Inbreeding load and purging: implications for the short-term survival and the conservation management of small populations

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SUMMARY

Using computer simulations we evaluate the effects of genetic purging of inbreeding load in small populations, assuming genetic models of deleterious mutations which account for the typical amount of load empirically observed. Our results show that genetic purging efficiently removes the inbreeding load of both lethal and non-lethal mutations, reducing the amount of inbreeding depression relative to that expected without selection. We find that the minimum effective population size to avoid severe inbreeding depression in the short term is of the order of $N_e \approx 70$ for a wide range of species´ reproductive rates. We also carried out simulations of captive breeding populations where two contrasting management methods are performed, one avoiding inbreeding (equalisation of parental contributions, EC), and the other forcing it (circular sib mating, CM). We show that, for the inbreeding loads considered, CM leads to unacceptably high extinction risks and, as a result, to lower genetic diversity than EC. Thus, we conclude that methods aimed at enhancing purging by intentional inbreeding should not be generally advised in captive breeding conservation programmes.

INTRODUCTION

One of the main issues currently discussed in conservation genetics refers to the joint consequences of inbreeding depression and purging, which determine the minimum size of a population to be viable over time (Jamieson and Allendorf, 2012; Frankham *et al*., 2014). This minimum size is strongly dependent on the inbreeding load, i.e. the load of deleterious recessive mutations concealed in heterozygotes (Morton *et al.*, 1956). This load, when exposed by inbreeding, is responsible for at least part of the decline in fitness traits (inbreeding depression) ubiquitous in all captive populations and species at the verge of extinction (Hedrick and Kalinowski, 2000; Boakes *et al*., 2007; Charlesworth and Willis, 2009). Inbreeding depression and the fixation of deleterious mutations due to the reduced efficiency of purifying selection in small populations (Lynch *et al*., 1995; Reed *et al*., 2003) are some of the main genetic factors which, combined with non-genetic ones, are likely to be responsible for the extinction of populations.

The inbreeding load and, thus, the decline in fitness through inbreeding depression, can be partially restrained by genetic purging, the action of natural selection

to remove recessive deleterious mutations exposed by inbreeding (see, e.g., Leberg and Firmin, 2008). Although the effects of genetic purging are not systematically detected in empirical studies (Ballou and Lacy, 1998; Crnokrak and Barret, 2002; Boakes *et al.*, 2007; Leberg and Firmin, 2008), theoretical studies have shown that purging can be rather effective for moderate size populations (García-Dorado, 2012). Experiments specifically designed to quantify genetic purging confirm this expectation (Bersabé and García-Dorado, 2013; López-Cortegano *et al.*, 2016). Theoretical and empirical evidence also suggests that purging is effective under subdivision of a population into small subpopulations, bottleneck cycles or forced inbreeding by partial full-sib mating (Hedrick, 1994; Miller and Hedrick, 2001; Swindle and Bouzat, 2006; Fox *et al.*, 2008; Ávila *et al.*, 2010).

The consequences of inbreeding and purging are fundamental to determine the minimum effective size (N_e; Wright, 1931) of a population to avoid inbreeding depression and to survive in the short term. In this respect, a consensus rule arising from animal breeding programmes is that the minimum N_e is 50, which corresponds to a rate of increase in inbreeding of 1% per generation (Soulé, 1980; Franklin, 1980). Because of the arbitrariness of this rule, Frankham *et al.* (2014) proposed a more specific definition, based on a maximum decline of 10% in fitness over 5 generations. This minimum effective population size will obviously depend on the reproductive nature of the species considered and the total inbreeding load, usually estimated from the regression of the natural logarithm of survival on the inbreeding coefficient. The inbreeding load (B) is often measured in terms of the number of lethal equivalents carried by a genome (Morton *et al*., 1956). In a review by O´Grady *et al.* (2006), it was found that the total deleterious inbreeding load in vertebrates is about $B = 6$ lethal equivalents per haploid genome, of which about 2 correspond to fecundity traits and 4 to survival traits. Similar or even larger estimates have been found by Kruuk *et al*. (2002), Grueber *et al*. (2010), Hedrick *et al.* (2015) and Hoeck *et al*. (2015), who obtained estimates of $B \approx 7.5$, 8, 6, and 7, respectively.

