

A simulation study on detecting purging of inbreeding depression in captive populations

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Summary

Inbreeding depression threatens the survival of small populations of both captive and wild outbreeding species. In order to fully understand this threat, it is necessary to investigate what role purging plays in reducing inbreeding depression. Ballou (1997) undertook such an investigation on 25 mammalian populations, using an ancestral inbreeding regression model to detect purging. He concluded that there was a small but highly significant trend of purging on neonatal survival across the populations. We tested the performance of the regression model that Ballou used to detect purging on independently simulated data. We found that the model has low statistical power when inbreeding depression is caused by the build-up of mildly deleterious alleles. It is therefore possible that Ballou's study may have underestimated the effects of ancestral inbreeding on the purging of inbreeding depression in captive populations if their inbreeding depression was caused mainly by mildly deleterious mutations. We also developed an alternative regression model to Ballou's, which showed an improvement in the detection of purging of mildly deleterious alleles but performed less well if deleterious alleles were of a large effect.

1. Introduction

Inbreeding depression, the reduction in fitness of the offspring of matings of close relatives, has been observed in many species of outbreeding plants and animals (e.g. Wright, 1977; Charlesworth & Charlesworth, 1987; Ralls *et al.*, 1988; Lacy *et al.*, 1993; Falconer & Mackay, 1996; Crnokrak & Roff, 1999; Frankham *et al.*, 2002). Inbreeding depression threatens the survival of small populations, both wild and captive, and so is a particular concern of breeding programmes in zoos. However, since inbreeding increases the frequency of the deleterious homozygous genotype, selection against these deleterious alleles is also increased (Crow & Kimura, 1970), providing an opportunity for a population to be 'purged' of its mutational load (Lande & Schemske, 1985; Barrett & Charlesworth, 1991; Hedrick, 1994). Such purging might cause fitness to increase, possibly returning to or even exceeding the fitness levels of a large, randomly

mating population at mutation-selection balance. Purging therefore has high conservation relevance. At present, the effects of purging in both wild and captive populations are little known and so it is not possible to fully gauge the long-term threat posed by inbreeding depression to small populations.

Templeton & Read (1984) reported that the deliberate inbreeding of Speke's gazelle and careful selection of healthy inbred individuals dramatically reduced inbreeding depression in only two to three generations. Based on these results, recommendations were made for the use of deliberate inbreeding in other captive populations suffering from severe inbreeding depression (Templeton & Read, 1984; Ralls & Ballou, 1986; Ballou, 1989). However, a reanalysis of the Speke's gazelle data by Kalinowski *et al.* (2000) did not find evidence of any genetic improvement in fitness due to inbreeding and selection. If purging is not occurring in captivity, deliberate inbreeding could be intensely damaging to small, often endangered, populations. There is a clear need to establish whether or not purging regularly occurs in captive populations.

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Some empirical data suggest that deleterious alleles of both large and small effect contribute equally to inbreeding depression in *Drosophila* (Simmons & Crow, 1977). Computer simulations have shown that semi-lethal and lethal alleles will be purged far more quickly than those that are only mildly deleterious (Hedrick, 1994; Schultz & Willis, 1995; Wang *et al.*, 1999). It therefore seems likely that successive generations of inbreeding will purge the lethal and semi-lethal alleles although the mutational load resulting from mildly deleterious alleles may persist (Hedrick, 1994; Wang *et al.*, 1999). Very little is known about purging in wild populations since it can be investigated only if the populations have a full pedigree spanning several generations and no immigration. In the laboratory, however, it is relatively straightforward to conduct investigations into the efficiency of purging and many such studies have been carried out. The results show that the effects of purging can be highly variable (e.g. Lacy & Ballou, 1998; Byers & Waller, 1999; Fowler & Whitlock, 1999; Frankham *et al.*, 2001; Reed & Bryant, 2001; Crnokrak & Barrett, 2002; Reed *et al.*, 2003). Significant purging has been detected in a variety of taxa (Streisinger *et al.*, 1981; Latter & Mulley, 1995; Pray & Goodnight, 1995; Saccheri *et al.*, 1996; Lacy & Ballou, 1998; Byers & Waller, 1999) but selection in these studies was fairly intense and the results cannot be extrapolated to populations of conservation concern. Selection in zoo conditions, for example, may not be sufficient to cause purging. The efficiency of purging also depends on the dominance of the deleterious alleles and the rate of inbreeding (Hedrick, 1994; Latter *et al.*, 1995; Wang *et al.*, 1999; Reed *et al.*, 2003). Populations of conservation concern usually have small effective population sizes and hence a more rapid rate of inbreeding, meaning that deleterious alleles may not be purged efficiently.

Ballou (1997) searched for evidence of purging in 25 captive mammalian populations which had inbred ancestry. He used a regression model to analyse the extent to which selection upon inbred ancestors of inbred individuals modifies those individuals' susceptibility to inbreeding depression. The logic behind the model is that an inbred animal with inbred ancestry should be less susceptible to inbreeding depression than an inbred animal with non-inbred ancestry because surviving and reproducing inbred ancestors are less likely to be carriers of deleterious alleles if purging is indeed occurring (Templeton & Read, 1984). Ballou found purging to be statistically significant in only one species, the Sumatran tiger, although, across species, ancestral inbreeding showed a highly significant trend toward reducing inbreeding depression in neonatal survival. This reduction was very small on average, however: a 2% change in

inbreeding depression at $f=0.25$. The observed extent of purging was not strong enough to advocate the use of deliberate inbreeding as a breeding strategy for captive populations.

