Effects of population structures and selection strategies on the purging of inbreeding depression due to deleterious mutations

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Summary

Stochastic simulations were run to compare the effects of nine breeding schemes, using full-sib mating, on the rate of purging of inbreeding depression due to mutations with equal deleterious effect on viability at unlinked loci in an outbred population. A number of full-sib mating lines were initiated from a large outbred population and maintained for 20 generations (if not extinct). Selection against deleterious mutations was allowed to occur within lines only, between lines or equal within and between lines, and surviving lines were either not crossed or crossed following every one or three generations of full-sib mating. The effectiveness of purging was indicated by the decreased number of lethal equivalents and the increased fitness of the purged population formed from crossing surviving lines after 20 generations under a given breeding scheme. The results show that the effectiveness of purging, the survival of the inbred lines and the inbreeding level attained are generally highest with between-line selection and lowest with within-line selection. Compared with no crossing, line crossing could lower the risk of extinction and the inbreeding coefficient of the purged population substantially with little loss of the effectiveness of purging. Compromising between the effectiveness of purging, and the risk of extinction and inbreeding coefficient, the breeding scheme with equal within- and between-line selection and crossing alternatively with full-sib mating is generally the most desirable scheme for purging deleterious mutations. Unless most deleterious mutations have relatively large effects on fitness in species with reproductive ability high enough to cope with the depressed fitness and thus increased risk of extinction with inbreeding, it is not justified to apply a breeding programme aimed at purging inbreeding depression by inbreeding and selection to a population of conservation concern.

1. Introduction

The phenomenon that inbreeding reduces fitness, known as inbreeding depression, has been widely observed in most species of plants and animals (Wright, 1977; Charlesworth & Charlesworth, 1987; Falconer & Mackay, 1996; Lynch & Walsh, 1998). Though still in dispute, substantial evidence from studies involving various fitness traits in diverse organisms has been accumulated, supporting the hypothesis that most inbreeding depression is due to many partially recessive and deleterious alleles maintained by mutation and selection balance, not to a few overdominant loci (e.g. Crow, 1993).

Inbreeding increases homozygosity, and thus exposes the harmful effects on fitness of the partially recessive and deleterious mutations. This results in inbreeding depression, which endangers the immediate survival of small populations, but at the same time provides potential opportunities for purging these mutations and the resultant depression in fitness. The latter was explored in conservation applications by Templeton & Read (1984), who suggested that inbreeding depression can be rapidly and effectively purged by selection with deliberate inbreeding. Since then, much debate has been focused on the effectiveness of purging inbreeding depression (Hedrick, 1994; Wang *et al.*, 1999), and diverse results have been obtained from various empirical studies (e.g. Frankham *et al.*, 1993; Garcia *et al.*, 1994; Saccheri *et*

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al., 1996; Willis & Wiese, 1997; Ballou, 1997; Lacy & Ballou, 1998; Willis, 2000).

The broad discrepancy about the effectiveness of purging inbreeding depression is understandable, considering, among other factors, the complex and variable combinations in the genetic base of inbreeding depression (such as distributions of selection coefficients and dominance coefficients of mutations), the rates of inbreeding and genetic drift, the pattern and strength of selection, the reproductive capacity and the organization of the genome (number and map lengths of chromosomes) of the species. As a result, the joint effects of inbreeding and selection are still incompletely understood (Hedrick, 1994), although they have been studied for many decades (e.g. Haldane, 1937; Wright, 1969; Hedrick, 1994).

From previous theoretical and empirical studies on inbreeding and selection, it is clear that the dynamics of deleterious mutations and the inbreeding depression from them in a small population are determined by the strength of selection relative to the rates of inbreeding and genetic drift (Hedrick, 1994; Wang et al., 1999). Lethals and semi-lethals can be quickly purged with inbreeding, because they are highly recessive and of large homozygous effects. In contrast, mildly deleterious mutations are difficult to eliminate but are likely to be fixed by genetic drift in populations with inbreeding, because they are only partially recessive and the efficiency of selection does not increase much with inbreeding, and because the strong genetic drift accompanying inbreeding relative to the weak selection against them dominates their fates. These results have been confirmed in empirical studies (e.g. Willis, 2000) and are used to design experiments for estimating mutation parameters (e.g. Keightley & Caballero, 1997) and for inferring the genetic base of inbreeding depression (e.g. Willis, 2000).

An important issue in purging inbreeding depression that has not been investigated appropriately in previous studies is the effect of selection between inbred lines. Except for extinctions of inbred lines, no between-line selection has been deliberately applied during the course of inbreeding in previous studies on purging (Hedrick, 1994; Schoen et al., 1998; Fu et al., 1998; Fu, 1999; Wang et al., 1999). Because genetic variation is progressively reduced within lines and increased between lines, especially for recessive genes (Robertson, 1952), between-line selection should become increasingly effective compared with within-line selection with inbreeding. Successful highly inbred lines with performance at least as good as the ancestral outbred population in organisms such as mice and maize are usually developed by inbreeding a large number of lines in parallel and applying between-line selection so that the worst lines are eliminated and the best retained (Falconer & Mackay, 1996). Recent studies generally report highly variable responses to

inbreeding for fitness components or fitness related traits among replicate lines with inbreeding (e.g. Pray & Goodnight, 1995; Koelewijn, 1998). Therefore, one possible strategy for more effectively purging inbreeding depression is to apply artificial between-line selection, in addition to within-line selection and extinction of lines due to natural selection. This possibility is explored in the present study in lines with full-sib mating, in combination with or without periodical crossing among inbred lines.