In the light of this high total inbreeding load, Frankham *et al.* (2014) questioned the classical minimum $N_e = 50$ to avoid short-term inbreeding depression. They estimated that the expected inbreeding depression accumulated in five generations for a population of effective size $N_e = 50$ would be $1 - \exp(-0.05 \times 6) = 26\%$, a too large decline according to the above definition of minimum effective size. With the same calculation they reckoned that N_e should be 142 to produce a maximum of 10% decline

in fitness in five generations. Thus, Frankham *et al.* (2014) suggested revising the N_e = 50 rule upwards to a minimum of N_e = 100. This proposal has, however, generated some debate because the above rough estimations ignore purging (Franklin *et al.*, 2014; Garcia-Dorado, 2015).

O´Grady *et al.* (2006) performed simulations to address the impact of an inbreeding load of 6 lethal equivalents on the time to extinction for a number of species, using the software Vortex (Miller and Lacy, 2003), which performs population viability analysis accounting for a wide number of genetic and demographic factors of extinction. They concluded that the median time to extinction of populations declines by an average of 37% with a mutational load of 6 lethal equivalents, relative to that with a mutational load of 1.57 lethal equivalents, which were usually assumed as default by the software and obtained from juvenile survival analysis of zoo populations (Ralls *et al.*, 1988).

Another issue of relevance in relation with the above arguments is the type of genetic management appropriate for conservation programmes. Managements based on equalisation of contributions from parents to progeny or minimum coancestry contributions (Ballou and Lacy, 1995; Wang, 1997; Caballero and Toro, 2000) are expected to maintain high levels of gene diversity, but have the collateral effect of reducing the strength of natural selection, particularly on fecundity traits, implying a diminished strength of genetic purging. Analytical and simulation studies indicate that equalisation of contributions is expected to produce higher fitness than random contributions for small populations (below about 50 individuals) in the short term (up to 10-20 generations) (Fernández and Caballero, 2001; Theodorou and Couvet, 2003; García-Dorado, 2012), and this is confirmed by empirical evidence (see Sánchez-Molano and García-Dorado, 2011 and references therein).

In contrast, other methods have been suggested to increase the strength of purging by applying different degrees of forced inbreeding (Crnokrak and Barret, 2002), such as mating between full-sibs (Hedrick, 1994; Fox *et al.*, 2008; Ávila *et al.*, 2010), and crosses between lines of sibs (Wang, 2000). More recently, Theodorou and Couvet (2010, 2015) suggested the possibility of applying circular sib mating (Kimura and Crow, 1963) in conservation programmes. These authors showed that circular sib mating produces an efficient purging of the inbreeding load, and maintains higher genetic diversity and higher population fitness than minimum coancestry contributions. The problem with any form of excessive inbreeding, as pointed out by Theodorou and

Couvet (2010, 2015) and other authors (Reed *et al*., 2003; Fox *et al*., 2008), is the high short-term inbreeding depression and risk of extinction. Nevertheless, Theodorou and Couvet (2015) concluded that, in the case of circular mating, this issue would not be too relevant for population sizes larger than 30 individuals and species with high reproductive rate, so that circular mating could be advisable in conservation management in these scenarios.

Genetic extinction of small populations thus depends on the inbreeding load and the efficacy of genetic purging. Previous simulation results assumed purging of only lethal mutations (O´Grady *et al.*, 2006), of only non-lethal ones (de Cara *et al.*, 2013), or of both but with lower inbreeding loads than observed (Wang *et al.*, 1999, Pérez-Figueroa *et al.*, 2009, Theodorou and Couvet, 2010, 2015). Here we simulate small populations with different reproductive rates and allow purging of both lethal and non-lethal mutations affecting viability and fecundity, to find the minimum population size used in conservation which aims to prevent fitness declining by more than 10% over a 5-generation period. We also evaluate the relative performance of equalisation of contributions and circular mating in conservation programmes under the more realistic parameter values of inbreeding load.

METHODS

Overview of the simulation design

Computer simulations were carried out of populations where deleterious alleles arise by mutations and are eliminated by selection and drift. The simulation procedure consisted of two steps. In the first, a large base population of size N_b = 1000 monoecious diploid individuals was simulated for 10,000 generations to reach a mutation–selection–drift balance. The details of the simulation procedure to obtain the base population can be found in Pérez-Figueroa *et al.* (2009). In the second, samples were taken from this base population to found populations of small sizes, which were maintained under different management schemes for a further number of generations. Two scenarios were considered for managing the small populations, a wild population scenario, with random mating of parents and variable contributions of offspring according to their fitness, and a captive breeding scenario, where two different management methods were followed as detailed below.