Using simulation studies, Kalinowski & Hedrick (1999) found that typical zoo pedigrees, i.e. those designed to avoid inbreeding depression, allow a low statistical power to detect inbreeding depression when analysed with a regression model. As far as we are aware, the statistical power of the regression model that Ballou (1997) used to investigate purging has not been tested. Since the zoo pedigrees he analysed were mostly managed to minimise inbreeding (such management plans were put into practice in the early 1980s; Frankham *et al.*, 2002), there is a possibility that his model may have failed to fully detect the inbreeding effects (depression) and ancestral inbreeding effects (purging).

In this study we use computer simulations to test the statistical power of Ballou's (1997) regression model. We investigate the effects of population size and number of generations on the regression model's ability to detect the purging of inbreeding depression caused separately by mildly deleterious alleles, by alleles of intermediate effect and by semi-lethal alleles. The model is also tested on simulated populations in which no purging occurs to see whether purging is falsely detected. The number of lethal equivalents in the simulated population is calculated at each generation in order to ascertain the actual degree of purging. Ballou's ancestral inbreeding model is fitted to the simulated pedigree and fitness data to investigate its power for detecting purging events.

2. Method

(i) Simulations

A stochastic simulation program was developed to simulate the dynamics of deleterious mutations at 5022 independent loci in a captive population (Fraser & Burnell, 1970). Each locus had two alleles: the wild-type *A* and the deleterious mutant *a*. There were three possible genotypes – *AA*, *Aa* and *aa* – with relative viabilities of 1, $1-hs$ and $1-s$ respectively, where s is the selection coefficient and h is the dominance coefficient of the deleterious allele. All loci were assumed to be unlinked and to segregate independently, which is a reasonable approximation for species with a large genome and a large number of chromosomes (Wang, 2000). No new mutations were assumed to occur during the short period of attempted purging.

A founding population of N individuals, half of each sex, was formed by sampling randomly from an infinitely large outbred population at mutation-selection balance. The number of lethal equivalents

carried by an individual, L , was calculated using the equation

$$L = s(n_1 + 2n_2) \quad (1)$$

where s is the selection coefficient, and n_1 and n_2 are the numbers of heterozygous and homozygous mutations carried by the individual, respectively. The N individuals drawn from the infinite population will have, in expectation, an equal number of lethal equivalents but different sets of loci with deleterious mutations.

In each generation the sequence of operations was mating, reproduction and selection. A male and female were picked at random and mated, producing a zygote. We focused on viability selection and assumed that different loci acted multiplicatively in determining viability. The survival of a zygote was determined by calculating the zygote's viability, v , and comparing it with a random number from a uniform distribution. If the random number was smaller than v then the zygote survived; otherwise, it died. The fitness function was

$$v = (1 - s)^{n_2} (1 - hs)^{n_1} \quad (2)$$

where n_1 and n_2 are the numbers of heterozygous and homozygous mutations in the genotype, respectively. This process of zygote formation and selection was repeated until N surviving zygotes were obtained. The first $N/2$ viable zygotes were given male sex and the next $N/2$ viable zygotes female sex. Generations did not overlap. In this way, the population was maintained with the same sex ratio and size as the initial population. The effective size (N_e) of the population under the breeding scheme described above would be

$$N_e = N + 1/2 \quad (3)$$

(Falconer & Mackay, 1996) if viability selection were absent. With viability selection, N_e is expected to be slightly smaller than N .

The mutation rate, u , in the infinitely large outbred population was assumed to be 10^{-4} per generation per gene or 0.5 per generation per genome for mildly deleterious alleles (selection coefficient, $s = 0.01 - 0.05$), 5×10^{-5} per generation per gene or 0.25 per generation per genome for alleles of intermediate effect ($s = 0.1$) and 7×10^{-6} per generation per gene or 0.035 for semi-lethal alleles ($s = 0.5 - 0.9$). These values were deliberately chosen to yield (for a given number of 5022 loci) a realistic number of lethal equivalents, close to the average of 3.14 found in captive populations (Ralls *et al.*, 1988). They are also within the range of the still controversial estimates from empirical data. Classical estimates of mutation rates per generation per genome are $> \sim 0.3$ (Mukai, 1964; Mukai *et al.*, 1972; Ohnishi, 1977) although more

recently estimates of about tenfold lower have been made (Fry *et al.*, 1999; Caballero *et al.*, 2002; Ávila & García-Dorado, 2002).

The effects of different census population sizes (20–200) and numbers of generations (6–20) were investigated, as were the effects of selection (0.01–0.05, 0.1 and 0.5–0.9) and dominance coefficients (0.3, 0.15 and 0.02) of deleterious mutations. Empirical studies have given similar values to those used in the simulations although there is some controversy over estimates of s and h . The selection and dominance coefficients of mildly deleterious alleles have been estimated from experiments using *Drosophila* (Mukai, 1964; Mukai *et al.*, 1972; Ohnishi, 1977) in which deleterious mutations were allowed to accumulate on intact second chromosomes, sheltered from natural selection by balancer chromosomes. These studies found the average selection coefficient of deleterious homozygous mutations to be 0.01–0.05 with a dominance coefficient of 0.2–0.4. Lynch *et al.* (1995) obtained similar estimates for mutations segregating in natural populations by using information on the relative fitness of chromosomal homozygotes. Estimates using information on inbreeding depression in naturally selfing plant populations (Charlesworth *et al.*, 1990; Johnston & Schoen, 1995) give similar results. However, more recently, higher estimates of the selection coefficient have been obtained from mutation accumulation experiments, which are 0.15–0.25 in *Drosophila* (Fry *et al.*, 1999; Caballero *et al.*, 2002; Ávila & García-Dorado, 2002) and 0.05–0.2 in *Caenorhabditis elegans* (Keightley & Bataillon, 2000; Vassilieva *et al.*, 2000). A reanalysis of Ohnishi's (1977) data gave an average dominance coefficient of 0.1 (García-Dorado & Caballero, 2000).

