

# Genetic Diversity at a Single Locus Under Viability Selection and Facultative Apomixis: Equilibrium Structure and Deviations from Hardy-Weinberg Frequencies

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## ABSTRACT

We extensively analyze the maintenance of genetic variation and deviations from Hardy-Weinberg frequencies at a diallelic locus under mixed mating with apomixis and constant viability selection. Analytical proofs show that: (1) at most one polymorphic equilibrium exists, (2) polymorphism requires overdominant or underdominant selection, and (3) a simple, modified overdominance condition is sufficient to maintain genetic variation. In numerical analyses, only overdominant polymorphic equilibria are stable, and these are stable whenever they exist, which happens for  $\sim 78\%$  of random fitness and mating parameters. The potential for maintaining both alleles increases with increasing apomixis or outcrossing and decreasing selfing. Simulations also indicate that equilibrium levels of heterozygosity will often be statistically indistinguishable from Hardy-Weinberg frequencies and that adults, not seeds, should usually be censused to maximize detecting deviations. Furthermore, although both censuses more often have an excess rather than a deficit of heterozygotes, analytical sign analyses of the fixation indices prove that, overall, adults are more likely to have an excess and seeds a deficit at equilibrium.

**M**ATING systems can have profound effects on plant population diversity and structure. For example, Hamrick and Godt (1989) found that predominantly selfing species contain less genetic variation than mixed mating or outcrossing species. In addition, populations of selfing species tend to show greater differentiation, presumably because gene flow is reduced. In contrast, species capable of both sexual and vegetative asexual reproduction have comparable, or even somewhat greater variation, within populations and comparable differentiation between populations, relative to sexually reproducing species (Hamrick and Godt 1989; Hamrick *et al.* 1992).

These empirical findings confirm key results of a number of theoretical investigations on the effects of mating system. For example, in mixed mating models with constant viability selection, selfing reduces the amount of heterozygosity possible at equilibrium and the range of fitness values over which maintenance of allelic variation is possible (Hayman 1953; Workman and Jain 1966; Marshall and Weir 1979). Asexual reproduction, such as apomixis (production of seeds without meiosis), can at least partially counteract the effect of selfing by maintaining diversity over a wider range of fitness values (Marshall and Weir 1979).

Although the existing selection models for nonrandom mating populations provide useful insight into the mainte-

nance of genetic variation, several important issues remain. For example, studies of the classic selection models with mixed mating, such as the one including apomixis by Marshall and Weir (1979), do not address the full equilibrium structure in terms of the precise conditions under which a stable polymorphic equilibrium exists and how often one is maintained. In addition, most of these investigations were restricted to overdominant fitnesses, assuming, without proof, that overdominance was the only form of selection that could maintain genetic variation. Finally, little is known about the combined effects of mating system and selection on the generation of detectable deviations from Hardy-Weinberg expectations. Here we address these issues by delimiting the complete equilibrium structure of Marshall and Weir's (1979) generalized mixed mating model under overdominant, underdominant, and directional selection. We then quantify the effect of each mating parameter on the potential for maintaining permanent genetic variation and generating statistically significant deviations from Hardy-Weinberg expectations.

## THE BASIC MODEL

We explore the effect of apomixis as well as mixed selfing and outcrossing on the maintenance and form of genetic variation under the diallelic version of the one-locus constant viability selection models introduced by Marshall and Weir (1979). These models of facultative apomixis offer the flexibility to study (1) the gener-

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alized mixed mating model with all three forms of reproduction; (2) the classic mixed mating model, which combines outcrossing with selfing; and (3–4) the two alternative mixed mating models, which combine apomixis with either selfing or outcrossing. Marshall and Weir's (1979) formulations are based on the standard Hardy-Weinberg assumptions, with the exceptions that selection is allowed between the zygote (or seed) stage and the adult stage and that in each generation, individuals outcross with probability  $t$ , self with probability  $s$ , and reproduce apomictically with probability  $a$ , where  $t + s + a = 1$ . Marshall and Weir (1979) considered two cases that differ in the time of censusing. Model I assumes an adult census followed by mating and then selection before the next generation of adults is censused. Model II, on the other hand, assumes a seed census followed by selection and then mating. We have reparameterized these models in terms of the allele frequencies and the fixation index, which measures the deviation from Hardy-Weinberg frequencies (Wright 1951). This parameterization greatly simplifies the analyses, as it also does for the standard mixed mating model (Workman and Jain 1966). Here we primarily focus on the seed census model, the easier case to analyze. The adult census model and key equilibrium relationships between the two censuses are presented in appendix a.

The seed census is represented by lowercase letters, with  $p$  the frequency of allele  $A_1$ ,  $q = 1 - p$  the frequency of allele  $A_2$ , and  $f = 1 - \text{freq}(A_1A_2)/2pq$ , the fixation index in seeds. The constant viability of genotype  $A_iA_j$  is denoted by  $w_{ij}$  ( $i, j = 1, 2$ ). The  $f$  and  $p$  recursions for the seed census are then

$$f' = a + s + \frac{(2a + s)(f - 1)w_{12}\bar{w}}{2w_1w_2} \quad (1)$$

and

$$p' = \frac{pw_1}{\bar{w}} \quad (2)$$

where

$$w_1 = p(1 - f)(w_{11} - w_{12}) + f(w_{11} - w_{12}) + w_{12} \quad (3)$$

$$w_2 = p(1 - f)(w_{12} - w_{22}) + w_{22} \quad (4)$$

are the marginal fitnesses of alleles  $A_1$  and  $A_2$ , respectively, and

$$\bar{w} = pw_1 + (1 - p)w_2 \quad (5)$$

is the mean fitness of the population. The prime symbol (') denotes a value in the next generation.

### ANALYTICAL METHODS AND RESULTS

**Analysis of equilibrium structure:** Marshall and Weir (1979) obtained polymorphic equilibria equation as a function of the equilibrium mean fitness  $\bar{w}$ . After correcting two signs in their linear term, their equilibrium

equation (10) can be simplified to

$$2(2w_{12} - w_{11} - w_{22})\hat{w}^2 + \{2(1 + a)(w_{11}w_{22} - w_{12}^2) - s[w_{12}(w_{11} + w_{22}) - 2w_{11}w_{22}]\}\hat{w} + (2a + s)w_{12}[w_{12}(w_{11} + w_{22}) - 2w_{11}w_{22}] = 0. \quad (6)$$

