Effect of Selection Against Deleterious Mutations on the Decline in Heterozygosity at Neutral Loci in Closely Inbreeding Populations

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ABSTRACT

Transition matrices for selfing and full-sib mating were derived to investigate the effect of selection against deleterious mutations on the process of inbreeding at a linked neutral locus. Selection was allowed to act within lines only (selection type I) or equally within and between lines (type II). For selfing lines under selection type I, inbreeding is always retarded, the retardation being determined by the recombination fraction between the neutral and selected loci and the inbreeding depression from the selected locus, irrespective of the selection coefficient (s) and dominance coefficient (h) of the mutant allele. For selfing under selection type II or full-sib mating under both selection types, inbreeding is delayed by weak selection (small s and sh), due to the associative overdominance created at the neutral locus, and accelerated by strong selection, due to the elevated differential contributions between alternative alleles at the neutral locus within individuals and between lines (for selection type II). For multiple fitness loci under selection, stochastic simulations were run for populations with selfing, full-sib mating, and random mating, using empirical estimates of mutation parameters and inbreeding load in Drosophila. The simulations results are in general compatible with empirical observations.

A consequence of inbreeding almost universally observed in diploid species is a reduction in fitness (inbreeding depression), which can be explained by the increased homozygosity at loci affecting fitness either with partially recessive deleterious alleles or with heterozygote advantage (Falconer and Mackay 1996). Because individuals vary in their actual degree of homozygosity and thus fitness even if they have the same expected inbreeding coefficient (Weir and Cockerham 1973), natural selection for fitness will inevitably occur, favoring heterozygotes and resisting the progress toward homozygosity at the loci affecting fitness. Furthermore, the rate of decrease in heterozygosity at neutral loci will also be affected by selection for fitness when there is a positive association between the homozygosity of fitness loci and neutral loci. This association, usually called identity disequilibrium, can arise if individuals vary in their expected inbreeding coefficients as computed from pedigree (Haldane 1950) or if the loci are linked even when all individuals have the same pedigree inbreeding coefficient (Weir and Cockerham 1973). Therefore, because of the positive association in homozygosity between loci, selection also tends to confer an apparent advantage on heterozygotes at neutral loci, resulting in the so-called associative overdominance (Ohta 1971; Ohta and Cockerham 1974; Strobeck 1980; Charlesworth 1991) and thus a possible delay in the progress toward homozygosity in an inbreeding population.

Selection for fitness also generates differences in the reproductive success among individuals, which tends to accelerate the progress toward homozygosity at neutral loci (Robertson 1961; Wray and Thompson 1990; Santiago and Caballero 1995). The effect of selection is further amplified when the neutral and selected loci are linked (Nordborg *et al.* 1996; Santiago and Caballero 1998), because the apparent advantage (or disadvantage) associated with a neutral allele in reproductive success will dissipate more slowly with tighter linkage.

Given the above counteracting effects of selection for fitness on the rate of inbreeding at neutral loci, what is the actual rate of inbreeding that can be realized in an inbreeding population? This question is important especially for close inbreeding, which is generally used to produce fixation or homozygosis within lines. Several experiments have indicated that the actual level or rate of inbreeding revealed by the observed heterozygosity at loci with enzyme or protein polymorphisms is lower than expected (Sing *et al.* 1973; Eriksson *et al.* 1976; Connor and Bellucci 1979; Strauss 1986; Mina *et al.* 1991; Frankham *et al.* 1993; Rumball *et al.* 1994; McGoldrick and Hedgecock 1997). Rumball *et al.* (1994) showed that the realized inbreeding coefficients as estimated from genotype frequencies at six enzyme loci were much lower than the theoretical predictions in two sets of full-sib mating lines and in one set of

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lines with double first-cousin mating in *Drosophila melanogaster*. They attributed the delay of inbreeding to natural selection favoring heterozygous chromosomal segments.

In this study, we construct an analytical model to investigate the effect of selection against partially recessive and deleterious mutations on the rate of inbreeding at neutral loci in selfing and full-sib mating lines. Extending these models to multiple loci under selection and using the empirical parameters for mutations to deleterious alleles and the inbreeding loads estimated from various experiments, we show by stochastic simulation that a delay in homozygosis at neutral loci is expected in Drosophila populations with inbreeding, and the predictions for full-sib mating are also quantitatively in agreement with the observations of Rumball *et al.* (1994). Other species and other regular or nonregular systems of inbreeding are also considered in the simulations. The results and their implications are discussed in the context of the establishment of inbred lines and other issues.

MODELS

One locus under selection: Two linked loci, each autosomal and biallelic, are considered. One of the loci (denoted as locus A) is assumed to be neutral, with the three genotypes AA, Aa, and aa having the same fitness. The other locus (B) is assumed to affect fitness, with the three genotypes BB, Bb, and bb having relative fitnesses 1, $1 - hs$, and $1 - s$, respectively. The mutant allele b is assumed to be partially recessive ($0 \leq h \leq 0.5$) and deleterious to fitness ($0 \leq s \leq 1$). No new mutation is assumed to occur at either locus over the generations of inbreeding. This is plausible if we consider only a few generations, because the mutation rate per locus is very small.

Selfing: If we are interested only in the progress of heterozygosity at the neutral locus, there is no need to distinguish between the two kinds of homozygotes at the neutral locus, or between the coupling and repulsion double heterozygotes. Therefore, six genotypes result from the two biallelic loci, and the inbreeding process can be described by a 6×6 transition matrix, with element t_{ij} being the probability of genotype i for the progeny given genotype j of its parent. The transition matrix is given in Table 1, in which $R = 2r(1 - r)$, where r is the recombination fraction between the two loci. If the recombination fractions are different in generating the gametes of the two sexes, *e.g.*, r_m and r_f, then $R = r_m + r_f - 2r_m r_f$.

In deriving the transition matrix in Table 1, two types of selection for fitness are considered. Type I is within-line selection, where one individual is randomly selected from the offspring that have survived to maturity from each selfing line. Therefore all lines have an equal chance of being retained, though they vary in homozygosity at the selected locus and thus in fitness. Obviously, within-line selection affects the probability of a given genotype in the offspring generation only when the parent is a heterozygote at the selected locus. In most practical inbreeding studies, reserve matings within inbred lines are used to avoid excessive loss of lines and to reduce selection among lines (Wallace and Maddern 1965; Mina *et al.* 1991; Rumball *et al.* 1994). Except for extinctions of lines, this kind of selection is similar to within-line selection used in this study.

Between-line selection will also occur if Bb or bb lines tend to be discarded in favor of BB lines, because of their lower fecundity, viability, or other undesirable characters. Between-line selection may be stronger or weaker than within-line selection. If the proportion of lines discarded each generation is sh for Bb lines and s for bb lines, selection occurs equally within and between lines. In the present study we consider this type of selection (called type II hereafter), which was used and discussed by Hayman and Mather (1953) and Haldane (1956) and generalized by Reeve (1957) for a locus with heterozygote advantage. In practice, between-line selection occurs when individuals for selfing or pairs of individuals for sib-mating are randomly selected from the progeny pool that survived the within-line fitness selection from all lines.

Our primary interest is in the rate of progress toward homozygosity at the neutral locus. At each generation, the frequencies of the six genotypes are normalized before the frequency of heterozygotes at the neutral locus (H_t) is calculated, and the inbreeding coefficient and rate of inbreeding are calculated as $F_t = 1 - H_t/H_0$ and $\lambda_t = 1 - H_t/H_{t-1}$, respectively. F_t and λ_t are compared with the corresponding values of $1 - 0.5^t$ and 0.5, the expectations in selfing lines in the absence of selection.

Full-sib mating: With full-sib mating, we have to distinguish between the two types of homozygotes at the neutral locus and the coupling and repulsion double heterozygotes. Therefore, 10 genotypes and 55 mating types, classified according to the genotypes of the two mates, are possible in full-sib mating lines.