Semi-lethal and lethal mutations have selection coefficients in the range 0.5–1.0. (A homozygous lethal is defined as viability being less than 10% of the wild-type value (Simmons & Crow, 1977).) In *Drosophila* these lethal mutations have an average dominance coefficient of 0.02–0.05 (Crow, 1993).

Two thousand replicates were run for each set of parameters in order to minimize sampling error. For every replicate, the average number of lethal equivalents (L) per surviving individual in the initial population (generation 0) and in the final population (generation t) was calculated. The actual effectiveness of purging was calculated as the proportional reduction in L , a widely used measure (Hedrick, 1994; Fu *et al.*, 1998; Fu, 1999; Wang *et al.*, 1999). L , however, could increase or decrease as a result of random genetic drift – although on average (over replicates) drift will have no effect on L . To disentangle the effects of purging and drift, we first obtained the (null) distribution of L assuming no selection. The

critical value of reduction in L , below which 95% of the replicates fell, was taken as the threshold. Only if the observed reduction in L was greater than this threshold was a purging event counted as actually occurring in a replicate.

The simulated pedigree and fitness data in each replicate were analysed by Ballou's regression model, as described below.

(ii) Ballou's logistic regression model

The effect of inbreeding on fitness is usually modelled as an exponential function of the inbreeding coefficient

$$S_f = S_0 e^{-Bf} \quad (4)$$

where S_f is the measurement of a fitness component (e.g. viability) of individuals with an inbreeding coefficient f , S_0 is the fitness component of outbred individuals and B is the number of lethal equivalents per gamete. In logistic regression analysis, a more convenient form of equation (4) is

$$u = u_0 + \beta_f f \quad (5)$$

where u is a measure of fitness, u_0 is the mean fitness of non-inbred animals, f is the inbreeding coefficient and β_f is the slope (regression coefficient) of fitness regressed against f (Ballou, 1997).

The regression model proposed by Ballou (1997) included the ancestral inbreeding coefficient as a modifier of the effect of inbreeding on fitness:

$$u = u_0 + \beta_f f + \beta_{f_a} f f_a \quad (6)$$

where u , u_0 , β_f and f are as described above, f_a is the ancestral inbreeding coefficient and β_{f_a} is the regression coefficient associated with the interactive term $f f_a$. It can be seen that the survival of non-inbred animals is independent of ancestral inbreeding since f_a is entered in the equation only as an interaction with the inbreeding coefficient. However, the inbreeding effect can be modified by the level of ancestral inbreeding. If purging has occurred then the coefficient β_{f_a} will be positive, thereby reducing the inbreeding effect. The larger the positive value of β_{f_a} , the greater is the effect of ancestral inbreeding on reducing inbreeding depression.

The fitness component used in this model was survival since the main focus of Ballou's analysis was neonatal survival. Every individual's survival (inviable = 0, viable = 1), inbreeding coefficient and ancestral inbreeding coefficient were calculated and recorded. An individual's inbreeding coefficient was calculated from the pedigree (Falconer & Mackay, 1996). The ancestral inbreeding coefficient, f_a , was defined as the cumulative proportion of an individual's genome that has previously been exposed

to inbreeding in its ancestors and was calculated according to Ballou (1997):

$$f_a = [(f_{a(s)} + (1 - f_{a(s)})f_{(s)}) + (f_{a(d)} + (1 - f_{a(d)})f_{(d)})]/2 \quad (7)$$

where f_a is the ancestral inbreeding coefficient for an individual, f is the inbreeding coefficient and the subscripts s and d represent these values for the sire and dam of the individual, respectively. An individual's f_a is thus the proportion of its parent's genome that has previously been exposed to inbreeding (f_a of the parent) plus the effect of the parent's inbreeding coefficient on the proportion that has not been previously exposed ($1 - f_a$), averaged across both parents; it ranges from 0 to 1.

The model Ballou (1997) used also included effects for year of birth (to control for changes in husbandry over time) and for inbreeding coefficient of the dam (this can be associated with poor offspring survival in mammals, independent of the inbreeding coefficient of the offspring (Brewer *et al.*, 1990)). These two effects were not relevant to our simulated data and hence were not included in our regression model.

(iii) Data and statistical analysis

Multiple logistic regression was used to estimate the regression coefficients in equation (6). In logistic regression the fitness function is of the form

$$u = \frac{e^x}{1 + e^x} \quad (8)$$

where x takes the form of the right-hand side of equation (6). Coefficients u_0 , β_f and β_{f_a} were estimated using Powell's quadratically convergent method (Press *et al.*, 2002 and references therein) and their significance tested with likelihood ratio tests (Hosmer & Lemeshow, 1989). Regression coefficients were calculated only in replicates in which an actual purging event occurred.

(iv) Measuring the effectiveness of the model

To assess the effectiveness of Ballou's model we measured the model's power at detecting a purging event. A decrease in L in a simulated data set (replicate) that was greater than would be expected as the result of genetic drift indicated that actual purging had occurred. Such an event was regarded as being detected by the model if both the regression coefficients, β_f and β_{f_a} , were statistically significant ($P < 0.05$). For each set of parameters, the power of the model was measured by calculating the number of replicates in which purging was detected by the model as a proportion of the number of replicates in which there was actual purging.

