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Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate

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Summary

A multilocus stochastic model is developed to simulate the dynamics of mutational load in small populations of various sizes. Old mutations sampled from a large ancestral population at mutation–selection balance and new mutations arising each generation are considered jointly, using biologically plausible lethal and deleterious mutation parameters. The results show that inbreeding depression and the number of lethal equivalents due to partially recessive mutations can be partly purged from the population by inbreeding, and that this purging mainly involves lethals or detrimentals of large effect. However, fitness decreases continuously with inbreeding, due to increased fixation and homozygosity of mildly deleterious mutants, resulting in extinctions of very small populations with low reproductive rates. No optimum inbreeding rate or population size exists for purging with respect to fitness (viability) changes, but there is an optimum inbreeding rate at a given final level of inbreeding for reducing inbreeding depression or the number of lethal equivalents. The interaction between selection against partially recessive mutations and genetic drift in small populations also influences the rate of decay of neutral variation. Weak selection against mutants relative to genetic drift results in apparent overdominance and thus an increase in effective size (N_e) at neutral loci, and strong selection relative to drift leads to a decrease in N_e due to the increased variance in family size. The simulation results and their implications are discussed in the context of biological conservation and tests for purging.

1. Introduction

Classical mutation accumulation studies in *Drosophila* have indicated a high rate of mutation to deleterious alleles: about one mutation per diploid genome per generation (Mukai, 1964; Simmons & Crow, 1977). A few recent investigations yielded similar results in various plant species (Charlesworth *et al.*, 1990, 1994; Johnston & Schoen, 1995), but much lower estimates were obtained from selfing lines of *Caenorhabditis elegans* (Keightley & Caballero, 1997) or full-sib mating lines of *Drosophila* (García-Dorado, 1997). If deleterious mutations occur at a high rate and are partially recessive, a large number of mutations will be maintained in a large population in mutation–selection balance, constituting the mutational load borne by the population. Mutational load is believed to be the main genetic basis for inbreeding depression (Charlesworth & Charlesworth, 1987; Barrett & Charlesworth, 1991; Crow, 1993; Dudash & Carr, 1998; but see Charlesworth, 1998), which has been observed for various fitness traits in nearly all diploid species investigated. It is important in relation to the evolution of mating systems, the breeding and improvement of domesticated species, and the conservation of endangered plant and animal species (Charlesworth & Charlesworth, 1987; Frankham, 1995*b*).

Given a high mutation rate, and thus substantial mutational load and inbreeding depression maintained in a large population, what is expected from a change in the level of inbreeding caused by a decrease in population size or an alteration of the mating system? This question is of practical concern in relation to caged populations captured from the wild and to populations used for conservation and improvement,

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which are usually maintained with a small effective size. Previous theoretical studies and empirical investigations showed that inbreeding has multiple effects on the dynamics of deleterious mutations and their population-level consequences. Inbreeding results in an increase in homozygosity and in the fixation probability of deleterious mutants, leading to decreased fitness and thus potentially to a higher risk of extinction of the population (Charlesworth *et al*., 1992; Hedrick, 1994; Lynch *et al*., 1995*a*; Schoen *et al*., 1998). It also enhances the effectiveness of selection against partially recessive mutants of deleterious effects, because they are more likely to be in homozygous state and thus exposed to selection, leading to the progressive purging of mutants (Lande & Schemske, 1985; Hedrick, 1994; Lynch *et al*., 1995*a*), thereby reducing inbreeding depression and allowing a possible recovery of fitness (Crow, 1970; Barrett & Charlesworth, 1991).

The overall outcome of the interacting effects of inbreeding and selection depends on many factors, including the nature of the mutational load (i.e. the mutation rate and the distributions of mutant selective effects and dominance coefficients), the rate of inbreeding, the reproductive capacity, and the organization of the genome (number of chromosomes and their map lengths) of the species. Hedrick (1994) showed that full-sib mating can quickly purge lethal mutations but is ineffective for mutations of small effect. Full-sib mating also results in a substantial decrease in fitness (inbreeding depression), and thus a high risk of extinction of the population. However, Hedrick did not consider new mutations arising during inbreeding and selection, and he ignored linkage and variation in selective effects and dominance coefficients. Schoen *et al*. (1998) investigated the purging of the plausible mutational load inferred from empirical estimates of mutation parameters in populations of various sizes. They assumed equal selection coefficients and dominance coefficients of all mutants and ignored lethal mutations and linkage. Linkage may be an important factor in relation to the purging of deleterious mutants, and also for determining the rate of decay of neutral variation in species with restricted recombination, such as *Drosophila*. A stochastic simulation study by Latter (1998) showed that many linked mutants with small deleterious effects could explain both the loss of fitness and the observed lower-than-expected rate of inbreeding at marker loci in *Drosophila* experiments with slow inbreeding (Latter & Mulley, 1995; Latter *et al*., 1995).

In this paper, we study by simulation the dynamics of deleterious mutants and their observed effects on inbreeding depression, extinction rate, and the genetic variation at neutral loci after a large population becomes restricted in size. Both detrimental and lethal mutants are considered, using mutation parameters estimated from various experiments. Old mutations inherited from the large ancestral population at mutation–selection balance and new mutations arising during the process of purging selection are considered jointly, and linkage and other complexities (such as identity disequilibrium) are taken into account in the stochastic simulations. The results are compared with experimental observations where possible, and are discussed in the context of the conservation of endangered species, testing for purging and other issues.

2. Methods

(i) *Mutation parameters*

By using balancer chromosomes with visible markers and exploiting the lack of recombination in males, Mukai and colleagues (Mukai, 1964; Mukai *et al*., 1972) accumulated deleterious mutations on intact second chromosomes of *Drosophila melanogaster* in the virtual absence of counter-selection. They obtained estimates of the rate of mutation to deleterious alleles of 0·5–1·0 per diploid genome per generation (U), a mean effect ($\bar{s}$) of 0·01–0·05 for homozygous mutations, and a mean dominance coefficient ($\bar{h}$) of 0·2–0·4. More recent indirect estimates using information on inbreeding depression in naturally selfing plant populations (Charlesworth *et al*., 1990; Johnston & Schoen, 1995) and *Drosophila* (Lynch *et al*., 1995*a*) yielded similar results, assuming mutation–selection balance.

The distributions of homozygous effects (s) and dominance coefficients (h) of mutations around their mean values are of crucial importance for the accumulation of mutations, inbreeding depression, and the short- or long-term survival of small populations. Unfortunately, however, little is known about these distributions. Using maximum likelihood, Keightley (1994) reanalysed the data from two published mutation accumulation experiments. The analysis indicated that the distribution of mutant effects is leptokurtic, with the vast majority of mutants having very small effects. Both sets of data in the reanalysis could be approximated by an exponential distribution of mutant effects, i.e. $P(s) = (1/\bar{s})\exp(-s/\bar{s})$ (Keightley, 1994). Although the exact shape of the distribution of s is very uncertain and open to further investigation, it seems highly likely that the distribution is leptokurtic. In this study we assume either an exponential distribution of effects (e.g. Mackay *et al*., 1992) or equal effect of all mutations (e.g. Schoen *et al*., 1998).

