Genet. Res., Camb. (1997), **70**, *pp.* 143–153. *With 7 figures.* Printed in the United Kingdom

Point estimation and graphical inference of marginal dominance for two viability loci controlling inbreeding depression

YONG-BI FU[1]*, DEBORAH CHARLESWORTH[2]** AND GENE NAMKOONG[1]
[1] *Department of Forest Sciences, University of British Columbia, 193-2357 Main Mall, Vancouver, BC, V6T 1Z4 Canada*
[2] *Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, IL 60637, USA*

(*Received 3 February 1997 and in revised form 8 July 1997*)

Summary

A deterministic analysis is conducted to examine marginal dominance for two linked viability loci influencing inbreeding depression and its graphical inferences. Four estimators of marginal dominance are derived, assuming a biallelic marker locus completely linked to one of the viability loci, and the biases in expected estimates due to the other deleterious locus are discussed. Three conditions under which apparent partial dominance or underdominance could occur are found, i.e. when two multiplicative, partially recessive loci are linked in coupling phase and when two synergistic, highly overdominant loci are linked in coupling or repulsion phases. Expected frequencies of the three marker genotypes in selfed progeny are derived, considering two linkage phases, two types of marker locus position with respect to the viability loci, and the multiplicative and synergistic fitness models. Segregation ratios are generated for the marker locus linked to either two overdominant or partially recessive loci and plotted in gene action graphs to examine the robustness of the graphical inferences of gene action due to the presence of an additional linked viability locus. Under a multiplicative fitness model, the presence of an additional partially recessive or overdominant locus in the vicinity of the marker locus does not greatly affect the graphical inferences of the relative role of partially recessive or overdominant genes in expression of inbreeding depression. A marker linked to two synergistic, highly overdominant loci can behave as though linked to a partially recessive, partially dominant or underdominant locus, even with relatively weak synergism.

1. Introduction

Understanding genetic mechanisms underlying inbreeding depression is important in many theoretical and applied aspects of genetics and evolution (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Crow, 1993). In spite of many genetic studies of inbreeding depression, the relative role of partially recessive genes versus overdominant genes in expression of inbreeding depression remains less than clear (Barrett & Charlesworth, 1991; Fu & Ritland, 1994*a*; Johnston & Schoen, 1995). Classical quantitative genetic approaches can provide estimates of the average dominance of many genes, but not individual genes (Wright, 1977; Mather & Jinks, 1982). Recent marker-based investigations appear to show promise for precise estimation of dominance at a resolution not obtainable by quantitative genetic methods, but so far most are still limited to a large chromosome segment (Lynch & Walsh, 1997). While it is possible in principle to estimate dominance for individual genes affecting a character when there is a large number of genetic markers spanning a chromosomal segment, such an estimation is still a challenging task for fitness (i.e. for inbreeding depression), especially for populations of wild species with a limited number of genetic markers (Ritland, 1996).

Fu & Ritland (1994*a*) recently developed a marker-based, graphical method for inferring the nature of gene action that requires only one generation of selfing and a handful of codominant neutral marker loci. By examining observed segregation ratios of marker loci in selfed progeny over the expected 'space' of segregation ratios plotted in a triangular plane (see below), one can estimate the relative roles of different genetic mechanisms. Such a graphical determination offers a simple and robust, although

* Corresponding author. Tel: +1(604)-822-8739. Fax: +1(604)-822-9102. e-mail: yongbifu@unixg.ubc.ca.
** Present address: ICAPB, University of Edinburgh, Ashworth Laboratories, West Mains Road, Edinburgh EH9 3JT, UK.

not precise, means to characterize the behaviours of deleterious genes in natural populations. When this method was applied to data on segregation of isozyme markers in a population of the plant *Mimulus guttatus*, Fu & Ritland found that, in the chromosomal segments identified by seven isozyme markers, partial recessivity/underdominance (i.e. effects greater than intermediate dominance) of alleles with low fitness appeared to play the predominant role in expression of genetic load. This finding, however, is inconsistent with the existence of inbreeding depression in this population (Ritland, 1990; Y. B. Fu & K. Ritland, unpublished data), which demands either partially dominant or overdominant alleles. With partially recessive alleles, no reduction in fitness of selfed progeny (the mean of homozygote fitnesses exceeds that of the heterozygote) is expected. This unexpected finding requires explanation.

One could argue that the observed pattern of gene action may reflect the presence in short chromosomal segments of several genes affecting fitness, as opposed to individual viability genes as assumed in deriving the method. Recent QTL studies show that clustering of selected genes is not uncommon in some chromosomal segments (for a review see Lynch & Walsh, 1997). The fact that a high proportion (9/15) of the allozyme markers studied by Fu & Ritland (1994*a*) showed evidence for linked viability loci suggests that loci with fitness effects are found in a high fraction of randomly picked intervals. This further suggests that it is not unlikely that multiple loci will be presented in the vicinity of a marker. This is especially likely if the marker is in a region with low recombination. It is well known that two partially recessive loci linked in repulsion phase can produce apparent overdominance (for discussion see Comstock & Robinson, 1952). Spurious partial dominance could arise if heterozygotes for a marker in a family produced by selfing have lower apparent fitness than expected from the homozygotes' fitness, because they are heterozygous not for just one selected locus but for two or more, such that the heterozygote is reduced in fitness more than the homozygotes. Such a condition could sometimes arise. To resolve this argument, a determination of the number of selected loci in a chromosome segment is certainly needed, but this is experimentally difficult, requiring some generations of crossing or many markers spanning the chromosome segment.

In this paper, we examine deterministically expected marginal dominance values when there are two linked viability loci influencing inbreeding depression, and the effects of such loci on its graphical estimation using linked neutral marker loci. This examination can shed some light on the robustness of the graphical method to an additional locus, and the observation in *Mimulus guttatus* of partial dominance/underdominance. In what follows, we first characterize the effects of an additional viability locus on point estimations of dominance and determine the conditions, if any, under which two linked partially recessive or overdominant viability loci behave like a partially dominant or underdominant locus. Second, we examine the expected patterns of apparent gene action inferred graphically in the case of a neutral marker locus with two alleles linked to two viability loci, considering both multiplicative and synergistic fitness models.

