Accumulation of deleterious mutations and equalization of parental contributions in the conservation of genetic resources

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Typical management strategies for the conservation of endangered species in captivity, such as equalization of family sizes, deal with the need for maintaining genetic variability and low levels of inbreeding, and for preventing the population from adapting to captivity. But they also produce a reduction in the intensity of natural selection, so that deleterious mutations can accumulate more easily in small populations. We have carried out computer simulations to investigate the effect of equalizing contributions on the accumulation of deleterious mutations. The models include effects on fecundity and viability, and account for different sets of mutational parameters and reproductive rates. The effect of relaxation of selection under captive conditions was also investigated. Our results suggest that equalization of family sizes does not produce a particularly high threat to small conserved populations, at least in the short term (up to about 20 generations), and the more efficient preservation of genetic variability seems to be a clear advantage of the procedure.

Keywords: genetic drift, genetic variation, inbreeding, mutational load.

Introduction

It is commonly accepted that the main genetic goals of a conservation programme are to avoid inbreeding, as it affects fitness performance; to retain the greatest genetic variation to guarantee the future evolvability of the population; and to preserve the conserved species from adaptation to captivity, in order to allow a possible reintroduction in the wild (Loebel *et al.*, 1992; Borlase *et al.*, 1993; Couvet & Ronfort, 1994). Because genetic resources are usually maintained with low census numbers, both in animal species kept in captivity and plant species under germplasm storage, genetic drift is the main source of loss of genetic diversity.

Several simple procedures using basic population genetics theory have been suggested to maintain the maximum possible genetic variation in conservation programmes (Ballou & Lacy, 1995; Caballero & Toro, 2000). One of the most widely used procedures is the equalization of parental contributions, i.e. each of the individuals in the population contributes exactly the same number of offspring to the following generation (Gowe *et al.*, 1959; Wang, 1997). This produces rates of inbreeding and genetic drift that are only

about half as large as those produced under random mating. The lower amounts of genetic drift diminish the probability of random loss of alleles, and the lower levels of inbreeding reduce the depression in reproductive traits. However, equalization of parental contributions has also the effect of reducing the intensity of natural selection, as differences in fecundity among parents are obviated, except for complete mating failures. Thus, the method makes it more likely that mild deleterious mutations accumulate in the genomes, particularly in small populations. Therefore, one could argue that standard practices in conservation programmes maximize the rate of accumulation of such alleles (Lange, 1981; Couvet & Ronfort, 1994); it is not immediately apparent that the benefits of preserving diversity by a particular programme are not offset by such negative side-effects.

Several authors have studied theoretically and through computer simulations the effects of the accumulation of deleterious mutations on the fitness of small populations (e.g. Gabriel & Bürger, 1994; Lande, 1995; Lynch *et al.*, 1995). They concluded that, depending on the population size, on the reproductive rate (i.e. the maximum number of offspring per individual) and on the action of stochastic demographical factors, this accumulation could represent a high risk of extinction

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for unmanaged populations. These studies, however, do not assess the performance of equalization of parental contributions.

Schoen *et al.* (1998) compared the theoretical loss in fitness occurring under a management method with variable contributions from parents against a method in which contributions were equalized. Their conclusion was, in general terms, that equalizing contributions leads to a higher accumulation of mutations and therefore to lower fitness in the population. According to this, management procedures based on equalization of family sizes should be discouraged. However, this study was restricted to mutations affecting differences in fecundity (with absence of selection within families through viability differences), constant mutational effects, a particular set of mutational parameters, and unlimited progeny per individual. In this paper we extend the previous work by taking into account the following factors.

1 Mutations affecting fecundity will not be purged under equalization of contributions provided the reproductive capacity of the species is not extremely low. However, mutations affecting viability will be purged even with equalization of parental contributions, through differences among the progeny of each family. Therefore, both principal components of fitness should be incorporated in the comparison. Moreover, other demographic parameters such as the reproductive rate (R) have been pointed out as critical factors in the fate of captive populations (see Lynch *et al.*, 1995), and should be considered.

2 The rate of mutation accumulation greatly depends on the distribution of mutational effects (Schultz & Lynch, 1997). This distribution and the dominance of mutations have not been completely elucidated, but there is current information that can be used (see, e.g. García-Dorado *et al.*, 1999; references therein).