Simulation model and mutational parameters

We assumed a model of deleterious mutations appearing with rate λ per haploid genome and generation with homozygous allelic effects obtained from a gamma distribution with mean $\bar{s}$ and shape parameter β, such that the genotypic fitness values for locus i are 1, $1 - h_i s_i$, $1 - s_i$, for the wild type homozygote, the heterozygote and the mutant homozygote, respectively. Dominance coefficients (h) were assumed to have negative correlation with selection coefficients by using the joint s and h model of Caballero and Keightley (1994), where h values are taken from a uniform distribution ranging between 0 and exp($-ks$), k being a constant to obtain the desired average value $\bar{h}$. Additionally, lethal mutations ($s_L = 1$) were assumed to occur with rate λ_L and dominance $h_L = 0.02$ (Simmons and Crow, 1977). The fitness of an individual was calculated as the product of genotypic fitness across all loci.

The mutational models considered are shown in Table 1. Model A assumed mutational parameters in agreement with empirical data: λ and $\bar{s}$ (Halligan and Keightley, 2009; Keightley, 2012), β (value appropriate to obtain a mutational variance of $V_M = 0.002$; García-Dorado *et al.*, 1999, 2004), $\bar{h}$ (García-Dorado and Caballero, 2000), λ_L (Simmons and Crow, 1977), and inbreeding load B (O´Grady *et al.*, 2006). Model B considered a larger rate of mutations with effects smaller than those for model A, but keeping the same values of V_M and B. Model C considered a much larger lethal mutation rate, thus providing a high lethal inbreeding load (about 2.5).

The above three models were used in the wild population scenario. For the captive breeding scenario we assumed model A and an additional model with the parameters used by Theodorou and Couvet (Model TC; Table 1), which implies an inbreeding load one third of that of model A.

– Table 1 –

In all cases, up to one thousand neutral loci were also simulated in order to obtain estimates of neutral gene diversity. All neutral and selected loci were assumed to be generally unlinked, but some simulations were carried out assuming a genome of L (5 to 30) Morgans in genetic map length where recombination occurred at random positions of the genome without interference.

Simulated populations of small size and systems of mating

From the large base population, random samples were taken to found small populations of maximum size N_{max}, ranging from 10 to 200 individuals, which were maintained for 50 generations under the evolutionary forces of mutation, selection and drift. One thousand replicates were carried out for each maximum population size and mutation parameter combination. In these small populations, fitness was partitioned into two traits, fecundity (W_f) and viability (W_v), which were assumed to be affected by 1/3 and 2/3 of the loci, respectively. At generation 0 of the small populations, fecundities and viabilities of individuals were scaled by their means, so that the initial average population fecundity and viability were at the maximum of $W_f = W_v = 1$.

We assumed species with different reproductive rates by fixing a maximum number of progeny per individual of $2K$, following a procedure similar to that used by Theodorou and Couvet (2015). The number of progeny from the mating between individuals i and j was then obtained as the product of $2K$ (wild scenario) or K (captive scenario; see Suppl. File 1) times $\sqrt{W_{f,i} \times W_{f,j}}$ truncated to the lower integer number, where $W_{f,x}$ is the fecundity of individual x ($x = i, j$). Values of K were varied from 1.5 to 10 to consider a range of species from low to high reproductive abilities. An individual progeny x survived or died according to its viability, $W_{v,x}$. A random number was drawn from a uniform distribution in the range [0, 1] and was compared with $W_{v,x}$. If it was lower (higher) than $W_{v,x}$, the individual would survive (die). Thus, because of the limited fecundity and survival, a small population with a high inbreeding load could diminish in census size and become extinct. However, the maximum population size would always be N_{max}, assuming a ceiling model where the maximum population size is limited by the resources. For some simulations, natural selection was assumed absent, what we call “no purging” scenario.

The mating models are described in detail in Supplemental File 1. Briefly, a random contribution model was assumed in the wild population scenario. In this case, a polygamous mating system was assumed where parents were taken from the population with probability proportional to their fecundity values. In the captive scenario, two management systems were carried out, equalisation of parents´ contributions (EC) and circular mating (CM). In this scenario, relaxation of selection in a benign environment

(Fox and Reed, 2010) was taken into account by halving the selection coefficients of all mutations except lethal ones.