Another issue that has not received much attention in previous studies on purging is the interactions among inbreeding, genetic drift and selection. Inbreeding facilitates the selective elimination of partially recessive deleterious mutations, while genetic drift hinders the purging process and may drive the deleterious mutations to fixation. Usually inbreeding and genetic drift cannot be separated and operate simultaneously at the same strength (measured by inbreeding and variance effective sizes) in a small population (Crow & Kimura, 1970). However, under certain circumstances such as partial inbreeding in a single population or subdividing the population into lines and limiting migration among them, inbreeding can occur at a higher rate than drift, at least temporarily (Wang, 1997; Wang & Caballero, 1999). In a highly subdivided population, a deleterious mutation quickly becomes either eliminated from or fixed in a line due to the high rate of inbreeding and genetic drift within lines. Even if the mutation is fixed in a line, however, it segregates and is subject to selection again upon migration or crossing among lines. Wright (1939, 1951) first noted the importance of population structure in evolution and artificial selection, and he proposed a structure of repeated cycles of subdividing the population and practising within- and between-line selection and crossing. The effectiveness of the structure was confirmed by simulation studies in which favourable recessive alleles at low initial frequencies were selected for (Madalena & Hill, 1972). An experiment with artificial selection for viability in *Drosophila* was conducted in a subdivided population and obtained promising results (Garcia et al., 1994). In this study, I investigate the effect of population subdivision combined with within- and between-line selection and line crossing on purging deleterious mutations, particularly those which are mildly deleterious and are known to be much more numerous than lethals, but also much more difficult to eliminate because of their small effects and partial recessivity.

2. Methods

(i) *The fitness trait and model*

Throughout this study, I concentrate on the fitness component viability, because most experiments on

estimating mutation parameters and inbreeding depression have been concerned with this trait.

Assume that viability is determined genetically by n loci. Each locus has two alleles, the wild type A and mutant a, and three genotypes, AA, Aa and aa with relative viability values of 1, $1-hs$ and $1-s$ respectively, where s is the selection coefficient and h is the dominance coefficient of the mutant allele. All the loci are assumed to be unlinked and segregate independently, which is reasonable as an approximation for most species of plants and animals with a large genome and a large number of chromosomes. No new mutations are assumed to occur at these loci during the short period of attempted purging.

I assumed that selection occurs in the diploid stage of the life cycle and different loci act multiplicatively in determining viability. The fitness function is

$$v = (1-s)^{n_2}(1-hs)^{n_1},$$

where n_1 and n_2 are the numbers of heterozygous and homozygous mutations in the genotype, respectively.

(ii) *Selection schemes and population structures*

A number of $2N$ individuals, half of each sex, were assumed to be sampled from a large outbred population at mutation–selection balance. For each individual, n loci are assumed to be heterozygous for deleterious mutations with dominance coefficient h and selection coefficient s, so that the initial number of lethal equivalents (calculated as the sum of s, the selection coefficient when homozygous, for all deleterious mutations; see Cavalli-Sforza & Bodmer, 1971) is $L_0 = ns$. Different individuals have the same value of L_0 but different loci at which mutations with the same values of s and h are heterozygous. This assumption is reasonable because deleterious mutations are very rare and mostly present in heterozygotes in the large outbred population at mutation–selection balance (Crow, 1993). A small number (say, 100) of individuals drawn at random from the large population generally have different sets of loci with deleterious mutations.

The $2N$ individuals formed a population of N full-sib mating lines. For each line, k zygotes were generated at each generation and their sexes allocated randomly with equal probability of occurrence of the two sexes. The survival of a zygote was determined by generating a random number and comparing it with the relative viability value of the zygote. It survived viability selection if the random number was smaller than its relative viability value, and vice versa. The mean number of surviving offspring per line (mating) is about ke^{-hL_0} initially and decreases over generations of inbreeding. For simplicity, k is called family size.

The N lines were maintained under each of the following selection and mating schemes:

Within-line selection (*WS*): From each line, two offspring of separate sexes are chosen at random from the surviving offspring as parents of the next generation. If there are no surviving offspring of any sex, then the line is considered extinct. Extinct lines are not replaced by surviving ones. For WS, therefore, selection against deleterious mutations occurs solely within lines if there is no extinction of lines. When line extinction happens, however, natural selection also operates between lines.

Between-line selection (*BS*): At each generation, N full-sib pairs (if possible) are selected by truncation selection on the surviving number of offspring per line. With BS, therefore, offspring are first naturally selected on their own survival, and then artificially selected on the surviving number of offspring of a mating (line).

Equal within- and between-line selection (*ES*): N pairs, if possible, of sibs for mating are taken at random from all surviving progeny of all extant lines at each generation. Selection pressures within and between lines are equal under this selection scheme if there is no line extinction.

No line crossing (*NC*): At any generation, mating is between full-sibs and no line crossing is allowed throughout the 20 generations.

Line crossing every 2 generations (*C2*): After each generation of full-sib mating, the surviving lines are intercrossed at random. N full-sib pairs for mating, if possible, are chosen equally from the surviving crossbred lines to start the next circle of full-sib mating and crossing.

Line crossing every 4 generations (*C4*): Similar to C2 except that line crossing occurs after every 3 generations of full-sib mating.

From the above 3 selection and 3 mating schemes, 9 breeding schemes are possible and are compared in the effectiveness of purging deleterious mutations. The breeding scheme WS:NC (within-line selection, no crossing among lines), for example, has been considered by Hedrick (1994). It serves as a reference scheme to compare with other breeding schemes in this study.

(iii) *Simulations and measurements of the effects of purging*

The simulation programme was adapted from a previous one (Wang *et al.*, 1999), incorporating between-line selection and crossing among lines. For each combination of parameters (values of s, h, N, L and k) and each of the 9 breeding schemes, 1000 replicates were run over 20 generations.