The element t_{ij} in the 55×55 transition matrix for full-sib mating is the probability of mating type i when the progeny are sibmated given the mating type j in parents, and it is obtained by writing out the relative frequency of each genotype in the progeny of mating type j after selection and finding the probability of a randomly sampled sib pair from the progeny being mating type i. Different recombination fractions in separate sexes were taken into account in constructing the transition matrix, which is complex and thus not shown here.

Using the transition matrix, the frequencies of the 55 types of full-sib families and thus the heterozygosity (H_t) at each generation can easily be calculated. The inbreeding coefficient (F_t) and rate of inbreeding (λ_t) can be calculated from H_t, as in the case of selfing shown

TABLE 1

Transition matrix for two loci with linkage and selection in selfing lines

Progeny genotypes	Parent genotypes					
	AABB aaBB	AABb aaBB	AAbb aabb	AaBB	AaBb	Aabb
AABB aaBB	1	v		0.5	$(1 - R)v$	
AABb aaBB		$2(1 - hs)v$			$2(1 - hs)Rv$	
AAbb aabb		$(1 - s)v$	u		$(1 - s)(1 - R)v$	$0.5u$
AaBB				0.5	Rv	
AaBb					$2(1 - hs)(1 - R)v$	
Aabb					$(1 - s)Rv$	$0.5u$

$R = 2r(1 - r)$. If the recombination fractions in generating male and female gametes are r_m and r_f, then $R = r_m + r_f - 2r_m r_f$. For selection type I (within-line selection only), $v = 1/(4 - 2hs - s)$, $u = 1$. For selection type II (within- and between-line selection), $v = 1/4$, $u = 1 - s$.

above. F_t and λ_t are compared with the standard values calculated from pedigree without selection, $F_t^* = (1 + 2F_{t-1}^* + F_{t-2}^*)/4$ and $\lambda_t^* = (F_t^* - F_{t-1}^*)/(1 - F_{t-1}^*)$. H_t is also compared with the corresponding standard heterozygosity in the absence of selection (H_t^*), calculated as $H_t^* = (1 - F_t^*)H_0$.

The change in the rate of inbreeding at the neutral locus compared with the theoretical rate without selection is caused by associative overdominance and the increased variation in reproductive success generated by selection at linked fitness loci. The apparent coefficient of selection for a given genotype at the neutral locus could be obtained as the proportional reduction in frequency of the genotype due to selection at any generation. The apparent fitness (1 − apparent selection coefficient) of genotype AA or aa (W_{AA}, W_{aa}) is expressed relative to that of genotype Aa. The mean fitness of a homozygote is the average of W_{AA} and W_{aa} weighted by the frequencies of the two genotypes. The above calculation could readily be made, given the frequencies of the 55 mating types at each generation.

When there is selection at a linked locus with deleterious mutations, different alleles at the neutral locus tend to contribute differentially to the next generation. The neutral allele linked to a deleterious mutation tends to contribute a smaller number of copies on average than the alternative allele linked to the wild-type allele at the selected locus. To quantify this effect of selection, we calculated the mean square difference between the contribution of a neutral allele and the expectation without selection (V_w, called the within-individual variance for simplicity hereafter). It consists of two parts, the first being the variance of the contribution of the allele around its mean and the second being the squared difference between the mean contribution and the expectation without selection. The first part is mainly due to sampling, and the second part comes entirely from selection. Without selection ($s = 0$) or linkage ($r = 0.5$), the mean contribution is equal to the expectation without selection, and V_w comes solely from binomial sampling. In this case, therefore, $V_w = 0.5$ is expected as each individual contributes two offspring or gametes on average to the next generation. With selection and linkage, $V_w > 0.5$ results, leading to a possible increased rate of inbreeding at the neutral locus. In this paper, V_w for each kind of family was calculated and weighted by its frequency to give the mean within-individual variance.

When there is between-line selection, different lines will tend to contribute differentially to the next generation. Compared with Bb lines, BB lines tend to contribute more and bb lines less offspring on average. This differential contribution among lines will also tend to accelerate the approach to homozygosity at the neutral locus if it is linked to the deleterious locus ($r < 0.5$) or if there is linkage disequilibrium in the initial lines. To quantify this effect of selection, we calculated the mean square difference between the number of offspring or gametes contributed to the next generation from each family (or equivalently line, since there is full-sib mating) and the expectation without selection (V_b, called the between-family variance for simplicity hereafter). Obviously, V_b is affected by both the intensity of selection (s and h) and the frequencies of different lines, and $V_b = 0$ is expected if there is no between-line selection. In this paper, V_b was calculated from the average contributions of offspring and the frequencies of the 55 mating types at each generation.

It is obvious that the genetic contribution of an advantageous (disadvantageous) allele or family in fitness accrues over generations (Robertson 1961), especially with tight linkage (Santiago and Caballero 1998).

Therefore if two or more generations are considered, within-individual and between-family variances will be larger than the V_w and V_b calculated as above and thus have greater effects on the rate of inbreeding at the neutral locus. For simplicity, we consider V_w and V_b over only one generation as indications of the effects of selection on accelerating inbreeding.

The initial lines (generation zero) are assumed to be sampled randomly from a large ancestral population with linkage and Hardy-Weinberg equilibria at the two loci. For the fitness locus, the ancestral population is also assumed to be at mutation-selection balance. Because the frequency of a deleterious allele is very small in the large ancestral population, it is found overwhelmingly in heterozygotes (Crow 1993). To a good approximation, therefore, the initial full-sib mating type with respect to the fitness locus would be BB × Bb if the deleterious allele is not lost from the sampling. Given the gene frequency and thus the genotype frequencies at the neutral locus, the initial frequencies of the mating types can be obtained by randomly combining the genotypes at the two loci for different individuals in a line.

Multiple loci under selection: The joint effect of multiple loci under selection on the rate of inbreeding at a neutral locus in selfing or full-sib mating lines can be obtained by extending the above analytical model, assuming multiplicative action among deleterious loci. However, some realistic complexities, such as extinctions of lines, variation in selection coefficient and dominance coefficient among mutations, new mutations arising each generation, and more complex mating systems than selfing or full-sib mating, are difficult to accommodate in the analytical model. We, therefore, use stochastic simulations instead.

Inbreeding loads: The inbreeding load can be expressed conveniently as B (Morton *et al.* 1956), the difference in fitness (measured in natural logarithms) between a large random mating population and the completely inbred population formed from it without selection. The empirical estimate of B for viability associated with a major autosome in several species of Drosophila is ~0.5–0.8 according to several studies reviewed by Simmons and Crow (1977). Roughly half the inbreeding load for viability is estimated to be from lethal mutations, and the other half from mildly deleterious mutations. Noting that a major autosome comprises approximately 40% of the Drosophila genome, the estimate indicates an average inbreeding load of three per individual.

For within-family and within-line selection schemes, only viability as a fitness component is relevant. When there is between-family or between-line selection, however, other fitness components, such as fecundity, should also be taken into account. A summary of several studies involving different species of Drosophila (Simmons and Crow 1977) indicates that B is about 1.5 for the net fitness effect of a single nonlethal autosome. The inbreeding load contributed by sterile or lethal mutations is small, only about 0.25 per second chromosome in *D. melanogaster* (Temin *et al.* 1969). Extrapolating to the whole genome, the inbreeding load for net fitness is about nine for a typical individual.

Estimates of inbreeding loads for some fitness traits in a few other species are reviewed by Lynch and Walsh (1998, pp. 278–283). Though variable, they are similar on average to the results for Drosophila.

Mutation parameters: Inbreeding depression can be explained by either the dominance model or the overdominance model, or any combination of both (Falconer and Mackay 1996), but the dominance model seems to be more biologically realistic as supported by an increasing number of empirical and theoretical studies (Crow 1993). In this study, we assume that all the inbreeding load comes from partially recessive mutations of deleterious effects on fitness.