Direct information on the distribution of the dominance coefficients of mutants is also scarce. Biochemical arguments suggest an inverse relationship between the magnitude of effect of a mutation and its degree of dominance, which is supported by evidence

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from mutation accumulation experiments (Simmons & Crow, 1977). A review of the data from several experiments (Caballero & Keightley, 1994) suggests that the mean dominance coefficient (h) of mutants with effect s can be described as $h = \exp(-ks)/2$, where constant k is chosen so that the average dominance coefficient of all mutants with various effects is $\bar{h}$. The predictions from this model fit well with empirical observations, although there are ascertainment problems for mutants with very small effects. In the present study we will use either this exponential model (e.g. Wang *et al*., 1998) or an equal dominance coefficient for all mutants (e.g. Schoen *et al*., 1998).

The above mutation parameters refer to deleterious mutants which are not lethal when homozygous individually. A mutant is usually classified as homozygous lethal if its viability is less than 10% of the wild-type value (Simmons & Crow, 1977). In *Drosophila*, these lethal mutations occur at a rate of about 0·03 per diploid genome per generation (U_L), with an average dominance coefficient of about 0·02–0·03 (Crow, 1993). Throughout the present study we will use these lethal mutation parameters in combination with the above mutation parameters for deleterious mutations of small effect (detrimentals).

(ii) *The stochastic simulation model*

A stochastic simulation program was developed to simulate the dynamics of mutations in a small population of N individuals (equal numbers of each sex) each generation. The initial population (generation zero) was formed by sampling randomly from an infinitely large population at mutation–selection balance. We assumed that the haploid genome consists of two chromosomes of the same length (L) of 1 M. This is roughly the case for *D. melanogaster*, which has two major autosomes with similar lengths and mutation loads for viability, a sex chromosome with little viability load, and a tiny chromosome 4 that can be neglected (Charlesworth & Charlesworth, 1987). A total number of 2900 loci ($\frac{1}{2}M$) mutable to deleterious or lethal alleles affecting viability were assumed to be spread along each chromosome with an equal space between adjacent loci. At each locus, a wild-type and a mutant allele were assumed, and the equilibrium mutant allele frequency (q) in the ancestral population was obtained from the equation $s(1-2h)\,q^2 + sh(1+u)\,q - u = 0$ (Crow & Kimura, 1970, p. 260), where u is the genic mutation rate given by $u = (U+U_L)/(2M)$. The genotype at each locus of each individual in the initial population was drawn at random from the genotypes of the ancestral population in Hardy–Weinberg proportions with the mutant allele frequency calculated above.

A total number of 200 neutral loci with four alleles at equal frequencies (0·25) were also included in the simulations. These loci were equally spaced along the chromosomes, with 29 selected loci between every two adjacent neutral loci. The heterozygosity and number of alleles at each locus, averaged over loci and replicate lines, were monitored over generations. The actual (observed) inbreeding coefficient at generation t at these loci was obtained in two ways. First, it was calculated from the frequency of heterozygotes of a particular allele averaged over replicate lines (H_t), relative to the level of heterozygosity in a large panmictic population with the same average gene frequency (q_t), i.e. $F_{H,t} = 1 - H_t/[2q_t(1-q_t)]$. Because of the absence of mutation and selection at neutral loci and their merely partial linkage to fitness loci, we have $q_t \approx q_0$, the initial allele frequency. Secondly, it was calculated from the variance in frequency of a neutral allele among replicate lines ($V_{q,t}$), i.e. $F_{V,t} = V_{q,t}/[q_t(1-q_t)]$. For both estimates, averages were taken over all neutral loci. The actual mean inbreeding ($N_{eI,t}$) and variance ($N_{eV,t}$) effective sizes over a period of t generations of inbreeding were calculated from $F_{H,t}$ and $F_{V,t}$ by $1/N_{eI,t} = 2[1-(1-F_{H,t})^{1/t}]^t$ and $1/N_{eV,t} = 2[1-(1-F_{V,t})^{1/t}]^t$ respectively. The theoretical inbreeding coefficient (F_t) and thus mean effective size ($N_{e,t}$) over a period of t generations were also calculated from the pedigree. As will be shown, $N_{eI,t}$, $N_{eV,t}$ and $N_{e,t}$ are not always the same.

The initial population was maintained for 50–100 generations, an upper limit to the maximum time scale usually considered in the conservation context or in laboratory populations. In each generation, the sequence of operations was mutation, mating, reproduction and selection, similar to that in previous studies (Kondrashov, 1985; Charlesworth *et al*., 1992). The number of new mutations arising in an individual at any generation was assumed to follow a Poisson distribution with mean $U+U_L$, and the loci where mutations occurred were chosen at random. Mutations were assumed to be unidirectionally from wild-type to mutant alleles only, which is reasonable because back-mutation rates are generally much lower than forward-mutation rates. It is possible that the locus randomly selected for mutation is homozygous for the mutant allele, resulting in a failure of mutation. However, the probability of mutation failure is very small, because we modelled small populations with a large number of loci, over only a small number of generations. To decrease further the probability of mutation failure, loci at which mutant alleles became fixed were re-set to the wild-type state, although fitness and other quantities were calculated including the contribution from these fixed loci. All neutral loci were assumed to be non-mutable.

After the mutations had been performed for all individuals of the population, males were randomly mated with females, forming $N/2$ families. Following

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mating, reproduction began, with each family contributing n offspring before selection ($n = 14$ in what follows, except where explicitly stated). The procedure for generating a new offspring was as follows. First, a gamete was generated from each mated parent. Male gamete creation involved no recombination within chromosomes. For maternal gametes, the number of crossovers on each chromosome was drawn from a Poisson distribution, with the mean number of crossovers being L. The locations where crossovers occurred were randomly chosen along the chromosome, without interference between different crossover events at different loci. Secondly, the male and female gametes were combined to form the new zygote. The viability of a zygote was calculated, using the multiplicative model, as $\nu = \prod_{i=1}^{M} \nu_i$, where ν_i is the relative viability at locus i and is 1, $1-h_i s_i$ or $1-s_i$ if it is a wild-type homozygote, a heterozygote or a mutant homozygote at the locus, respectively. For selection, ν was re-scaled so that the mean viability of the initial population was 0·8, roughly the mean egg-to-adult viability of *Drosophila* populations recently captured from the wild in several experiments (see Wang *et al.*, 1998). The re-scaling reduced the computing time without changing the simulation results. A random number was generated and compared with ν to determine whether the zygote survived (it survived if the random number was between 0 and ν). Therefore, the number of surviving offspring varied among families, though they generated an equal number of zygotes. The sex of each surviving offspring was determined by a random binary number, with an equal probability of being male or female. If the number of surviving offspring of any sex was zero, then the population (replicate) became extinct. Extinct populations were not replaced by viable ones. If the number of surviving offspring of any sex was larger than $N/2$, then only $N/2$ offspring were randomly selected as the next generation. Crosses were made among replicate populations at a given generation (inbreeding coefficient). The genotypes of each replicate population were stored and used to create crossbreds with the succeeding replicate population.

For each combination of parameters, 3000 replicates were run for 50 or 100 generations (unless extinction occurred). The variables calculated were: the numbers of heterozygous and homozygous mutations per individual, the numbers of loci fixed and segregating for mutant alleles in the population, the number of lethal equivalents per individual (the sum of the s values for every mutant allele carried by an individual, whether at heterozygous or homozygous loci; see Cavalli-Sforza & Bodmer, 1971, p. 364), inbreeding depression (expressed as the decrease in viability of completely homozygous (ν_I) individuals relative to outbred individuals (ν_O), i.e. $D = 1-\nu_I/\nu_O$), mean viability of the population relative to that of the initial population, extinction rate (proportion of lines extinct), theoretical (N_e), inbreeding (N_{eI}) and variance (N_{eV}) effective sizes.