2. Point estimation of marginal dominance for two linked viability loci

Many studies have demonstrated that a neutral genetic marker may show distorted segregation ratios when linked to loci affecting viability (Sorensen, 1967; Hedrick & Mouna, 1990; Fu & Ritland, 1994*b*). Hedrick & Muona (1990) first showed how such distorted segregation ratios can be used to characterize viability alleles in self-fertile organisms. If a plant with a heterozygous marker locus (M_1M_2) linked to a heterozygous viability locus (Aa) is selfed, the expected frequencies of its progeny marker genotypes, M_1M_1, M_1M_2 and M_2M_2, are, respectively,

$$\left.\begin{aligned} p_{11} &= \{1-s_a[r_m+2h_a r_m(1-r_m)]\}/p \\ p_{12} &= 2\{1-s_a[r_m(1-r_m)+h_a(1-2r_m(1-r_m))]\}/p \\ p_{22} &= \{1-s_a[(1-r_m)^2+2h_a r_m(1-r_m)]\}/p, \end{aligned}\right\} \quad (1)$$

where $p = 4-s_a(1+2h_a)$, s_a and h_a are the selection and dominance coefficients at the viability locus, and r_m is the recombination fraction between the marker and viability loci (Fu & Ritland, 1994*a*). Since (1) is a system of two independent equations with three unknowns, the selection and dominance coefficients cannot be simultaneously estimated. As a result, Hedrick & Muona (1990) proposed a maximum likelihood procedure to estimate s_a and r_m by assuming that the linked deleterious gene is completely recessive (i.e. $h_a = 0$), but these estimates can be seriously biased when the viability allele is partially recessive (Fu & Ritland, 1994*b*). In contrast, Fu & Ritland (1994*a*) proposed a graphical method that allows inference of the dominance level, instead of the strength of gene effect and the linkage, to distinguish between dominance and overdominance hypotheses.

These single-marker estimation procedures assume the presence of just one viability locus in the vicinity of the marker locus, which may not always be true. If the marker is linked to more than one viability locus, estimation bias may occur. Fu & Ritland (1994*b*) conducted a computer simulation to examine the bias of estimating s_a and r_m due to an additional viability locus (assuming that the deleterious genes of both loci are completely recessive). They showed (*a*) that the presence of two viability loci in coupling generally results in estimates not representative of either viability locus (both average s_a and r_m are usually overestimated) and (*b*) that extreme biases occur for two

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

Table 1. *Expected estimates of marginal dominance under two multiplicative, partially recessive viability loci*

Case	True value					Estimated value of h_a	
	r_{ab}	h_a	s_a	h_b	s_b	Loci in coupling	Loci in repulsion
1	0·05	0·02	0·90	0·02	0·90	0·074	−8·538
2	0·05	0·02	0·90	0·20	0·60	0·148	−1·149
3	0·05	0·02	0·90	0·40	0·30	0·138	−0·232
4	0·05	0·20	0·60	0·02	0·90	0·182	−2·023
5	0·05	0·20	0·60	0·20	0·60	0·282	−5·975
6	0·05	0·20	0·60	0·40	0·30	0·308	−0·167
7	0·05	0·40	0·30	0·02	0·90	0·194	−0·256
8	0·05	0·40	0·30	0·20	0·60	0·338	−0·280
9	0·05	0·40	0·30	0·40	0·30	0·445	−2·051
10	0·45	0·02	0·90	0·02	0·90	0·074	−0·056
11	0·45	0·02	0·90	0·20	0·60	0·057	−0·026
12	0·45	0·02	0·90	0·40	0·30	0·038	0·000
13	0·45	0·20	0·60	0·02	0·90	0·255	0·108
14	0·45	0·20	0·60	0·20	0·60	0·239	0·146
15	0·45	0·20	0·60	0·40	0·30	0·220	0·178
16	0·45	0·40	0·30	0·02	0·90	0·435	0·281
17	0·45	0·40	0·30	0·20	0·60	0·428	0·340
18	0·45	0·40	0·30	0·40	0·30	0·416	0·378
19	0·05	0·45	0·30	0·45	0·30	0·494	−1·179
20	0·05	0·45	0·30	0·45	0·60	0·513	0·210
21	0·05	0·45	0·60	0·45	0·60	0·553	−2·241
22	0·45	0·45	0·30	0·45	0·30	0·462	0·434
23	0·45	0·45	0·30	0·45	0·60	0·476	0·405
24	0·45	0·45	0·60	0·45	0·60	0·473	0·421

viability loci linked in repulsion, with apparent overdominance of the neutral marker. However, the magnitude of the bias in estimating the dominance level due to an additional linked locus was not studied.

In this section, we examine deterministically the expected effects of an additional deleterious locus on the dominance estimates. Here we consider a simple case and assume complete linkage ($r_m = 0$) between the first viability locus (A) and the marker locus. If no other viability locus is present, the estimator of dominance for the locus A can be obtained as an exact solution of (1) as:

$$\hat{h}_a = 0{\cdot}5\left(1+\frac{1-2p_{12}}{|p_{22}-p_{11}|}\right). \tag{2}$$

To examine the behaviour of this estimator in the presence of an additional viability locus, we need to derive the expected frequencies of the three marker genotypes under the linkage of one marker locus and two viability loci and substitute the expected frequencies into (2) to obtain the estimator of marginal dominance under two viability loci.

The expected genotype frequencies of the marker locus linked to two viability loci are derived by multiplying the expected frequencies of the 27 possible progeny genotypes before selection by the corresponding fitnesses, summing over genotype frequencies at the two viability loci, and weighting them by the total fitness for the marker locus. Such a derivation is simplest with $r_m = 0$, but in general depends on the linkage phase (coupling versus repulsion) and fitness model (multiplicative versus synergistic). For the linkage of two multiplicative viability loci in coupling phase, the expected frequencies of the progeny marker genotypes, M_1M_1, M_1M_2 and M_2M_2, are, respectively,

$$p_{11} = p'_{11}/p, \quad p_{12} = p'_{12}/p, \quad \text{and} \quad p_{22} = p'_{22}/p, \tag{3}$$

where

$$p'_{11} = 2h_b s_b(r_{ab}^2 - r_{ab}) - s_b r_{ab}^2 + 1),$$
$$p'_{12} = 2(h_a s_a - 1)(h_b s_b(2r_{ab}^2 - 2r_{ab} + 1) - s_b(r_{ab}^2 - r_{ab}) - 1),$$
$$p'_{22} = 2h_b s_b(1-s_a)(r_{ab}^2 - r_{ab}) - (1-s_a) \times (s_b(1-r_{ab})^2 - 1) \text{ and } p = p'_{11} + p'_{12} + p'_{22}.$$