Pedigrees were saved in all cases. The following parameters were calculated each generation and averaged over replicates: (1) the probability of extinction, computed as the percentage of replicated populations extinct at a given generation; (2) the time to extinction, computed as the average generation number when populations become extinct; (3) the number of individuals (N) in the population at each generation (excluding extinct populations); (4) the average fecundity (W_f) and the average viability (W_v) of the population; (5) the inbreeding load for fecundity (B_f) and viability (B_v), calculated as $B = \Sigma s_i(1 - 2h_i)p_iq_i$ (Morton *et al*., 1956), where q_i is the mutant frequency, $p_i = 1 - q_i$, and the summation is for all loci affecting fecundity or viability; (6) the inbreeding coefficient estimated from pedigrees (F); (7) the average expected frequency of heterozygotes at generation t for neutral loci; and (8) the average effective size (N_e) calculated from the inbreeding coefficient as $N_e = 1 / (2\Delta F)$, where ΔF is the average rate of change in inbreeding between generations 5 and 10, i.e. $\Delta F = (F_{10} - F_5) / [5\times(1 - F_5)]$.

RESULTS

Minimum population size to avoid excessive inbreeding depression in a wild population scenario

Figure 1 illustrates the evolution of populations over 50 generations with a maximum size $N_{max} = 50$ and three reproductive rates (K) under mutation model A (see Table 1). A model with no purging is also displayed as a reference. Extinctions and reductions in population size were more extensive with a lower reproductive rate of the species (K), as expected (Fig. 1A-B). Some recoveries in census size were observed for all K values, but these still finally led to extinction in most cases. The average drop in fecundity and viability was considerably lower under selection than that expected with no purging (Fig. 1C-D), clearly showing the impact of purging in removing inbreeding load (Fig. 1E-F). Supplemental Figure S1 gives the corresponding results for $N_{max} = 100$, with the same qualitative results.

– Figure 1 –

The drop in fitness during the first 5 generations is given in Figure 2A for different maximum population sizes (N_{max}) and K values under mutation model A. The horizontal line at 10% drop indicates the limiting value suggested for a population to avoid severe short-term fitness decline due to inbreeding depression. With no purging, the drop in fitness was substantially larger than 10% for all N_{max} values (Fig. 2A). However, when purging selection was taken into account the drop became smaller than 10% for maximum population sizes above $N_{max} \approx 130$.

The ratio of the effective population size (N_e) computed in the initial generations to the maximum population size (N_{max}) is given in Fig. 2B, showing a tendency towards a value of $N_e/N_{max} \approx 0.5$ with no purging and a bit over with purging. Therefore, the populations showing a drop in fitness smaller than 10% were those with effective size above $N_e \approx 70$.

– Figure 2 –

The average time until extinction and the probability of extinction at generations 10, 25 and 50 for the different K values are shown in Figure 3. Except for species with very low reproductive rate (K = 1.5, a maximum of 3 progeny per individual), extinction rate in the first 50 generations was low for maximum population sizes over $N_{max} \approx 130$ ($N_e \approx 70$). From Figure 3, and assuming the asymptotic ratios of $N_e/N_{\max}$ observed in Fig. 2B, the average times until extinction for K = 1.5, 2.5, 3.5 and 10 were about $0.3N_e$, $0.8N_e$, $1.2N_e$ and $2.9N_e$ generations.

– Figure 3 –

The drop in fitness in the first five generations was slightly lower for mutational models B and C than for model A, and the effective population sizes were very similar for the three models (Suppl. Fig. S2). In addition, extinction rates were similar for models A and B but somewhat lower for model C (Suppl. Fig. S3), as this latter implied many more lethal genes than models A and B and, therefore, a more efficient purging was allowed. Thus, in what follows we will focus on model A as the most conservative one in terms of purging efficiency. The above results assumed free recombination among mutations. Assuming a short genome length (L = 5 Morgans), the probability of extinction was somewhat larger than for free recombination or L = 20, but the effect was small (Suppl. Fig. S4).