Because the mutation parameters required for modelling are mostly concerned with viability in *Drosophila*, we will concentrate on this genus throughout, except where otherwise stated.

3. Results

(i) *Effects of mutation parameters on genetic loads and inbreeding depression*

First, we examine the effects of the distributions of selective effects and dominance coefficients of deleterious mutants. Fig. 1 shows the changes in mean viability relative to that of the initial population, the average numbers of homozygous and heterozygous mutations per individual, the numbers of segregating and fixed loci for deleterious mutations in the population, and the decreases in the number of lethal equivalents and inbreeding depression over generations in populations with 50 individuals. When mutant effects are exponentially distributed, there is a large proportion of mutants with very small effects. These mutants are held at a substantial frequency in the large ancestral population at mutation–selection balance, and therefore a large number of loci with mutants of small effects are sampled and segregate in the initial populations (Fig. 1 *d*). Although the average number of homozygous mutations per individual and the number of fixed loci increase much faster over time with the exponential distribution of mutant effects (Fig. 1 *b*, *e*) than with equal effects, the rate of decrease in viability is nevertheless lower (Fig. 1 *a*). This is because, with the exponential distribution of s, most of the mutants homozygous or fixed are of very small effect and thus have little effect on the mean viability.

Compared with the other two cases, exponential distributions of both s and h result in a higher initial rate of decrease in viability, and a lower rate later on (Fig. 1 *a*). The non-linear change in viability also holds when viability is transformed to its natural logarithm and plotted against inbreeding coefficient (Fig. 1 *h*). This non-linear decrease in viability with inbreeding reflects the inverse relationship between s and h; mutations of larger effect are more recessive and thus all mutants, including lethals, tend to have a similar heterozygous effect and also a similar equilibrium frequency in the large ancestral population (Crow, 1993). With inbreeding, mutants of larger effect and smaller dominance coefficient are exposed to selection because of the increased homozygosity, leading to both a sharp decrease in viability and more effective purging of them. After a few generations of purging, the viability therefore decreases at a lower rate.

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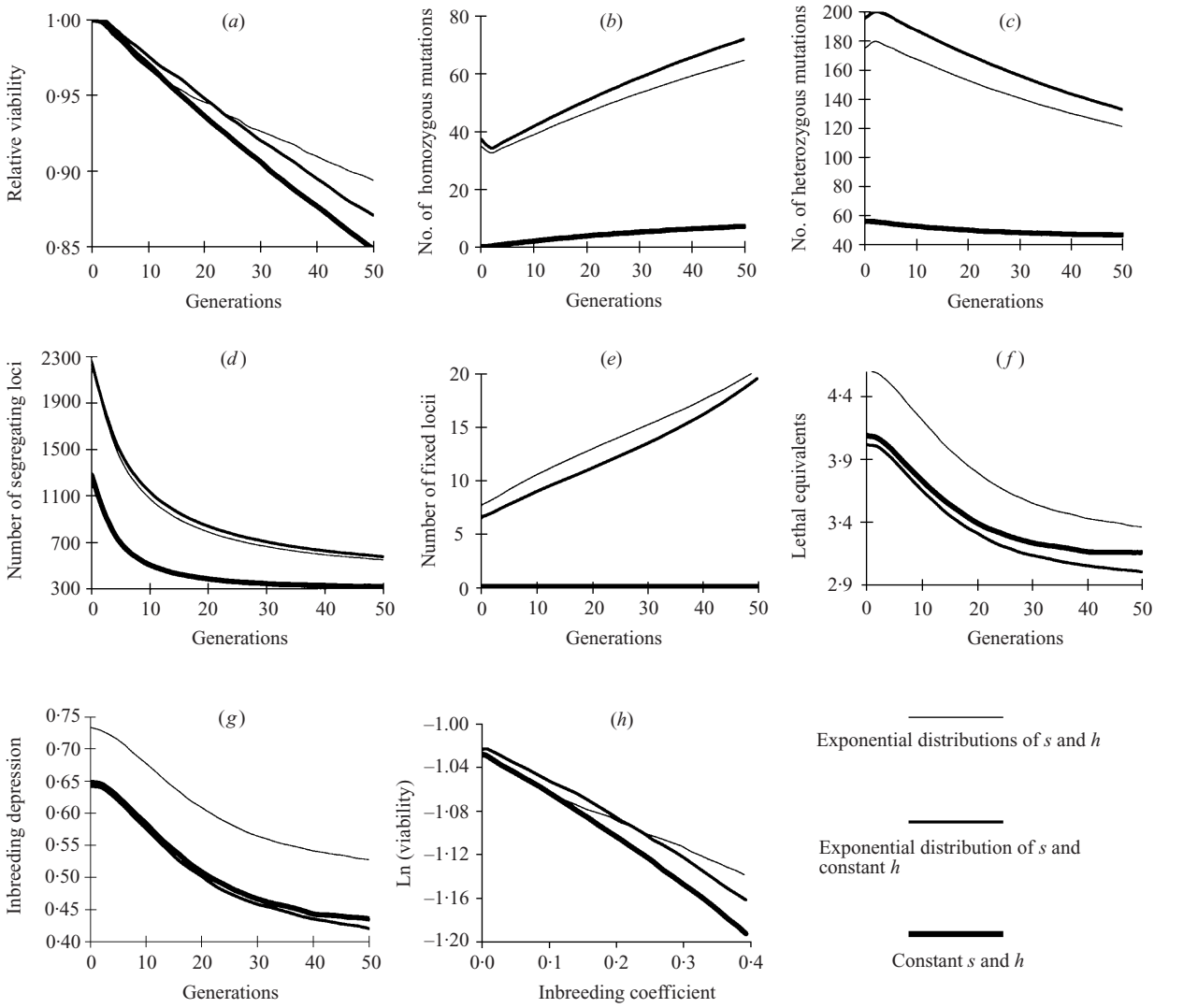

Fig. 1. Changes in genetic properties of a population of 50 individuals (half of each sex) over 50 generations of inbreeding and selection. (*a*) Mean viability relative to the initial population. (*b*) Mean number of homozygous mutations per individual. (*c*) Mean number of heterozygous mutations per individual. (*d*) Number of segregating loci. (*e*) Number of loci fixed for mutations. (*f*) Number of lethal equivalents. (*g*) Inbreeding depression. (*h*) Natural log of mean viability plotted against inbreeding coefficient. The mutation parameters are $U = 1{\cdot}0$, $\bar{s} = 0{\cdot}05$ and $\bar{h} = 0{\cdot}36$ for detrimentals, and a mutation rate of 0·03 and a dominance coefficient of 0·02 for lethals.