Note that s_b and h_b are the selection and dominance coefficients at the viability locus B and r_{ab} is the recombination between the two viability loci (A and B). It should also be mentioned that (3) applies only for $r_{ab} > 0$; with $r_{ab} = 0$, the linkage situation becomes a one-locus configuration, not a two-locus case as assumed in (3). Thus, the estimator of marginal dominance under the two viability loci is readily obtained simply by substituting into (2) the expected marker frequencies as in (3), although it is a rather

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

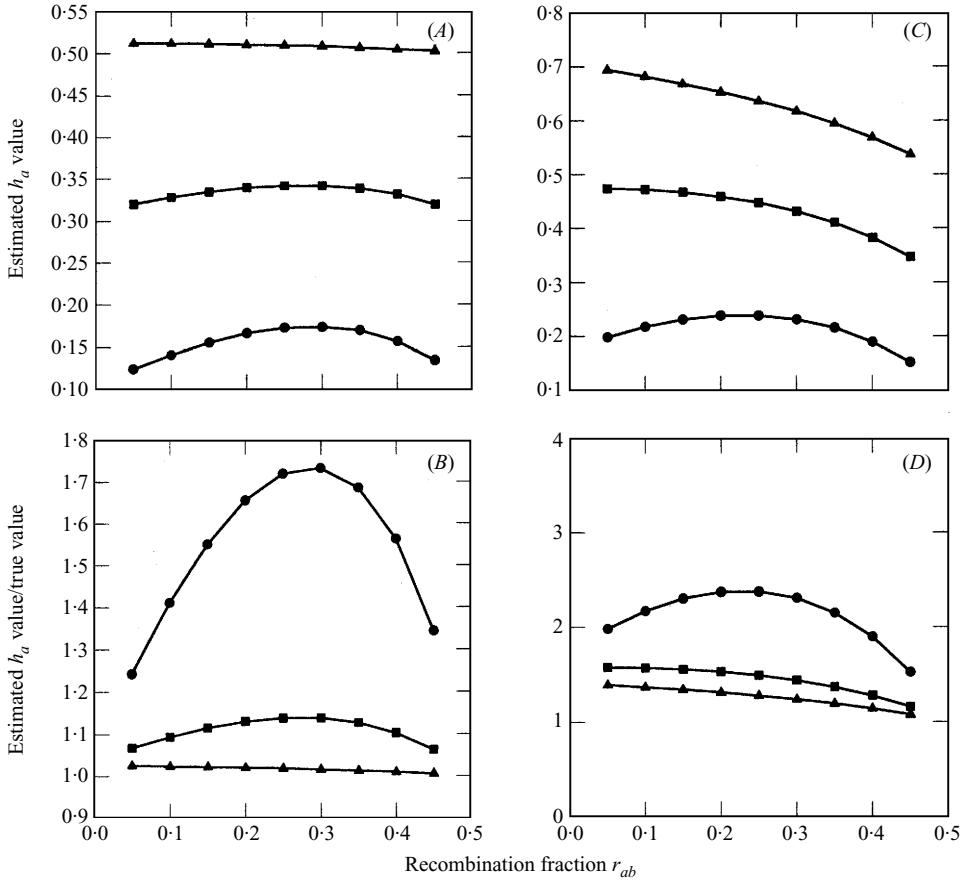

Fig. 1. Expected estimates of marginal dominance and their biases for two identical deleterious loci, as a function of their true dominance levels and recombination fraction. (*A*) Estimated marginal dominance value for $s = 0{\cdot}1$; (*B*) the bias for $s = 0{\cdot}1$; (*C*) the estimated marginal dominance value for $s = 0{\cdot}9$; and (*D*) the bias for $s = 0{\cdot}9$. Note that the scales of the *Y*-axes differ. ●, $h = 0{\cdot}1$; ■, $h = 0{\cdot}3$; ▲, $h = 0{\cdot}5$.

lengthy equation. If one considers only two identical viability loci, i.e. $s_a = s_b = s$, $h_a = h_b = h$, the estimator can be reduced to

$$\hat{h}_a = 0{\cdot}5 \left\{1 - \frac{[r_{ab}^2 + (1-r_{ab})^2](2h^2 s - 4h - s + 2) + r_{ab}^2 s}{|(1-r_{ab})[r_{ab}(s-2hs) - s + 2]|}\right\}. \quad (4)$$

Similarly, the estimators of marginal dominance are derived for the other three linkage situations, i.e. two multiplicative viability loci linked in repulsion phase and two synergistic viability loci in either coupling or repulsion linkage phases.

In this paper, we use the synergistic fitness model of Kimura & Maruyama (1966), $w_i = 1 - an - bn^2$, where a is the linear coefficient for selection, b is the quadratic coefficient for synergism, h is the average dominance level, $n = hy + z$ (the effective number of deleterious alleles), and y and z are the numbers of heterozygotes and deleterious homozygotes (although other models are also available; for discussion see Charlesworth *et al.*, 1991). If $b > 0$, the harmful effect of deleterious loci on fitness increases non-linearly with the number of effective loci present in the individual. Clearly, with $b > 0$, the effect is more harmful than under multiplicativity (i.e. $b = 0$). In general, the degree of synergism is commonly defined as b/a. Empirical data obtained so far seem to suggest that such synergism affecting viability exists in natural populations, but is fairly weak (for discussion see Fu & Ritland, 1996). With this model, we obtain the estimator of marginal dominance for two synergistic loci linked in coupling phase as

$$\hat{h}_a = 0{\cdot}5 \left\{1 + \frac{[2r_{ab}^2 - 2r_{ab} + 1][a(2h-1) + b(4h^2 - 2)] + br_{ab}^2}{|(r_{ab} - 1)[a + b(2hr_{ab} - r_{ab} + 2)]|}\right\}. \quad (5)$$

The estimators for the two repulsion linkage situations are not shown, but are available on request from the first author.