3 The rate at which spontaneous deleterious mutations appear is a matter of current debate. Some studies with *Drosophila*, *Daphnia* and plants (see review by Lynch *et al.*, 1999) suggest detected mutation rates per haploid genome as high as $\lambda = 0.5$; but others with nematodes, plants and reanalysis of *Drosophila* estimates (see reviews by García-Dorado *et al.*, 1999; Keightley & Eyre-Walker, 1999; Bataillon, 2000) suggest a different scenario with much smaller mutation rates. Therefore, different sets of parameters should be tested to broaden the study.

4 Equalization of parental contributions is likely to imply monogamous matings. Thus, in order to allow a comparison in equivalent conditions, the unmanaged procedure with which the former should be compared has to involve not only polygamous but also monogamous matings.

5 Other additional factors, such as relaxation of selection in conditions of captivity, may increase the rate of accumulation of deleterious mutations. Selection coefficients that are responsible for the equilibrium allele frequencies in a natural population are likely to be different from those applicable in captive conditions (Kondrashov & Houle, 1994). Because of the relaxed conditions in zoological parks and botanical gardens (e.g. avoidance of predators, competitors and pathogens, provision of balanced diets, assistance in child rearing, etc.), this may result in the relaxation of selection against alleles that otherwise would be harmful when expressed in nature (Schoen *et al.*, 1998).

We used stochastic computer simulations to investigate the effect of the above factors on the accumulation of deleterious mutations in a population under equalization of parental contributions, in comparison with other under unmanaged variable contributions.

Model assumptions and simulations

We carried out simulations for a monoecious cross-fertilizing species. Fitness was controlled by a large number (up to 5800) of biallelic loci with effects, s, in homozygosis and dominance coefficients, h, that can be constant or variable (see mutational models below). For each locus, the genotypic fitnesses were 1, $1 - sh$, $1 - s$ for the AA, Aa, aa genotypes, respectively. Fitness was assumed to be multiplicative across loci. An ancestral population was first set up in which gene frequencies were at mutation–selection balance. For a given allele with values s and h, the frequency was calculated from eqn 6.2.6 in Crow & Kimura (1970; p. 260), and the allele was randomly allocated among the individuals of the base population according to this frequency. Two-hundred neutral multiallelic loci were also simulated interspersed between the selected loci in order to monitor changes in neutral genetic variation. Neutral and selected loci were equally spaced in a genome length of 20 morgans, with cross-overs without interference occurring at randomly chosen places.

Samples of 25 or 100 individuals, the two considered population sizes (N), were randomly taken from the ancestral population and used for reproduction during a total of 50 generations. Every generation the fitness of the population was calculated and averaged over 50 replicates. As the number of offspring per individual (R) was limited, sometimes the number of surviving individuals did not reach the fixed population size, and this diminished. If the number of surviving offspring in the whole population was less than two, we considered that the line had become extinct and zero fitness was assigned to this line in the remaining generations when calculating the average fitness for all replicates.

Mutational models

We investigated a model in which λ mutations (Poisson distributed) appeared per haploid genome and generation with mean effect $\bar{s}$ and mean dominance coefficient $\bar{h}$. Every generation, mutations were randomly assigned to non-polymorphic positions of the genome. The selection coefficients were sampled from a gamma distribution with shape parameter β (the smaller β, the more leptokurtic the distribution of effects). Lethal mutations were not considered, as they would be expected to be efficiently purged from the population (this is an assumption made in most previous studies). Different values of λ and $\bar{s}$ were assumed (see Table 1), and the values of β were taken to give a mutational variance around 0.0016, the observed estimate for fitness in *Drosophila* (see Table 5 in García-Dorado *et al.*, 1998). The parameters $\lambda = 0.5$, $\bar{s} = 0.05$, $\beta = 1$ (exponential distribution) have been frequently assumed in mutation studies. A model with these parameters but constant effects ($\beta = \infty$) and dominance ($h = 0.35$) was assumed by Schoen *et al.* (1998). The parameters $\lambda = 0.03$, $\bar{s} = 0.264$, $\beta = 2.3$ were obtained through minimum distance estimation of *Drosophila* fitness data (García-Dorado *et al.*, 1999) and imply a small number of mutations with large effects.