The effectiveness of purging was expressed by two

measurements. One is the reduction of the number of lethal equivalents (L). Because inbreeding and genetic drift *per se* without selective elimination of deleterious mutations (no purging) do not change L, the proportional decrease in L signifies the effectiveness of purging for a given breeding scheme. In the simulations I measured the average number of lethal equivalents over individuals of surviving lines and replicates at each generation as a proportion of the initial value. Though very useful and widely used as a direct measurement of the effectiveness of purging (e.g. Hedrick, 1994; Fu *et al.*, 1998; Fu, 1999; Wang *et al.*, 1999), this measurement does not describe exclusively the changes in mutational load in a population, especially the inbreeding load which depends also on the recessivity of mutations and the homozygosity of the population. If a mutation is fixed in a population or has an additive effect on fitness, then it does not result in inbreeding depression but contributes to L.

The other measurement is the mean viability of the random mating population formed from crossing the surviving lines after the purging period (denoted as v_r). From the practical point of view, the lines extant at the end of the purging period are highly likely to be crossed to form a random mating population (called purged population hereafter), whose fitness is the primary concern of a purging programme because it determines the fate of the population in the future. If, after the period of attempted purging, the purged population is greatly improved in fitness such that the risk of extinction due to genetic and demographic factors is substantially reduced, then the population can more safely be reintroduced into the wild.

During the period of purging deleterious mutations, increased homozygosity with inbreeding may reduce the fitness of the lines and, as a result, increase the probability of extinction. The probability of extinction of a replicate population can be assessed and compared among breeding schemes. It is, however, not very informative because a surviving population may have different numbers of surviving lines. In this paper, the average number of lines surviving the period of purging over replicate populations as a proportion of the initial number of lines (denoted as p_s) was used to assess the risk of extinction. The ideal breeding scheme would be to decrease L and increase v_r without much decrease of p_s below one.

Another concern with purging by inbreeding in conservation programmes is the amount of genetic variation that can be maintained for further adaptation in the future. To address this issue, 10 neutral loci were included in the simulations and used to calculate the inbreeding coefficient (F) of the purged population. The magnitude of F signifies roughly the decrease in genetic variation due to the attempted purging.

3. Results

The purging of deleterious mutations involves a complex interaction of many factors, such as the strength of selection relative to that of inbreeding and genetic drift, the pattern of selection, the reproductive ability and inbreeding load of the organism, the population size and structure. To comprehend the influence of these factors, I will examine them one by one. First, I compare the breeding schemes in terms of their purging effects for mutations of various selection coefficients and dominance coefficients, assuming a given family size (10 zygotes generated for each mating), and initial numbers of lethal equivalents ($L_0 = 2$) and lines ($N = 30$). Second, the breeding schemes are compared for different family sizes and different initial numbers of lethal equivalents, given the selection coefficients and dominance coefficients of all mutations. Third, different population sizes ($2N$) are considered.

(i) *Selection coefficients of mutations*

Table 1 compares the 9 breeding schemes for the number of lethal equivalents (L), viability (v_r) and inbreeding coefficient (F) of the purged population, and the mean number of surviving lines at the end of the purging period (p_s), assuming a family size of 10, an initial number of 30 lines, an initial number of 2 lethal equivalents due to mutations of various selection coefficients and a fixed dominance coefficient ($h = 0.3$). Except for F, the quantities are expressed relative to the initial values.

When mutations have a very small deleterious effect ($s < 0.03125$), all breeding schemes have little influence on v_r, though they decrease L slightly (Table 1). After 20 generations of inbreeding, selection and genetic drift, mutations at most loci are lost while others are driven to much higher frequencies than the initial value (1/120). Though between-line selection leads to fewer loci segregating or fixed for deleterious mutations, because less fit lines and the mutations carried by them tend to be lost, it also results in a higher average frequency of existing mutations in the purged population than within-line selection (data not shown). Since v_r is much more sensitive to the frequency of partially recessive mutations than L, it is not increased though L is substantially decreased by between-line selection compared with within-line selection. It is clear that any purging scheme with full-sib mating becomes undesirable if most mutations have a very small effect due to the ineffectiveness of purging and the substantially increased risk of extinction and inbreeding.

Any breeding scheme becomes increasingly more effective for purging with increasing s. Compared with within-line selection (WS), between-line selection (BS)

Table 1. *Comparison of the 9 breeding schemes regarding the effectiveness of purging deleterious mutations of various selection coefficients[a]*

Breeding Schemes	$s = 0.03125$				$s = 0.0625$				$s = 0.125$				$s = 0.25$			
	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F
WS:NC	0.79	0.98	0.01	0.47	0.68	1.09	0.03	0.42	0.52	1.24	0.08	0.35	0.35	1.44	0.19	0.20
WS:C4	0.83	1.05	0.63	0.15	0.70	1.16	0.72	0.14	0.53	1.29	0.82	0.13	0.32	1.49	0.90	0.11
WS:C2	0.84	1.05	0.83	0.13	0.72	1.15	0.88	0.13	0.55	1.29	0.92	0.12	0.33	1.47	0.96	0.12
ES:NC	0.72	1.02	0.87	0.61	0.54	1.16	0.93	0.66	0.29	1.44	0.97	0.69	0.05	1.76	1.00	0.68
ES:C4	0.74	1.05	0.90	0.37	0.57	1.20	0.94	0.42	0.32	1.44	0.98	0.48	0.09	1.71	1.00	0.47
ES:C2	0.78	1.05	0.91	0.27	0.62	1.18	0.94	0.29	0.39	1.40	0.97	0.31	0.16	1.64	1.00	0.31
BS:NC	0.65	1.00	0.90	0.83	0.42	1.24	0.96	0.91	0.13	1.62	0.99	0.95	0.00	1.82	1.00	0.96
BS:C4	0.65	1.07	0.92	0.60	0.40	1.31	0.97	0.73	0.11	1.65	0.99	0.85	0.00	1.82	1.00	0.90
BS:C2	0.68	1.09	0.92	0.44	0.45	1.29	0.97	0.53	0.16	1.62	0.99	0.68	0.00	1.82	1.00	0.76