Mutation accumulation experiments using balancer chromosomes in Drosophila yielded a lower-bound estimate for the rate of mutation to deleterious alleles affecting viability of about 1.0 per diploid genome per generation. The mutations are estimated to have a mean selection coefficient ($\bar{s}$) of ~0.03–0.05 and a mean dominance coefficient ($\bar{h}$) of ~0.2–0.4 (*e.g.*, Mukai *et al.* 1972). The distribution of the selection coefficients, however, could be leptokurtic, with most mutations having much smaller homozygous effects on viability than the mean value (Keightley 1994). Although some more recent studies (*e.g.*, Keightley 1996; García-Dorado 1997; Fry *et al.* 1999) indicated a much lower mutation rate than the classical estimates, they do not rule out the possibility of a high rate of occurrence of mutations with very small effects (*e.g.*, $s < 0.001$). As will be shown, mutations of small effects are more likely to delay the inbreeding process at linked neutral loci for a given amount of inbreeding depression.

The above mutation parameters refer to deleterious mutants that are not lethal when homozygous individually. A mutation is usually classified as homozygous lethal if its viability is <10% of the wild-type value (Simmons and Crow 1977). In Drosophila, these lethal mutations occur at a rate of ~0.03 per diploid genome per generation, with an average dominance coefficient of ~0.02–0.03 (Crow 1993).

Recent experiments in plant species (Johnston and Schoen 1995) yielded similar estimates for mutations affecting total fitness, except that the mutations tend to be more recessive with respect to fitness. It is possible that deleterious mutations are pleiotropic in their effects on all fitness components (Crow 1993).

Stochastic simulation model: Throughout the study, the length of the chromosome was assumed to be 1 M ($L = 1$). This is roughly the case for a major autosome in Drosophila and for an average chromosome in many other species. The total inbreeding load per haploid genome was assumed to be $B = 1.5$ for viability when

only within-family selection was considered and $B = 4.5$ for net fitness when there was also between-line or between-family selection. Therefore different numbers of chromosomes per haploid genome correspond to different amounts of inbreeding load associated with a single chromosome.

The simulation program has been described previously (Wang *et al.* 1999) and details are not given here. The genotypes at loci with deleterious mutations in initial lines were randomly sampled from an infinitely large population at mutation-selection balance, and genotypes at neutral loci were generated assuming Hardy-Weinberg and linkage equilibria. The number of new deleterious mutations on the chromosome arising each generation was assumed to follow a Poisson distribution with mean U, the mutation rate per chromosome. Mutations were assumed to be unidirectional from wild-type to mutant alleles, which is reasonable because back-mutation rates are generally much lower than forward-mutation rates.

A total number of 100 neutral loci with two alleles at equal frequencies were also included in the simulations. These loci were assumed to be nonmutable. The heterozygosity (H_t), averaged over loci and replicate lines, was calculated at each generation. F_t was calculated from H_t as shown in the analytical model, and it was also calculated from the variance in frequency of a neutral allele among replicate lines ($V_{q,t}$), *i.e.*, $F_t = V_{q,t}/[q(1 - q)]$, where t was corrected because genetic drift precedes inbreeding by one or more generations. H_t and F_t were then compared with the pedigree values in the absence of selection.

For generating a gamete, the number of crossovers on the chromosome was drawn from a Poisson distribution with mean $2L$. The locations where crossovers occurred were randomly chosen along the chromosome, without interference between different crossover events. For Drosophila, no recombination was involved in generating male gametes. The fitness of a zygote was calculated assuming multiplicative effects of different loci.

RESULTS

One locus with deleterious alleles under selection in selfing lines: The inbreeding coefficients at generations three and six and the asymptotic rate of inbreeding when there is selection against deleterious mutations of various selection coefficients and dominance coefficients are listed in Table 2. These are compared with the corresponding values without selection, listed at the bottom of the table.

The inbreeding depression from a single deleterious locus can be measured as the difference between the fitness of a heterozygote and the mean fitness of the two homozygotes formed from it, relative to the fitness of the heterozygote, *i.e.*,

$$D = 1 - \frac{1 - s/2}{1 - hs} = \frac{s(1 - 2h)}{2(1 - hs)}.$$

For selection type I, inbreeding is always delayed compared with the standard case without selection. The inbreeding process is, however, not affected by the values of s and h for a given value of D (Table 2). The larger the depression from the selected locus and the tighter its linkage with the neutral locus, the greater the decrease in the realized level of inbreeding.

Without selection, the rate of inbreeding is constant (0.5) over generations of selfing. With within-line selection at a linked deleterious locus, the rate of inbreeding at the neutral locus is decreased and varies over generations except for complete linkage. Eventually, an asymptotic rate of inbreeding will be reached, although many generations may be required, depending on the values of D and r. When the value of D is large enough and that of r is small enough, an asymptotic rate of inbreeding <0.5 results, *i.e.*, the rate of inbreeding is permanently decreased by selection for fitness.

For selection type II, the inbreeding process is no longer independent of the intensity of selection (values of s and h) against the deleterious mutation for a given value of inbreeding depression (D). Except for weak selection (*e.g.*, $s < 0.2$), inbreeding increases with increasing values of s and h for a fixed value of D (Table 2). When s and h are large enough, inbreeding is accelerated rather than delayed compared with the standard case without selection. This is because mutations can be effectively purged from the selfing lines by between-line selection if their effects are large. During the process of purging, the heterozygosity at both the selected locus and the linked neutral locus decreases faster than the standard case without selection.

In contrast to selection type I, the asymptotic rate of inbreeding under selection type II can be larger than the standard rate of 0.5. This happens when the neutral locus is completely linked ($r = 0$) to the selected locus with a noncompletely recessive mutant allele ($h > 0$), and the larger the heterozygous effect (hs) the higher the asymptotic rate of inbreeding. Similar to within-line selection, however, many generations may be required to reach the steady state.

The asymptotic rate of inbreeding can be obtained analytically from the eigenvalues of the transition matrix in Table 1. For selection type I, the eigenvalues are

$$\beta_1 = 1, \quad \beta_2 = \frac{2(1 - hs)}{4 - 2hs - s},$$

$$\beta_3 = \tfrac{1}{2}, \quad \beta_4 = \frac{2(1 - hs)(1 - R)}{4 - 2hs - s}.$$

One minus the dominant nonunit eigenvalue gives the asymptotic rate of inbreeding. β_2 is the dominant (nonunit) eigenvalue when linkage is complete, and $\beta_2 = \beta_4$ if $R = 0$. The condition for β_3 to be the dominant

TABLE 2

Inbreeding coefficients at generations three and six and the asymptotic rate of inbreeding with selfing

				Selection type I			Selection type II		
r	D	s	h^a	$F_3{}^b$	F_6	λ_A	F_3	F_6	λ_A
0	0.01	0.02	0	0.873	0.984	0.498	0.872	0.984	0.5
		0.05	0.30						0.508
	0.05	0.10	0	0.865	0.982	0.487	0.862	0.980	0.5
		0.20	0.26				0.864	0.982	0.526
	0.25	0.50	0	0.813	0.965	0.429	0.810	0.971	0.5
		0.75	0.22				0.853	0.988	0.584
	0.50	1.00	0	0.704	0.912	0.333	0.778	0.969	0.5
0.05	0.01	0.02	0	0.874	0.984	0.5	0.873	0.984	0.5
		0.05	0.30						
	0.05	0.10	0	0.868	0.983	0.5	0.865	0.982	0.5
		0.20	0.26				0.867	0.983	
	0.25	0.50	0	0.830	0.973	0.483	0.827	0.976	0.5
		0.75	0.22				0.860	0.988	
	0.50	1.00	0	0.751	0.942	0.397	0.807	0.976	0.5
0.1	0.01	0.02	0	0.874	0.984	0.5	0.874	0.984	0.5
		0.05	0.30						
	0.05	0.10	0	0.870	0.983	0.5	0.868	0.983	0.5
		0.20	0.26				0.869	0.984	
	0.25	0.50	0	0.843	0.977	0.5	0.841	0.980	0.5
		0.75	0.22				0.866	0.987	
	0.50	1.00	0	0.787	0.959	0.453	0.828	0.980	0.5
Expectation: $s = 0$ or $r = 0.5$				0.875	0.984	0.5	0.875	0.984	0.5