The dominance coefficient of mutations, h, was obtained from an exponential function of the gene effects. The model is that proposed by Caballero & Keightley (1994), for which h is taken from a uniform distribution between 0 and $\exp(-ks)$, where k is a constant allowing the mean dominance coefficient to be the desired one. A value $\bar{h} = 0.35$ was generally used, but for the model of $\lambda = 0.03$ we also considered a value of $\bar{h} = 0.2$, a figure recently suggested by García-Dorado & Caballero (2000) for viability mutations in *Drosophila*.

Relaxation of selection in benign conditions

In order to assess the effect of relaxation in the selection pressure because of the benign conditions in captive populations, we investigated the effect of a reduction in the selection coefficient of mutations. The above models and parameters were used to set the initial natural population, so the initial distribution of gene frequencies would be as above. However, when the population was subject to genetic management, gene effects for initially segregating or new mutations were reduced by a given proportion. Fecundity or viability of individuals was calculated with relaxed selection coefficients, but the population was evaluated for fitness using the original ones. This was aimed at evaluating the performance of the population if it were brought back into the wild (a final objective in conservation programmes). Because the magnitude of the relaxation of selection in captive conditions is unknown, we investigated reduction factors of 50 and 10%.

Management procedures

Every generation, adults mated randomly (except by self-fertilization) following three procedures.
- *Random.* Parents had a probability of contributing to the next generation that depended on their fecundity and/or the viability of their offspring (see selection models below). Each new individual was obtained as follows. Two parents were randomly chosen among all potential parents (25 or 100) according to their

Table 1 Fitness at generation 50 (% of that in the ancestral population) for selection on fecundity, viability or fecundity and viability

Case	N	λ	$\bar{s}$	β	$\bar{h}$	r	Fecundity			Fec. + Via.			Viability		
							R	RP	E	R	RP	E	R	RP	E
a	25	2	0.01	0.07	0.35	0.6	24	17	19	28	23	28	31	30	38
b	25	1	0.025	0.25	0.35	0.6	34	18	15	39	29	26	42	42	40
c	25	0.5	0.05	1	0.35	0.6	51	34	23	54	47	39	60	59	52
d	25	0.1	0.1	0.5	0.35	0.6	88	77	59	91	87	76	92	92	86
e	25	0.03	0.264	2.3	0.35	0.6	98	91	70	99	96	87	99	99	95
f	25	0.5	0.05	1	0.35	0	–	–	–	58	44	36	–	–	–
g	25	0.5	0.05	1	0.35	1	–	–	–	56	49	40	–	–	–
h	25	0.03	0.264	2.3	0.20	0.6	97	91	72	98	96	84	99	98	95
i	100	0.5	0.05	1	0.35	0.6	84	60	43	84	72	56	83	84	69
j	100	0.03	0.264	2.3	0.20	0.6	98	94	83	99	98	91	99	99	96

R, Random method; RP, Random–Paired method; E, Equal method; N, population size; λ, haploid mutation rate per generation; $\bar{s}$, average selection coefficient; $\bar{h}$, average dominance coefficient; r, correlation between fecundity and viability in the model including both traits. Reproductive rate $R = 50$.

fecundity values. These parents produced an off-spring. The viability of this offspring was evaluated, and the individual could survive or die according to it. If it died, two new parents were randomly chosen, again according to their fecundity values. A parent was still eligible if the number of offspring it had produced (surviving or not) had not exceeded the determined reproductive rate (R). This process went on until N offspring survived or all parents but one had spent all their R opportunities.

- *Random–Paired*. This is similar to the previous method except that pairs of parents were randomly fixed at the beginning of every generation, so pairs instead of individuals were chosen. This management procedure was performed to mimic conserved populations of monogamous species. It also allowed a comparison in similar terms with the Equal method below.

- *Equal*. In this method couples of parents were randomly determined. Then the offspring were obtained in two steps. First we tried to produce two offspring per couple; when an offspring died, depending on its fitness (viability), another offspring from the same pair was produced unless one or the two parents had reached the R limit. If the population size had not been regenerated after this step, couples were taken randomly to produce offspring until N was reached or all possibilities had been exhausted.

Selection models

Fitness was assumed to act through differences in fecundity, differences in viability or both simultaneously maintaining the same mutational pressure (λs) for total fitness.