(i) *Expected marginal dominance and its estimation bias*

To appreciate the magnitude of the effect of an additional nearby viability locus on estimates of dominance for the locus of interest, we examine the four derived estimators of marginal dominance in detail and focus on two partially recessive loci. Table 1 gives the expected estimates of marginal dominance with two multiplicative, partially recessive loci linked in coupling and repulsion phases, in 24 representative cases. These estimates are generated, based on the

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

Table 2. *Expected estimates of marginal dominance under two synergistic, partially recessive viability loci*

Case	True value				Estimated value of h_a	
	r_{ab}	h	a	b	Loci in coupling	Loci in repulsion
1	0·05	0·02	0·02	0·10	0·016	−7·884
2	0·05	0·02	0·10	0·10	0·023	−8·000
3	0·05	0·02	0·10	0·02	0·034	−8·124
4	0·05	0·02	0·20	0·02	0·038	−8·153
5	0·05	0·20	0·02	0·10	0·070	−4·845
6	0·05	0·20	0·10	0·10	0·109	−4·873
7	0·05	0·20	0·10	0·02	0·169	−4·909
8	0·05	0·20	0·20	0·02	0·188	−4·918
9	0·05	0·40	0·02	0·10	0·196	−1·313
10	0·05	0·40	0·10	0·10	0·252	−1·312
11	0·05	0·40	0·10	0·02	0·339	−1·311
12	0·05	0·40	0·20	0·02	0·367	−1·311
13	0·45	0·02	0·02	0·10	0·035	−0·034
14	0·45	0·02	0·10	0·10	0·043	−0·036
15	0·45	0·02	0·10	0·02	0·053	−0·037
16	0·45	0·02	0·20	0·02	0·056	−0·038
17	0·45	0·20	0·02	0·10	0·129	0·092
18	0·45	0·20	0·10	0·10	0·157	0·113
19	0·45	0·20	0·10	0·02	0·197	0·143
20	0·45	0·20	0·20	0·02	0·209	0·152
21	0·45	0·40	0·02	0·10	0·283	0·285
22	0·45	0·40	0·10	0·10	0·317	0·313
23	0·45	0·40	0·10	0·02	0·370	0·357
24	0·45	0·40	0·20	0·02	0·386	0·370

estimators derived, by specifying all the five genetic parameters. When two partially recessive loci are linked in coupling, the estimates of h_a are biased either upward or downward, depending on the selection and dominance coefficients at the other locus. For example, if the gene at the first locus is highly deleterious, the estimates are biased upward by the presence of an additional deleterious locus, as shown in cases 1–3. A deleterious gene of small effect but with high dominance will display a decrease in apparent dominance if linked closely to a sublethal gene, and an increase in dominance with the linkage of an additional deleterious gene of small effect, as indicated in cases 7–9. To visualize these biases, we plot some of the results in Fig. 1. Clearly, the lower the true dominance value, the larger the bias in estimates of h_a, when the two deleterious loci have equal selection and dominance coefficients.

Further examination of cases 19–24 in Table 1 shows that a partially recessive gene of small effect can behave like a partially dominant gene if it is closely linked to an additional deleterious gene. When two partially recessive loci are linked in repulsion, the estimates of h_a are biased downward as shown in all 24 cases. In these cases, two linked, partially recessive loci can behave like an overdominant one.

Table 2 gives the expected estimates for two synergistic, partially recessive loci. With the synergistic fitness model of Kimura & Maruyama (1966), we cannot specify the dominance level for either locus as in the multiplicative fitness model, but we assume that $h_a = h_b = h$, for ease of a comparison. In coupling linkage cases, the estimates of h_a are biased upward when the true h value is low, but downward when the true h is higher, as can be seen by comparing cases 1–4 with 9–12. The higher the synergism, the smaller the bias when the true h value is low (see cases 13–16), and the larger the bias when the true h value is higher (as in cases 21–24), which is just the opposite to the selection coefficient (a). The closer the linkage (i.e. the smaller r_{ab}), the bigger the bias with a lower h (see cases 1 and 13), and the smaller the bias for a higher h value (as seen in cases 12 and 24). In repulsion linkage cases, the estimates of h_a are biased downward as under multiplicativity. In these cases, the linkage intensity seems to have a great impact on the estimates. When the recombination fraction is larger (i.e. looser linkage), the bias becomes smaller as shown in cases 9–12 and 21–24.

In summary, in the presence of an additional, partially recessive locus in the vicinity of the marker locus, the estimates of dominance can be severely biased, either upward or downward depending on selection and dominance coefficients at both loci as well as the linkage intensity. The estimates under various combinations of overdominant loci as well as

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

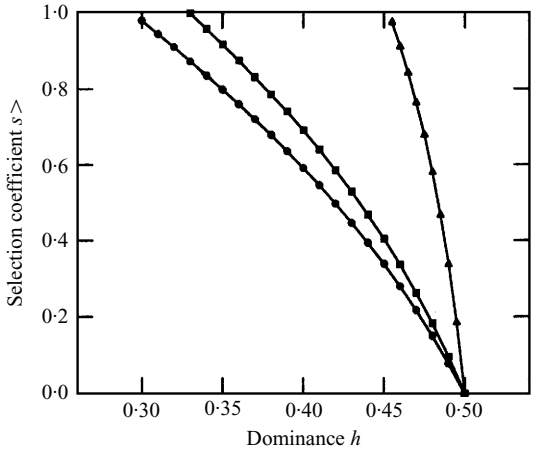

Fig. 2. Graphical presentation of the condition under which partial dominance or underdominance can occur, i.e. when two multiplicative, partially recessive loci are linked in coupling phase. ●, $r_{ab} = 0{\cdot}05$; ■, $r_{ab} = 0{\cdot}25$; ▲, $r_{ab} = 0{\cdot}45$.

for different marker locus positions with respect to the viability loci (i.e. $r_m \neq 0$) are also biased, but are complicated and thus are not shown.

(ii) *Conditions for apparent partial dominance or underdominance*

Detailed examination of the estimates of marginal dominance reveals that there are three situations in which apparent partial dominance or underdominance could occur due to the linkage of an additional viability locus. There are (*a*) when two multiplicative, partially recessive loci are linked in coupling phase and (*b*) when two synergistic, overdominant loci are linked in coupling phase or (*c*) in repulsion phase. In what follows, we present the condition for each situation, which is obtained by finding the condition for the estimator of marginal dominance to be greater than 0·5 when the true dominance levels (*h*) at both loci are less than 0·5.