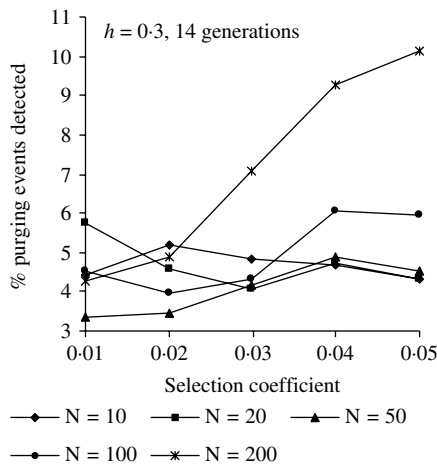


Fig. 1. Effects of the selection coefficient of the mutant alleles on the number of replicates in which purging was detected as a percentage of the number of replicates in which purging actually occurred. Population sizes are $N=10, 20, 50, 100, 200$. The dominance coefficient of the mutant alleles is 0.3 . Results are for 2000 replicates after 14 generations of breeding.

3. Results

We examined the model's ability to detect purging in four sets of simulated data. First, mutant alleles were assumed to be mildly deleterious (selection coefficient $s=0.01-0.05$ and dominance coefficient $h=0.3$). Second, alleles were assumed to be of intermediate effect ($s=0.1, h=0.15$). Third, mutant alleles were assumed to be semi-lethal ($s=0.5-0.9, h=0.02$). Fourth, mutant alleles were assumed to be nearly neutral ($s=0.001, h=0.5$). Pedigree and fitness data were simulated for populations of size $N=10, 20, 50, 100$ and 200 over a period of 6–20 generations. Two thousand replicates were run for each set of parameters.

(i) Mildly deleterious alleles

When mutant alleles were mildly deleterious ($s=0.01-0.05, h=0.3$), the percentage of replicates in which purging occurred in the simulated data increased with Ns from 50% to 100%; when $Ns > 1$, i.e. when selection dominated over genetic drift, purging occurred in over 95% of the replicates. The proportion of lethal equivalents purged, as measured at the last generation (i.e. generation 6–20), was not affected by population size but increased approximately linearly from 3% to 19% as s increased. Fig. 1 shows how the power of the regression model changed with s in populations of 10, 20, 50, 100 and 200 individuals after 14 generations. It can be seen that the model regularly failed to detect purging; purging was detected in only 3–10% of instances. Detection rate increased with N and s . As would be expected, the model's power of detection increased with both the frequency and the extent of actual purging.

Fig. 2 shows how the power of the model was affected by 6–20 generations of inbreeding when $s=0.03$ and $h=0.3$. Actual purging occurred in 65–100% of the replicates, actual purging events increasing with number of generations and with N . The proportion of lethal equivalents purged increased with number of generations (i.e. with inbreeding) from 5% to 16%. The model detected purging in only 1–10% of replicates (Fig. 2). Detection increased with number of generations and with N (Fig. 2a). It is helpful to plot these results against the average inbreeding coefficient of the last generation of the populations, f (Fig. 2b). Clearly, populations of larger N had lower f values after breeding for the same number of generations. Detection of purging increased with f and this increase became more rapid as N increased. The model therefore became more powerful as selective forces dominated drift and as inbreeding level increased.

As generation numbers increased, ancestral inbreeding increased and its effects became more pronounced, being detected as significant in up to 54% of replicates. Inbreeding effects were found to be significant in a maximum of only 10.5% of replicates. The model's poor detection of inbreeding effects therefore seems to have limited the detection of purging of mildly deleterious alleles.

(ii) Alleles of intermediate effect

Fig. 3 shows how the power of the model was affected by 6–20 generations of inbreeding when alleles were of intermediate effect ($s=0.1, h=0.15$). Purging actually occurred in 75–100% of replicates. Between 9% and 33% of lethal equivalents were purged from the simulated data, the proportion increasing with f . The model detected purging in 2–30% of replicates (Fig. 3). Detection increased with f and N . Although the detection of purging follows a similar pattern to that in Fig. 2, the signal is less noisy – since selection is higher, the effect of genetic drift is much weaker relative to the effect of selection. Once again, the detection of purging seemed to be limited by the model's detection of inbreeding depression; inbreeding depression was detected in up to 31% of replicates but ancestral inbreeding effects in up to 67% of replicates.

(iii) Semi-lethal alleles

As would be expected, the model was more powerful in detecting the purging of depression caused by semi-lethal alleles ($s=0.5-0.9, h=0.02$) as opposed to alleles of a mildly deleterious or intermediate effect. Fig. 4 shows how the power of the regression model changed with s in populations of 10, 20, 50, 100 and 200 individuals after 14 generations. The model's

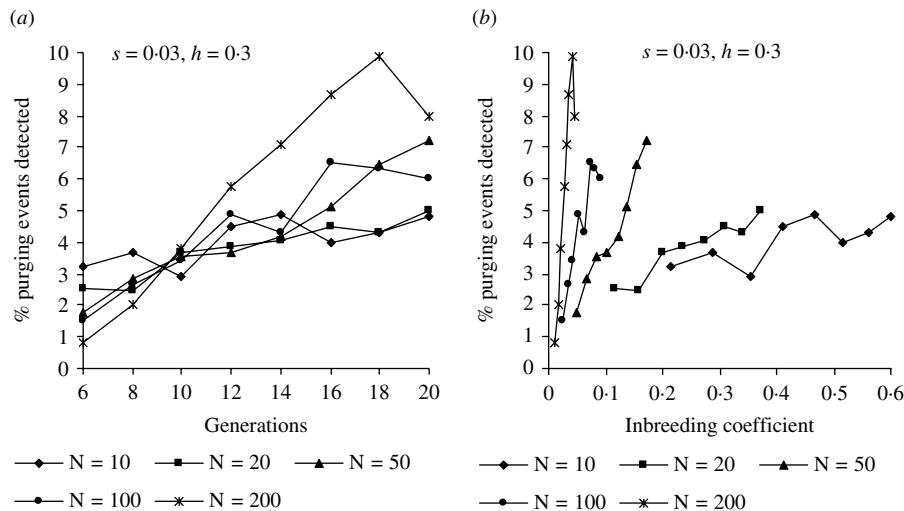


Fig. 2. Effects of (a) the number of generations of breeding and of (b) the average inbreeding coefficient of the surviving individuals in the last generation on the number of replicates in which purging was detected as a percentage of the number of replicates in which purging actually occurred. Population sizes are $N = 10, 20, 50, 100, 200$. The selection coefficient of the mutant alleles is 0.03 and the dominance coefficient is 0.3. Results are for 2000 replicates.