- *Differences in fecundity*. All genes were assumed to act through differences in fecundity among individuals. For the Random method, when a potential parent was chosen, its fitness (fecundity) was evaluated. A random number between 0 and 1 was taken. If the number was smaller or equal to the fecundity value, the parent was used, otherwise the parent was not used and another parent (with replacement) was randomly chosen. For the Random–Paired method the procedure was the same except that the average fecundity of the couple was used and couples instead of individuals were sampled. Unless the reproductive capacity of the species was low, no selection occurred under this model for the Equal method, because each couple necessarily contributed two offspring.

- *Differences in viability*. All genes were assumed to act through differences in viability among individuals. When an offspring was produced, its fitness (viability) was evaluated. A random number between 0 and 1 was taken. If the number was smaller or equal to the

viability value, the individual survived, otherwise the individual died. This selection model applied similarly for all management methods.

- *Differences in fecundity and viability*. Genes were assumed to act through differences in fecundity and viability with different correlations (r) between traits. In some runs, half of the genes and mutations affected fecundity and the other half affected viability, giving $r = 0$. For others, all genes affected both fecundity and viability ($r = 1$), with effect $\sqrt{s}$ on each trait. Finally, an intermediate case ($r \approx 0.6$) was obtained assuming that three-quarters of the genes affected both fecundity and viability, while the remainder affected only one or the other trait, in equal proportions.

Results

Figure 1 shows the evolution of fitness for different selection models and management procedures with mutational parameters $\lambda = 0.5$, $\bar{s} = 0.5$. The observed drop in fitness occurs for two reasons. First, the inbreeding depression due to the bottleneck to which the ancestral population is subjected. Second, the accumulation of mutations arising during the conservation programme. The relative effect of these two factors depends on the population size and the management method. Under equalization of parental contributions inbreeding depression is expected to be lower, as the method produces a larger effective population size. However, the purging of mutations will be slowed down at different rates depending on the models of selection.

Graph (a) of Fig. 1 refers to the fecundity model with constant mutational effects considered by Schoen *et al.* (1998). Because, with this model, selection does not operate under the Equal method, there is a substantial drop in fitness relative to the Random method. However, an unmanaged method with monogamy (Random–Paired method) is closer to the Equal method. The reason is that with monogamy the amount of purging is much smaller than with polygamy. If an individual with few mutations is randomly mated to another with many mutations, the first will suffer from a reduction in its probability of leaving offspring. However, under polygamy (Random method) the individual will be able to mate to different individuals, so its probability of contributing offspring to the next generation may be greater. This effect occurs for fecundity selection (see Fig. 1 and below), and depends on the mating requirements of the species, not on the parental contributions being equal or variable. Therefore, part of the difference between management and no management observed by Schoen *et al.* (1998) is explained by the difference between polygamous and monogamous systems, the first one allowing more purging of deleterious mutations.
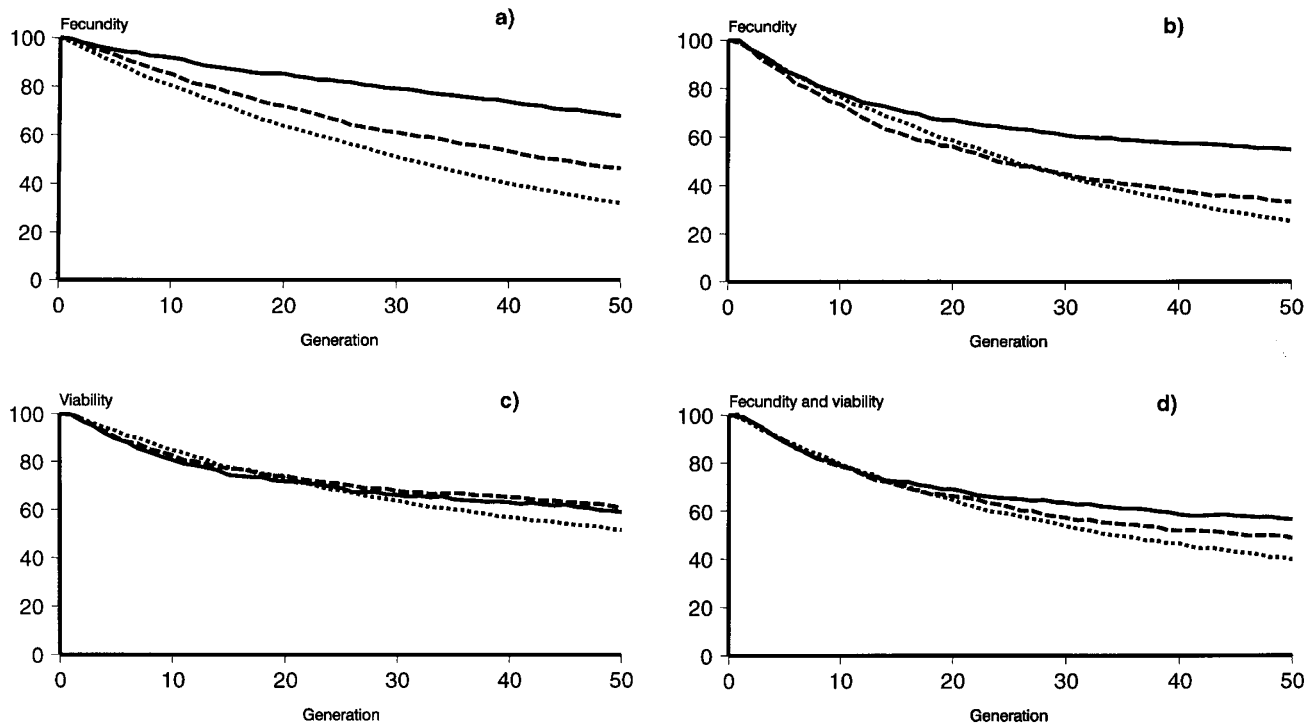
Fig. 1 Mean population fitness (% of that in the ancestral population), plotted against generations, for a population of size $N = 25$, reproductive rate $R = 50$, and different models of selection (effects on fecundity, viability or both with correlation $r = 0.6$). Solid lines represent performance of Random method, broken lines Random–Paired method and dotted ones the Equal method. Mutational parameters: $\lambda = 0.5$, $\bar{s} = 0.05$, $\beta = \infty$ (graph a) or $\beta = 1$ (graphs b, c and d).