For two multiplicative, partially recessive loci linked in coupling phase, the condition for the case of two identical viability loci is

$$s > \frac{2-4h}{|2h^2-(1-r_{ab})^2/[(1-r_{ab})^2+r_{ab}^2]|}. \qquad (6)$$

If the loci differ in selection and dominance coefficients, this condition will be only approximate. Fig. 2 shows this condition under three linkage intensities. Clearly, a high level of dominance (i.e. $h > 0{\cdot}3$) is required to produce apparent partial dominance. As the true dominance increases, the range of selection coefficients against the deleterious gene decreases dramatically. For example, apparent partial dominance is seen only with $s > 0{\cdot}8$ when $h = 0{\cdot}35$, but with $s > 0{\cdot}2$ when $h = 0{\cdot}47$, assuming $r_{ab} = 0{\cdot}05$. In other words, linked deleterious genes of relatively small effect with a higher level of dominance can cause

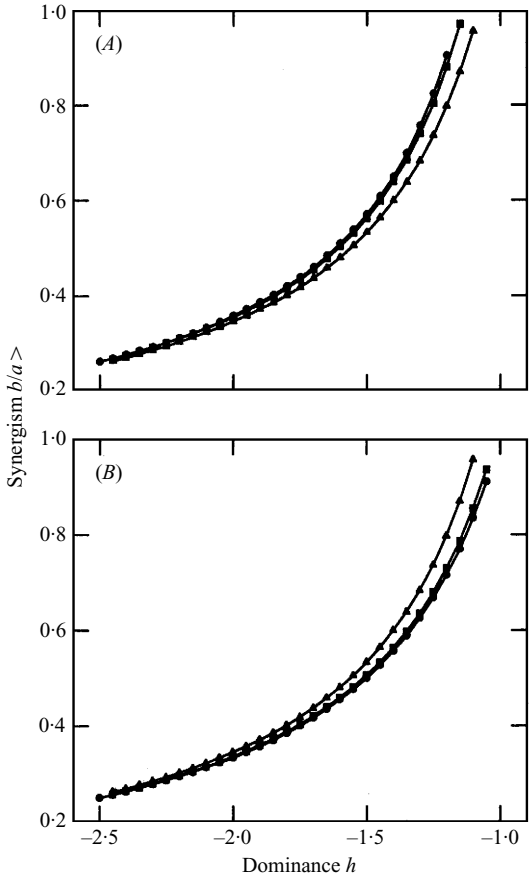

Fig. 3. Graphical presentation of the conditions under which partial dominance or underdominance can occur: (*A*) when two synergistic, highly overdominant loci are linked in coupling phase; and (*B*) when two synergistic, highly overdominant loci are linked in repulsion phase. ●, $r_{ab} = 0{\cdot}05$; ■, $r_{ab} = 0{\cdot}25$; ▲, $r_{ab} = 0{\cdot}5$.

apparent partial dominance. It is also clear that the linkage has a great impact on the relation between *s* and *h*, and that such a linkage must exist (i.e. $r_{ab} < 0{\cdot}5$) for a possible occurrence of partial dominance.

The conditions for two synergistic, overdominant loci linked in coupling and repulsion phases are, respectively,

$$b/a > \frac{1-2h}{\left|4h^2-\frac{2(1-r_{ab})^2+r_{ab}^2}{(1-r_{ab})^2+r_{ab}^2}\right|}$$

and

$$b/a > \frac{1-2h}{\left|4h^2-\frac{(1-r_{ab})^2+2r_{ab}^2}{(1-r_{ab})^2+r_{ab}^2}\right|}, \qquad (7)$$

where only $h < 0$ is considered. Fig. 3 shows the conditions under three linkage intensities. The degree of synergism required for the occurrence of partial dominance or underdominance becomes smaller as the dominance level decreases from -1. For example, the required magnitude of the synergistic interaction between two overdominant loci with $h = -2$ is 0·37

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

(Fig. 3*A*). Interestingly, the linkage does not greatly affect the relation between the magnitude of the synergistic interaction and the dominance level (Fig. 3). In other words, even two *unlinked* overdominant loci that interact synergistically could still produce partial dominance or underdominance. As a result, (7) can be approximately merged into one equation as:

$$b/a > \frac{1-2h}{|4h^2-1{\cdot}5|} \quad \text{for } h < 0, \tag{8}$$

to simplify the conditions under the synergistic fitness model.

3. Graphical inference of gene action for two linked viability loci

To deal with insufficient degrees of freedom for a single marker to estimate the selection coefficient, dominance level and recombination fraction of the viability locus, Fu & Ritland (1994*a*) introduced a graphical method for inferring dominance. By plotting two of the three marker genotype frequencies (the heterozygote and less frequent homozygote) in a triangle, given all possible linkages of the marker to a single locus affecting fitness for the range of possible selection coefficients, one can obtain the region in the triangular plane corresponding to each of seven selection models (overdominance, $h < 0$; complete recessivity, $h = 0$; partial recessivity, $0 < h < 0{\cdot}5$; additivity, $h = 0{\cdot}5$; partial dominance, $0{\cdot}5 < h < 1$; complete dominance, $h = 1$; and underdominance, $h > 1$). The results in Figs. 4 and 5, in conjunction with the analyses below, show that different modes of gene action can be distinguished to the extent that they occupy different spaces. Thus, one can evaluate, albeit roughly, the relative importance of the seven genetic mechanisms, simply by plotting observed segregation ratios of marker loci in selfed progeny over the expected regions in a triangle. Clearly, this graphical method is different from the point estimation procedure discussed above, at least in that no assumption of either selection coefficient or recombination fraction is required for the former, but whether the graphical inference is as biased as point estimates in the presence of an additional linked viability locus remains to be determined.

In this section, we examine the robustness of this graphical inference to an additional deleterious locus in distinguishing dominance versus overdominance