[a] The dominance coefficient h is calculated from $D = s(1 - 2h)/2(1 - hs)$, given values of s and D.
[b] F_3, F_6, and λ_A are inbreeding coefficients at generations three and six and the asymptotic rate of inbreeding at the neutral locus.

eigenvalue is $\beta_4 \geq \beta_3$, which, after some algebra, reduces to $R \geq D/2$. Therefore, β_4 is the dominant eigenvalue when $R < D/2$. $R = D/2$ is the critical value that determines whether the asymptotic rate of inbreeding is equal to or smaller than 0.5. Summarizing the above results, the asymptotic rate of inbreeding is given by

$$\text{Min}(1 - \beta_4, \tfrac{1}{2}) = \text{Min}\left(\frac{1 - D + R}{2 - D}, \frac{1}{2}\right).$$

Similarly, the dominant eigenvalues for selection type II can also be obtained from the transition matrix in Table 1. The asymptotic rate of inbreeding is 0.5 when $r > 0$ and is $0.5(1 + hs)$ when $r = 0$.

One locus with deleterious alleles under selection in full-sib mating lines: The inbreeding coefficients at generations 5, 10, and 20 of full-sib mating when there is selection against mutations of various values of s, h, and r were obtained by using the transition matrix and are listed in Table 3.

For a given value of D, the realized inbreeding coefficient in full-sib mating lines increases with increasing values of s for both selection types. This is evident for selection type I, however, only when s exceeds about 0.2. Mutants with smaller s values are effectively neutral in full-sib mating lines under within-line selection.

For a given value of h, the extent of delay in inbreeding first increases then decreases with increasing values of s. This is true for both types of selection, but the maximum delay is reached at a lower value of s for selection type II than for type I. For $h = 0$ and $r = 0$, for example, the inbreeding coefficient at generation 10 is minimized at about 0.855 (96.5% of the standard value) when s is about 0.6 for selection type I, and the corresponding value is 0.874 (98.6% of the standard value) when s is about 0.2 for selection type II. For both types of selection, the value of s resulting in the maximum delay of inbreeding decreases with increasing values of h. The reason for this nonlinear relationship between s and the extent of delay of inbreeding is that both inbreeding depression and the variances of genetic contribution within and among individuals due to selection against deleterious mutations increase with increasing values of s for a given value of h. Inbreeding depression tends to delay inbreeding due to associative overdominance, while variation in genetic contribution tends to accelerate inbreeding. At appropriate values of s, which are also dependent on the values of h and r as well as the selection type, therefore, the delay of inbreeding is maximized.

With full-sib mating, an asymptotic rate of inbreeding

TABLE 3

Inbreeding coefficients at generations 5, 10, and 20 with full-sib mating

				Selection type I			Selection type II		
r	D	s	h[a]	F_5[b]	F_{10}	F_{20}	F_5	F_{10}	F_{20}
0	0.01	0.02	0	0.670	0.885	0.986	0.669	0.884	0.986
		0.05	0.30	0.670	0.885	0.986	0.670	0.885	0.986
	0.05	0.10	0	0.664	0.880	0.985	0.660	0.877	0.985
		0.20	0.26	0.665	0.881	0.985	0.667	0.887	0.988
	0.25	0.50	0	0.634	0.858	0.978	0.645	0.881	0.988
		0.75	0.22	0.663	0.886	0.987	0.693	0.906	0.990
	0.50	1.00	0	0.640	0.875	0.985	0.663	0.897	0.990
0.05	0.01	0.02	0	0.671	0.886	0.986	0.670	0.885	0.986
		0.05	0.30	0.671	0.886	0.986	0.670	0.885	0.986
	0.05	0.10	0	0.667	0.884	0.986	0.664	0.881	0.986
		0.20	0.26	0.667	0.884	0.986	0.669	0.887	0.987
	0.25	0.50	0	0.649	0.872	0.983	0.655	0.884	0.987
		0.75	0.22	0.667	0.886	0.987	0.687	0.899	0.988
	0.50	1.00	0	0.653	0.881	0.986	0.667	0.893	0.988
0.1	0.01	0.02	0	0.671	0.886	0.986	0.671	0.885	0.986
		0.05	0.30	0.671	0.886	0.986	0.671	0.885	0.986
	0.05	0.10	0	0.669	0.884	0.986	0.667	0.883	0.986
		0.20	0.26	0.669	0.884	0.986	0.670	0.887	0.987
	0.25	0.50	0	0.658	0.879	0.985	0.661	0.885	0.987
		0.75	0.22	0.670	0.887	0.987	0.683	0.894	0.988
	0.50	1.00	0	0.661	0.883	0.986	0.669	0.890	0.987
Expectation: $s = 0$ or $r = 0.5$				0.672	0.886	0.986	0.672	0.886	0.986

[a] The dominance coefficient h is calculated from $D = s(1 - 2h)/2(1 - hs)$, given values of s and D.
[b] F_5, F_{10}, and F_{20} are inbreeding coefficients at generations 5, 10, and 20 of full-sib mating, respectively.

different from the standard value without selection is possible only for selection type I with very small values of r, small to intermediate values of s, and small values of h. For given values of s and h, the critical value of r or R to give an asymptotic inbreeding rate smaller than expectation is much smaller for full-sib mating than for selfing. For a deleterious mutation with $s = 0.5$ and $h = 0$, for example, the critical value of r is about 0.07 for selfing and 0.03 for full-sib mating.

Different recombination fractions in separate sexes with a given mean value have little effect on the inbreeding process. When $s = 0.5$ and $h = 0$, for example, the inbreeding coefficients at generation five are 0.64852 and 0.65505 for selection types I and II, respectively, if $r = 0.05$ is assumed for both sexes, and the corresponding values are 0.64847 and 0.65497 if $r_m = 0$ and $r_f = 0.1$ are assumed.

Figure 1, A and B, shows the heterozygosity after 20 generations of full-sib mating when there is selection against deleterious alleles with various dominance coefficients and selection coefficients, relative to the corresponding values in the absence of selection. The recombination fraction between the selected and neutral loci is assumed to be 0.1. For selection type I (Figure 1A), a realized heterozygosity higher than expected results from the selection against deleterious alleles of various values of s and h, except for strong selection (large s and h). For selection type II (Figure 1B), only highly recessive alleles of small effects lead to a delayed decrease in heterozygosity. The relative heterozygosity is larger for selection type II than that for selection type I when selection is weak (*e.g.*, $s < 0.05$, $h < 0.3$). Otherwise, selection type I gives a larger relative heterozygosity.

Selection against deleterious alleles can generate an apparent selection at the linked neutral locus, which tends to affect the realized rate of inbreeding. The mean fitness of a homozygote relative to a heterozygote at the neutral locus, averaged over 20 generations, is shown in Figure 1, C and D, for selection types I and II, respectively. When the deleterious alleles are partially recessive ($h < 0.5$) and are of small to medium homozygous effects, an apparent heterozygote advantage at the neutral locus (associative overdominance) is present for selection type I. With selection type II, only highly recessive deleterious alleles could result in apparent overdominance; otherwise, an apparent heterozygote disadvantage is generated at the neutral locus. The magnitude of the apparent relative fitness of a homozygote at the neutral locus is less sensitive to the values of s for selection type II than that for selection type I.