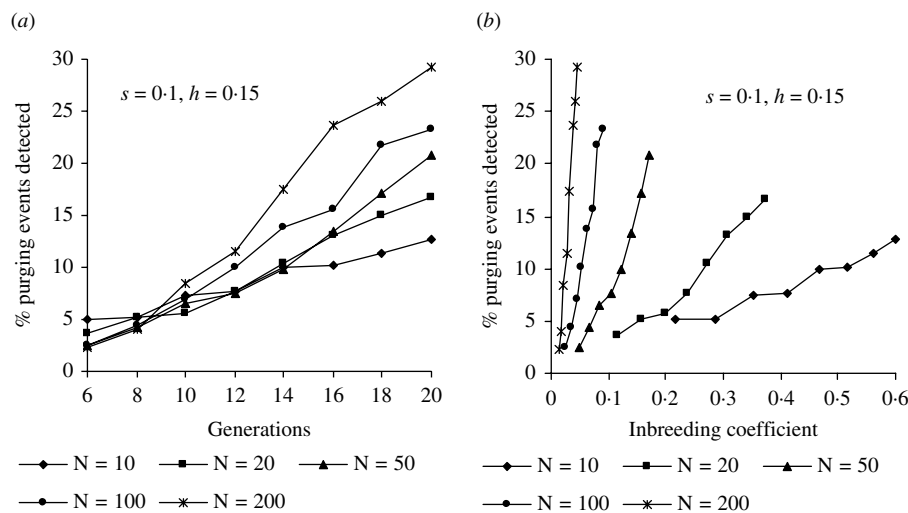


Fig. 3. Effects of (a) the number of generations of breeding and of (b) the average inbreeding coefficient of the surviving individuals in the last generation on the number of replicates in which purging was detected as a percentage of the number of replicates in which purging actually occurred. Population sizes are $N = 10, 20, 50, 100, 200$. The selection coefficient of the mutant alleles is 0.1 and the dominance coefficient is 0.15. Results are for 2000 replicates.

detection rate of purging increased from 11% to 55% as s and f increased. Actual purging in the simulated data increased from 98.1% to 100%. Between 21% and 79% of lethal equivalents were purged from the simulated data, the proportion increasing with f .

After 6–20 generations when $s = 0.7$ and $h = 0.02$, purging occurred in 83–100% of replicates, increasing with f and N . The proportion of lethal equivalents purged increased with f from 10% to 76%. The model detected purging in 4–78% of replicates (Fig. 5). The model's power to detect purging increased with f and N .

The model is clearly more powerful when alleles are semi-lethal than when they are mildly deleterious or

of intermediate effect. The model detected significant inbreeding effects caused by semi-lethal alleles in 42–95% of replicates. Ancestral inbreeding effects were found to be significant in 4–81% of replicates. When mutations are semi-lethal the detection of purging is therefore limited by the model's ability to detect ancestral inbreeding effects.

(iv) Nearly neutral alleles

Finally, we checked that the model did not give a false positive estimate of purging when no purging had occurred. When mutant alleles were nearly neutral ($s = 0.001$, $h = 0.5$), actual purging occurred, on

average, in 2.5% of replicates. The model detected purging in 0.6–2% of replicates. The rate of detection was not affected by f . Since significance measurements are one-tailed at a 5% significance level, these results show that the model does not falsely detect purging.

4. Discussion

The power of Ballou's (1997) model to detect purging generally increased as the proportion of lethal equivalents actually being purged increased. Up to 78% of purging events were detected when mutations

were semi-lethal as opposed to a maximum of 10% when mutations were mildly deleterious or a maximum of 30% when mutations were of intermediate effect. When detecting purging events, the model was most powerful when mutations were semi-lethal, inbreeding level was high and, for a fixed level of inbreeding, population size was large. The model was weakest when mutations were mildly deleterious, inbreeding had occurred for only a few generations and population size was small, i.e. when genetic drift dominated over selection and hence the proportion of lethal equivalents actually purged was low. The model did not falsely detect purging. The results show that the model is conservative at detecting purging, particularly when inbreeding depression is caused by mildly deleterious alleles.

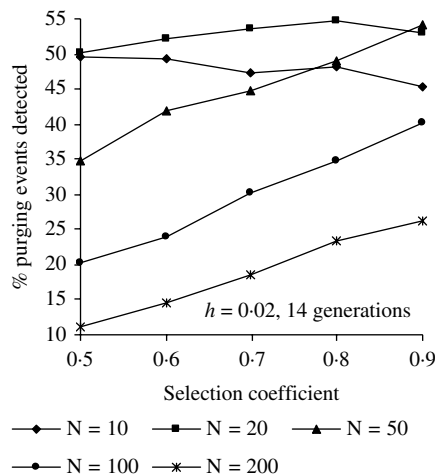


Fig. 4. Effects of the selection coefficient of the mutant alleles on the number of replicates in which purging was detected as a percentage of the number of replicates in which purging actually occurred. Population sizes are $N=10, 20, 50, 100, 200$. The dominance coefficient of the mutant alleles is 0.02. Results are for 2000 replicates after 14 generations of breeding.