The above explanation for the non-linear decline in viability when s and h are both exponentially distributed is made clearer in Fig. 2, which shows the changes in viability over 100 generations in a population of 50 individuals, relative to that of the ancestral population, for five classes of mutants (classified by the magnitude of their homozygous effects). The viability, ν_j, due to mutants of class j is the product of the relative viabilities from all loci at which the mutant's selective effect falls into class j. Therefore the total viability is $\nu = \prod_{j=1}^{5} \nu_j$. The deleterious mutation parameters used in Fig. 2 are $U = 1{\cdot}0$, $\bar{s} = 0{\cdot}05$, $\bar{h} = 0{\cdot}36$, and s and h follow exponential distributions. Lethal mutants are purged rapidly and the viability due to them increases from the first generation. With the parameters assumed,

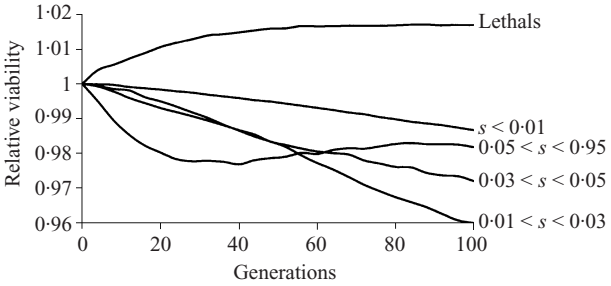

Fig. 2. Changes in mean viability relative to the initial population due to various classes of mutants in a population of 50 individuals over 100 generations. The deleterious mutation parameters are $U = 1{\cdot}0$, $\bar{s} = 0{\cdot}05$ and $\bar{h} = 0{\cdot}36$; both s and h have exponential distributions. The lethal mutation rate is 0·03 and the dominance coefficient of lethal alleles is 0·02.

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Table 1. *Number of lethal equivalents and inbreeding depression contributed by different classes of mutations at different generations* (t)[a]

	No. of lethal equivalents at generation			Inbreeding depression at generation		
Mutant effects (s)	$t = 0$	$t = 25$	$t = 50$	$t = 0$	$t = 25$	$t = 50$
$< 0{\cdot}01$	0·35	0·32	0·30	0·007	0·006	0·005
0·01–0·03	0·62	0·58	0·56	0·045	0·038	0·034
0·03–0·05	0·52	0·50	0·50	0·070	0·061	0·057
$> 0{\cdot}05$	1·78	1·58	1·48	0·420	0·358	0·329
Lethals	1·34	0·68	0·52	0·479	0·281	0·224
Total	4·61	3·65	3·36	0·734	0·583	0·527

[a] The population size is 50, mutation parameters for detrimentals are $U = 1$, $\bar{s} = 0{\cdot}05$, $\bar{h} = 0{\cdot}36$, and both s and h have exponential distributions.

deleterious mutants with effects larger than 0·05 cause a large decrease in viability for the first 40 generations, but the trend is reversed in later generations. The implication is that, if s and h are indeed negatively related, mutants of large effect are more harmful for the short-term survival of small populations than mutants of small effects, especially with fast inbreeding, though the former are expected to be purged from the population and thus pose little threat to the long-term survival of small populations. Fig. 1*h* and Fig. 2 also imply that, while a non-linear decline in fitness might suggest purging selection, a linear decrease in log-transformed fitness with inbreeding does not preclude the possibility of purging selection. When only a small proportion of the mutational load is due to mutants of large effects which can be purged, the decrease in fitness with inbreeding still appears to be linear.

The distribution of dominance coefficients has a large effect on the absolute number of lethal equivalents and amount of inbreeding depression (Fig. 1*f*, *g*). More mutants of large effect and small dominance coefficient are harboured in the large ancestral population when s and h are inversely correlated than without such a correlation, leading to an elevated number of lethal equivalents and inbreeding depression in the ancestral population. Charlesworth & Charlesworth (1987) summarized data on inbreeding depression for egg-to-adult viability in four species of *Drosophila*. The total depression is about 0·63–0·77, and about half the depression is from lethals. These results are fairly similar across species. The predictions for the three cases in Fig. 1*g* are consistent with the empirical results for total depression, but a slightly higher proportion of inbreeding depression ($\sim 60\%$) is from lethals when all detrimental mutants are assumed to have the same dominance coefficient (0·36). The exponential relation between h and s also results in a slightly lower rate of decrease in inbreeding depression over the later period of 50 generations (Fig. 1*g*), but it has little effect on the rate of change in the number of lethal equivalents (Fig. 1*f*). In contrast, the exponential distribution of s leads to a slightly higher rate of decrease in the number of lethal equivalents in the later period (Fig. 1*f*), but it has no consistent effect on the purging of inbreeding depression (Fig. 1*g*).

Mutants of different effects are not proportionally purged from the population with inbreeding. The number of lethal equivalents and inbreeding depression from different classes of mutants in a population of 50 individuals at generation 0, 25 and 50 are compared in Table 1, when s and h have exponential distributions. The number of lethal equivalents and the inbreeding depression from all classes of mutants are decreased by inbreeding, but most of the decrease occurs by elimination of lethal mutations and mutations of large effects in the first 25 generations of inbreeding. In the initial population, 29% of the lethal equivalents and 50% of the inbreeding depression are due to lethal mutations. After 50 generations of slow inbreeding in a population restricted to 50 individuals, the corresponding values fall to 16% and 36%, respectively. However, the load from lethal mutations cannot be completely purged from the population, because they are highly recessive, and also because new lethal mutations arise each generation. The number of lethal equivalents and inbreeding depression from deleterious mutations of small effects are only slightly reduced by inbreeding, and the reductions are mainly due to genetic drift rather than selection. Genetic drift does not in itself change the mean frequency of alleles, but it results in the dispersion of allele frequencies around this mean, leading to an increased homozygosity for deleterious alleles and thus a decrease in the number of lethal equivalents and amount of inbreeding depression remaining in the population. In *Drosophila*, the genetic loads from lethals and detrimentals can be measured

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Table 2. *The viability and number of lethal equivalents relative to those of the large ancestral population in inbred and outbred populations*[a]

Generations	Inbreeding coefficient	Viability		No. of lethal equivalents	
		Inbreds	Outbreds	Inbreds	Outbreds
20	0·17	0·943	1·025	0·826	0·858
30	0·26	0·926	1·019	0·774	0·822
40	0·34	0·907	1·010	0·745	0·805
50	0·41	0·893	1·002	0·729	0·801
100	0·63	0·798	0·924	0·721	0·835

[a] The population size is 50, mutation parameters for detrimentals are $U = 1$, $\bar{s} = 0{\cdot}05$, $\bar{h} = 0{\cdot}36$, and both s and h have exponential distributions.

separately in experiments using balancer chromosomes (Simmons & Crow, 1977), and the disproportionate effects of inbreeding on these components of the load would indicate purging of lethals.

In Table 2, the number of lethal equivalents and the mean viability of crossbreds generated by crosses between replicate inbred populations, relative to those of the large ancestral population, are compared with the corresponding values for inbreds in different generations. The same population size and mutation parameters as in Table 1 are used in the calculations. Crossbreds always have a higher viability and a larger number of lethal equivalents than inbreds, and the differences increase with increasing levels of inbreeding. Crossbreeding combines separate sets of deleterious mutations from different populations, resulting in a great increase in the number of heterozygous loci and a large decrease in the number of homozygous loci per individual, and thus an increase in the number of lethal equivalents and viability.