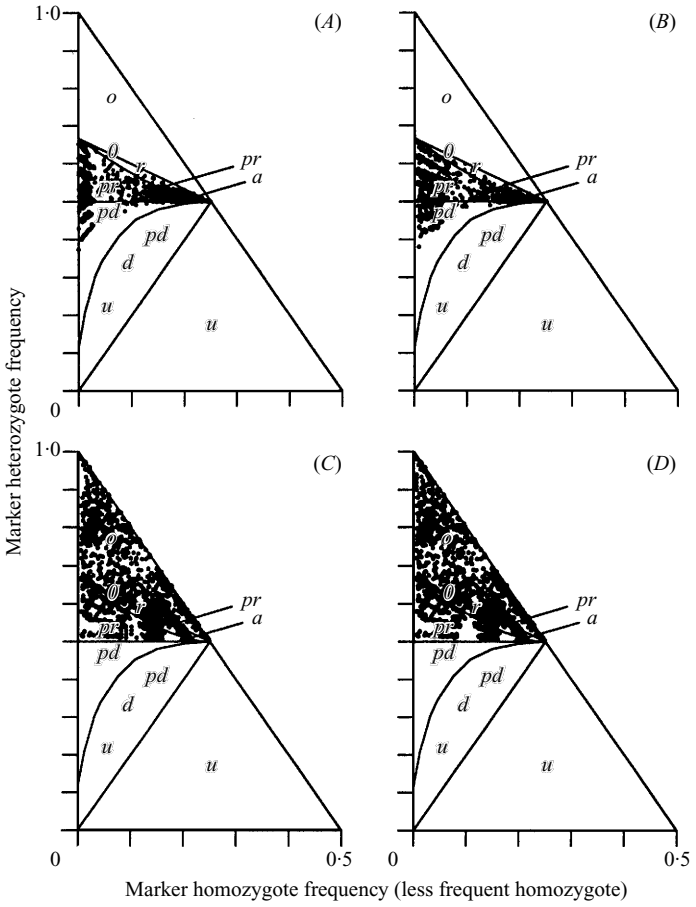

Fig. 4. Regions of apparent gene action under linkage of two multiplicative, partially recessive viability loci ($0 < h < 0{\cdot}5$), where four linkage situations are considered: (*A*) A–M_1–B (flanking coupling); (*B*) M_1–A–B (one-side coupling); (*C*) A–M_1–b (flanking repulsion); and (*D*) M_1–A–b (one-side repulsion). *o*, overdominance; *r*, complete recessivity; *pr*, partial recessivity; *a*, additivity; *pd*, partial dominance; *d*, complete dominance, *u*, underdominance.

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

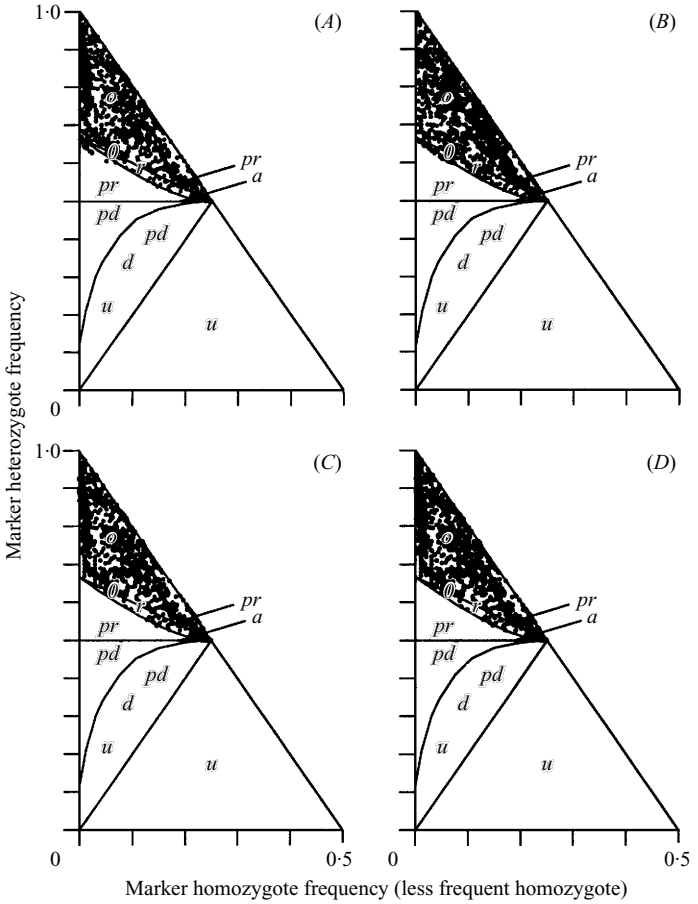

Fig. 5. Regions of apparent gene action under linkage of two multiplicative, overdominant viability loci ($h < 0$), where four linkage situations are considered. See the legend to Fig. 4 for further details.

hypotheses. This is done by comparing the expected regions in the triangle corresponding to the behaviours of linkage to two deleterious loci with those for a single viability locus. First we derived, following the same procedure described above, the expected frequencies of the three marker genotypes in selfed progeny for all eight possible linkage situations, i.e. the two marker positions with respect to the deleterious loci (both on one side versus flanked by two viability loci), the two linkage phases of the two deleterious loci (coupling versus repulsion) and the two fitness models (multiplicative versus synergistic). Since this derivation involves five or six genetic parameters, lengthy equations are expected, but they are still tractable by present analytical equation solvers. These expected frequencies are not shown but are available on request from the first author. Second, we wrote a PC Pascal program, based on the expected frequency equations, to generate the segregation ratios for a range of h values at each viability locus, with a range of values of each of the other genetic parameters (i.e. $0 < r < 0{\cdot}5$; $0 < s < 1$; $0{\cdot}02 < a < 0{\cdot}2$; and $0{\cdot}02 < b < 0{\cdot}2$). Lastly, the segregation data were plotted in the triangular plane. We focus on two major modes of gene action at the two viability loci, i.e. overdominance ($h < 0$) and partial recessivity ($0 < h <$ 0·5), as we are concerned mainly with distinguishing between these two hypotheses. The patterns for the other two modes of gene action, i.e. partial dominance ($0{\cdot}5 < h < 1$) and underdominance ($h > 1$), are not shown.

(i) *Apparent gene action for two multiplicative, partially recessive loci*

Fig. 4 shows the regions of apparent gene action assuming two partially recessive viability loci, for the four linkage cases. These regions for partially recessive loci are sensitive only to the linkage phase between the two viability loci, not to their positions with respect to the marker locus. For the coupling linkage phase, the linked marker data points largely fall within the region suggesting partial recessivity, with some 'invasion' of the region for the partially dominant mode (Fig. 4*A*, *B*). This 'invasion' supports the condition described in (6). Clearly, the graphical inference gives estimates that are biased towards the partial dominance mode if this condition is met.