[a] L, v_r and F are the number of lethal equivalents, the viability and inbreeding coefficient of the purged population, and p_s is the average number of surviving lines after 20 generations. L, v_r and p_s are expressed relative to the corresponding initial values (at generation zero). The results are obtained assuming 10 zygotes generated per mating (line), 2 lethal equivalents per individual initially, 30 initial lines and $h = 0.3$.

decreases L and increases v_r and p_s, signifying more effective purging with less risk of extinction. At the same time, however, it results in a great increase in inbreeding level of the purged population, endangering the long-term potential of adaptation. Under the same mating scheme, BS gives the highest and WS the lowest F, except for the case of no line crossing and small s where most lines become extinct under WS. Equal within- and between-line selection generally gives results intermediate between WS and BS, as expected.

An interesting observation from Table 1 is that, under any given selection scheme and for any value of selection coefficient of mutations, line crossing changes little of the effectiveness of purging, while it increases the survival of lines and decreases the inbreeding coefficient of the purged population substantially. The result is of significance in practical purging programmes which aim to purge inbreeding depression, improve fitness performance without much risk of extinction and loss of genetic variation. Therefore, alternative full-sib mating and line crossing is the best mating strategy for purging under any selection scheme.

(ii) *Dominance coefficients of mutations*

Table 2 compares the 9 breeding schemes, using the same parameters as Table 1 except for a fixed selection coefficient of 0.125 and various dominance coefficients of mutations. As can be seen, the effectiveness of purging for each breeding scheme increases with increasing values of h, as reflected by the decreasing number of lethal equivalents (L) and increasing fitness (v_r) of the purged population. This is because a higher h signifies a larger selective disadvantage and thus a greater chance of mutations being eliminated in heterozygotes. Generally, v_r is much more sensitive to

the changes of h than L for any breeding scheme. Though L is decreased for any value of h, v_r is increased only when h is relatively large. This is understandable because all mutations are initially in heterozygotes and only their heterozygous effects (hs) determine the initial fitness and the room for improvement. In the extreme case of $h = 0$, the initial lines have the maximum fitness and further improvement in fitness is impossible, though L could still be decreased by the purging process.

The risk of line extinctions also increases with increasing h for breeding schemes with within-line selection. The reason is that a higher h generally results in a lower mean viability and thus a smaller number of surviving offspring per mating (line) at any generation. F changes little with h, except for the breeding scheme of WS:NC where the proportion of line extinction increases with increasing h.

For any selection scheme under any value of h, line crossing does not change the effectiveness of purging (indicated by L and v_r) appreciably, but it decreases risk of extinctions (indicated by p_s) and the inbreeding level (F) of the purged population substantially. Alternative full-sib mating and line crossing seems to be the best population structure for purging mutations, irrespective of the values of s and h.

(iii) *Initial numbers of lethal equivalents*

From several studies on various species of *Drosophila* (reviewed by Simmons & Crow, 1977), the number of lethal equivalents is estimated to be about 2 or 3 for the whole genome. The estimates vary greatly among other species and reports (Lynch & Walsh, 1998). Table 3 compares the 9 breeding schemes for their purging effects in populations with different initial numbers of lethal equivalents, assuming 30 initial lines per population, a number of 10 zygotes generated

Table 2. *Comparison of the 9 breeding schemes regarding the effectiveness of purging deleterious mutations of various dominance coefficients*[a]

Breeding schemes	$h = 0.05$				$h = 0.1$				$h = 0.2$				$h = 0.4$			
	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F
WS:NC	0.57	0.92	0.14	0.27	0.56	0.98	0.13	0.28	0.54	1.09	0.11	0.30	0.51	1.41	0.06	0.38
WS:C4	0.66	1.00	0.87	0.10	0.63	1.04	0.86	0.11	0.58	1.15	0.84	0.11	0.48	1.48	0.80	0.16
WS:C2	0.70	0.99	0.95	0.10	0.68	1.03	0.94	0.11	0.61	1.13	0.92	0.12	0.48	1.48	0.91	0.14
ES:NC	0.32	0.94	0.97	0.70	0.31	1.02	0.97	0.70	0.30	1.21	0.97	0.69	0.28	1.70	0.98	0.69
ES:C4	0.40	0.98	0.98	0.41	0.38	1.06	0.98	0.41	0.36	1.23	0.98	0.43	0.30	1.69	0.98	0.49
ES:C2	0.51	0.99	0.98	0.26	0.48	1.05	0.98	0.27	0.43	1.21	0.98	0.29	0.36	1.63	0.97	0.31
BS:NC	0.12	1.04	0.99	0.96	0.13	1.13	0.99	0.95	0.13	1.35	0.99	0.95	0.13	1.93	0.99	0.95
BS:C4	0.12	1.07	0.99	0.85	0.12	1.17	0.99	0.85	0.11	1.39	0.99	0.85	0.12	1.97	0.99	0.86
BS:C2	0.20	1.05	0.99	0.66	0.18	1.15	0.99	0.68	0.16	1.37	0.99	0.67	0.14	1.93	0.99	0.68

[a] L, v_r and F are the number of lethal equivalents, the viability and inbreeding coefficient of the purged population, and p_s is the mean number of surviving lines after 20 generations. L, v_r and p_s are expressed relative to the corresponding initial values (at generation zero). The results are obtained assuming 10 zygotes generated per mating (line), 2 lethal equivalents per individual initially, 30 initial lines and $s = 0.125$.

per mating (line), and mutations with $s = 0.0625$ and $h = 0.3$, which are typical for the vast majority of mildly deleterious mutations (Crow, 1993).