Compared with the ancestral population, however, crossbreds have a very limited mean increase in viability at low levels of inbreeding, although their number of lethal equivalents is lower. First, let us consider lethals and mutations of large effect. Viability is little affected by these mutations in both the ancestral and crossbred populations, because they are few and mostly found in heterozygotes which have a similar viability to that of heterozygotes for mildly deleterious mutations. On the other hand, these mutations contribute substantially to the number of lethal equivalents, because in this case they are evaluated as homozygotes. Therefore, the partial purging of these mutations by selection results in an evident decrease in the number of lethal equivalents but little change in viability of the crossbreds. Secondly, let us consider mutations of small effects. These mutations are the vast majority and mainly determine the viabilities in the ancestral and crossbred populations. Because of their small effects they are not purge by selection but are driven to fixation or lost by drift, with their mean frequencies over replicate populations remaining unchanged. On crossing, therefore, the viability and the number of lethal equivalents due to them return to the original values of the ancestral population.

The mean viability of crossbreds decreases with increased inbreeding. At high levels of inbreeding, the viability of crossbreds is even lower than that of the ancestral population (Table 2). This is because the load due to mildly deleterious mutations is not purged from the population, but is increasing over generations due to new mutations. After some generations, the increased load from the accumulation of mildly deleterious mutations surpasses the decreased load from the purging of lethals and mutations of large effect, resulting in a lower viability of crossbreds compared with that of the ancestral population. Results similar to Table 2 for crossbreds are obtained with different distributions of selection coefficients and dominance coefficients of mutations.

(ii) *Effects of selection against deleterious mutations on neutral variation*

The behaviour of neutral variation in small populations is affected by selection against deleterious mutations. Changes in neutral genetic variation (such as heterozygosity and number of alleles) can be conveniently summarized by the parameters N_{eI} and N_{eV}, which are shown in Fig. 3 together with N_e, the theoretical effective size calculated from the pedigree. The conclusions from the simulations are as follows.

1. In the first few generations, N_{eV} is smaller than N_{eI}, but the two values converge to the same asymptotic value. In an idealized Fisher–Wright population with the additional assumption of separate sexes, N_{eV} and N_{eI} reach the same value in two generations (see Caballero, 1994). With selection, however, the realization of the asymptotic effective size is delayed (Fig. 3). Therefore, the behaviour of neutral variation in small populations cannot be predicted accurately in the initial generations using the asymptotic effective size.

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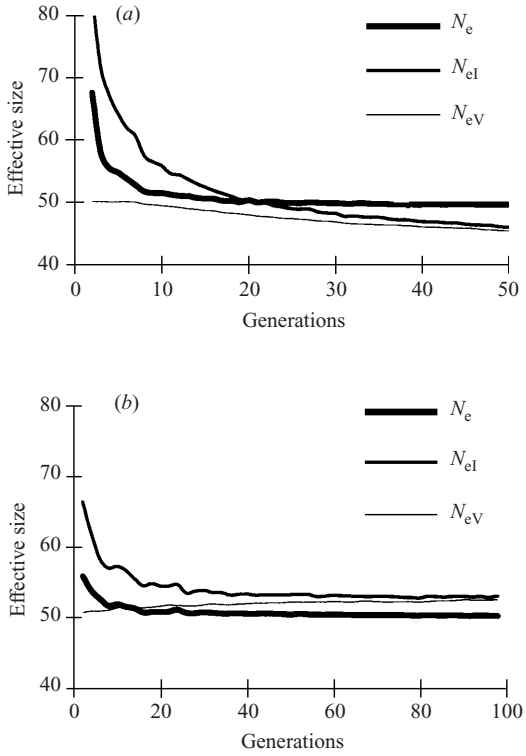

Fig. 3. Changes over generations in inbreeding (N_{eI}) and variance (N_{eV}) effective sizes for linked neutral loci, and theoretical effective size (N_e), of a population of 50 individuals. The lethal mutation rate is 0·03 and the dominance coefficient of lethal alleles is 0·02. The deleterious mutation parameters are $U = 1{\cdot}0$, $\bar{h} = 0{\cdot}36$, and $\bar{s} = 0{\cdot}05$ in (*a*) and $\bar{s} = 0{\cdot}01$ in (*b*). Both *s* and *h* have exponential distributions.

2. Compared with N_e, the asymptotic value of N_{eV} and N_{eI} is smaller for strong selection against deleterious mutations (Fig. 3*a*) and is larger for weak selection (Fig. 3*b*). Two forces affect the inbreeding and genetic drift at neutral loci linked to loci under selection. First, a detrimental mutation under strong selection will soon become lost from the population, eliminating linked neutral variation (Charlesworth *et al.*, 1993; Hudson & Kaplan, 1995; Nordborg *et al.*, 1996; Santiago & Caballero, 1998). Secondly associative overdominance at neutral loci, caused by the segregation of linked deleterious mutations, impedes the decay of neutral variation, resulting in a possible increase in effective size (Ohta, 1971; Charlesworth, 1991; Latter, 1998). The relative importance of these two counteracting forces depends on the relative strengths of selection and drift. When $N\bar{s}$ is small, the fate of mutants is mainly determined by drift. In such a case, associative overdominance is the main factor if $\bar{h} < 0{\cdot}5$, and therefore the asymptotic effective size is increased (Fig. 3*b*).

3. N_e is also affected by linked deleterious mutants. If there is no selection against deleterious mutants in a population under the same breeding system as that in Fig. 3, the simulated N_e is 51·8 and the simulated asymptotic value of N_{eV} and N_{eI} is 51·6, which are in close agreement and are also close to the prediction (51·6) from standard equations (Crow & Kimura, 1970, p. 351), as expected. Selection against mutations is predicted to decrease N_e, due to the increased variance in family size (Santiago & Cabellero, 1995, 1998; Lynch *et al.*, 1995*b*). For the two cases shown in Fig. 3, the asymptotic values of N_e are 49·7 and 50·2 respectively, both slightly smaller than the corresponding value without selection. When mutant effects are large, N_e is substantially reduced compared with the corresponding value without selection (data not shown).

4. With given values of $\bar{s}$ and $\bar{h}$, the distributions of *s* and *h* also influence N_e, N_{eV} and N_{eI}, but the effects are not large (data not shown). This is because the distributions of *s* and *h* affect the proportion of mutations under effective selection.

(iii) *Effects of the rate of inbreeding on mutational load*

The mean viability relative to the initial population, the number of lethal equivalents and the inbreeding depression are plotted against inbreeding coefficient in Fig. 4 for various population sizes. The inbreeding

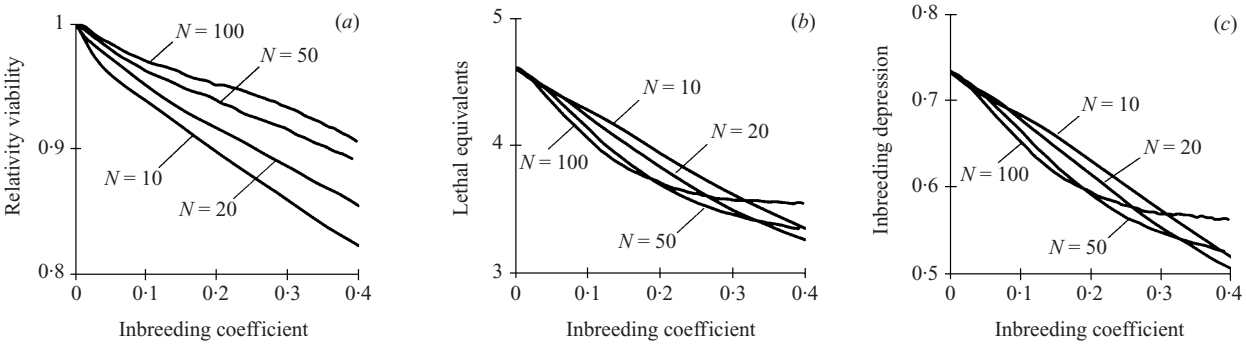

Fig. 4. Effects of the rate of inbreeding. (*a*) Mean viability relative to the initial population. (*b*) Number of lethal equivalents. (*c*) Inbreeding depression. The deleterious mutation parameters are $U = 1{\cdot}0$, $\bar{s} = 0{\cdot}05$ and $\bar{h} = 0{\cdot}36$; both *s* and *h* have exponential distributions. The lethal mutation rate is 0·03 and the dominance coefficient of lethal alleles is 0·02.