Graph (b) incorporates a more realistic model of mutations, where these are variable (exponential distribution; $\beta = 1$). For this model, mutations of very small effect are more abundant. Thus, the purging is less apparent under the random method (and, particularly, the Random–Paired method), and the differences between methods are reduced, especially in the earlier generations.

Graph (c) represents a model of viability selection. In this case, selection also occurs for the Equal method and the differences between methods are further reduced. Finally, graph (d) represents a compound model where fitness effects act both through differences in fecundity and viability. The results are approximately intermediate between the models for each of the fitness components alone.

Table 1 shows the relative fitness at generation 50 for a range of parameters. A comparison of cases (a) to (e) indicates that the largest relative differences between Random and Equal methods occur with $\lambda = 0.5$ or 1 and $\bar{s} = 0.05$ or 0.025. For models with very large λ and very small $\bar{s}$ purging has very low efficiency, the mutational damage is high, and the difference between management methods is generally reduced. The higher effective size produced by the Equal method may render even larger fitness than the Random method with viability selection. For models with very small λ and large $\bar{s}$, purging is very effective, the mutational damage is low, and the differences between methods are again reduced.

An increasing correlation between fecundity and viability increases purging under the Equal method, but the effect is almost negligible [see cases (c) (f) and (g) in Table 1]. For the model of few mutations of large effect, a reduction in the average coefficient of dominance has also very little effect [cf. cases (e) and (h)]. The differences between Random and Equal methods are increased with increasing population size for models of high mutation rate [cf. cases (c) and (i)]. This occurs because the relative effect of inbreeding depression vs. accumulation of mutations is lowered with high