A more informative method is to express the internal (*i.e.*, polymorphic) equilibria in terms of the equilibrium fixation index  $\hat{f} = 1 - \hat{v}/2\hat{p}\hat{q}$ , where  $\hat{v}$  is the equilibrium frequency of heterozygotes in seeds. These formulae can be derived directly from the genotypic and allelic recursions or via an extension of a technique developed by Kimura and Ohta (1971) for the standard mixed mating model without apomixis. In either case, the equilibrium fixation index satisfies

$$g(\hat{f}) = 2i\hat{f}^2 - (2 - 2a - s - 2it)\hat{f} + s - 2i(a + s) = 0 \quad (7)$$

which determines the equilibrium allele frequencies via

$$\hat{p} = \frac{w_{12} - w_{22} - \hat{f}(w_{12} - w_{11})}{(2w_{12} - w_{11} - w_{22})(1 - \hat{f})} \quad (8)$$

where

$$i = \frac{(w_{12} - w_{11})(w_{12} - w_{22})}{w_{12}(2w_{12} - w_{11} - w_{22})}. \quad (9)$$

The equilibrium genotypic frequencies can then be calculated from the standard relationships,

$$a = \hat{p}^2 + \hat{f}\hat{p}(1 - \hat{p}) \quad (10)$$

$$v = 2\hat{p}(1 - \hat{p})(1 - \hat{f}) \quad (11)$$

and

$$w = (1 - \hat{p})^2 + \hat{f}\hat{p}(1 - \hat{p}) \quad (12)$$

which require (for admissibility) that

$$-\min\left[\frac{\hat{p}}{1 - \hat{p}}, \frac{1 - \hat{p}}{\hat{p}}\right] \leq \hat{f} \leq 1. \quad (13)$$

Note that the above formulae also apply to the special case of no outcrossing ( $t = 0$ ), for which  $\hat{f} = (s/2i) - 1$  or  $\hat{f} = 1$ ; whereas, as noted by Marshall and Weir (1979), the approach based on  $\hat{w}$  leads only to the single root  $\hat{f} = 1$ . [The latter can determine valid polymorphic equilibria only when the two homozygotes have equal fitness (*i.e.*,  $w_{11} = w_{22}$ ).]

In analyzing the full equilibrium structure of this system, the first issue is the exact number of polymorphic equilibria and when they exist. Our approach uses the Intermediate Value Theorem from calculus to bracket the roots of the  $\hat{f}$  quadratic in (7) based on its sign at five critical points, and the basic facts that a quadratic equation has at most two real roots and all frequencies must be in the interval [0,1]. Details of this analysis are given in appendix b. The results provide a formal analytical proof that at most one polymorphic equilib-

rium can exist for a given set of fitnesses and mating system parameters and that such equilibria exist only for overdominant and underdominant selection. [The case of no outcrossing ( $t = 0$ ) is a slight exception in that when  $w_{11} = w_{22}$  it has an infinite number of (neutrally stable) equilibria.]

More important, this approach reveals exactly when a polymorphic equilibrium will occur. For overdominant or underdominant fitnesses, (7) always has exactly one root ( $\hat{f}$ ) in the maximal admissible interval  $[-1, 1]$ . This root determines a valid internal equilibrium if and only if the corresponding allele frequency in (8) is in the interval (0,1) and the bounds in (13) are satisfied. Consequently, equilibrium frequencies will be valid and a polymorphic equilibrium will exist, if and only if

$$\hat{f} < \min \left[ \frac{w_{12} - w_{11}}{w_{12} - w_{22}}, \frac{w_{12} - w_{22}}{w_{12} - w_{11}} \right] = f^*. \quad (14)$$

Graphing the quadratic in (7) shows that inequality (14) holds if and only if  $g(f^*) < 0$ . Thus, a polymorphic equilibrium will exist for overdominant selection ( $w_{12} > w_{ii} > w_{jj}$ ) if and only if

$$sw_{12}(w_{ii} - w_{jj}) - 2(w_{12} - w_{ii})[w_{ii} - (1 - t)w_{jj}] < 0 \quad (15)$$

and for underdominant selection ( $w_{ii} > w_{jj} > w_{12}$ ) if and only if

$$sw_{12}(w_{jj} - w_{ii}) - 2(w_{12} - w_{jj})[w_{jj} - (1 - t)w_{ii}] > 0. \quad (16)$$

**Sign of the equilibrium fixation index:** The equilibrium analysis based on  $\hat{f}$  shows exactly when the frequency of heterozygotes at an internal equilibrium will be above ( $\hat{f} < 0$ ), below ( $\hat{f} > 0$ ), or equal to ( $\hat{f} = 0$ ) the Hardy-Weinberg frequency  $2\hat{p}\hat{q}$ . In particular, although with underdominant selection  $\hat{f}$  is always positive, with overdominant selection the sign of  $\hat{f}$  is given by the sign of the quantity  $s/2(a + s) - i$ , where  $i$  is the function of fitness defined in (9). The adult census gives the same results, except that for overdominance the sign of  $\hat{F}$ , the equilibrium fixation index in adults, is given by the sign of the quantity  $s/(2 + s) - i$ . Comparing these two sign criteria reveals that  $s/2(a + s) \geq s/(2 + s)$  (with equality only if  $s = 0$ ) and hence suggests that as long as some selfing occurs, the adult census is the more likely to have a negative fixation index, whereas the seed census is the more likely to have a positive value (Table 1). If  $s = 0$  and  $a > 0$ , however,  $\hat{f}$  and  $\hat{F}$  are both negative at overdominant polymorphisms. Note also that when  $t = 0$  an excess of heterozygotes requires  $s < 1$  for seeds but not for adults (appendix b). Finally, although the sign criteria are invalid for completely random mating populations ( $a = s = 0, t = 1$ ), inspection of this case shows that the adult census will always have an excess of heterozy-

**TABLE 1**  
Sign of the equilibrium fixation indices

Criterion	Sign of $\hat{F}$	Sign of $\hat{f}$
$0 < i < \frac{s}{2 + s}$	+	+
$i = \frac{s}{2 + s}$	0	+
$\frac{s}{2 + s} < i < \frac{s}{2(a + s)}$	-	+
$i = \frac{s}{2(a + s)}$	-	0
$\frac{s}{2(a + s)} < i < \frac{1}{2}$	-	-

$\hat{F}$  is the equilibrium fixation index for the adult census and  $\hat{f}$  the corresponding value for the seed census under the generalized mixed mating model, and  $i$  is the function of fitness defined in (9).

gotes ( $\hat{F} < 0$ ), whereas seeds will necessarily be in Hardy-Weinberg equilibrium ( $\hat{f} = 0$ ).

The overdominant results also reveal an important distinction between the generalized mixed mating model and the model with only mixed selfing and outcrossing. When all three forms of reproduction are present, both the adult and seed census can have either negative or positive fixation indices. For the standard mixed mating model ( $a = 0$ ), this is true only when adults are censused [as Workman and Jain (1966) illustrated for specific values via phase diagrams] because  $i < 1/2$  ensures the seed census always has a positive fixation index. In seeds, apomixis is clearly necessary to produce an excess of heterozygotes at equilibrium ( $\hat{f} < 0$ ) when selfing is present; however, apomixis itself is not sufficient because in the absence of outcrossing ( $t = 0$ ) other conditions ( $s < 2t$ ) must hold.