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coefficient is calculated from the pedigree, but the figure does not change much if F_H or F_V is used. At the same level of inbreeding, larger populations give a consistently higher fitness than smaller populations (Fig. 4*a*). This is presumably due to three factors. First, a larger population requires more generations to reach a given level of inbreeding, and so it has a greater opportunity to purge deleterious mutations. Secondly, with a given set of mutation parameters, a higher proportion of mutants will be under effective selection, and thus possibly purged, in a larger population. Thirdly, at the same expected level of inbreeding, selection causes the realized level of inbreeding at deleterious loci to vary among populations of different sizes. With the same expected inbreeding coefficient, a larger population tends to have a smaller extent of drift at the selected loci (see below), resulting in a higher viability. Therefore, slow inbreeding results in a higher fitness than fast inbreeding, when populations are compared at the same level of inbreeding. This is supported by empirical evidence from a *Drosophila* experiment (Ehiobu *et al*., 1989). However, this is not a good test for purging, because the realized level of inbreeding or drift still varies among populations of different sizes at the same expected level of inbreeding.

The patterns of changes in the number of lethal equivalents and inbreeding depression (Fig. 4*b*, *c*) are similar. A lower rate of inbreeding (larger population size) gives rise to a smaller number of lethal equivalents or inbreeding depression when the inbreeding coefficient is small, but the trend is reversed at higher levels of inbreeding. Most of the purging occurs during the initial period of inbreeding (Table 1), and therefore larger populations have a smaller number of lethal equivalents or inbreeding depression due to their more effective purging. After this, however, drift dominates the dynamics of deleterious mutations. For the same level of inbreeding at the neutral locus, the realized level of inbreeding at loci with deleterious alleles tends to be smaller in larger populations than in smaller populations. The difference increases over generations. This is reflected by a larger number of heterozygous loci and a smaller number of homozygous loci per individual in a larger population, for the same expected level of inbreeding at the neutral locus (data not shown). A smaller population therefore gives rise to a faster decrease in, and eventually a lower value of, the number of lethal equivalents or inbreeding depression, due to its stronger genetic drift. At a given level of inbreeding, an optimum rate of inbreeding (or population size) therefore exists that gives the population the lowest level of lethal equivalents and inbreeding depression.

The changes in the viability of crossbreds over generations (inbreeding coefficient) show a similar pattern to Fig. 4*b*, *c*, but the magnitude of the difference among populations of different sizes is much smaller (data not shown).

(iv) *Effects of family size on the mutational load and population extinction*

For a fixed number of offspring per family (n), the probability of population extinction is increased with inbreeding because the number of surviving offspring per family is decreased due to depressed viability. For populations of a moderate or large size, however, the probability of extinction due to genetic reasons during 50 generations is small. In what follows, we consider full-sib mating populations ($N = 2$) as an example of very small populations.

The dynamics of mean viability relative to that of the initial population, together with the number of lethal equivalents, inbreeding depression and proportion of extinction over 30 generations of full-sib mating, are shown in Fig. 5 for various numbers of offspring per family (n). At a given generation, the frequency of extinctions is greatly increased with decreasing family size, as expected (Fig. 5*d*). However, larger family size results in a greater decrease in mean viability (Fig. 5*a*) and slightly higher levels of lethal equivalents (Fig. 5*b*) and inbreeding depression (Fig. 5*c*) remaining in the surviving full-sib lines. The reason for these seemingly paradoxical results is that a population with larger family size can endure more severe accumulation of mutants, and a greater decrease in viability, without extinction. Between-line selection becomes more and more important with decreasing family size and mean viability. For a given family size, mutations can accumulate only to a point where mean viability is so low that the line will become extinct with further decline in viability. It is obvious that extreme between-line selection (extinction) is effective in purging mutation load without much decrease in fitness of the extant lines. However, this is accomplished at the expense of sacrificing a large number of lines.

Empirical studies of continuous full-sib mating have shown quite high frequencies of extinction, varying from 100% extinction in Japanese quail over four generations (Sittman *et al*., 1966), 95% extinction over 20 generations in house mice (Bowman & Falconer, 1960) to 80% extinction over six generations in *Drosophila* (García *et al*., 1994). These empirical results cannot readily be compared with the simulation results, because, in addition to other complications in these experiments, all fitness components (not only viability) are involved in extinction. In García *et al*.'s experiment, viability decreased linearly with inbreeding coefficient until it was 0·5. After that (the third generation of full-sib mating), the viability changed little with further inbreeding, in agreement with the predictions (Fig. 5*a*).

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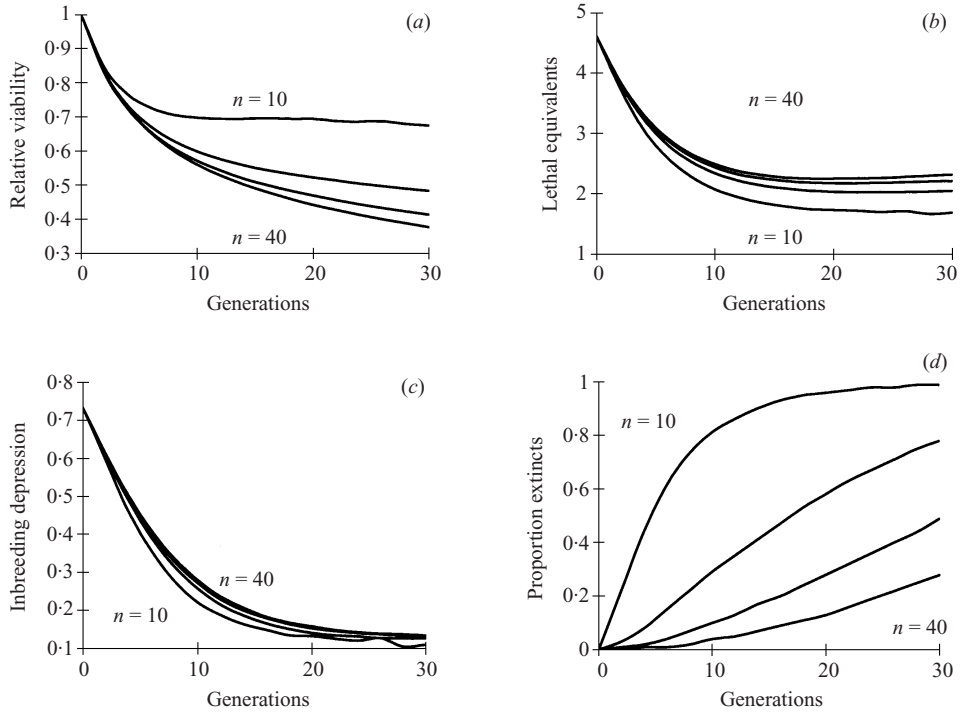

Fig. 5. Effects of family size in full-sib mating lines. (*a*) Mean viability relative to the initial population. (*b*) Number of lethal equivalents. (*c*) Inbreeding depression. (*d*) Proportion of extinction. The number of offspring per family (*n*) is 10, 20, 30 or 40. The deleterious mutation parameters are $U = 1{\cdot}0$, $\bar{s} = 0{\cdot}05$ and $\bar{h} = 0{\cdot}36$; both s and h have exponential distributions. The lethal mutation rate is 0·03 and the dominance coefficient of lethal alleles is 0·02.

