \) and 7.642 for \( b_i \)'s vs. 2.257 for \( b_2 \).

In addition, further regression analyses of the effects of various informative marker loci across 31 arrays on the estimates and their variances revealed that, as expected, only variances of estimates for synergism were significantly decreased for progeny arrays with more heterozygous marker loci (results not shown).

Table 1 also shows that some linear coefficients \( (b_i) \) were significantly positive. Two positive estimates \( (b_i = 0.0221 \) and 0.1750) in univariate regressions are expected if the average level of dominance is greater than 0.5 (see Equation 8). This accords with Fu and Ritland (1994a) analyses of the same data, from which partial dominance/underdominance were found to be the major single-locus modes of gene action in these selfed progeny. This is further supported by the observation of the significantly negative estimate in \( b_i (-0.0090) \) in bivariate linear regression, which was unaffected by the level of dominance (see Equation 9). However, the real unexpected is the positive estimate of \( b_i (=0.0830) \) in bivariate quadratic regression. This might be still due to the effect of dominance contributing to the covariance of two variables in the bivariate quadratic regression.

Table 2 gives the results for the fecundity trait, plant dry weight. Synergism was not detected: all estimates of nonlinear regression coefficients were not significant at the 0.05 level. But there was a significant \( (P < 0.05) \) linear regression coefficient in bivariate regression \( (b_i = -0.0090 \) and \( b_j = 0.0217) \), although the linear coefficient for deleterious homozygote in univariate regression was not significant at the 0.05 level. Also, power increased when the partial recessive model was included. The results for other four fecundity traits were essentially the same as plant dry weight (not shown). Overall, our results suggest that multiplicative gene interaction is predominant for fecundity selection.

### DISCUSSION

Inference of epistasis for genes affecting inbreeding depression is generally difficult. Previous approaches usually require enforced inbreeding over generations, which may distort true interactions due to selective purging over this time. In this paper, we introduced an experimental method that suited for self-fertile organisms. It is based upon selfing a parent heterozygous for several unlinked, codominant markers and analyzing the fitness of multilocus progeny genotypes using regressions. This method requires selfing only for one generation, so that purging is minimized. Also, this method examines gene interactions among specific chromosomal segments, as opposed to gene interactions averaged over the genome. We applied this method to empirical data from \( M. guttatus \) and found a predominance of multiplicative fitness gene interac-
Results of six regressions of fecundity component (plant dry weight; $x$) on the number of marker homozygotes ($i$) showing lower fitness values (ADHs) and/or number of marker heterozygotes ($j$)

<table>
<thead>
<tr>
<th>Regression model/coefficient</th>
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<th>$t$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplicative recessive on ADHs</td>
<td>[ln($x$) = $b_i + b_{ij}$]</td>
<td>$-0.0033$</td>
<td>$0.0022$</td>
</tr>
<tr>
<td>Synergistic recessive on ADHs</td>
<td>[ln($x$) = $b_i + b_{ij} + b_{ij}^2$]</td>
<td>$-0.0298$</td>
<td>$0.0186$</td>
</tr>
<tr>
<td>Multiplicative recessive on heterozygotes</td>
<td>[ln($x$) = $b_i + b_{ij}$]</td>
<td>$0.0077$</td>
<td>$0.0049$</td>
</tr>
<tr>
<td>Synergistic recessive on heterozygotes</td>
<td>[ln($x$) = $b_j + b_{ij} + b_{ij}^2$]</td>
<td>$0.0568$</td>
<td>$0.0414$</td>
</tr>
<tr>
<td>Multiplicative partial recessive</td>
<td>[ln($x$) = $b_i + b_{ij} + b_{ij}^2$]</td>
<td>$-0.0909$</td>
<td>$0.0044$</td>
</tr>
<tr>
<td>Synergistic partial recessive</td>
<td>[ln($x$) = $b_i + b_{ij} + b_{ij}^2 + b_j + b_{ij}^2$]</td>
<td>$-0.0200$</td>
<td>$0.0219$</td>
</tr>
</tbody>
</table>

* $P < 0.05$. 

Limitations of the inferences: There are several limitations to our marker-based method. First, this method provides no simple tests for validity of the assumption of a single selected locus to a marker, when indeed it is possible for two or more selected loci to be linked. In this light, our inferences strictly hold for short chromosomal segments about the marker and not for individual selected loci. Second, we can detect only the sign of epistasis (i.e., positive or negative interaction), as in the absence of linkage estimates, we cannot estimate the strength of synergism (multiple marker loci spanning a selected locus should give sufficient degrees of freedom to estimate such linkage). Third, our inferences provide no information on higher order gene interactions. In addition, lack of epistasis after log-transformation of fitness does not exclude epistasis in nontransformed fitness scale (Seager and Ayala 1982).