**Local stability of the equilibria:** In the absence of mutation and gene flow, polymorphism maintained in adults must be maintained in seeds and vice versa. Consequently, although genotypic frequencies and fixation indices for internal equilibria differ between the two census times, the criteria for existence of such equilibria, and the stability properties of all equilibria, apply to both models. The analytical conditions under which an internal equilibrium will be locally stable are very complex. The local stability conditions for the boundary equilibria, that is, the fixation of one allele, however, are much more tractable, especially when derived from the genotypic recursions (appendix c). These show that fixation of allele  $A_i$  will be locally stable if

$$w_{12} < \frac{2w_{ii}}{2 - s} \quad (17)$$

and

$$stw_{12}w_{jj} - [(1 - t)w_{jj} - w_{ii}][(2 - s)w_{12} - 2w_{ii}] < 0 \tag{18}$$

for  $j \neq i = 1, 2$ . Note that these two inequalities imply that  $w_{ii} > (1 - t)w_{jj}$  when fixation for  $A_i$  is stable.

Two general observations are evident from the above criteria. First, the stability of each fixation state depends on all three genotypic fitnesses, as long as the population is not mating completely at random ( $t < 1$ ). [If  $t = 1$ , we have the classical selection model for which  $a = s = 0$  and (17) and (18) reduce to  $w_{12} < w_{ii}$ .] Second, the criterion (18) corresponds to the fixation boundary curves in Marshall and Weir's (1979) phase diagrams [their equation (15)]. However, because this criterion does not necessarily imply the first [in fact, it implies the reverse of (17) when  $w_{ii} < (1 - t)w_{jj}$ ], the phase diagram curves do not appear to completely describe the local stability conditions of the fixation states for this system.

**Conditions for protected polymorphism:** These formal stability conditions determine the exact conditions under which genetic variation is preserved through a protected polymorphism (PP), which prevents the loss of either allele. In particular, a PP exists for the generalized mixed mating model with selection if for both boundary equilibria (fixation of  $A_1$  or  $A_2$ ) either (17) or (18) is reversed, because both fixation states are then unstable. Closer examination of these local stability conditions, in conjunction with the conditions for a polymorphic equilibrium in (15) and (16), reveals that for overdominance a PP exists if and only if a valid internal equilibrium also exists (see appendix d). Similar analysis indicates that for underdominance both of the boundary equilibria will always be stable whenever a polymorphic equilibrium exists, and therefore a PP never exists under these conditions. Because two adjacent equilibria are unlikely to be both stable or both unstable, these results also suggest that (in the absence of cycling) overdominant polymorphic equilibria will be stable whenever they exist, whereas underdominant polymorphic equilibria will always be unstable.

Interpretation of the biological implications of the precise PP conditions is difficult; however, sufficiently strong overdominance,

$$w_{12} > \frac{2w_{11}}{2 - s}, \frac{2w_{22}}{2 - s} \tag{19}$$

will clearly [see (17) and (18)] always be sufficient to maintain both alleles in the population through a PP under any combination of selfing, random outcrossing, and apomixis. Analysis of the coefficient  $2/(2 - s)$  as a function of  $s$  shows that as the selfing rate increases from 0 to 1, this fitness condition increases from simple overdominance ( $w_{12} > w_{11}, w_{22}$ ) to "double overdominance" ( $w_{12} > 2w_{11}, 2w_{22}$ ), in which the fitness of heterozygotes exceeds twice that of both homozygotes. Although this condition for protected polymorphism is

only sufficient and not necessary to prevent the loss of either allele, it does suggest that simple overdominance will not always maintain variation when any selfing occurs. Moreover, ignoring the complications of the unlikely case of equality in (17) or (18), the modified overdominance criterion (19) is, in fact, the exact condition for a protected polymorphism under the special conditions of no selfing ( $s = 0$ ), complete selfing ( $s = 1$ ), no outcrossing ( $t = 0$ ), and complete random mating ( $t = 1$ ).

For comparison to the numerical results below, we also calculated analytically how often the sufficient PP condition (19) holds by evaluating the appropriate multiple integrals under a uniform distribution on the relevant parameter space. The results reveal that, on average, the modified overdominance condition (19) holds for 17/72 (23.6%) of all sets of fitnesses and mating parameters and for 17/24 (70.8%) of the random overdominant parameter sets. These two fractions are somewhat lower, 7/36 (19.4%) and 7/12 (58.3%), for both the standard mixed mating model with selfing and outcrossing ( $s + t = 1$ ) and the alternate mixed mating model with selfing and apomixis ( $a + s = 1$ ). Similar analysis shows that a given degree of selfing, (19) holds for  $(2 - s)^2/12$  of random fitnesses and for  $(2 - s)^2/4$  of random overdominant fitnesses. As  $s$  increases from 0 to 1, the fraction of fitnesses meeting the sufficient PP condition (19) decreases from 33.3 to 8.3% of random fitnesses and from 100 to 25% of random overdominant fitnesses, a fourfold drop in each case.

#### NUMERICAL ANALYSIS

Computer simulations provided further insight into the number, stability pattern, and nature of the equilibria. The program randomly generated fitnesses (overdominant, underdominant, or directional) and associated sets of mating system parameters using a random number generator with a uniform distribution on the interval [0,1]. Random fitness sets of a specific form were generated by choosing  $w_{11}$ ,  $w_{12}$ , and  $w_{22}$  at random and then interchanging their values as necessary to achieve the desired pattern (e.g.,  $w_{12} = \max\{w_{ij}\}$  for overdominance). When all three modes of reproduction were present, a uniform distribution of the mating parameters was ensured by first choosing two random numbers to divide the interval [0,1] into three pieces and then using the lengths of these pieces as the values of the three mating parameters (Karl in 1969, pp. 241–242). Adapting the approach of Asmussen and Basnayake (1990), we determined how many (if any) polymorphic equilibria exist [from (7)–(13)], the stability of all equilibria (using the analytically derived local stability conditions), and the value of the fixation index at polymorphic equilibria, for each combined set of parameters. Each run of the program included 10,000 such

parameter sets, and reported values are means of 100 such runs.

**Analysis of equilibrium structure:** The simulations confirmed the analytical predictions that fitnesses must be overdominant to produce a stable polymorphic equilibrium and that such overdominant equilibria are stable whenever they exist. Moreover, a stable polymorphic equilibrium exists if and only if both fixation equilibria are unstable and selection is overdominant. Consequently, only four equilibrium patterns are possible in these systems: SU, US, SUS, and USU [where the end entries indicate the stability of fixation for  $A_1$  and  $A_2$  (S = locally stable, U = unstable) and the intermediate entry refers to a polymorphic equilibrium (when present)]. The equilibrium structure, in terms of the number and stability patterns of the equilibria, is thus the same as for the classical selection model for random mating populations.