(i) Assumptions made in the simulations

Several assumptions were made about both the individuals and the population dynamics in order to simplify the simulation study. However, the relaxation of these assumptions should not alter our conclusions although, in some cases, it would result in a slight reduction in the model's power. We assumed that all deleterious alleles had equal selection and dominance coefficients. This situation is unlikely to occur in a real genome – studies indicate that mildly deleterious alleles and lethals contribute simultaneously to inbreeding depression (Simmons & Crow, 1977; Sperlich *et al.*, 1977; Willis, 1999). However, this assumption does not alter our conclusion that inbreeding depression caused by mutations with a large selection coefficient and small dominance coefficient, i.e. semi-lethals or lethals, is

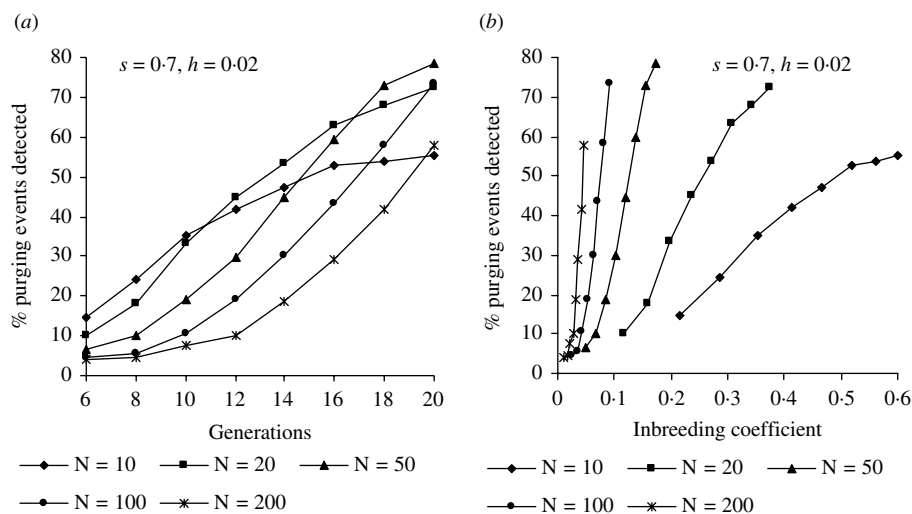


Fig. 5. Effects of (a) the number of generations of breeding and of (b) the average inbreeding coefficient of the surviving individuals in the last generation on the number of replicates in which purging was detected as a percentage of the number of replicates in which purging actually occurred. Population sizes are $N=10, 20, 50, 100, 200$. The selection coefficient of the mutant alleles is 0.7 and the dominance coefficient is 0.02. Results are for 2000 replicates.

more likely to be purged and hence that purging is more likely to be detected by the model. The mutations in the simulated individuals were assumed to be unlinked. Again, this situation would not be mirrored in real populations. It has been shown that selection against many linked deleterious mutations of small effects could result in apparent overdominance and thus reduce the efficacy of purging (Wang *et al.*, 1999). However, unless a small genome size or lack of recombination causes linkage to be tight, the reduction of the efficacy of selection should be small (Hill & Robertson, 1966; Wang *et al.*, 1999). For typical mammalian genomes (which are usually large), ignoring linkage will give a good approximation.

In the simulations, all data points were included in the analysis. In a real population, however, many zygotes might die unnoticed, e.g. those aborted early in pregnancy. The model's power might therefore be decreased when analysis is of empirical data.

Population size was constant and generations did not overlap in the simulated populations – this scenario will rarely occur in zoo populations. The simulated pedigrees differed from real zoo pedigrees since mating was random whereas, in managed zoo pedigrees, inbreeding is usually minimized. Contemporary animals in real pedigrees will therefore have a different distribution of inbreeding coefficients to the simulated animals. A more uniform distribution of lower inbreeding coefficients would decrease the power of detection of inbreeding depression (Kalinowski & Hedrick, 1999) and hence we would expect the power to detect purging to be decreased as well.

(ii) *The power of the model*

We thought that one of the reasons that Ballou's model had a low power in detecting purging might be due to the second regression term, $\beta_{f_a} f f_a$. When $f=0$, $\beta_{f_a} f f_a=0$, resulting in information about individuals' who have inbred ancestors but are not inbred themselves being used inefficiently in the analysis. These individuals' genomes may have experienced purging due to the inbred lineages from which they descend. The individuals may therefore have a greater fitness than non-inbred individuals with non-inbred ancestors. However, since $f=0$ for these individuals, any information about purging is discarded by the model. We therefore adapted the regression model to

$$u = u_0 + \beta_f f + \beta_{f_a} f_a. \quad (9)$$

We repeated the analysis using this new model. The detection of significant ancestral inbreeding increased to up to 89% of replicates when alleles were mildly deleterious. In contrast, Ballou's model detected significant ancestral inbreeding effects in a maximum of 54% of replicates when alleles were

mildly deleterious. The detection of significant inbreeding effects only increased by 1–3% of replicates, however. For mildly deleterious alleles, the application of the new model resulted in an improvement in purging detection of 1–3% of replicates, i.e. up to approximately 30% relative improvement over Ballou's model. When alleles were of an intermediate effect, the new model detected purging in 1–7% more of replicates, i.e. up to approximately 23% relative improvement over Ballou's model. When alleles were semi-lethal, the new model increased the detection of inbreeding effects but, when $N < 200$, actually decreased the detection of significant ancestral inbreeding effects relative to Ballou's model. This resulted in the new model detecting up to 46% less of the replicates in which purging occurred when $N = 10$ (a relative decrease of 84%) but 3% more when $N = 200$ (a relative increase of 5%). The new model did not falsely detect purging.

We also investigated several additional alternative models including

$$u = u_0 + \beta_f f + \beta_{f_a} f_a + \beta_{f f_a} f f_a \quad (10)$$

(unpublished data) but all were less powerful than both Ballou's (equation 6) model and the new model (equation 9).