The apparent fitnesses of the two homozygotes at the

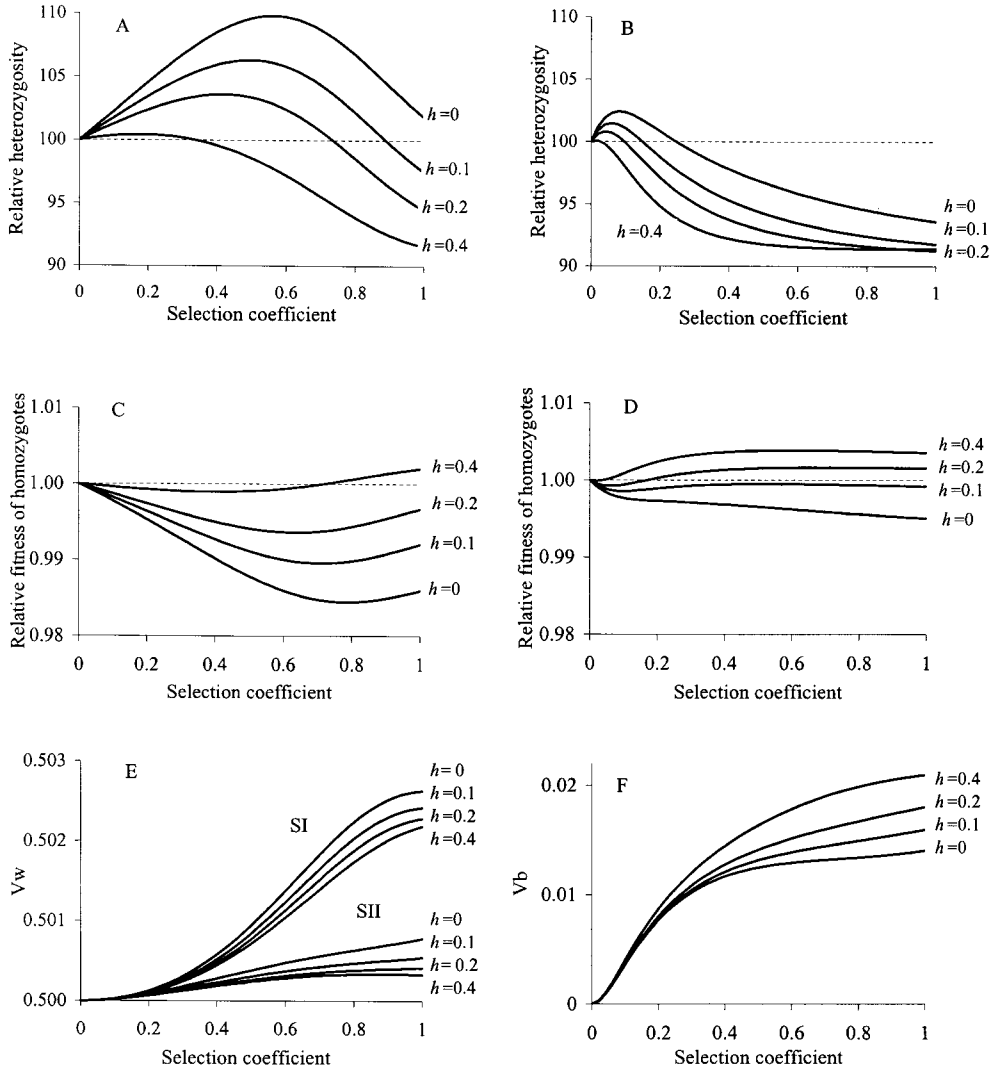

Figure 1.—Effects of selection against deleterious mutations in full-sib mating lines. (A) The heterozygosity at generation 20 as a percentage of the expected value without selection, for selection type I. (B) The same as A but for selection type II. (C) The relative fitness of homozygotes for selection type I. (D) The relative fitness of homozygotes for selection type II. (E) Within-individual variance (V_w) for selection types I and II. (F) Between-family variance (V_b) for selection type II. The relative fitness, V_w, and V_b are calculated as averages over 20 generations of full-sib mating. The recombination fraction between the selected and neutral loci is assumed to be 0.1.

neutral locus are different if the gene frequency departs from 0.5. Figure 2 shows the changes in their apparent fitnesses over 20 generations, when there is selection against a lethal mutation ($s = 1$, $h = 0.02$). The frequency of the common allele at the neutral locus is assumed to be 0.99, and the recombination fraction is 0.05. The apparent fitness of homozygotes for the common allele is always larger than that of the homozygotes for the rare allele if associative overdominance is present. The same results were obtained in previous theoretical (*e.g.*, Ohta 1971; Charlesworth 1991) and empirical (Rumball *et al.* 1994) studies. In contrast, when values of both *s* and *h* are large so there is an apparent heterozygote disadvantage, homozygotes for the rare allele always appear fitter than homozygotes for the common allele (data not shown). The difference in fitness between the two kinds of homozygotes diminishes over generations. The gene frequency at the neutral locus remains constant over generations.

Selection against deleterious alleles can also generate increased differential contributions from alternative alleles within individuals and from different lines (for selection type II), resulting in a possible increase in the realized rate of inbreeding. The mean square difference between the contribution of a neutral allele and the expectation without selection (V_w), averaged over 20 generations, increases with increasing selection coefficients and recessiveness of the deleterious alleles under selection (Figure 1E). For a mutant allele with a given value of *s*, a higher *h* value gives a larger within-individual variance in the first few generations, but the variance diminishes faster because of the stronger selection and thus the earlier elimination of the allele. Averaged over 20 generations, therefore, a higher *h* value gives rise to a smaller V_w. Selection type I results in a consistently larger V_w than type II, for a deleterious allele of any *s* and *h* values. This is because only lines with heterozygotes at the selected locus give an elevated within-individual variance. These lines have smaller mean contributions and thus smaller within-individual variances under selection type II than those under type I.

Though between-line selection gives a smaller in-

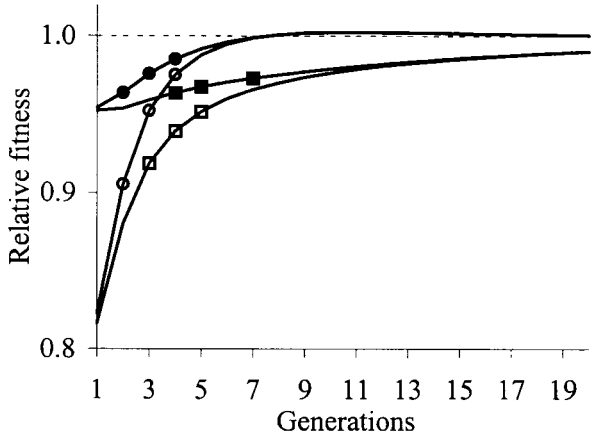

Figure 2.—The changes over 20 generations of full-sib mating in the apparent fitness at the neutral locus of the two homozygotes relative to that of a heterozygote. The parameters used in the calculations are $s = 1$, $h = 0.02$, and $r = 0.05$. The frequencies of the common and rare alleles at the neutral locus are assumed to be 0.99 and 0.01, respectively. Lines with solid squares and circles represent relative fitnesses of a homozygote for the common allele under selection types I and II, respectively, and lines with open squares and circles refer to the relative fitnesses of a homozygote for the rare allele under selection types I and II, respectively.

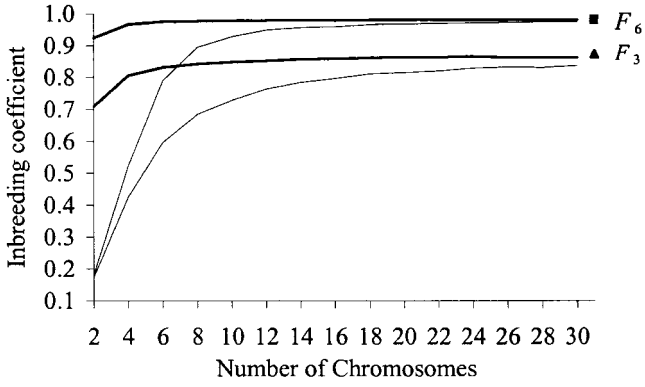

Figure 3.—Inbreeding coefficients at generations three and six with selfing. The inbreeding load per haploid genome is $B = 1.5$ for selection type I (thick lines) and is $B = 4.5$ for type II (thin lines); the length of each chromosome is 1 M. The triangle and square represent the expected inbreeding coefficients at generations three and six without selection. The selection and dominance coefficients assumed for mutations are 0.05 and 0.2, respectively.

crease in V_w than within-line selection, it results in an additional increase in between-family variance. The between-family variance averaged over 20 generations (V_b) is shown in Figure 1F for selection against deleterious mutations of various values of s and h. As expected, the between-family variance changes over generations. Deleterious alleles under strong selection (large s and h) give a higher between-family variance initially, but the variance decreases much faster than for weakly selected alleles because of the more effective purging. Averaging the between-family variance over 20 generations, however, shows that strong selection still results in a larger V_b than weak selection (Figure 1F), in contrast to within-individual variance (Figure 1E).