Because only overdominance will maintain genetic variation, further analysis of the equilibrium structure was restricted to this case. The results reveal that, on average, when all three mating parameters are chosen at random, 78% of random overdominant parameter sets retain both alleles at equilibrium. Of the three subsumed models with only two forms of reproduction, mixed apomixis and outcrossing ( $s = 0$ , and  $a$  and  $t$  chosen at random) preserves genetic variation 100% of the time if selection is overdominant, as predicted by the PP condition (19). This percentage decreases to 70% for the standard mixed mating model ( $a = 0$ , with  $s$  and  $t$  chosen at random) and to 58% for mixed selfing and apomixis ( $t = 0$ , with  $a$  and  $s$  chosen at random), indicating the extent to which simple overdominance is insufficient to maintain genetic diversity when selfing occurs. These results also show, in conjunction with the analytical calculations above, that condition (19) is a fairly accurate predictor of when genetic polymorphism will be retained. This criterion is actually a perfect predictor in the absence of outcrossing ( $t = 0$ ) or selfing ( $s = 0$ ), when it is the exact condition for a PP. On average, the modified overdominance condition (19) underestimates the maintenance of genetic variation by 17% when apomixis is absent ( $a = 0$ ; *i.e.*, the standard mixed mating model) and by <10% when all three forms of reproduction are present.

Next, we explored the average effect of each of the three forms of reproduction on the maintenance of genetic diversity by setting one of the mating parameters to a constant value (from 0 to 1 in increments of 0.1) and generating 10,000 sets of the other two at random, as described above. The results in Figure 1 show that, on average, the likelihood of obtaining a stable polymorphic equilibrium steadily increases to 100% as apomixis or outcrossing increases to 1 and is always at least 58% along these two curves ( $0 \leq a \leq 1$ ;  $0 \leq t \leq 1$ ). As selfing increases from 0 to 1, however, the potential for permanent genetic variation decreases dramatically

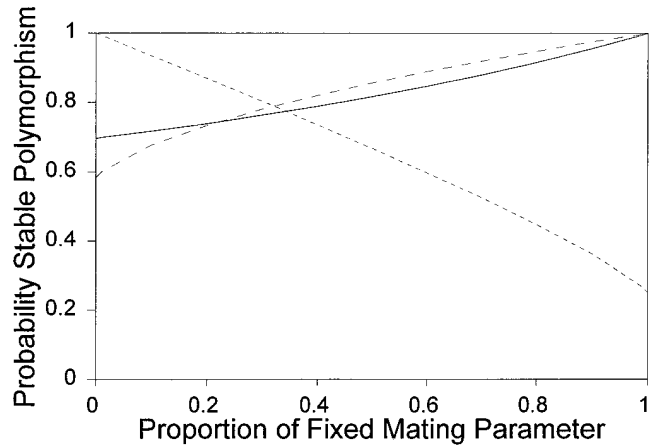


Figure 1.—Mean probability of a stable polymorphism for random overdominant selection with fixed rates of apomixis (solid line), selfing (short-dashed line) or outcrossing (long-dashed line). Each curve was generated by setting one of the mating parameters to a constant value (from 0 to 1 in increments of 0.1), while 10,000 sets of the other two and the fitnesses were chosen at random, and then checking for a stable polymorphic equilibrium using (7)–(13) and the analytical local stability criteria. Points are means of 10,000 random parameter sets obtained using a random number generator with a uniform distribution on the interval [0,1].

from 100% to only ~25%. (Note that the 25% for complete selfing agrees with the analytically derived fraction of overdominant fitnesses with a PP, which occurs in the case of  $s = 1$  if and only if  $w_{12} > 2w_{11}, 2w_{22}$ .)

**Significant deviations from Hardy-Weinberg expectations:** The nature of genetic diversity in this system was characterized by computing the proportion of polymorphic equilibria meeting Hardy-Weinberg expectations (HWE) as well as the sign distribution of the equilibrium fixation indices at both census times. To facilitate comparisons with studies of natural populations, these calculations were based on values that are statistically distinguishable from HWE using Li and Horvitz's (1953) test statistic  $\chi^2 = f^2 N(k-1)$  with  $k(k-1)/2$  degrees of freedom, where  $N$  is the sample size,  $k$  is the number of alleles, and  $f$  is the equilibrium fixation index ( $\hat{f}$  for seeds,  $\bar{f}$  for adults). Values are reported for a sample size of  $N = 100$  and  $k = 2$  alleles, which requires  $|f| > 0.2$  for significance at the 0.05 level. A lower  $N$  of course would mean that fewer fixation indices would be distinguishable from HWE (and that the curves in Figure 2 would be higher and those in Figure 3 would be lower).

On average, with overdominant fitnesses and all mating parameters chosen at random, 32% of equilibria for adults and 60% for seeds are statistically indistinguishable from HWE. Qualitatively similar results were obtained for the three subsumed models ( $a = 0$ ,  $s = 0$ , or  $t = 0$ , with the other two mating parameters chosen at random). In addition, the mixed selfing and apomixis models ( $t = 0$ ) have the lowest expected fraction indistinguishable from HWE (15% for adults and 27% for seeds), whereas the standard mixed mating model ( $a = 0$ )