When the size of the line is larger than two individuals, the proportion extinct within 50 generations is greatly reduced. With $N = 10$ (half in each sex), for example, the extinction proportion is only 2% at generation 50 when family size is 14, compared with 64% for full-sib mating at generation 12, when the same level of inbreeding is reached.

(v) *Organisms other than* Drosophila

Throughout our study, we have concentrated on a model based on *Drosophila*, because most of the available information necessary for modelling is from this genus. For most species of domesticated plants and animals, the number of chromosomes and the total genome size are larger than in *Drosophila* and so linkage is not as important. With increasing independence among loci with deleterious mutants, purging becomes slightly more effective. For instance, using the same parameters as in Table 1 and Fig. 3*a*, but with 10 chromosomes in the haploid genome and recombination in both sexes, the number of lethal equivalents and inbreeding depression are decreased to 3·29 and 0·52 respectively by 50 generations of inbreeding – both slightly smaller than the corresponding values in Table 1 for the case of *Drosophila*. Similar results were obtained assuming different population sizes with random mating, and different numbers of chromosomes per haploid genome. Although associative overdominance generated by selection against linked deleterious mutations with inbreeding was proposed to explain why the purging effect observed in several studies was small (e.g. Ballou, 1997; Lacy & Ballou, 1998), our results suggest that it does not significantly affect the dynamics of inbreeding load in a small random mating population. However, for non-random mating that results in a large amount of identity disequilibrium, such as mixed outbreeding and selfing at medium proportions (Ohta & Cockerham, 1974; Charlesworth, 1991), associative overdominance may become important.

Linkage has a far more significant effect on the rate of decay in neutral variation than on genetic load. In the above example, the simulated value of asymptotic effective size is 44·3, only about 89% of the corresponding value (in Fig. 3*a*) for *Drosophila*. This is expected, because apparent overdominance diminishes quickly with decreasing linkage, and so the increased variation in family size due to selection against deleterious mutants becomes the dominating force in determining effective size.

The reproductive capacity of a species is also important in determining the dynamics of deleterious mutations and their population-level consequences in small populations. Most animal species considered in conservation have much lower reproductive ability than *Drosophila*, and therefore they are more vul-

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nerable to extinction (see Fig. 5) as a result of inbreeding designed to purge deleterious mutations.

4. Discussion

(i) *The model used in the simulation study*

Some of the assumptions made in this study, such as an infinite size of the ancestral population, can be relaxed with little change in the results. The expected mutant frequency in the ancestral population changes little if its size is large enough (say 10000) (Crow & Kimura, 1970). The number of loci in the genome mutable to deleterious alleles (M) also has little effect on the simulation results, provided M is large enough. The number of deleterious loci assumed (5800) in this study may be implausibly small for real organisms. In *Drosophila*, it has been estimated that roughly 5000 loci may mutate to lethal alleles alone (Simmons & Crow, 1977). When larger numbers of loci (say, 23200) were used in the simulations, however, essentially the same results were obtained (data not shown). This is because the important quantity is the number of segregating loci with mutants having some effect on fitness, which is always much smaller than the value of $M = 5800$ assumed in this study.

Violation of other assumptions, however, may alter the conclusions. Like most previous studies, we assumed a multiplicative model of fitness. If the joint effect of a number of deleterious mutants is greater than the product of the individual mutants (reinforcing synergistic epistasis), selection becomes more effective and therefore the equilibrium mutation load will be decreased in the large ancestral population (Crow, 1993) and the purging effect in small populations might be larger. Further work is needed on such models.

The deleterious mutation parameter assumed in this study are also in dispute. Some recent experiments using inbred lines of *Drosophila* (García-Dorado, 1997) and *C. elegans* (Keightley & Caballero, 1997) yielded estimates of the deleterious mutation rate about 1–2 orders of magnitude smaller, and of the mean homozygous effect about 1 order of magnitude larger, than earlier estimates. If these new estimates are correct, then purging selection will be effective and mutations will not endanger the long-term survival of populations as small as only dozens of individuals. However, these new estimates do not rule out the possibility of a high rate of occurrence of mutations with very small effects (say $s < 0{\cdot}001$). These would be undetectable in mutation accumulation experiments, but nevertheless pose a threat to the long-term survival of small populations. Mutations with very small effects could also be important for several evolutionary issues, such as the maintenance of neutral variation, evolution of sex, and mating system evolution (Charlesworth & Charlesworth, 1987).

Inbreeding increases the effectiveness of selection against recessive or partially recessive mutants, but at the same time the extent of genetic drift (dispersion of allele frequency) is also enhanced. Thus inbreeding as a purging strategy should be used in combination with strong selection; otherwise genetic drift and weak selection will lead to fixation rather than elimination of recessive mutants. In this and previous studies (e.g. Hedrick, 1994; Schoen *et al*., 1998), breeding individuals for the next generation are randomly selected from surviving offspring. Several artificial selection strategies could be used to increase the effectiveness of purging. Partial inbreeding, such as partial full-sib mating in a large population (Lande & Barrowclough, 1987), might also be effective in eliminating deleterious mutations. In a follow-up study we plan to examine the effect of different selection and mating strategies on the mutational load in small populations.

(ii) *Purging deleterious alleles from conserved populations by purposeful inbreeding*

Is inbreeding effective in purging the genetic load from naturally outbreeding populations? Empirical studies give mixed answers to this question (Ballou, 1997; Lacy & Ballou, 1998; and references therein). Even in the same study, different populations or different fitness components show different responses to inbreeding. This is not surprising, considering the complex and variable genetic basis of inbreeding load over fitness traits and populations, different environments and patterns of inbreeding and selection, various methods of statistical analysis and (perhaps more importantly) the usually small sample sizes in these experiments. In a study of 25 captive pedigreed mammal populations, Ballou (1997) found that purging effects were weak within any one species. The overall trend in the sign (direction) of the purging effect on neonatal survival across species, however, was highly significant. He concluded that purging effects are probably not strong enough to be of practical use in eliminating inbreeding depression. These results could be explained if lethals and deleterious mutations of large effects constitute only a small part of the inbreeding load for neonatal survival, as in this simulation study. Lacy & Ballou (1998) observed strong, weak or no purging for various fitness traits in three populations (subspecies) of *Peromyscus polionotus* during 10 generations of laboratory inbreeding. These results seem to be compatible with the prior inbreeding histories of the populations. Previous inbreeding before the establishment of the laboratory population tends to result in lower inbreeding depression, and vice versa.