For the repulsion linkage phase, the segregation ratios of the linked marker locus are spread out over the regions for both partial recessivity and overdominance modes, as seen in Fig. 4*C* and *D*, regardless of

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

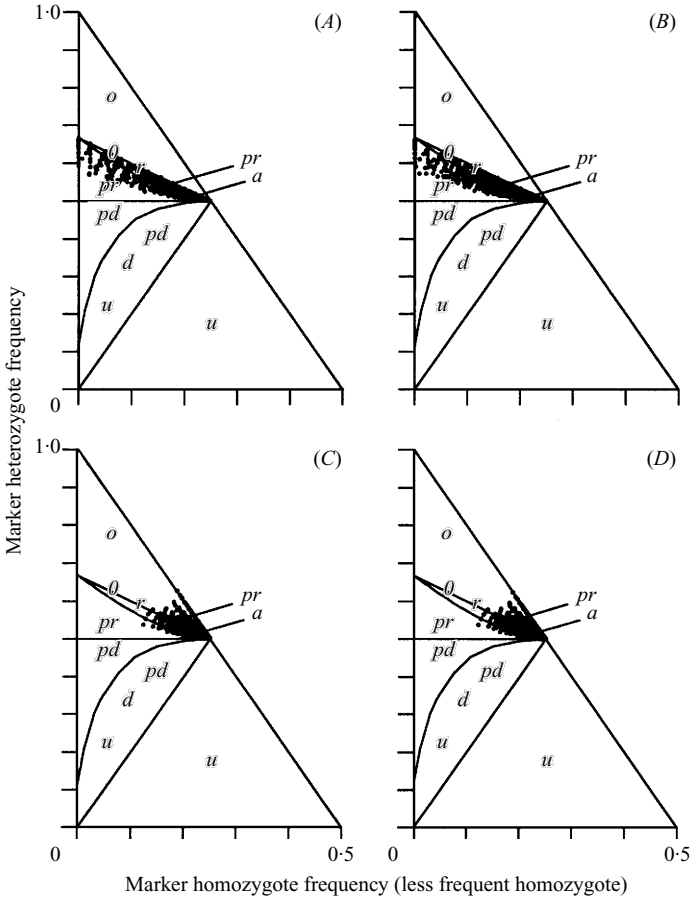

Fig. 6. Regions of apparent gene action under linkage of two synergistic, partially recessive viability loci ($0 < h < 0{\cdot}5$), where four linkage situations are considered. See the legend to Fig. 4 for further details.

the positions of the viability loci. As already explained, this represents the well-known fact that linkage of two partially recessive alleles in repulsion phase can produce a false inference of linkage of an overdominant locus.

(ii) *Apparent gene action for two multiplicative, overdominant loci*

Fig. 5 shows the regions of apparent gene action when there are two true overdominant viability loci. It is clear that linkage of an additional overdominant viability locus in repulsion phase does not change the patterns from those with linkage of only one viability locus, regardless of its position with respect to the marker locus (Figs. 5*C*, *D*). Also clear is that most segregation ratios for the marker in the coupling linkage phase are located in the space expected under linkage of a single overdominant locus, although some fall into the space where partial recessivity would be inferred (Figs. 5*A*, *B*). This slight 'invasion' occurs only when the two linked viability loci display large effects with a low level of overdominance ($s > 0{\cdot}7$ requires $h > -0{\cdot}05$) and tight linkage ($r < 0{\cdot}15$). This implies that the graphical inference of partial recessivity can be biased if two sublethal loci with a low level of overdominance (i.e. almost complete recessivity) are located in a short chromosomal segment close to the marker locus.

(iii) *Apparent gene action for two synergistic, partially recessive loci*

Fig. 6 shows the regions of apparent gene action for two synergistic, partially recessive loci ($0 < h < 0{\cdot}5$). When the viability loci are linked in coupling phase, the results still fall in the region for partial recessivity (Fig. 6*A*, *B*), although in a much smaller area than in the case of two multiplicative loci (Fig. 4*A*, *B*), indicating that synergism tends to reduce the observed dominance level (i.e. draws the linkage points away from the partial dominance region). This is consistent with the results present in Table 2. If the two viability loci are in repulsion phase, the partially recessive loci can still generate pseudo-overdominance (Fig. 6*C*, *D*). However, under the synergistic fitness model the region of parameter space where this occurs becomes smaller than under multiplicativity, suggesting that synergism can reduce the power of detecting the linkage.

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

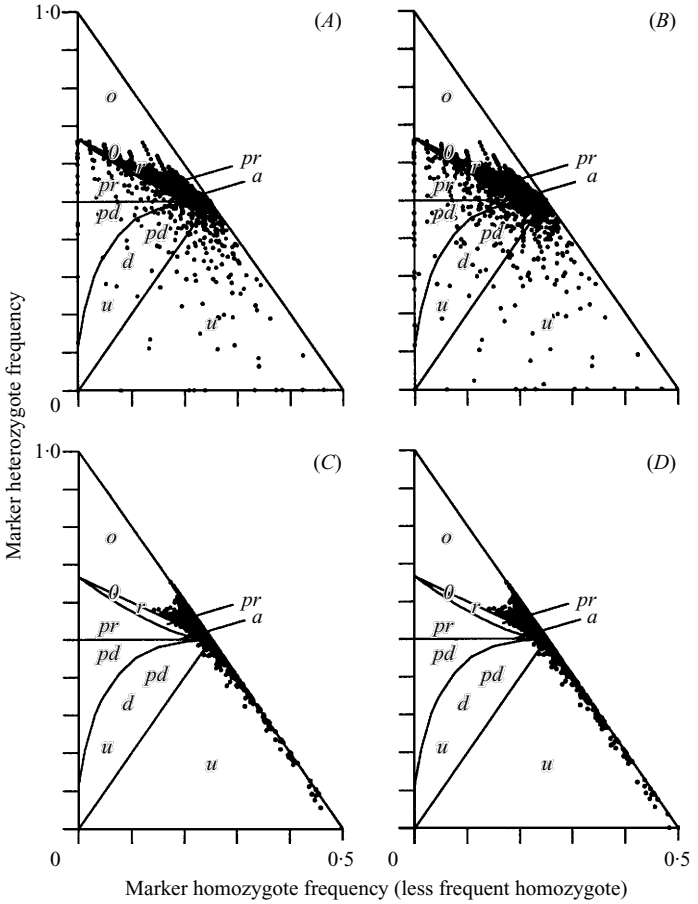

Fig. 7. Regions of apparent gene action under linkage of two synergistic, overdominant viability loci ($h < 0$), where four linkage situations are considered. See the legend to Fig. 4 for further details.

(iv) *Apparent gene action for two synergistic, overdominant loci*

Fig. 7 shows the regions of apparent gene action for two synergistic, overdominant loci ($-2 < h < 0$). Clearly, when the two overdominant loci are in coupling phase, regardless of their positions with respect to the marker, apparent partial recessivity, partial dominance and underdominance can be generated (Fig. 7 *A*, *B*). Under the repulsion linkage phase, the marker can behave as though there is either overdominance or underdominance (Fig. 7 *C*, *D*). These patterns are consistent with the conditions described in (7). Figs. 6 and 7 also show that the expected patterns of gene action for overdominant and partially recessive loci are sensitive to the phase of linkage between the loci, not the position of the loci, as with the multiplicative fitness model.