Increasing the power of detecting synergism: The power to detect gene interaction via the traditional pairwise analysis of fitness loci (Hastings 1986; Clark 1987) is generally low. In our regression approach, the fitness interactions are effectively averaged over all loci, resulting in more power than if just one pair of loci were examined. However, our analytic results show that the power of detection via multiple loci approach still is relatively low. Perhaps such averaging may not significantly increase power when loci have small effects (cf. Karlin 1979). Incomplete linkage also limits the power to distinguish nonlinearity from linearity, as linkage contributes to the linear term of the regression in proportion to $(1 - 2\rho)(1 - 3\rho)$ and the quadratic term in proportion to $(1 - 2\rho)^2(1 - 3\rho)^2$. Also, when the number of progeny is small, the unknown linkage phase between marker and selected loci reduces power. Variation in selection intensity among loci may also reduce power.

To increase the statistical power to detect synergism, we have considered two alternatives. First, we have found that inclusion of heterozygotes in the analysis (i.e., bivariate regression) increases power. Second, in investigating the number of unlinked markers needed to obtain a reasonable probability of detection, we concluded that 10 or more loci is desirable (see Figure 2).

Extensions to total fitness: To extend inferences to total fitness (viability times fecundity), one weights the observed marker genotype frequencies by the fecundity of each respective marker genotype, normalizes the genotypic frequencies at each locus, then applies the viability selection method described above. Estimation of total fitness, however, may be impossible (see Lewontin 1974 and Endler 1986 for detailed discussion). For example, it is difficult to determine which of several fecundity traits most closely reflects fitness. For this rea-
son, inferences using some total fitness measure may not be any more informative than inferences made separately for various fitness components.

**Evidence for synergistic gene interaction:** Negative synergism occurs when the reduction of multilocus fitness is greater than that expected from the product of single locus fitnesses. Our empirical results provide evidence for negative synergism of genes affecting viability but not for those affecting fecundity. Lack of synergism for fecundity selection is consistent with two recent studies in this species (WILLIS 1993; LATTA and RITLAND 1994). In the investigation of the effect of four levels of inbreeding on four fitness components in _M. guttatus_, WILLIS (1993) found a weak synergistic interaction for pollen viability, among four fitness components examined. In a study of the relationship between inbreeding depression and prior inbreeding among populations of four _Mimulus_ taxa, LATTA and RITLAND (1994) found one significant association (for germination rate) and several nonsignificant negative associations between inbreeding depression and the inbreeding coefficient. These findings, along with those in Drosophila, seem to suggest a predominance of synergism for viability selection but not for fecundity selection. Certainly more empirical data are needed to confirm this pattern, as “it is possible that such epistatic interactions are found only in some but not in all populations” (SPASSKY et al. 1965).

However, several critical points should be raised about our empirical study. First, prior selection may have occurred during the generation of multiple heterozygotes (via intercrosses) used as parents of the selfed progeny arrays (FU and RITLAND 1994c). This may explain, in part, the observed lack of synergistic interactions affecting fecundity. Ideally, selfed progeny should be obtained directly from the parental plants in the field (via hand pollination) and grown in the field environment. Second, some significantly positive estimates were found in this study (Table 1). This can be explained, in large part, by the high level of dominance (partial dominance/underdominance) revealed by the single-locus, graphical method of FU and RITLAND (1994a) applied to the same data. Such a high level of dominance is unexpected in selfed progeny (FU and RITLAND 1994a) and its explanation is still being questioned. Among possible factors are gametic selection, background selection against slightly deleterious genes, and/or the presence of genotype by environment interactions. Third, the isozyme loci used in our study mark only ~3% of the _Mimulus_ genome (based on the calculation following BISHOP et al. 1980), so that much genetic information is missing, and our power to detect synergism correspondingly reduced. Current efforts are being directed at obtaining simple sequence repeat loci (SSRs, or microsatellites) in _Mimulus_ (P. AWADALLA and K. RITLAND, unpublished results; J. H. WILLIS, unpublished results). With highly polymorphic and dispersed markers, a large portion of the genome could be surveyed in field conditions, albeit at the increased work involved with DNA assays.

**Prospects:** The use of genetic markers to infer the genetic basis of inbreeding depression opens new avenues to addressing long standing questions concerning the genetic basis of inbreeding depression (CHARLESWORTH 1994; RITLAND 1996). The multilocus method presented herein, as well as our previous, single-locus method for inferring gene action (FU and RITLAND 1994a), are not without their limitations, but in many respects they are feasible and more powerful than classical quantitative genetics approaches. With their features of simplicity and minimal experimental manipulations, genetic marker methods should allow new insights into genes affecting inbreeding depression, particularly in natural populations.

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