For each breeding scheme, the risk of line extinction increases with increasing initial numbers of lethal equivalents (L_0). This is expected because a larger L_0 means a smaller number of surviving offspring per mating on average and thus a higher probability of line extinction. The effect of L_0 on F is dependent on the selection scheme. For within-line selection (WS) or equal within- and between-line selection (ES), F increases with increasing L_0 due to the decreasing population size. For between-line selection (BS), F first decreases and then increases with increasing L_0. This is understood because, with a small L_0, the mean surviving number of offspring per line is large and the population in the next generation comes mainly from a small number of parent lines under BS. With a large L_0, most lines become extinct and the population size is substantially decreased. Both cases result in a large F, while intermediate L_0 results in the smallest F.

The number of lethal equivalents decreases faster with a larger L_0 for within-line selection, due to a higher proportion of line extinction (natural between-line selection). In contrast, lethal equivalents decrease slower with a larger L_0 for between-line selection (BS), except for BS:NC with $L_0 = 4$ which results in an extremely high proportion of line extinction. This is because, with increasing L_0, there is a decreasing chance for artificial between-line selection to act. Equal within- and between-line selection does not change its effect much on lethal equivalents with various values of L_0, due to the contrary trends of within- and between-line selections.

The viability of the purged population is increased by the purging process, and the magnitude of the relative increase is generally larger for a higher L_0.

As can be seen from Table 3, line crossing is advantageous for any selection scheme and any initial inbreeding load. It generally decreases F and the risk of line extinction without greatly changing the purging effect.

(iv) *Fecundity of the species*

Different species vary a great deal in fecundity. Some species have more than 10 progeny as assumed above, while others, such as large mammals, have fewer. Table 4 compares the 9 breeding schemes under different numbers of zygotes per mating (k), assuming $L_0 = 2$, $s = 0.0625$, $h = 0.3$ and 30 initial lines. The mean number of surviving progeny per mating (line) is about $0.55k$ initially.

As can be seen from Table 4, the effectiveness of purging (reflected by L and v_r), the risk of extinction and inbreeding decrease with increasing family sizes for the 3 schemes with within-line selection. This is because the selection occurring between lines as a result of line extinction diminishes with increasing family size. When the family size is large enough that no line is extinct, selection acts solely within lines. At the other extreme, where the inbreeding process is dominated by events of extinctions due to a very small family size, there will be little difference among the 3 selection schemes.

For the 3 schemes with equal within- and between-line selection (ES), there is little change in the effectiveness of purging, risk of extinction and inbreeding with family sizes. With much smaller family sizes (say $k < 8$), the same relationships as shown above for within-line selection apply to ES (data not shown).

For the 3 schemes with between-line selection (BS), a larger family size results in more effective purging

Table 3. *Comparison of the 9 breeding schemes regarding the effectiveness of purging deleterious mutations for populations with different initial numbers of lethal equivalents* (L_0)[a]

Breeding schemes	$L_0 = 1$				$L_0 = 2$				$L_0 = 3$				$L_0 = 4$			
	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F
WS:NC	0·81	1·05	0·44	0·08	0·68	1·09	0·03	0·42	—	—	0·00	—	—	—	0·00	—
WS:C4	0·78	1·05	0·90	0·09	0·70	1·16	0·72	0·14	0·62	1·19	0·05	0·53	—	—	0·00	—
WS:C2	0·78	1·05	0·96	0·10	0·72	1·15	0·88	0·13	0·66	1·25	0·64	0·25	0·55	1·36	0·01	0·62
ES:NC	0·51	1·10	0·98	0·59	0·54	1·16	0·93	0·66	0·55	1·21	0·65	0·73	0·47	1·38	0·01	0·92
ES:C4	0·57	1·10	0·98	0·38	0·57	1·20	0·94	0·42	0·56	1·34	0·86	0·42	0·51	1·42	0·02	0·69
ES:C2	0·64	1·09	0·98	0·26	0·62	1·18	0·94	0·29	0·59	1·34	0·88	0·28	0·55	1·40	0·08	0·56
BS:NC	0·29	1·18	0·99	0·94	0·42	1·24	0·96	0·91	0·52	1·22	0·68	0·81	0·48	1·36	0·01	0·93
BS:C4	0·29	1·19	0·99	0·86	0·40	1·31	0·97	0·73	0·48	1·43	0·90	0·54	0·52	1·40	0·03	0·68
BS:C2	0·36	1·17	0·99	0·68	0·45	1·29	0·97	0·53	0·51	1·43	0·91	0·39	0·55	1·41	0·08	0·55

[a] L, v_r and F are the number of lethal equivalents, the viability and inbreeding coefficient of the purged population, and p_s is the mean number of surviving lines after 20 generations. L, v_r and p_s are expressed relative to the corresponding initial values (at generation zero). The results are obtained assuming 10 zygotes generated per mating (line), 30 initial lines and $s = 0.0625$ and $h = 0.3$ for all mutations. — indicates that all lines in a population become extinct.

with a lower risk of extinction, but also a higher level of inbreeding, due to a smaller number of lines contributing to the next generation.

For each selection scheme with any family size, line crossing decreases the risk of extinction with little change in the effectiveness of purging. For ES and BS, line crossing also reduces the level of inbreeding of the purged population. For WS, line crossing decreases inbreeding by reducing line extinctions when family size is small, and increases inbreeding when family size is large.

(v) *Number of initial lines* (N)

The size of the initial population in a conservation programme could vary considerably. Table 5 compares the 9 breeding schemes under different numbers of initial lines (N), assuming $L_0 = 2$, $k = 10$, $s = 0.0625$ and $h = 0.3$.