With very weak selection against deleterious mutations (*e.g.*, $s < 0.05$, $h < 0.3$), V_b (Figure 1F) is small and V_w is essentially the same under selection types I and II (Figure 1E). Selection type II, however, gives a larger magnitude of associative overdominance than type I (Figure 1, C and D) and therefore a more significant delay in the decrease of heterozygosity (Figure 1, A and B). With increasing values of selection coefficient, V_b increases dramatically, while the associative heterozygote advantage changes little or even an apparent heterozygote disadvantage is generated, under selection type II. Therefore, it results in a lower heterozygosity than selection type I when selection against mutations is strong (Figure 1, A and B).

Multiple loci under selection: In the previous analysis, we considered the impact of a single locus with deleterious alleles on the inbreeding at a linked neutral locus, whereas in reality many linked loci are simultaneously under selection and therefore have joint effects on a neutral locus. The results for multiple loci under selection were obtained by stochastic simulations and are shown below.

Selfing: With a fixed inbreeding load per haploid genome ($B = 1.5$ for within-line selection for viability and $B = 4.5$ for within- and between-line selection for fitness), the changes in the inbreeding coefficients at generations three and six of selfing with numbers of chromosomes per haploid genome are shown in Figure 3. The selection and dominance coefficients of the mutations are assumed to be 0.05 and 0.2, respectively, and no extinction of the lines is assumed. As can be seen, inbreeding is substantially delayed by selection only when the inbreeding load per chromosome is large. Selection type II gives a lower inbreeding than type I mainly because of the higher load assumed. If extinctions are allowed, a further decrease in inbreeding for both types of selection should be expected (see below).

When the inbreeding load per chromosome is high enough, the inbreeding process with selfing can be completely arrested. For four chromosomes per haploid genome with selection type II ($B = 1.125$ per chromosome), for example, the final inbreeding coefficient is about 0.75 calculated from heterozygosity and about 0.87 calculated from the variance in gene frequency among replicate lines. This is because a balanced lethal system can develop with a high inbreeding load per chromosome. With such a system, identity by descent of any particular chromosome is lethal, but an individual can survive if its homologues are nonidentical. Recombination will break the balanced system, and therefore higher inbreeding load is required for larger chromosomes to maintain the system. With complete linkage,

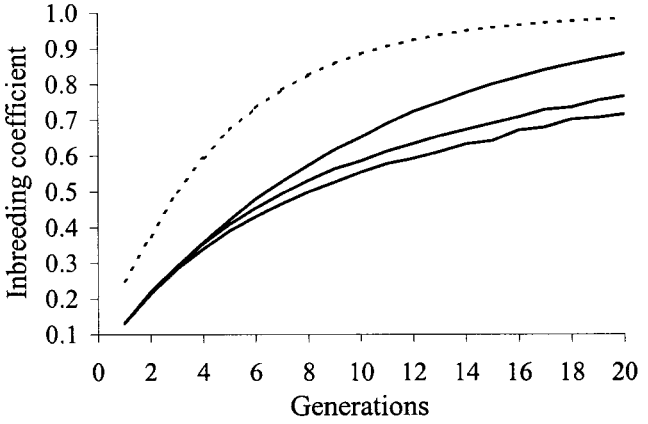

Figure 4.—Inbreeding coefficients over 20 generations of full-sib mating in Drosophila. The inbreeding load is assumed to be $B = 1.5$ per haploid genome ($B = 0.6$ per autosome of length 1 M), half being due to lethal mutations ($s = 1$, $h = 0.02$) and half due to mildly deleterious mutations with selection coefficient 0.05 and dominance coefficient 0.2. The dotted line shows the expected values without selection, and the continuous lines from bottom to top represent the corresponding values when there is selection for fitness with different numbers of offspring per family resulting in an accumulated extinction of 81, 53, and 0% at generation 10, respectively.

an inbreeding load of only ln(0.5) is enough to arrest the inbreeding with selfing.

The inbreeding coefficients shown in Figure 3 are calculated from heterozygosity and could be different from those calculated from the variance in gene frequency. The latter is usually larger than the former, and the larger the inbreeding load the greater the difference. This difference, however, diminishes over generations and converges to the same value if the inbreeding or drift process is not completely arrested.

The results are essentially the same as shown in Figure 3 for other values of s and h used in the simulation, provided $s < 0.2$, as is true for the vast majority of deleterious mutations (Crow 1993). This is because mutations with $s < 0.2$ are effectively neutral in selfing lines, no matter whether within- or between-line selection is applied (Table 2).

Full-sib mating: Figure 4 shows the simulation results for the inbreeding coefficients (calculated from heterozygosity) over 20 generations of full-sib mating in Drosophila with various family sizes (number of offspring produced per parent) and thus various accumulated proportions of extinctions of the lines. The inbreeding load is assumed to be $B = 1.5$ per haploid genome, half being due to lethal mutations ($s = 1$, $h = 0.02$) and half due to mildly deleterious mutations with selection coefficient 0.05 and dominance coefficient 0.2. Similar results were also obtained using other values of s and h with a given value of B, so long as $s < 0.2$. When $s > 0.2$, the realized inbreeding increases with s.

As can be seen from Figure 4, the realized level of inbreeding after 20 generations of full-sib mating is only ~70–90% of the standard value without selection. With more severe inbreeding depression, inbreeding will be further delayed. Extinctions of lines result in a further delay of inbreeding, especially in early generations. Extinct lines are expected to have a higher homozygosity than surviving ones, so their exclusion in calculating the inbreeding coefficient will lead to a lower estimate.

In Rumball *et al.*'s (1994) experiment, full-sib mating was conducted in two sets of lines for 18 and 11 generations, respectively. One set of lines with first-cousin mating was also maintained for 17 generations. At the end of the period of inbreeding, about 80% of the full-sib lines and 40% of the first-cousin lines were extinct, and the realized inbreeding coefficients estimated from five to six polymorphic enzyme loci were about 80% of the pedigree values without selection. These empirical results are in agreement with the predictions shown in Figure 4.

Drosophila is a case of particularly close linkage, because it has a small genome with few chromosomes and there is no recombination within chromosomes in males. The major domestic animal or crop species have much larger genome sizes and chromosome numbers than Drosophila, and there is recombination in both sexes. For example, the pig has a haploid chromosome number of 19, and the minimum genome size is estimated to be about 23 M (Rohrer *et al.* 1996). Assuming an inbreeding load of 1.5 and 10–30 chromosomes of length 1 M per haploid genome, the realized inbreeding coefficient is only slightly decreased by selection (data not shown), similar to the case of within-line selection for selfing shown in Figure 3. Therefore, except for neutral loci closely linked with lethals or mutations of large s and small h, the difference between the realized and expected inbreeding at most neutral loci is likely to be too small to detect in a reasonably scaled experiment with full-sib mating of species with large genomes. If the proportion of extinctions is large or the inbreeding load is higher than assumed above, however, inbreeding could be further delayed.