census numbers. Thus, for larger population size, inbreeding depression is less important, and the benefit from an increased effective population size due to equalization of parental contributions is diminished. For models of low mutation rate and large effects, an increase in population size may reduce the difference between Random and Equal methods [cf. cases (h) and (j)], because the higher selection pressure eliminates mutations more efficiently under all methods.
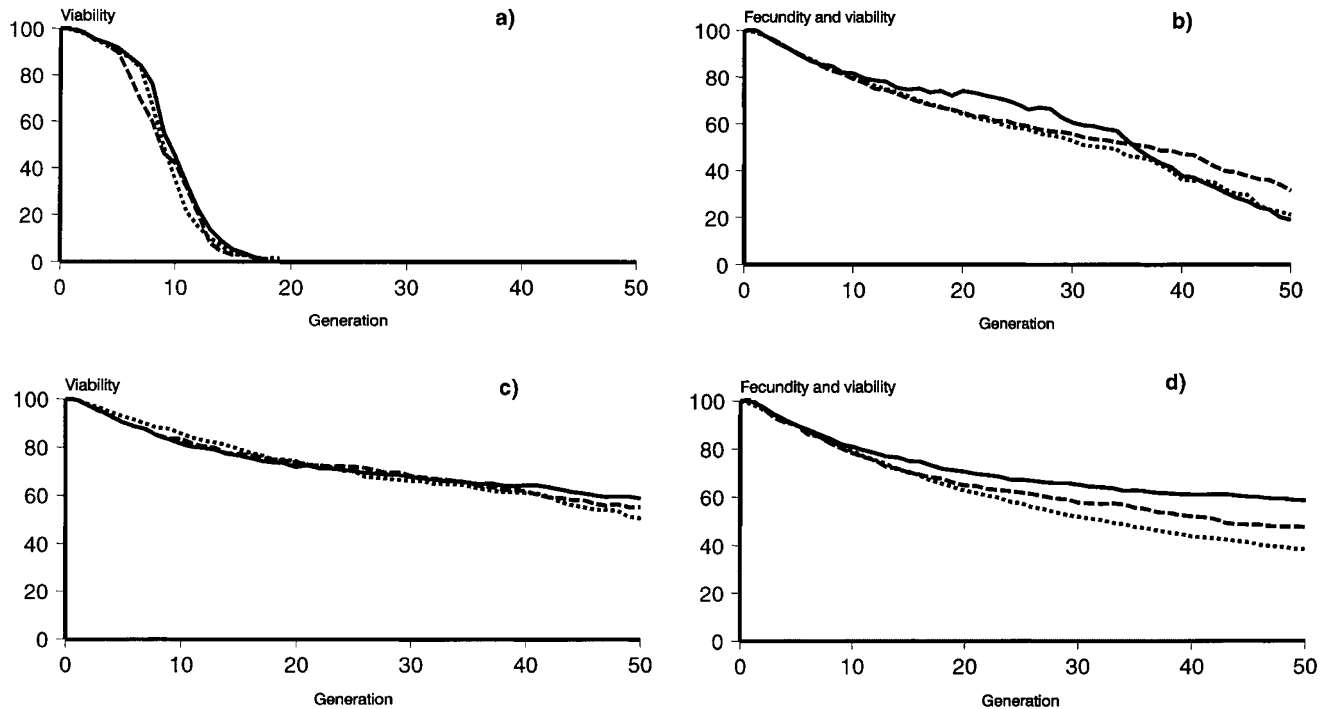
Fig. 2 Mean population fitness against generations for variable reproductive rates. Model of selection affecting only viability, or both fecundity and viability with correlation $r = 0.6$. Definitions as in Fig. 1. $\lambda = 0.5$, $\bar{s} = 0.05$, $\beta = 1$. $R = 5$ (graphs a and b) or $R = 10$ (graphs c and d).

Figure 2 shows the evolution of relative fitness considering smaller reproductive rates for the species. As these become smaller the discrepancy between methods is reduced (cf. $R = 5$ in Fig. 2a,b; $R = 10$ in Fig. 2c,d; $R = 50$ in Fig. 1c,d). In the extreme case of $R = 2$ (not shown) populations run to extinction very quickly (in around five generations) irrespective of the management system and population size.

To assess the effect of a reduction in the selection pressure in captive conditions we investigated the fitness performance of populations when the selection coefficients of mutations in captive conditions are a given proportion of those in natural environment. The effect of a reduction in the selection coefficient is double. First, the mean time to extinction obviously increases, because populations with many mutations that would become extinct in nature are able to survive in benign conditions. Second, purging is less effective and therefore more mutations are accumulated in captive conditions. The first effect is more apparent for low reproductive rates, for which extinction occurs very rapidly. The second one is observed for large reproductive rates, the population fitness in nature being smaller the lower the selection coefficient in captive conditions.