For breeding scheme WS:NC, different lines in a population are independent and therefore N does not affect L, v_r and p_s. The inbreeding level (F) of the purged population formed from crossing the surviving lines after 20 generations depends on the number of surviving lines and therefore decreases with increasing N.

For the other 8 breeding schemes, the effectiveness of purging increases and the risk of extinction and inbreeding level decrease with an increasing number of initial lines. This is because different lines in a population are no longer independent, and more lines result in less genetic drift and stronger selection against the deleterious mutations.

Compared with WS:NC, any of the other 8 breeding scheme loses its advantage in purging effects when N is small. For $N = 5$ in Table 5, all breeding schemes

have roughly the same effectiveness of purging (indicated by L and v_r). Crossing among lines decreases the risk of extinction, and increases for WS and decreases for ES or BS the inbreeding level of the purged population. Compared with WS, ES or BS decreases the risk of extinction but incurs a higher level of inbreeding.

The population size at which a breeding scheme involving ES, BS or line crossing loses its purging advantage over WS:NC depends on the homozygous and heterozygous effects of the mutations. With $N = 5$ and the same parameters as in Table 5 except for $s = 0.25$, for example, the simulated values of L are 0·34, 0·27 and 0·20 and the simulated values of v_r are 1·42, 1·50 and 1·59 for breeding schemes WS:NC, ES:C2 and BS:C2 respectively. From the simulations it is clear that the smaller the effects of mutations, the larger the initial number of lines is required for ES, BS or line crossing to become substantially advantageous in purging effects over WS:NC.

4. Discussion

The simulation results show that mildly deleterious mutations can be partially purged from an outbreeding population by full-sib mating and selection. The effectiveness of purging is reflected in a reduced number of lethal equivalents and, more readily observed, by an increased viability of the purged population. After some generations of effective purging, therefore, the population attains a higher fitness, becomes less vulnerable to further inbreeding, and can be more safely reintroduced into the wild. However, the process of purging also results in an increased risk of extinction and loss of genetic variation. Taking all these possible desirable and undesirable effects of the

Table 4. *Comparison of the 9 breeding schemes regarding the effectiveness of purging deleterious mutations for populations with different family sizes*[a]

Breeding schemes	$k = 10$				$k = 20$				$k = 40$			
	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F
WS:NC	0·68	1·09	0·03	0·42	0·86	1·07	0·65	0·05	0·92	1·04	0·99	0·03
WS:C4	0·70	1·16	0·72	0·14	0·82	1·09	0·97	0·07	0·84	1·08	1·00	0·06
WS:C2	0·72	1·15	0·88	0·13	0·81	1·09	0·99	0·07	0·82	1·09	1·00	0·07
ES:NC	0·54	1·16	0·93	0·66	0·57	1·14	1·00	0·66	0·56	1·14	1·00	0·67
ES:C4	0·57	1·20	0·94	0·42	0·59	1·18	1·00	0·43	0·60	1·17	1·00	0·41
ES:C2	0·62	1·18	0·94	0·29	0·66	1·16	1·00	0·27	0·67	1·15	1·00	0·25
BS:NC	0·42	1·24	0·96	0·91	0·37	1·28	1·00	0·98	0·34	1·36	1·00	0·99
BS:C4	0·40	1·31	0·97	0·73	0·38	1·29	1·00	0·94	0·34	1·36	1·00	0·98
BS:C2	0·45	1·29	0·97	0·53	0·40	1·29	1·00	0·84	0·37	1·33	1·00	0·96

[a] L, v_r and F are the number of lethal equivalents, the viability and inbreeding coefficient of the purged population, and p_s is the mean number of surviving lines after 20 generations. L, v_r and p_s are expressed relative to the corresponding initial values (at generation zero). The results are obtained assuming 2 lethal equivalents per individual initially, 30 initial lines, and $s = 0.0625$ and $h = 0.3$ for all mutations.

purging process into account, it is not easy to decide whether a purging programme with inbreeding is in general advantageous or not. Several factors need to be taken into account when considering a breeding program for purging in conservation practice.

(i) *Breeding schemes*

Because genetic drift generally accompanies inbreeding and can drive deleterious mutations to fixation if they are not effectively selected against, a programme of purging by purposeful inbreeding usually risks an immediate extinction of the population. To increase the purging effect without endangering the survival, efforts should be made to intensify selection and reduce drift if possible. Previous theoretical investigations on purging have considered different rates of inbreeding (such as selfing, half- or full-sib mating and slow inbreeding), but population structure or line crossing and between-line selection have not been considered (e.g. Hedrick, 1994; Fu *et al.*, 1998; Fu, 1999; Wang *et al.*, 1999). In this study, I have shown that between-line selection with alternative full-sib mating and crossing among lines is the most effective breeding schemes for purging deleterious mutations in all cases considered. If the loss of genetic variation or inbreeding level is also of concern, equal within- and between-line selection combined with crossing every other generation (ES:C2) is the most appropriate breeding system for purging. This is especially evident for mutations with relatively large effects (say, $s > 0.1$). For mutations of small effect (say, $s < 0.05$), all breeding schemes with full-sib mating become ineffective for purging with much increased risk of extinction and inbreeding.

Though the importance of population structure in evolution and artificial selection was noted by Wright (1939), empirical studies have been scarce. Garcia *et al.* (1994) conducted artificial selection experiments for viability in *Drosophila* in a single population and in subdivided populations. They found that medium-term (generations 5–13) response was higher in subdivided than in unsubdivided populations. However, short-term (1–4) and long-term (14–26) responses were similar for different population structures. This is because inbreeding depression and selection response were possibly confounded in their experiment. During the medium period, between-line crosses were made and thus there was little inbreeding depression in the subdivided population; while in the other two periods no cross was made and thus the higher responses in subdivided populations might be offset by inbreeding depression. The effectiveness of population structure was confirmed in simulation studies in which recessive alleles at low initial frequencies were modelled (Madalena & Hill, 1972).