Slow inbreeding: For less intense inbreeding than selfing or full-sib mating (*e.g.*, a random mating population with a few dozen individuals), the inbreeding process is also affected by the accompanying inbreeding depression and thus selection for fitness. The simulation results (data not shown) indicate that, with weak selection against deleterious mutations relative to genetic drift (*i.e.*, sN_e and shN_e are small, where N_e is the effective population size), the realized rate of inbreeding is smaller than expected without fitness selection. Otherwise, an increase in the realized rate of inbreeding is observed because of the elevated variances in genetic contributions within and between individuals. For example, for a random-mating Drosophila population of 50 individuals (half of each sex) with within-family selection, the asymptotic rate of inbreeding is ~92% of the

standard value if $s = 0.01$ and is ~112% if $s = 0.1$, assuming $B = 1.5$ per haploid genome (half due to lethal mutations) and $h = 0.3$. If individuals are randomly selected from the progeny pool that survived from all families, the corresponding values are 82 and 142%, respectively. Obviously, within-family selection minimizes the impact of deleterious mutations on inbreeding.

With larger population sizes, the direction and magnitude of effect of selection against deleterious mutations on inbreeding become sensitive to the distributions of s and h among mutations. By using a gamma function, different shapes of the distribution of s have been considered in the simulations (data not shown). The qualitative conclusion is that inbreeding is delayed if most mutations have small effects (shN_e small) and the depression from them is substantial. Otherwise, inbreeding is accelerated by selection against deleterious mutations due to increased variation in contribution between alternative genes within individuals and between families.

For species with the same inbreeding load but much larger genome sizes and chromosome numbers than Drosophila, simulations show that the inbreeding process is much less delayed by selection for fitness. This is expected because the apparent overdominance at neutral loci created by selection against recessive deleterious mutations diminishes much more quickly than within-individual (V_w) and between-family (V_b) variances with decreasing linkage. In the extreme case of no linkage, natural selection does not generate associative overdominance in a random-mating population and has no effect on V_w, but V_b could still be elevated, resulting in an acceleration of inbreeding in a small population. Assuming one chromosome of length 1 M with $B = 0.2$ due to mutations with $s = 0.05$ and $h = 0.2$, for example, the rate of inbreeding in a random-mating population of 20 individuals is ~95% of that expected without selection if within-family selection is applied and is ~90% if both within- and between-family selections occur.

DISCUSSION

The classical theory of inbreeding, mainly developed by Wright (reviewed in 1969), is concerned with a single neutral locus without mutation and without selection at other loci. In real populations, however, the neglect of selection is an unrealistic simplification, because natural selection cannot be wholly avoided even in laboratory experiments. The realized level and rate of inbreeding at a neutral locus, therefore, may deviate from those predicted by the classical theory. The deviation becomes serious with close inbreeding, because an association in homozygosity between the neutral and selected loci is generated (Weir and Cockerham 1973) and because deliberate inbreeding is usually accompanied by some artificial selection for characters subject to inbreeding depression.

Drosophila and other species with tight linkage: The present study shows that when inbreeding depression is caused by partially recessive mutations of deleterious effects on fitness, the realized inbreeding in Drosophila with either full-sib mating or random mating in a small population is usually delayed by natural selection for fitness. This is because apparent overdominance is created at the neutral locus, due to the positive correlation in homozygosity between the selected and neutral loci. Using the mutation parameters and inbreeding load from empirical studies, the realized inbreeding coefficient after 20 generations of full-sib mating in Drosophila is predicted to be ~70–90% of the expected value, in agreement with the empirical results of Rumball *et al.* (1994). A small experimental population of *D. melanogaster* has been maintained for about 200 generations at a harmonic mean size of 65–70, and its rate of inbreeding estimated by using the enzyme polymorphism data was only ~70% of that expected (Latter and Mulley 1995; Latter *et al.* 1995). The delay of inbreeding could be explained by assuming that many mutations of small effects were selected against during the period (Latter 1998). Frankham *et al.*'s (1993) experiment with full-sib mating in *D. melanogaster* indicated that the inbreeding process was further delayed by artificial selection for fitness. Our prediction assuming $B = 1.5$ per haploid genome is roughly in agreement with the empirical results. Because the number of loci involved in estimating the inbreeding coefficient in most empirical studies is usually small, the sampling variance may be substantial. The comparison with predictions, therefore, can be used only as a rough guide.

Selection against deleterious mutations also results in an increase in within-individual (V_w) and between-family (V_b) variances in genetic contributions to the next generation. These increased variances tend to counteract the effect of the apparent overdominance and lead to an accelerated rate of inbreeding. V_w and V_b increase much faster than the magnitude of apparent overdominance (Figure 1) with increasing intensity of selection against mutations. Compared with the standard case without selection, therefore, the decrease in heterozygosity with inbreeding is delayed with weak selection against deleterious mutations and is accelerated when s and sh are large enough (Figure 1). Because of the additional between-family variance (V_b), selection type II (within- and between-line selection) is more likely to cause an increase in the rate of inbreeding than type I, when s and sh are large. Mutation accumulation studies indicate an average homozygous effect of $\bar{s} = 0.03$–0.05 (*e.g.*, Mukai *et al.* 1972) for deleterious mutations, but most of the mutations may have a value well below this mean (Keightley 1994). Therefore, even when between-family selection is applied to full-sib mating lines of Drosophila, a delay is expected in the realized rate of inbreeding (Table 1). With a much larger size of the population and random mating, however, an accelera-

tion of inbreeding is possible if most mutations are under effective selection.

Species with loose linkage: The magnitude of the apparent overdominance depends critically on the tightness of linkage. It declines much faster than V_w and V_b with decreasing linkage. It also depends critically on the inbreeding load per chromosome. For species with a much larger genome size and chromosome number, therefore, the realized rate of inbreeding is decreased much less than in Drosophila.

Some studies provide evidence, mainly circumstantial, that the realized rate of inbreeding is smaller than that predicted by classical theory. These studies used rats, house mice, and chickens (Eriksson *et al.* 1976; Connor and Bellucci 1979; Mina *et al.* 1991). Falconer and Mackay (1996, pp. 70–72) described a mouse experiment involving 18 lines, each consisting of eight pairs of parents and maintained by minimal inbreeding (within-family selection) for 27 generations. The exact effective size (half the inverse of the rate of inbreeding) calculated from pedigree is 28.6. The effective size estimated from the heterozygosity, corrected for the small line size, at five polymorphic enzyme loci is 30.3 and is 28.7 when it is estimated from the variance in gene frequency among lines at these loci. More appropriately, the observed uncorrected heterozygosity should be used in the estimation and at least two more generations should be included in using the variance in gene frequency. Taking these into account, the estimated effective size is 32.7 from heterozygosity and is 30.8 from the variance in gene frequency, an 8 to 14% increase over the expected value. McGoldrick and Hedgecock (1997) found that, after one generation of self-fertilization of the normally outcrossing Pacific oyster *Crassostrea gigas* (Thunberg), the average inbreeding coefficient estimated from allozyme data at 14 loci was only 0.462, significantly lower than the expectation of 0.5. They also detected a substantial deficiency of homozygotes for rare alleles as compared with the expected segregation ratios, supporting the associative overdominance hypothesis.

Sex-linked loci: For full-sib mating, we also constructed a transition matrix to investigate the inbreeding process at a sex-linked neutral locus linked with another locus under selection. If the fitness of an individual in the heterogametic sex is $1 - s$ if it carries the deleterious allele and 1 if it does not, the inbreeding process is always delayed less by selection than in the autosomal case. Compared with the standard case without selection, the rate of inbreeding is decreased by selection in the first few generations and increased in later generations for within-line selection in the sex-linked case. With $r = 0.05$, $s = 0.5$, and $h = 0$, for example, the inbreeding coefficients are 0.668 and 0.888 at generations 5 and 10, respectively. The corresponding values are 0.649 and 0.872 for the autosomal case and 0.672 and 0.886 for the standard case without selection (Table 3). The acceleration of inbreeding in later generations of within-line selection at a sex-linked locus is actually similar to within- and between-line selection in the autosomal case. The reason for this is that the deleterious allele is more strongly selected against when it is at a sex-linked locus, because it is exposed in the heterogametic sex.