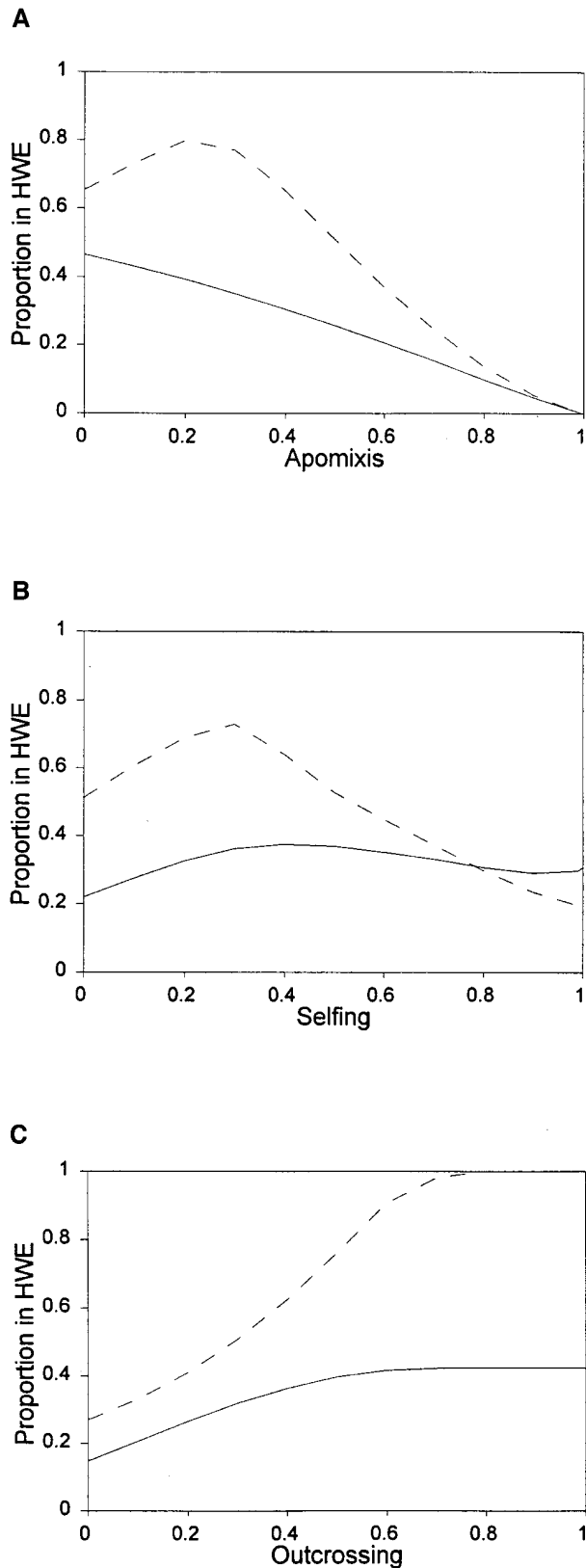


Figure 2.—Mean frequency of overdominant polymorphic equilibria that are statistically indistinguishable from Hardy-Weinberg expectations (HWE) for fixed rates of apomixis (A), selfing (B), or outcrossing (C). Fixation indices were considered insignificant if  $|f| < 0.2$ , where  $f$  is  $\hat{f}$  for seeds (dashed line) and  $\hat{F}$  for adults (solid line). Curves were generated as described for Figure 1.

has the highest (47% for adults and 65% for seeds). The average values for mixed apomixis and outcrossing ( $s = 0$ ) are intermediate, with 22% for adults and 51% for seeds.

For both censuses of the complete model, the average proportion of equilibria meeting HWE ultimately decreases to 0 as apomixis increases (Figure 2A). This makes sense intuitively, because with more apomixis the favored heterozygotes are producing more heterozygous offspring. Seeds are much more likely to meet HWE than adults until the rate of apomixis approaches 0.7, after which the difference between the two census times rapidly disappears. As  $s$  increases to  $\sim 0.3$ , the proportion meeting HWE increases under both censuses (to 35% for adults and 70% for seeds) and then decreases sharply in seeds (to  $\sim 20\%$ ) but only slightly in adults (Figure 2B). As a result, at high levels of selfing ( $s > 0.8$ ), fewer equilibria meet HWE when seeds, rather than adults, are censused, contrary to the results for all other conditions studied. This is consistent, however, with the greater deviation from HWE expected with high selfing rates because of the loss of heterozygotes in seeds. As  $t$  increases, the proportion of equilibria indistinguishable from HWE steadily increases to  $\sim 40\%$  in adults and to 100% in seeds, reflecting the greater effect of viability selection upon adult frequencies at high levels of outcrossing (Figure 2C).

**Sign of the equilibrium fixation index:** The results from the numerical sign analysis of the equilibrium fixation indices are in accord with analytical predictions: adults have a lower frequency of significantly positive fixation indices and a higher frequency of negative ones than seeds because of increased heterozygosity following overdominant viability selection. More specifically, in the general model the average proportion of significantly positive fixation indices is 15% for seeds *vs.* 4% for adults, whereas for negative values the average proportions are 25% for seeds and 64% for adults. Under the standard mixed mating model ( $a = 0$ ), the seed census has no negative fixation indices and a substantial proportion (35%) of statistically positive ones, as expected. The adult census, however, has a substantial proportion of negative values (46%) and a small proportion of positive ones (8%). Both of the two alternate mixed mating models have a fairly high proportion of negative fixation indices in seeds (49% for  $s = 0$  and 56% for  $t = 0$ ) and adults (78% for  $s = 0$  and 81% for  $t = 0$ ). However, although the mixed apomixis and outcrossing models ( $s = 0$ ) never have a significant deficit of heterozygotes, the mixed apomixis and selfing models ( $t = 0$ ) do have a low average frequency of such equilibria (4% in adults and 17% in seeds).

The effect of each mating system parameter on the sign distribution of the fixation indices under the generalized model is shown in Figure 3 as the average frequency of significantly positive (Figure 3, A–C) or negative (Figure 3, D–F) equilibrium fixation indices. Increasing apomixis