4. Discussion

In the deterministic analysis above, we examined the bias in both point estimation and graphical inference of dominance when there is an additional viability locus present in the vicinity of a marker locus. Clearly, the linkage of an additional viability locus can severely bias point estimations of dominance, with a magnitude depending on the linkage situation (the recombination fraction, coupling versus repulsion linkage phases, and marker positions) and the selection and dominance levels of both viability loci as well as their fitness functions. For the graphical inference, biases could occur, but only in certain special situations. As is well known, a marker linked to two multiplicative, partially recessive loci in repulsion phase could behave as though linked to an overdominant locus, which is critical for the inference only if more overdominant loci are observed than partially recessive ones. Also, partial recessivity could be falsely inferred if two sublethal loci with low levels of overdominance (i.e. almost complete recessivity) are closely linked. Moreover, two highly overdominant loci that interact synergistically can cause apparent partial recessivity. If these situations are infrequent in nature, the graphical method will be rather robust in distinguishing dominance from overdominance.

In this study, we also found three linkage conditions under which two partially recessive or overdominant loci behave like a partially dominant or underdominant locus. These occur when two multiplicative, partially recessive loci are linked in coupling phase or when two synergistic, highly overdominant loci are linked in coupling or repulsion phases. As shown in Fig. 3 *A* and *B*, relatively weak synergism can make a

https://doi.org/10.1017/S0016672397002966 Published online by Cambridge University Press

marker locus linked to two highly overdominant loci behave like a partially recessive, partially dominant, or even underdominant locus. This interesting finding could explain the observation of apparent partial dominance/underdominance in *Mimulus guttatus* (Fu & Ritland, 1994*a*). Recent multilocus regression analyses of the same *Mimulus guttatus* data as in Fu & Ritland (1994*a*) showed evidence for weak, negative synergism for viability (Fu & Ritland, 1996), which seems to support the explanation. However, it is not certain whether there really were any highly overdominant loci present in the proximity of the marker loci.

Another factor that can cause apparent partial dominance/underdominance is gametic selection. Previous theoretical examination of gametic selection (Fu & Ritland, 1994*a*) showed that if it occurs in the same direction in both sexes, it can contribute to the appearance of partial dominance observed in zygote viability. Thus, which explanation is correct remains to be tested experimentally. One could perform controlled matings to test for various forms of gametic selection, by selfing and multiple testcrosses of known genotypes and determining gametic contributions at each marker locus. Directional selection in favour of one homozygous genotype in selfed progeny and an excess of the same allele in the testcrossed progeny would suggest gametic selection in one or both sex.

In this paper, we considered only the linkage of two selected loci. It is possible that this analysis can be extended to the multilocus case, at least by computer simulations. In the multilocus case, the marker loci can be considered as part of a genome with many selected loci, so that the assumption of the linkage to only one selected locus in Fu & Ritland's graphical method may be relaxed. One more realistic simulation scenario is to simulate the actual experiment performed in *Mimulus guttatus*, given various combinations of partially recessive and overdominant loci, with various selfing rates. Starting with an equilibrium population under mutation and selection, a genotype for a selfing experiment could be picked and the same marker analysis could be performed as described. It would be of interest to know how robust the graphical method is in distinguishing between alternative hypotheses, and whether partial dominance or underdominance often occurs in these multilocus cases. We are currently investigating these multilocus cases.

The linkage situations we examined here could exist in nature, but how frequently each of these situations occurs is poorly known. While the graphical inference of gene action is apparently quite robust to the presence of an additional deleterious locus in the vicinity of the marker locus, a point estimate of dominance level is still desirable. Such an estimation can reliably be made if there are more marker loci spanning a short chromosomal segment, as the degrees of freedom are then increased (Fu & Ritland, 1994*b*). However, even this estimation may not be free from bias, especially when there are closely linked selected loci present in a short chromosomal region. This presents a great challenge for statistical geneticists and deserves more attention, especially for studies of natural populations.

We thank Drs Kermit Ritland and Daniel Schoen for their comments on the early version of the manuscript. This research is partially supported by an NSERC Postdoctoral Fellowship to Y. B. F.

References

Barrett, S. C. H. & Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**, 522–524.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. (1991). Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genetical Research* **57**, 177–194.

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.

Comstock, R. E. & Robinson, H. F. (1952). Estimation of average dominance of genes. In *Heterosis* (ed. J. W. Gowen), pp. 494–519. Iowa, USA: Iowa State College Press.

Crow, J. F. (1993). Mutation, mean fitness, and genetic load. *Oxford Survey in Evolutionary Biology* **9**, 3–42.

Fu, Y. B. & Ritland, K. (1994*a*). Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. *Genetics* **136**, 323–331.

Fu, Y. B. & Ritland, K. (1994*b*). On estimating linkage of marker genes to viability genes controlling inbreeding depression. *Theoretical and Applied Genetics* **88**, 925–932.

Fu, Y. B. & Ritland, K. (1996). Marker-based inferences about epistasis for genes influencing inbreeding depression. *Genetics* **144**, 339–348.

Hedrick, P. W. & Muona, O. (1990). Linkage of viability genes to marker loci in selfing organisms. *Heredity* **64**, 67–72.

Johnston, M. O. & Schoen, D. J. (1995). Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* **267**, 226–229.

Kimura, M. & Maruyama, T. (1966). The mutational load with epistatic gene interactions in fitness. *Genetics* **54**, 1337–1351.

Lande, R. & Schemske, D. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40.

Lynch, M. & Walsh, B. (1997). *Fundamentals of Quantitative Genetics*, vol. 1. Sunderland, Mass.: Sinauer Associates.

Mather, K. & Jinks, J. L. (1982). *Biometrical Genetics*. London: Chapman & Hall.

Ritland, K. (1990). Inferences about inbreeding depression based upon changes of inbreeding coefficient. *Evolution* **44**, 1230–1241.

Ritland, K. (1996). Inferring genetic basis of inbreeding depression in plants. *Genome* **39**, 1–8.

Sorensen, F. C. (1967). Linkage between marker genes and embryonic lethal factors may cause disturbed segregation ratios. *Silvae Genetica* **16**, 132–134.

Wright, S. (1977). *Evolution and the Genetics of Populations*, vol. 3, chap. 2. Chicago: University of Chicago Press.