As inbreeding depression is caused by mutations of both large and small effect, it is difficult to know which model would be more appropriate to use on real pedigree data. Most zoo populations have effective population sizes of between 10 and 20 (Frankham *et al.*, 2002), suggesting that purging (if any) would be predominantly of lethals or semi-lethals. Ballou's model might therefore be more appropriate in an investigation of purging in captive breeding programmes. However, since both models are conservative and do not falsely detect purging, perhaps both should be used and the less conservative answer taken to be the more accurate estimate. Further, the comparative analysis of a real data set by the two alternative models may shed light on the genetic architecture of inbreeding depression.

Kalinowski & Hedrick (1999) found from simulation studies that there is low statistical power to detect inbreeding depression from typical zoo pedigrees, i.e. those designed to avoid inbreeding depression. They showed that the statistical power to detect inbreeding depression increases with the number of lethal equivalents and the viability of non-inbred births. The number of lethal equivalents in the simulations was similar for populations containing both mildly deleterious alleles and semi-lethal alleles. However, the viability of non-inbred births was much higher in populations containing semi-lethal alleles than those with mildly deleterious alleles (~ 0.9 as opposed to ~ 0.35). This could account for why, at

a fixed level of purging, the model had less power at detecting inbreeding depression if mutations were mildly deleterious than if they were semi-lethal. Kalinowski & Hedrick (1999) also found that a pedigree with many (14) founders had more power to detect inbreeding depression than a pedigree with few (3) founders. Ballou's model might therefore be less powerful if used on a pedigree with few founders than one with many.

(iii) Ballou's results

Ballou found inbreeding depression in neonatal survival to be significant in 7 of 19 of the mammalian populations that he investigated. Information is not given about the number of founders for each pedigree, but it seems reasonable to assume that a much higher proportion of the species were suffering from inbreeding depression (Kalinowski & Hedrick, 1999) but that the combination of the model and the data were not sufficiently powerful to detect this inbreeding depression. Indeed, inbreeding decreased survival in 17 of the 19 populations. Ancestral inbreeding was found to increase survival in 15 of the populations but the effect was statistically significant in only one of them. The simulations carried out in this paper showed that Ballou's model is poor at detecting the purging of mildly deleterious alleles but that it does not detect spurious purging when purging is absent. It therefore seems probable that Ballou's single detection of purging is real. It is possible that other populations may have experienced purging but that the model failed to detect it.

Several factors affect the efficiency of purging: the genetic basis of inbreeding depression (e.g. dominance or overdominance), selection and dominance coefficients of deleterious mutations, effective population size and the breeding history of a population previous to captivity. It could well be that Ballou detected only very low levels of purging in captive populations because purging simply had not occurred. Studies have shown that purging is dependent upon population structure (Fu *et al.*, 1998; Wang, 2000; Reed & Bryant, 2001; Reed *et al.*, 2003) – it seems probable that selection levels in zoos are too low and population sizes too small for purging to occur. However, we should not ignore the possibility that purging had occurred but that it was not detected due to the low statistical power of Ballou's regression model.

In order to fully understand the risks that inbreeding depression poses to captive breeding programmes, it would be preferable to reanalyse purging in captive populations using a more powerful method. However, due to the nature of zoo pedigree data (i.e. small population sizes, small numbers of generations and small variations in inbreeding and ancestral inbreeding coefficients), it seems likely that, in an empirical

analysis, any regression model would lack statistical power, particularly when mildly deleterious alleles contribute significantly to inbreeding depression.

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References

- Ávila, V. & García-Dorado, A. (2002). The effects of spontaneous mutation on competitive fitness in *Drosophila melanogaster*. *Journal of Evolutionary Biology* **15**, 561–566.
- Ballou, J. D. (1989). Inbreeding and outbreeding depression in the captive propagation of black-footed ferrets. In *Conservation Biology and the Black-footed Ferret* (ed. U. S. Seal, E. T. Thorne, M. A. Bogan & S. H. Anderson). New Haven, CN: Yale University Press.
- Ballou, J. D. (1997). Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *Journal of Heredity* **88**, 169–178.
- Barrett, S. C. H. & Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**, 522–524.
- Brewer, B. A., Lacy, R. C., Foster, M. L. & Alaks, G. (1990). Inbreeding depression in insular and central populations of *Peromyscus* mice. *Journal of Heredity* **81**, 257–266.
- Byers, D. L. & Waller, D. M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* **30**, 479–513.
- Caballero, A., Cusi, E., García, C. & García-Dorado, A. (2002). Accumulation of deleterious mutations: further *Drosophila melanogaster* estimates and a study of the effects of selection. *Evolution* **56**, 1150–1159.
- Charlesworth, B., Charlesworth, D. & Morgan, M. T. (1990). Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* **347**, 380–382.
- Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.
- Crnokrak, P. & Roff, D. A. (1999). Inbreeding depression in the wild. *Heredity* **83**, 260–270.
- Crnokrak, P. & Barrett, S. C. H. (2002). Perspective: purging the genetic load: A review of the experimental evidence. *Evolution*, **56**, 2347–2358.
- Crow, J. F. (1993). Mutation, mean fitness, and genetic load. *Oxford Surveys in Evolutionary Biology* **9**, 3–42.
- Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper and Row.
- Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. Harlow, UK: Longman.
- Fowler, K. & Whitlock, M. C. (1999). The variance in inbreeding depression and the recovery of fitness in bottlenecked populations. *Proceedings of the Royal Society of London* **266**, 2061–2066.
- Frankham, R., Gilligan, D. M., Morris, D. & Briscoe, D. A. (2001). Inbreeding and extinction: effects of purging. *Conservation Genetics* **2**, 279–285.
- Frankham, R., Ballou, J. D. & Briscoe, D. A. (2002). *Introduction to Conservation Genetics*, 1st edn. Cambridge: Cambridge University Press.