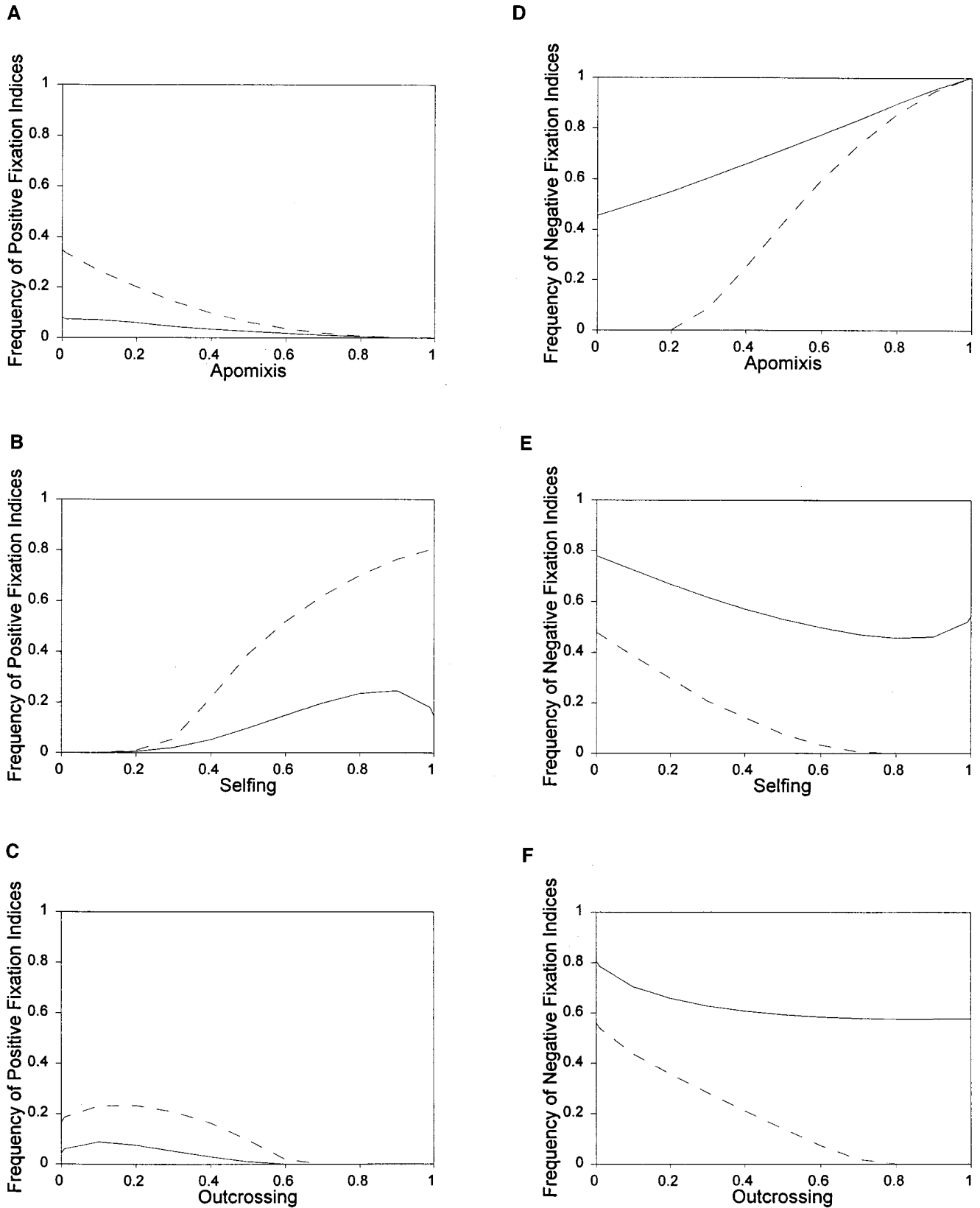


Figure 3.—Mean frequency of statistically significant positive (A, B, C) and negative (D, E, F) equilibrium fixation indices with fixed rates of apomixis (A, D), selfing (B, E), or outcrossing (C, F) for adults (solid line) and seeds (dashed line) under random overdominant selection. Curves were generated as described for Figure 1, based on the significance criteria used for Figure 2.

(Figure 3, A and D) naturally increases the proportion of negative values at both census times because the favored heterozygotes produce exact copies of themselves when they reproduce apomictically. For selfing (Figure 3, B and E), the frequency of significantly positive fixation indices is essentially zero at low selfing rates and only increases as selfing increases above 0.2. Adults, however, show a decrease in this frequency at very high levels of selfing ( $s > 0.9$ ), consistent with Hayman's (1953) individual phase diagrams for the standard mixed mating model. In the case of increased outcrossing, rather than becoming more positive or more negative, the average value of the equilibrium fixation index appears to be approaching 0 in seeds and some small negative number in adults (Figure 3, C and F, and data not shown).

### DISCUSSION

The comprehensive analysis here provides the exact conditions that maintain genetic variation under a generalized mixed mating model with apomixis, selfing, outcrossing and constant viability selection at a single, diallelic locus. A combination of analytical and numerical results shows that the full system, as well as the three subsumed cases with only two forms of reproduction, has the same general equilibrium structure as the classical selection model with random mating: (1) at most one polymorphic equilibrium exists; (2) a polymorphic equilibrium exists only with overdominance or underdominance; (3) a stable polymorphic equilibrium exists only when selection is overdominant; and (4) a protected polymorphism, with both fixation states unstable, exists whenever a stable internal equilibrium exists, and vice versa. The one critical difference is that when any self-fertilization occurs, simple overdominant selection may not be sufficient to maintain both alleles in the population.

In addition to delimiting the full equilibrium structure of the models, we have obtained a simple, sufficient condition for the existence of a protected polymorphism. This shows that genetic variation will be maintained whenever  $w_{12} > 2w_{11}/(2-s)$ ,  $2w_{22}/(2-s)$ . Furthermore, numerical analyses demonstrate that this modified overdominance condition is an excellent predictor of the maintenance of genetic polymorphism, because for the full model it holds for >90% of the parameter sets that produce a stable polymorphic equilibrium. In fact, under the alternate mixed mating models in which apomixis is mixed with either just outcrossing ( $s = 0$ ) or just selfing ( $t = 0$ ), the overdominant criterion is a perfect predictor. Under the standard mixed mating model, without apomixis, the predictive power is somewhat less than that for the full model (83%), but it is still substantial.

Together, our analytical and numerical investigations of the equilibrium structure of this system formally quantify and significantly extend the qualitative findings

of Marshall and Weir (1979) based on individual phase diagrams. For instance, we are able to compare the full model with the three subsumed models and gauge the relative effects of the different mating parameters on the overall likelihood of maintaining genetic diversity. Only mixed apomixis and outcrossing will always maintain genetic variation with overdominant selection. The full model does so for 78% of random overdominant parameter sets, followed by the standard mixed mating model (70%) and, finally, mixed apomixis and selfing (58%). Clearly, the presence of selfing in the mating system reduces the overall potential for maintaining variation.

In interpreting the numerical results here, however, it must be realized that they are based on random fitnesses and mating parameters drawn from a uniform distribution. How often genetic variation or significant deviation from Hardy-Weinberg frequencies is actually maintained in natural populations depends on the unknown, true distribution of fitnesses and mating system parameters in nature. By quantifying the fraction of the parameter space with the specified evolutionary outcome, our results instead provide a baseline for comparing different biological scenarios and developing hypotheses. For example, the numerical investigation of the average effect of each mode of reproduction indicates that an increase in the selfing rate dramatically reduces the possibility of maintaining variation. In contrast, increasing apomixis or outcrossing has a positive, but smaller, effect on the maintenance of genetic diversity. Therefore, contrary to the traditional view that apomictic species should be genetically depauperate (reviewed in Asker and Jerling 1992), we would expect that apomictic species capable of some sexual reproduction should have levels of genetic diversity comparable to their sexual relatives. Recent allozyme studies suggest that this may indeed be the case (Bayer 1989; Hamrick *et al.* 1992; Overath and Hamrick 1998).

In addition to motivating hypotheses, our numerical results lead to several practical guidelines for those studying natural populations. For instance, our discovery of little deviation from Hardy-Weinberg expectations (HWE) quantitatively reinforces previous cautions that the fixation index can be quite unreliable in detecting the presence of evolutionary forces (Wallace 1958; Lewontin and Cockerham 1959; Li 1959; Workman 1969; Schaap 1980; Li 1988; Lessios 1992). This equilibrium analysis also revealed an important difference between the complete and classical mixed mating models: when apomixis is present, excesses of heterozygotes can occur at both seed and adult censuses. Without apomixis, this can occur only in adults. Perhaps finding a significant excess of heterozygotes in studies using a seed census could be used as an indication that apomixis is occurring; however, apomixis would need to be fairly high under most conditions for the equilibrium fixation index in seeds to be statistically different from



HWE (Figure 2A). Finally, our results also indicate that adults should usually be censused, except in highly selfing species, in order to maximize the possibility of detecting any significant deviation from HWE.