Table 2 shows the mean fitness of the population in natural conditions assuming it has spent 50 generations

in captivity. As pointed out above, the population fitness in nature is generally smaller the lower the selection coefficient in captive conditions. It is also observed that differences between Random and Equal methods almost disappear when $s' < s$. Furthermore, for strong relaxation ($s' = s/10$), the Equal method yields higher fitness when brought back to the wild. This occurs because genetic drift becomes more important than selection as the deleterious effects of mutations get smaller in captive populations. Thus, fixation of mutations is basically driven by genetic drift. Because the Equal method produces a larger effective population size, fixation of mutations will be lower than in the Random method.

Finally, neutral genes were used to measure the gene diversity and the allelic diversity maintained by each method under the different models and management methods. As expected, the advantage of the Equal method in maintaining genetic diversity was apparent (not shown), keeping higher levels of heterozygosity and delaying the loss of alleles.

Discussion

Strategies to diminish genetic drift by enlarging effective population size, such as equalization of contributions, are usually recommended in conservation programmes.

Table 2 Fitness in natural conditions (% of that in the ancestral population) after 50 generations under relaxed selection pressure

			$N = 25$			$N = 100$		
			$s' = s$	$s' = s/2$	$s' = s/10$	$s' = s$	$s' = s/2$	$s' = s/10$
Model I								
	$R = 5$							
		R	19	37	21	78	60	42
		E	21	31	26	61	50	44
	$R = 50$							
		R	54	37	18	84	61	40
		E	39	31	26	56	50	44
Model II								
	$R = 5$							
		R	98	91	70	99	94	82
		E	90	81	69	90	88	84
	$R = 50$							
		R	98	90	67	99	94	81
		E	84	81	69	91	88	84

All data for a compound fitness model with $r \approx 0.6$ between fecundity and viability.
R, Random method; E, Equal method; s, selection coefficient in natural conditions; s', selection coefficient in captive conditions; N, population size; R, reproductive rate.
Model I: $\lambda = 0.5$, $\bar{s} = 0.05$, $\beta = 1$, $\bar{h} = 0.35$.
Model II: $\lambda = 0.03$, $\bar{s} = 0.264$, $\beta = 2.3$, $\bar{h} = 0.2$.

These procedures, however, may lower the strength of selection and allow deleterious mutations to accumulate, with a detriment in population fitness. In this study we compared equalization of parental contributions with an unmanaged population with variable ones. Our general conclusion is that the potential risk from accumulation of deleterious mutations in populations under equalization of contributions is not very different from that in unmanaged ones, at least in the short term (say, up to 20 generations). This is particularly so for small populations and low reproductive rates. In small populations genetic drift and inbreeding depression play a more important role, so the effect of selection as a purging mechanism is reduced in relative terms. Low reproductive rates tend to diminish the variance of contributions even with large differences between individual fitnesses. Thus, the two methods, Random or Random–Paired and Equal contributions, tend to converge.

Schoen *et al.* (1998) carried out computer simulations to mimic the evolution of fitness in a seed collection (germplasm bank). Management of the population included a method with contributions proportional to parents' fecundity (equivalent to our Random method under fecundity selection and $R = 50$; see Fig. 1a) and another method where contributions from all parents were equalized. They found that the decline in fitness under the equalization strategy was much greater than under unmanaged conditions. In their theoretical assumptions they used a model of fecundity selection, such that, in the case of the Equal method, it did not allow for any natural selection at all. As pointed out by Couvet & Ronfort (1994) and Wang & Hill (2000), although differences in fecundity among parents are avoided with equalization of family sizes, selection within the offspring of a family or an individual always takes place. This selection must be present in the form of viability selection, as less viable seeds will have a smaller probability of being used in the following cycle. Therefore, it is not justified to ignore this type of selection.

We have shown that part of the discrepancy between the Random and Equal methods resides in the system of reproduction. A polygamous system allows more purging of deleterious mutations. When the Random method is carried out with fixed pairs (Random–Paired method), the difference from the Equal method is reduced. Although the reference system in many plants may be the Random method, it is likely that the corresponding one in animals will be the Random–Paired method. In addition, the use of a viability model allows more mutations to be purged under the Equal method and the differences between Random and Equal methods mostly disappear.