The results from this study show that deleterious mutations and thus inbreeding depression can be

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partially purged from the population by inbreeding. However, severe side-effects arise with this purging. First, during the period when lethal mutants and mutants of large effect are being purged, the fitness of the population is reduced (Fig. 1*a*), resulting in immediate extinction if the population size is small and the reproductive rate is low (Fig. 5). Secondly, some mutants of small effect are fixed in small populations due to genetic drift, permanently lowering the fitness of the population, and probably endangering its long-term survival. Frankham *et al.* (1993) showed that artificial selection for reproductive fitness during seven generations of full-sib mating in *D. melanogaster* failed to prevent 88% of the inbreeding depression. Hence, breeding programmes designed to purge inbreeding depression by purposeful inbreeding in conserved populations (e.g. Templeton & Read, 1984) may not be as effective as expected, and intentional inbreeding may well do more harm than good to either the short- or long-term survival of small populations.

Furthermore, even lethal mutants and mutants of large effect are only partially purged, not eradicated, from the population. This is because these mutants are highly recessive, and they are introduced into the population by mutation in each generation. If mutations indeed occur at a high rate and mostly have small effects on fitness (Crow, 1993), as assumed in this study, then it seems that breeding strategies designed to increase effective size would be preferable to those designed to purge inbreeding depression by intentional inbreeding (Hedrick, 1994). Given the small magnitude of N_e/N, of the order of one-tenth, estimated from various species in the wild (Frankham, 1995*a*), genetic management can potentially increase the N_e of populations by almost 20-fold (Frankham, 1995*b*). Some of the effective measures to increase N_e, however, need to be re-considered in the context of mutation accumulation. For instance, equalization of family size could double the effective size (Falconer & Mackay, 1996). However, this measure also excludes between-family selection against deleterious mutations. Schoen *et al.* (1998) showed that equalization of family size increases the accumulation of mutations, because of the absence of selective elimination of mutations. They did not, however, consider within-family selection against mutations, which is still possible under equalization of family size. Further work is needed to evaluate the overall effect of equalization of family size on the accumulation of deleterious mutations and the short- and long-term survival of small populations.

Hedrick (1994) suggested that there may exist an optimum inbreeding rate that is high enough to eliminate lethals but low enough that the fixation of detrimentals (and thence the decrease in fitness and the increase in probability of extinction) is kept to a minimum. From this study there does not appear to be an obvious optimum inbreeding rate as far as mean viability is concerned. When measured at the same level of inbreeding, viability decreases monotonically with increasing rate of inbreeding (Fig. 4*a*). However, for the number of lethal equivalents or inbreeding depression, an optimum rate of inbreeding does exist, at any given level of homozygosity. If the maximum inbreeding coefficient is allowed to reach 0·2–0·3, for example, a population size of 50 individuals gives maximum purging of the mutational load (see Fig. 4*b*, *c*). Obviously, the optimum population size in this sense varies with inbreeding level, as well as with the mutation parameters.

(iii) *Testing for purging*

With the multiplicative model, the log-transformed viability will decrease linearly with inbreeding coefficient (F) if there is no purging selection (Morton *et al.*, 1956). Therefore, when a negative quadratic function of F is significant in fitting quadratic (or cubic) regressions of log-transformed viability on F, purging is suggested. However, this is not an effective way to detect purging because the non-linearity is not strong if only a small proportion of load is purged. In Fig. 1*h*, lethals and detrimentals of large effects are partially purged by inbreeding for all three distributions of s and h assumed. However, the non-linear relationship is evident only in the case when s and h are inversely correlated. Even in this case it would still be difficult to detect purging, because the data sets from experiments are usually too small.

Another method to detect purging would be to inbreed the population at different rates and compare the fitnesses at the same level of inbreeding (Fig. 4*a*). Without selection, the mean fitnesses of inbred lines of different sizes would be the same, evaluated at the same level of inbreeding and ignoring the small bias introduced by new mutations if the number of generations is small. Any difference in fitness among inbred lines of different sizes would indicate purging, as in the experiments of Ehiobu *et al.* (1989). However, complications arise with this method. The expected level of inbreeding predicted from the actual population size and mating system, or estimated from marker information, is different from the realized level of inbreeding at the selected loci. It is obviously the latter which is relevant to the changes in load due to drift. A large part of the difference in mean viability among populations of different sizes (Fig. 4*a*) at a given expected level of inbreeding comes from the difference in realized level of inbreeding at selected loci, which is difficult to predict and varies among loci. A more plausible way to test for purging is to compare the viability among crossbreds obtained from inbred populations of different sizes at the same expected

level of inbreeding. For this method, the effect of drift on the mean viability of crossbreds is mostly eliminated and the difference among crossbreds in viability will indicate different extents of purging. This method, however, underestimates the extent of purging, because the mutations purged are mainly lethals and those with large homozygous effects, which are highly recessive and therefore have little effect on the viability of crossbreds. The most reasonable way to test for purging seems to be to inbreed the crossbreds by selfing or full-sib mating and compare the inbreeding depression among populations of different sizes. To minimize environmental difference in this experimental design, fitness assays should be carried out simultaneously on populations of different sizes. This is possible for some species by adjusting generation intervals (e.g. through freezing individuals or keeping seeds).

Another single-population method is to compare the loads from lethals before and after some generations of inbreeding (as shown in Table 1). This is feasible for all lethals in *Drosophila*, and for lethal mutations of known phenotypic effects (e.g. chlorophyll-deficient lethals) in other species. The extent of purging of non-lethal mutations of large effects cannot, however, be detected in this way.

In some empirical studies, comparisons of fitness between crossbreds and the outbred ancestral population have been used to test for purging. This test, however, is very limited in its power to detect purging. Even if the fitness of crossbreds is not increased, purging of highly recessive lethals and mutations of large effects may still have occurred during the inbreeding process, as argued above. On the other hand, an increase in fitness of crossbreds does not necessarily imply purging, because adaptation over the generations of inbreeding can not be ruled out. Increased fitness components of crossbreds have been observed in some experiments (e.g. Barrett & Charlesworth, 1991) but not in others (e.g. Dudash *et al*., 1997).

(iv) *Effects on genetic variability at neutral loci*

A major concern in conservation programmes is the amount of genetic variation that can be maintained. The present study shows that selection against deleterious mutants may increase or decrease the rate of decay of genetic variation at other loci, depending on the magnitude of mutant effects relative to the population size. If the effect of deleterious mutants is small in a small population, so that their fate is mainly determined by genetic drift, the rate of decrease in heterozygosity at neutral loci is decreased by selection, due to the development of associative overdominance. This can be generated via linkage disequilibrium (Ohta, 1971) or more likely identity disequilibrium (Ohta & Cockerham, 1974) between the neutral loci and loci with (partially) recessive mutants under selection, both being possible in a small population. Several inbreeding experiments with *Drosophila* and other species (e.g. Rumball *et al*., 1994; Latter *et al*., 1995; and reference therein) found that the inbreeding coefficient estimated from gene frequency data at enzyme loci was significantly smaller than predicted, supporting the associative overdominance theory. If, however, deleterious mutant effects are large or population size is large, so that the fate of deleterious mutations is mainly controlled by selection, a decreased effective size at the neutral loci is observed in the simulations.

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