One aspect of the maintenance of genetic variation not considered here is the potential for long-lived transient polymorphism. If the time to fixation is rather long, polymorphism may be effectively maintained, even if fixation is the expected outcome. Given that apomixis is known to slow the approach to equilibrium in the absence of selection (Marshall and Weir 1979), it would not be surprising to find that the duration of polymorphism can be quite long in cases with high levels of apomixis. Variation may be maintained in such cases because natural populations are not at or near equilibrium and before an allele can be lost, selection changes (see Lynch 1987) or new alleles enter the population through migration or mutation. Consequently, the possibility of long-lived transient polymorphism merits careful study to fully understand the dynamics of the models presented here and to better interpret data from natural populations.

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#### APPENDIX A

**Recursions for the adult census model:** Using uppercase letters to designate adults, the adult census recursions are

$$F' = 1 + \frac{[(2a + s)F + s - 2]w_{12}\bar{W}}{2W_1W_2} \quad (\text{A1})$$

and

$$P' = \frac{PW_1}{\bar{W}} \quad (\text{A2})$$

where

$$W_1 = \frac{1}{2}P(w_{12} - w_{11})[(2a + s)F + s - 2] + \frac{1}{2}(w_{11} - w_{12})[(2a + s)F + s] + w_{12} \quad (\text{A3})$$

$$W_2 = \frac{1}{2}P(w_{22} - w_{12})[(2a + s)F + s - 2] + w_{22} \quad (\text{A4})$$

$$\bar{W} = PW_1 + (1 - P)W_2. \quad (\text{A5})$$

At equilibrium, the two censuses are related by  $\hat{P} = \hat{p}$ ,  $\hat{W} = \hat{w}$ , and  $\hat{F} = (2\hat{f} - s)/2(a + s)$  [Equation A.11 in Marshall and Weir (1979)].

#### APPENDIX B

**Existence of polymorphic equilibria and the sign of their fixation indices:** Most of the results follow by using the Intermediate Value Theorem to bracket the roots of the  $\hat{f}$  quadratic (7) in the maximal admissible interval  $[-1, 1]$ , based on the sign of  $g(\hat{f})$  at five critical points:  $g(-1) = 2(1 - a)$ ,  $g(0) = s - 2i(a + s)$ ,  $g(1) = 2i(2i - 1)$ , and  $g(\pm\infty) = (\text{sign of } \lambda)\infty$ , where the latter is shorthand for

$$\lim_{\hat{f} \rightarrow \pm\infty} g(\hat{f}).$$

The sign patterns for informative cases under the gen-

**TABLE B1**  
**The sign of  $g(\hat{f})$  at five critical points**  
**under three selection schemes**

	$g(-\infty)$	$g(-1)$	$g(0)$	$g(1)$	$g(+\infty)$
Overdominance ( $w_{12} > w_{11}, w_{22}$ )					
$\frac{s}{2(a+s)} < i$	+	+	-	-	+
$\frac{s}{2(a+s)} = 1$	+	+	0	-	+
$\frac{s}{2(a+s)} > i$	+	+	+	-	+
Underdominance ( $w_{12} < w_{11}, w_{22}$ )	-	+	+	-	-
Directional selection ( $w_{11} < w_{12} < w_{22}$ )					
$w_{12} > \frac{w_{11} + w_{22}}{2}$	-	+	+	-	-

eral seed census model with all three forms of reproduction present are summarized in Table B1.

When selection is overdominant,  $0 < i < 1/2$  and the sign changes of  $g(\hat{f})$  across  $(-\infty, \infty)$  show that one root of the quadratic is in  $(-1, 1)$  and the other is in  $(1, \infty)$ . Exactly one solution for  $\hat{f}$  therefore exists in the maximal admissible interval for any set of overdominant fitnesses, and it has the sign of  $s/2(a+s) - i$ . With underdominant selection,  $i < 0$ ; again, only one root lies in  $(-1, 1)$ , but in this case it is always positive. The overdominant and underdominant roots in  $[-1, 1]$  determine valid polymorphic equilibria if and only if (14) holds. Directional selection with  $w_{11} < w_{12} < w_{22}$  and  $w_{12} > (w_{11} + w_{22})/2$  yields the same sign pattern as underdominance, but evaluation of (8) shows that the root in  $(0, 1)$  is inadmissible because  $\hat{p} < 0$ . The Intermediate Value Theorem is uninformative when  $w_{11} < w_{12} < w_{22}$  and  $w_{12} < (w_{11} + w_{22})/2$ ; however, (8) and (12) show that in this case either  $\hat{p} > 1$  or  $\hat{w} < 0$  for any  $\hat{f} \in (-1, 1)$ . Therefore, no polymorphic equilibria exist under directional selection.

A few differences arise for the three subsumed systems with only two of the three forms of reproduction (*i.e.*,  $a = 0$ ,  $s = 0$ , or  $t = 0$ ). For  $t = 0$ , the underdominant sign pattern differs from Table B1 in that  $g(1) = 0$ , indicating that  $\hat{f} = 1$  is the only admissible root. In addition, the case  $t = 0$  has two roots when selection is overdominant:  $\hat{f} = 1$  and  $\hat{f} = (s/2i) - 1$ . Note that the latter implies that when  $t = 0$ , an excess of heterozygotes can occur in seeds only if  $s < 1$  because  $2i < 1$ . The root  $\hat{f} = 1$  determines a valid equilibrium for underdominance and overdominance only if  $w_{11} = w_{22}$  (in which case every state in which  $u + w = 1$  is an equilibrium). The distinctions of  $a = 0$  and  $s = 0$  are that with overdominance,  $\hat{f} > 0$  for  $a = 0$ , because then  $g(0) = s(1 - 2i) > 0$ , whereas  $\hat{f} < 0$  for  $s = 0$ , because  $g(0) = -2ai < 0$ .

The sign patterns for the  $\hat{F}$  quadratic under the adult census,

$$(2a + s)i\hat{F}^2 + [2(1 - a)(i - 1) + s]\hat{F} + s - (2 + s)i = 0$$

differ from those for  $\hat{f}$  with overdominance in two ways. First, under the complete model and the two subsumed models  $a = 0$  and  $t = 0$ , the sign of  $\hat{F}$  is that of  $s - i(2 + s)$ . Second, the exact solutions for  $t = 0$  are  $\hat{F} = 1$  and  $\hat{F} = [s - i(2 + s)]/i(2 - s)$ , which implies that for adults, obtaining a negative  $\hat{F}$  for  $t = 0$  does not require  $s < 1$ ; in particular, if  $s = 1$  an excess of heterozygotes will occur whenever  $i > 1/3$ .