We have investigated a wide range of mutational models (Table 1). In the worse scenario (a large amount

of mutations with an effect of a few percentage) it is predicted that the fitness of the conserved population will be largely reduced, leading in some cases to extinction. Most of the comparisons have been made on this scenario (Figs 1 and 2). For an alternative model of few mutations of large effect, the predicted decline in fitness is quite small (Table 1) and should not be a concern in conservation programmes. It should be noted that most data pointing to the former model come from egg-to-adult viability in *Drosophila*, whereas data from other fitness components and species usually point to the latter model (see, e.g. Bataillon, 2000). Because, as we have shown, mutations affecting viability are easily purged with the Equal method, this suggests that the differences found between Equal and Random methods using fecundity models are exaggerated.

Usually the conserved populations are kept under benign conditions and the relaxation of selection can increase the accumulation of mutations. Bryant & Reed (1999) performed an experiment with flies (*Musca domestica*) reducing the selection pressure on late-fitness traits. They found greater declines in fitness when measured in natural conditions. We have shown (Table 2) that under management in relaxed conditions there is a greater probability for deleterious mutations to become fixed under the Random method than under Equal contributions. The greater the relaxation the more mutations become fixed under the former method and the better the performance of the Equal method.

In captive populations of most animals the idea of practising some type of artificial selection for removing deleterious mutations seems to be unrealistic. However, this possibility deserves some consideration in domestic animals (e.g. Toro *et al.*, 1999), as well as in plants. In the particular case of seed banks in plants, one can think that several seeds per plant can be tested (when adults) for fecundity, and the one with the best performance (that producing more seeds) used for regeneration. The selection method can also be aided by the use of genetic markers (Toro *et al.*, 1999; Wang & Hill, 2000). The effectiveness of artificial selection on fitness in removing deleterious genes has been experimentally proved by Frankham *et al.* (1993).

Few experimental data are available to test theoretical results on the above issues. In a work by Shabalina *et al.* (1997), where large populations were maintained under equalization of family sizes, a substantial decline in fitness was observed. This decline was ascribed to accumulation of mutations, but other additional explanations can be considered (Keightley *et al.*, 1998; see also a similar experiment by Gilligan *et al.*, 1997). Moreover, the effective population size in the experiment of Shabalina *et al.* was 400. In *ex-situ* conservation

programmes it is expected that the population numbers are much smaller. Thus, genetic drift and inbreeding depression are expected to be more important, in relative terms, than accumulation of mutations. Experiments with low population sizes have been run by Loebel *et al.* (1992) and Borlase *et al.* (1993). In these experiments populations with random contributions from parents showed no significantly different, or even lower, fitness than those obtained by equalizing individual or founder contributions.

The issue of adaptation to captivity may be another important factor to be considered in conservation, when the final aim is the reintroduction into the wild. Allendorf (1993) studied, through computer simulation, the effect of equalization of family size on the frequency, and eventual fixation, of an allele deleterious in natural conditions, but responsible for adaptation to some captive condition. His conclusions were that frequencies of such kind of alleles increase faster in unmanaged populations than in populations with equalized contributions. So equalization of parental contributions is a potentially important technique to retard adaptation to captivity of populations undergoing a conservation programme.

An assumption in our simulations and the ones from different studies (Gabriel & Bürger, 1994; Lynch *et al.*, 1995; Schoen *et al.*, 1998) is the multiplicative relation between mutations. When synergistic interactions are considered the mutational load is lower, as the effect of new mutations is magnified by the existing ones, so selection can easily purge them. This has been assessed both theoretically (Schultz & Lynch, 1997) and using simulations (Charlesworth *et al.*, 1993; Couvet & Ronfort, 1994). Another issue to consider is the fact that beneficial mutations may actually occur (Bataillon, 2000), although at a much lower rate than deleterious ones. This may be of importance when thinking about long-term performance of conserved populations (Schultz & Lynch, 1997), but the problem arises as to what mutational rate can be realistic.

Another point of interest is the number of generations that should be considered. We have run simulations with up to 50 generations, but most species under conservation programmes have long generation intervals, so it may not be realistic thinking about schedules of such a long time. In fact, one recommended strategy would be to enlarge as much as possible the time between generations to slow down the rise of mutations by keeping individuals in dormant states as plant seed or cryopreserved embryos (Schoen *et al.*, 1998; Gandini & Oldenbroek, 1999). Therefore, the problems of accumulation of mutations may be, in most practical situations, less worrying than one would expect after so many generations.

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