Empirical studies show that there is little load for viability on the sex chromosome in Drosophila, but the load for total fitness is substantial (see review by Simmons and Crow 1977). It might be interesting to compare the inbreeding coefficients at sex-linked and autosomal loci estimated by genetic markers in lines practicing full-sib mating and within-line selection. According to the predictions made here, the inbreeding coefficient in an appropriate generation (*e.g.*, 10) at the sex-linked loci would be larger and that at the autosomal loci would be smaller than the expectation without selection. To detect such a difference, however, a large data set would be needed.

Dominance or overdominance: Although both dominance and overdominance can cause inbreeding depression and their relative importance is still in dispute, it seems plausible that depression is mainly due to partially recessive mutations (Crow 1993). Therefore we have concentrated on the mutational model throughout this study. Overdominance, however, can also be considered in the present analytical model. If we set s to a very small value (*e.g.*, $s = 10^{-6}$) and $h = -d/s$, then the two homozygotes have a selection coefficient of $1/(1 + d)$. For a single locus with an equal disadvantage of the two homozygotes in full-sib mating lines, the results turn out to be the same as those of Reeve and Gower (1958). The present model, however, allows different selection coefficients of the two kinds of homozygotes.

Though dominance and overdominance are difficult to distinguish as potential causes of inbreeding depression in empirical studies, their effects on the rate of inbreeding are different if the two homozygotes have a similar disadvantage. Overdominance always results in a decrease in the rate of inbreeding at linked neutral loci, irrespective of the type of selection and the strength of selection (s). The effect on the neutral locus also persists much longer for overdominance than for dominance. For a given inbreeding load, overdominance always leads to a smaller rate of inbreeding than dominance when there is between-family selection. These differences between dominance and overdominance might be helpful in designing inbreeding experiments to distinguish the two causes of inbreeding depression in Drosophila. However, if the difference in the magnitude of disadvantage between the two homozygotes is large, the behavior of the inbreeding process with overdominance becomes similar to that with dominance shown in this article.

Interactions among mutations at different loci: A multiplicative model of fitness effects among loci was

assumed, and epistatic interactions among mutations were not considered in the simulations. In general, however, there is little compelling evidence for epistasis from experiments, except for Mukai's (1969) mutation accumulation experiment with Drosophila. Because of this and its simplicity, most previous studies adopted the multiplicative model. Furthermore, Latter (1998) has shown that, with the same amount of inbreeding depression, epistatic and multiplicative fitness models give similar results for the rate of inbreeding at linked neutral loci. Qualitatively the results from this article are unlikely to be altered by epistatic interactions among mutations.

Mating system and population size: In our analytical model, only selfing and full-sib mating are considered. Other regular systems of inbreeding, such as double first-cousin mating, can also be tackled in a similar way. For a small population with random mating, the results were obtained by stochastic simulations. Two factors in determining the effect of selection for fitness on the inbreeding process are affected by mating systems.

First, mating systems affect the relative importance of selection and genetic drift on the mutant alleles. Under within-line selection, for example, all mutants are effectively neutral with selfing, but only mutants with $s < 0.2$ approximately are effectively neutral with full-sib mating.

Second, mating systems also affect the amount of identity disequilibrium. For mating systems resulting in the same expected inbreeding coefficient for all individuals at any generation (*e.g.*, selfing, full-sib mating), identity disequilibrium is created only between linked loci. For other mating systems in which individuals differ in their expected inbreeding coefficients, identity disequilibrium is possible even if the loci are not linked (Haldane 1950). Thus in a small population with partial inbreeding, all deleterious loci, no matter whether linked to the neutral locus or not, are expected to cause associative overdominance (Charlesworth 1991) and therefore a decrease in the rate of inbreeding. When the proportion of close inbreeding is intermediate and thus the identity disequilibrium is maximized in the population, the inbreeding could be much delayed even if no linkage is assumed.

From the present analytical and simulation study, it is clear that selection against deleterious mutations has two counteracting effects on the neutral variation at linked loci, associative overdominance and elevated variance in genetic contribution. The relative importance of the two effects depends mainly on the strength of selection against deleterious mutations relative to genetic drift, sN_e and shN_e. With strong selection (sN_e and shN_e large), elevated variance in genetic contribution is the dominating effect and the effective population size is decreased by selection, resulting in smaller genetic diversity at linked neutral loci than that predicted by the neutral theory (Charlesworth *et al.* 1993; Nordborg *et al.* 1996). This process has been termed background selection (Charlesworth *et al.* 1993). It can be used to account for the positive relationship between recombination rate and genetic variability over regions of chromosomes observed in *D. melanogaster* (*e.g.*, Charlesworth 1996) and other species (*e.g.*, Stephan and Langley 1998), although hitchhiking or selective sweeps (Maynard Smith and Haigh 1974) due to strongly selected favorable mutations at linked loci could provide similar satisfactory explanations.

With weak selection (sN_e and shN_e small), associative overdominance could override the effects of background selection, resulting in an increase in effective size and thus higher than expected genetic variability at linked neutral loci and a positive association of individual heterozygosity and fitness. Many empirical studies have shown a positive correlation between individual heterozygosity at marker loci and fitness traits, mostly in species with either a substantial heterozygote deficiency (putatively due to inbreeding) or a small and structured population (*e.g.*, David *et al.* 1997). Associative overdominance as well as inbreeding could contribute to the observed relations. The delay of inbreeding in experiments cited above is likely to be due to associative overdominance built up at neutral loci linked to the numerous fitness loci with deleterious mutations under weak selection.

Given the uncertainty about the means and distributions of s and h for deleterious mutations and their rates of occurrence, as well as N_e of natural populations, it is difficult to predict the effect of selection against mutations on linked neutral variation. Roughly speaking, however, background selection is the dominating force in shaping the neutral variation in large random-mating populations. In populations with substantial inbreeding due to their small sizes, subdivision, or partial inbreeding, associative overdominance may become important and should be considered together with background selection. Most previous formulations considered either background selection (*e.g.*, Charlesworth *et al.* 1993; Nordborg *et al.* 1996; Santiago and Caballero 1998) or associative overdominance (*e.g.*, Ohta 1971; Ohta and Cockerham 1974), but not both. Further work is needed to incorporate background selection and associative overdominance, particularly for nearly neutral mutations.

Implications: Most inbreeding studies in Drosophila and other species adopt the inbreeding coefficient predicted from classical theory as a measurement of the level of homozygosity (*e.g.*, Garcia *et al.* 1994; Saccheri *et al.* 1996). While the predicted value may provide a rough guide, it may well be an underestimation with close inbreeding, especially for species with restricted recombination in the genome such as Drosophila. Typically in these experiments with close inbreeding, a large number of lines are lost due to inbreeding depression (Garcia *et al.* 1994). The delay in realized inbreeding,

therefore, might be substantial. To account for the bias of prediction, a number of molecular marker loci could be used to estimate the realized rate or level of inbreeding. This, however, incurs much additional work in the inbreeding experiments.

The results also have implications for the establishment of inbred lines. For Drosophila and other species in which linkage is important, more generations of inbreeding than predicted by theory are required to attain a given level of homozygosity. For example, to decrease the heterozygosity by 90%, 11 generations of full-sib mating are required if there is no selection for fitness during the process of inbreeding (Falconer and Mackay 1996), but ~20–30 generations of full-sib mating, predicted from the present model, are necessary to reach the same goal in Drosophila because of the inevitable selection against partially deleterious mutations and therefore against homozygosity.

For species with a much larger genome size than Drosophila, this problem is less important. If, however, partial inbreeding is applied to the population so that the increase in homozygosity is realized mainly due to an intermediate proportion of matings between close relatives, then the inbreeding process could also be substantially delayed by selection against deleterious mutations.

In large outbreeding populations, on the contrary, inbreeding and genetic drift are likely to be accelerated by background selection, resulting in lower genetic diversity than that predicted by the neutral theory.

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