Compared with within-line selection, between-line selection becomes increasingly effective for purging deleterious mutations with inbreeding. The reason is that genetic variation decreases within lines and increases between lines with inbreeding (Wright, 1952), especially for recessive genes (Robertson, 1952). Intrafamily selection, which is similar to within-line selection considered in this paper, has been shown to increase the accumulation of deleterious mutations and inbreeding depression compared with mass selection (Campbell, 1988; Couvet & Ronfort, 1996).

Between-line selection, however, also greatly increases inbreeding compared with within-line selection (Tables 1–5). Equal within- and between-line selection generally increases the effectiveness of purging and reduces extinction risk substantially compared with within-line selection, while it does not increase inbreeding greatly when combined with crossing

Table 5. *Comparison of the 9 breeding schemes regarding the effectiveness of purging deleterious mutations for populations with different numbers of initial lines*[a]

Breeding schemes	$N = 5$				$N = 10$				$N = 20$				$N = 40$			
	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F	L	v_r	p_s	F
WS:NC	0·69	1·08	0·01	0·47	0·68	1·08	0·01	0·46	0·68	1·08	0·02	0·46	0·68	1·09	0·01	0·41
WS:C4	0·71	1·05	0·37	0·63	0·72	1·08	0·62	0·41	0·71	1·14	0·71	0·21	0·70	1·16	0·73	0·11
WS:C2	0·74	1·03	0·68	0·60	0·73	1·09	0·86	0·34	0·72	1·13	0·87	0·18	0·72	1·15	0·87	0·10
ES:NC	0·70	0·96	0·69	0·95	0·64	1·02	0·89	0·87	0·57	1·12	0·92	0·75	0·51	1·20	0·94	0·60
ES:C4	0·70	1·00	0·79	0·84	0·64	1·08	0·92	0·67	0·59	1·16	0·93	0·51	0·55	1·23	0·94	0·35
ES:C2	0·72	1·02	0·83	0·75	0·67	1·09	0·93	0·54	0·63	1·16	0·93	0·36	0·61	1·21	0·94	0·24
BS:NC	0·69	0·98	0·71	0·98	0·61	1·05	0·90	0·96	0·49	1·16	0·94	0·93	0·39	1·28	0·96	0·88
BS:C4	0·69	0·99	0·74	0·95	0·59	1·09	0·92	0·89	0·45	1·24	0·96	0·80	0·36	1·37	0·97	0·69
BS:C2	0·69	1·01	0·79	0·89	0·59	1·12	0·93	0·77	0·49	1·24	0·96	0·62	0·42	1·34	0·97	0·47

[a] L, v_r and F are the number of lethal equivalents, the viability and inbreeding coefficient of the purged population, and p_s is the mean number of surviving lines after 20 generations. L, v_r and p_s are expressed relative to the corresponding initial values (at generation zero). The results are obtained assuming 10 zygotes generated per mating (line), 2 lethal equivalents per individual initially, and $s = 0.0625$ and $h = 0.3$ for all mutations.

among lines. Summarizing the simulation results over all the cases investigated and considering jointly the effectiveness of purging, risk of extinction and inbreeding, ES:C2 seems to be the best breeding scheme for a programme aimed at purging deleterious mutations.

Empirical studies of continuous full-sib mating have shown quite high frequencies of extinction, varying from 100 % extinction in Japanese quail over 4 generations (Sittman *et al.*, 1966), 95 % extinction over 20 generations in house mice (Bowman & Falconer, 1960) to 80 % extinction over 6 generations in *Drosophila* (García *et al.*, 1994). Most line extinctions occur after 2 or 3 generations of full-sib mating (e.g. Bowman & Falconer, 1960; García *et al.*, 1994). Therefore, alternative full-sib mating and crossing (C2) should be very effective in avoiding extinctions in practice. The present paper shows that this is realized without greatly sacrificing the effectiveness of purging deleterious mutations (Tables 1–5).

Bowman & Falconer (1960) showed that selection within inbred lines of mice was ineffective in preventing both inbreeding depression of litter size and extinctions of lines. Three of the 20 initial lines survived 10 generations of full-sib mating with little change in mean litter size compared with the outbred large population, indicating the effectiveness of between-line selection as a result of line extinction. The cross made from the three surviving lines had a heterosis of about two more mice per litter than the outbred control population. The results are generally in line with the conclusions drawn from the simulations. They also showed, however, that later cycles of inbreeding and crossing made little further improvement to litter size. This is perhaps partly due to the fact that only the 3 or 4 best lines were selected to

cross in a cycle, and therefore inbreeding was substantial in the crossbreds of the second and later cycles.

An important assumption made in this and previous theoretical investigations (e.g. Hedrick, 1994; Fu *et al.*, 1998; Fu, 1999; Wang *et al.*, 1999) on purging deleterious mutations by inbreeding is that there is no strong genotype–environment interaction. Empirical studies showed, however, that inbreeding depression in a stressful (Miller, 1994) or natural environment (Jimenez *et al.*, 1994; Keller *et al.*, 1994) is greater than that of the same population when measured in a controlled laboratory environment. If mutations are less harmful to fitness in benign conditions (e.g. in captivity or laboratory) than in harsh conditions (e.g. in the wild), as supported by empirical studies (Kondrashov & Houle, 1994; Koelewijn, 1998; Bryant & Reed, 1999), then any breeding scheme aimed at purging deleterious mutations by inbreeding from a captive population for future reintroduction into the natural habitat would be even more limited in effectiveness.