- Fraser, A. & Burnell, D. (1970). *Computer Models in Genetics*. New York: McGraw-Hill.
- Fry, J. D., Keightley, P. D., Heinsohn, S. L. & Nuzhdin, S. V. (1999). New estimates of the rates and effects of mildly deleterious mutations in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **96**, 574–579.
- Fu, Y., Namkoong, G. & Carlson, J. E. (1998). Comparison of breeding strategies for purging inbreeding depression via simulation. *Conservation Biology* **12**, 856–864.
- Fu, Y. B. (1999). Patterns of the purging of deleterious genes with synergistic interactions in different breeding schemes. *Theoretical and Applied Genetics* **98**, 337–346.
- García-Dorado, A. & Caballero, A. (2000). On the average coefficient of dominance of deleterious spontaneous mutations. *Genetics* **155**, 1991–2001.
- Hedrick, P. W. (1994). Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* **73**, 363–372.
- Hill, W. G. & Robertson, A. (1966). The effect of linkage on limits to artificial selection. *Genetical Research* **8**, 269–294.
- Hosmer, D. W. & Lemeshow, S. (1989). *Applied Logistic Regression*. New York: Wiley.
- Johnston, M. O. & Schoen, D. J. (1995). Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* **267**, 226–229.
- Kalinowski, S. T. & Hedrick, P. W. (1999). Detecting inbreeding depression is difficult in captive endangered species. *Animal Conservation* **2**, 131–136.
- Kalinowski, S. T., Hedrick, P. W. & Miller, P. S. (2000). Inbreeding depression in the Speke's gazelle captive breeding program. *Conservation Biology* **14**, 1375–1384.
- Keightley, P. D. & Bataillon, T. M. (2000). Multigeneration maximum-likelihood analysis applied to mutation-accumulation experiments in *Caenorhabditis elegans*. *Genetics* **154**, 1193–1201.
- Lacy, R. C. & Ballou, J. D. (1998). Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution* **42**, 900–909.
- Lacy, R. C., Petric, A. & Warneke, M. (1993). Inbreeding and outbreeding in captive populations of wild animal species. In *The Natural History of Inbreeding and Outbreeding* (ed. N. W. Thornhill), pp. 352–374. Chicago and London: University of Chicago Press.
- Lande, R. & Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40.
- Latter, B. D. H. & Mulley, J. C. (1995). Genetic adaptation to captivity and inbreeding depression in small laboratory populations of *Drosophila melanogaster*. *Genetics* **139**, 255–266.
- Latter, B. D. H., Mulley, J. C. & Reid, D. (1995). Reduced genetic load revealed by slow inbreeding in *Drosophila melanogaster*. *Genetics* **139**, 287–297.
- Lynch, M., Conery, J. & Bürger, R. (1995). Mutation accumulation and the extinction of small populations. *The American Naturalist* **146**, 489–518.
- Mukai, T. (1964). The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics* **50**, 1–19.
- Mukai, T., Chigusa, S. I., Mettler, L. E. & Crow, J. F. (1972). Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 335–355.
- Ohnishi, O. (1977). Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effect of polygenic mutations. *Genetics* **87**, 529–545.
- Pray, L. & Goodnight, C. J. (1995). Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum*. *Evolution* **49**, 176–188.
- Press, W. H., Teukolsky, S. A., Vetterling, W. T. & Flannery, B. P. (2002). *Numerical Recipes in C++*. Cambridge: Cambridge University Press.
- Ralls, K. & Ballou, J. D. (1986). Captive breeding programs for populations with a small number of founders. *Trends in Ecology and Evolution* **1**, 19–22.
- Ralls, K., Ballou, J. D. & Templeton, A. R. (1988). Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conservation Biology* **2**, 185–193.
- Reed, D. H. & Bryant, E. H. (2001). Fitness, genetic load and purging in experimental populations of the housefly. *Conservation Genetics* **2**, 57–62.
- Reed, D. H., Lowe, E. H., Briscoe, D. A. & Frankham, R. (2003). Inbreeding and extinction: effects of rate of inbreeding. *Conservation Genetics* **4**, 405–410.
- Saccheri, I. J., Brakefield, P. M. & Nichols, R. A. (1996). Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution* **50**, 2000–2013.
- Schultz, S. T. & Willis, J. H. (1995). Individual variation in inbreeding depression: the roles of inbreeding history and mutation. *Genetics* **141**, 1209–1223.
- Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.
- Sperlich, D., Feuerbach-Mravlag, H., Lange, P., Michaelidis, A. & Pentzos-Daponte, A. (1977). Genetic load and viability distribution in central and marginal populations of *Drosophila subobscura*. *Genetics* **86**, 835–848.
- Streisinger, G., Walker, C., Dower, N., Knauber, D. & Singer, F. (1981). Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature* **291**, 293–296.
- Templeton, A. R. & Read, B. (1984). Factors eliminating inbreeding depression in a captive herd of Speke's Gazelle (*Gazella spekei*). *Zoo Biology* **3**, 177–199.
- Vassilieva, L. L., Hook, A. M. & Lynch, M. (2000). The fitness effects of spontaneous mutations in *Caenorhabditis elegans*. *Evolution* **54**, 1234–1246.
- Wang, J. (2000). Effects of population structures and selection strategies on the purging of inbreeding depression due to deleterious mutations. *Genetical Research* **76**, 75–86.
- Wang, J., Hill, W. G., Charlesworth, D. & Charlesworth, B. (1999). Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genetical Research* **74**, 165–178.
- Willis, J. H. (1999). The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* **53**, 1678–1691.
- Wright, S. (1977). *Evolution and Genetics of Populations*, vol. 3, *Experimental and Evolutionary Deductions*. Chicago: University of Chicago Press.