APPENDIX C

**Genotypic recursions and local stability analysis for the seed census:** Letting  $u = \text{freq}(A_1A_1)$  and  $v = \text{freq}(A_1A_2)$  in zygotes (seeds), the genotypic recursions for the seed census model are

$$u' = \frac{(a + s)w_{11}u}{\bar{w}} + \frac{sw_{12}v}{4\bar{w}} + t(p')^2$$

and

$$v' = \frac{(2a + s)w_{12}v}{2\bar{w}} + 2tp'(1 - p')$$

where

$$p' = \frac{w_{11}u}{\bar{w}} + \frac{w_{12}v}{2\bar{w}}$$

and

$$\bar{w} = w_{22} + (w_{11} - w_{22})u + (w_{12} - w_{22})v.$$

An equilibrium  $(\hat{u}, \hat{v})$  is locally stable if both eigenvalues of its local stability matrix have magnitude  $< 1$ . These eigenvalues are given by the roots of the characteristic equation  $\lambda^2 - B\lambda + C = 0$  where

$$B = \frac{\partial u'}{\partial u} + \frac{\partial v'}{\partial v} \tag{C1}$$

and

$$C = \left(\frac{\partial u'}{\partial u}\right)\left(\frac{\partial v'}{\partial v}\right) - \left(\frac{\partial u'}{\partial v}\right)\left(\frac{\partial v'}{\partial u}\right) \tag{C2}$$

with the partials evaluated at  $(\hat{u}, \hat{v})$ . At the fixation equilibria,  $B > 0$ , and the two roots are real numbers (because  $B^2 - 4C > 0$ ); therefore, fixation for  $A_i$  will be locally stable if  $B_i < 2$  and  $B_i - C_i < 1$ , where for  $i \neq j = 1, 2$ ,

$$B_i = (1 - t)\frac{w_{ji}}{w_{ii}} + \left(1 - \frac{s}{2}\right)\frac{w_{12}}{w_{ii}}$$

and

$$C_i = \left(1 - \frac{s}{2} - t\right)\frac{w_{12}w_{jj}}{w_{ii}^2}$$

are (C1) and (C2), respectively, evaluated at the fixation of  $A_i$ . Rewriting the two stability criteria as

$$2[(1 - \delta)w_{jj} - w_{ii}] + (2 - s)w_{12} - 2w_{ii} < 0 \quad (\text{C3})$$

and

$$stw_{12}w_{jj} - [(1 - \delta)w_{jj} - w_{ii}][(2 - s)w_{12} - 2w_{ii}] < 0 \quad (\text{C4})$$

it follows that fixation of  $A_i$  is locally stable if (C4) holds together with

$$w_{12} < \frac{2w_{ii}}{2 - s} \quad (\text{C5})$$

and

$$w_{ii} > (1 - \delta)w_{jj}. \quad (\text{C6})$$

This reduces to the requirement that (C4) holds together with (C5), because this ensures (C6) is satisfied.

#### APPENDIX D

**Co-occurrence (or not) of a valid internal equilibrium and a protected polymorphism:** In the case of the seed census, recall that a polymorphic equilibrium exists for  $w_{12} > w_{ii} > w_{jj}$  if and only if inequality (15) holds and for  $w_{ii} > w_{jj} > w_{12}$  if and only if inequality (16) holds ( $i \neq j = 1, 2$ ). Showing that a protected polymorphism (PP) and a valid internal equilibrium always simultaneously exist or not is facilitated by rewriting the local stability conditions in (17) and (18) as

$$sw_{12} > 2(w_{12} - w_{11}) \quad (\text{D1})$$

and

$$sw_{12}(w_{11} - w_{22}) > 2(w_{12} - w_{11})[w_{11} - (1 - \delta)w_{22}] \quad (\text{D2})$$

for the fixation of  $A_1$  ( $\hat{p} = 1$ ) and as

$$sw_{12} > 2(w_{12} - w_{22}) \quad (\text{D3})$$

and

$$sw_{12}(w_{22} - w_{11}) > 2(w_{12} - w_{22})[w_{22} - (1 - \delta)w_{11}] \quad (\text{D4})$$

for the fixation of  $A_2$  ( $\hat{p} = 0$ ).

Consider the case of overdominance with  $w_{12} > w_{11} > w_{22}$ , which implies, by (15), that a polymorphic equilibrium exists if and only if

$$sw_{12}(w_{11} - w_{22}) < 2(w_{12} - w_{11})[w_{11} - (1 - \delta)w_{22}]. \quad (\text{D5})$$

Now suppose that an internal equilibrium exists; thus, (D5) holds. This condition is the reverse of inequality (D2) and therefore implies that fixation of  $A_1$  is unstable whenever a polymorphic equilibrium exists. Fixation of  $A_2$  will also be unstable and is so whenever  $w_{12} > w_{11} > w_{22}$ , because then either  $w_{22} \geq (1 - \delta)w_{11}$ , in which case (D4) fails, or  $w_{22} < (1 - \delta)w_{11}$ , in which case (D3) and (D4) cannot both hold. By symmetry,  $\hat{p} = 1$  and  $\hat{p} = 0$  are also both unstable if a polymorphic equilibrium exists when  $w_{12} > w_{22} > w_{11}$ . Thus, a PP exists whenever a valid internal equilibrium exists for overdominant fitnesses. Now suppose a PP exists when  $w_{12} > w_{11} > w_{22}$ . The instability of  $\hat{p} = 1$  implies that either inequality (D1) or inequality (D2) is reversed (where we are ignoring the complications posed by the unlikely event of strict equality). For this fitness order, however, inequality (D2) is reversed whenever inequality (D1) is reversed, and hence (D5) always holds when  $\hat{p} = 1$  is unstable. Therefore a PP implies a valid polymorphic equilibrium exists. By symmetry, the same is true for the case  $w_{12} > w_{22} > w_{11}$ . This completes the proof that a polymorphic equilibrium exists whenever a PP exists and vice versa when selection is overdominant.

Consider next the case of underdominance with  $w_{11} > w_{22} > w_{12}$ . Note first that this fitness order implies that (D1), (D2), and (D3) hold. Also, by (16), a polymorphic equilibrium exists if and only if (D4) holds. Therefore, in this case, both fixation states are stable, and there is no PP whenever an internal equilibrium exists. By symmetry, the same is true for  $w_{22} > w_{11} > w_{12}$ . Thus, in the case of underdominance, a polymorphic equilibrium implies that both  $\hat{p} = 1$  and  $\hat{p} = 0$  are stable, which in turn ensures that a PP cannot occur.