(ii) *The essence of mutational load*

The magnitudes of both homozygous and heterozygous effects of mutations influence the effectiveness of purging for any breeding scheme. Regardless of the rate and form of inbreeding (such as selfing, sib mating or slower inbreeding), lethal mutations can easily be eliminated (Hedrick, 1994; Fu *et al.*, 1998; Wang *et al.*, 1999), as confirmed by empirical studies (e.g. Willis, 2000). These lethal mutations are believed to be much fewer than mildly deleterious mutations (Crow, 1993), though they constitute roughly half the inbreeding depression for viability in *Drosophila* (see, e.g., Charlesworth & Charlesworth, 1987). The

difficulty for purging is with the vast majority of mildly deleterious mutations, which are the main concern of this study.

For mildly deleterious mutations, the means and distributions of their homozygous and heterozygous effects are difficult to determine and are currently under debate. Classical mutation accumulation studies indicate an average homozygous effect of 0·03–0·05 (e.g. Mukai *et al.*, 1972) for deleterious mutations, but some recent experiments using inbred lines of *Drosophila* (García-Dorado, 1997) and *C. elegans* (Keightley & Caballero, 1997) yielded estimates of about 0·1–0·2. Without knowing the means and distributions of *s* and *h* for mutations, it is impossible to predict the extent of purging and the accompanying risk of extinction with inbreeding. The simulation results show that, with increasing values of *s* and *h* of mutations, each breeding scheme becomes more effective for purging, and between-line selection with crossing becomes increasingly more powerful than the other breeding schemes.

Though different organisms and different fitness components may have different architectures of the mutational load, it is clearly not realistic to assume that all deleterious mutations have equal effects on fitness. It is possible to relax this assumption, and a mixture of mutant effects can be included in this simulation study. However, the more complex model does not change the basic conclusions. If both lethals and mutations of very small effects (say *s* = 0·03) are taken into account simultaneously, for example, the first will be eliminated with full-sib mating while the second will not (Hedrick, 1994). This is expected because, without linkage and with linkage and identity equilibria, mutations at loci acting multiplicatively are independent.

(iii) *The initial mutational load and reproductive ability of the species*

The initial mutational load and reproductive ability of the species influence the purging process in a similar way by affecting the probability of extinction of the lines with inbreeding. The higher the initial mutational load and the lower the reproductive ability of the species, the smaller the average number of surviving progeny per family, and the higher the risk of extinction during the period of purging due to depressed fitness with inbreeding. Therefore it is disappointing that, in conservation, species with a higher mutational load and/or lower reproductive ability are in more urgent need of purging but the purging is less effective with a greater risk of extinction during inbreeding. Most species of mammals have small reproductive ability. Except when the majority of mutational load is due to lethals or mutations of large effects, purging by full-sib mating would be very

limited in effectiveness with much increased risk of extinction.

The total level and constitution of mutational load change over time during the purging period (Wang *et al.*, 1999). The initial population without previous inbreeding generally has a high level of mutational load, a substantial part of which might be due to lethals. This is true for *Drosophila* (Crow, 1993) and *Mimulus guttatus* (Willis, 1992) and a few other species well studied for their genetic architectures of mutational load. One possible way to purge the mutational load gradually without a high risk of extinction is to change the rate of inbreeding during the whole purging period. Initially, the rate of inbreeding should be low so that the inbreeding depression is not severe enough to cause extinction while some genetic load (mainly due to recessive lethals) is being purged. Then the rate of inbreeding is gradually increased to purge more effectively the lethals and perhaps some detrimentals. Further studies are needed in this respect.

(iv) *Population size*

The simulations demonstrate that the larger the number of initial lines, the more effective the purging that can be realized by line crossing and between-line or equal within- and between-line selection. Population size is especially important for purging mutations of small effects. The reason is that mutations of large effects are easily eliminated even by solely within-line selection, while the removal of mutations of small effects relies mainly on between-line selection, whose effectiveness depends on the number of lines available for selection.

(v) *Conclusion*

The effectiveness of purging inbreeding depression by inbreeding and selection is highly variable over empirical studies. Some investigations suggest that only a small fraction of the inbreeding load is eliminated after a period of inbreeding (e.g. Ehiobu *et al.*, 1989; Frankham *et al.*, 1993; Ballou, 1997; Lacy & Ballou, 1998). However, very effective purging has been documented in developing inbred lines. Some high-yielding inbred lines of maize have now been developed, which may be not as good as the best current hybrids but are better than hybrids in the past (Crow, 1993). Some successful inbred mice lines as fecund as their parental outbred population are developed by a few generations of full-sib mating (Bowman & Falconer, 1960; Falconer & Mackay, 1996), indicating perfect purging of the genetic load. It is still not clear why the broad differences in effectiveness of purging with inbreeding occur among these studies.

The present study demonstrated that, considering the effectiveness of purging, the risk of extinction and loss of genetic variation associated with the purging process, equal within- and between-line selection with alternative full-sib mating and crossing seems to be better than other breeding schemes with full-sib mating. In general, however, purging with inbreeding increases the risk of immediate extinction due to depressed fitness, especially when most mutations are only slightly deleterious, the initial mutational load is high and the reproductive ability of the species is low. The attempted purging also incurs a substantial increase in inbreeding level and loss of genetic variation. Considering the wide discrepancy in the effectiveness of purging from empirical studies, the generally unknown architecture of mutational load for the species in question, and the disadvantageous effects accompanying the purging process, caution should be exercised in planning any purging programme with close inbreeding. Without knowing the relevant information for predicting the purging process, it is better to minimize inbreeding in the short term or to maximize effective population size in the long term by means of appropriate breeding strategies.

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