Conservation management in captive breeding populations

Figure 4 illustrates the evolution of a population over 50 generations for N_{max} = 40 under two conservation management methods, equalization of contributions (EC) and circular mating (CM). Extinction and decline in population size was larger for the CM scheme (Fig. 4A-B) than for EC scheme due to the sharp decline in fitness occurred during the first generations with CM (Fig. 4C-D). CM method resulted in a substantial amount of purging of inbreeding load (Fig. 4E-F) because of the large initial increase in inbreeding (Fig. 4G). This elimination of inbreeding load produced a higher fecundity than EC in the final generations (Fig. 4C). Expected heterozygosity for neutral genes declined more under CM than under EC (Fig. 4H).

– Figure 4 –

The probability of extinction with the two management methods is given in Figure 5 for different values of N_{max} and K. For low reproductive rates, method CM showed substantial extinction even during the first 10-25 generations. It also showed a substantially larger extinction rate than for EC with higher values of K (see also Suppl. Table S1). This method produced a remarkable drop in fitness in the early generations (Suppl. Fig. S5). The effective population size for CM was larger than that for EC under a no purging model, but the opposite was true when selection was taken into account (Suppl. Fig. S5). Thus, the amount of diversity maintained by CM was always lower than that maintained by EC when purging was accounted for (Suppl. Fig. S6). Simulations with restricted recombination did not alter qualitatively the above results (Suppl. Fig. S7).

– Figure 5 –

These results contrast with those obtained using mutational models with lower inbreeding loads, such as model TC (Table 1), for which extinction rates were considerably lower and CM maintained a higher neutral expected heterozygosity than EC under some scenarios (Suppl. Table S1). In fact, for this model, EC incurred more extinctions than CM on some occasions. This did not occur, however, for the model with a higher inbreeding load of $B \approx 6$ (Suppl. Table S1).

DISCUSSION

Minimum population size to avoid excessive inbreeding depression and extinction in the short term

When the minimum effective population size in conservation is defined as the one which results in the rate of fitness decline being below 10% in the first five generations (Frankham *et al.* 2014), our results suggest that this number is of the order of $N_e \approx 70$ for a range of species´ reproductive rates. The above result is based on a genetic model accounting for an inbreeding load of the order of $B = 6$. Thus, our results are midway between the classical rule of $N_e = 50$, originally suggested by Soulé (1980) and Franklin (1980), and the latest proposal from Frankham *et al.* (2014), who suggested to double that figure. Assuming absence of purging, Frankham *et al.* (2014) calculated that an effective size higher than 142 was necessary to limit fitness decline to less than 10% in five generations. This agrees with our simulations, which show that, without purging a drop in fitness below 10% would be reached with $N_{max} > 200$ (Fig. 2A), corresponding to $N_e > 100$. However, the full consideration of purging of both lethal and non-lethal genes reduces these values (Fig. 2A).

Several caveats should be mentioned regarding these results. First, our model assumes absence of stochastic demographic changes in the population other than the reduction in population size due to inbreeding depression and fixation of mutations. If other factors occur, such as biases in the sex ratio or sudden reductions in population size due to genetic or non-genetic causes, the deduced minimum effective size could be larger. Second, we have assumed that the whole inbreeding load is due to recessive or partially recessive deleterious mutations. It is possible that part of this load is due to overdominant loci or other types of balancing selection which would not be purged by inbreeding. However, whereas partial recessive deleterious mutations are ubiquitous and are considered the predominant cause of inbreeding depression (Charlesworth and Willis, 2009), heterozygote advantage should be a minority (Hedrick, 2012). Third, we have assumed a model with inbreeding load $B = 6$, but other recent experimental data suggest larger values of $B = 7\text{-}8$ (Kruuk *et al*., 2002; Grueber *et al*., 2010; Hoeck *et al*., 2015). The impact of inbreeding depression on the fitness decline, extinction risk and loss of genetic variation in small populations could thus be larger, which calls for a higher minimum effective population size.

We have focused in this paper on short term-inbreeding and survival of small-medium sized populations. There are other aspects regarding survival of populations which are out of the scope of the paper. For example, it has been proposed that $N_e = 500$ is the minimum population size to retain evolutionary potential in perpetuity (Franklin,

1980). This figure has been recently suggested to be twice as large by Frankham *et al.* (2014). In addition, a long-term minimum viable population (MVP) size is also frequently discussed, which refers to the number of individuals required for a high probability of population persistence over the long run (Shaffer, 1981; Soulé, 1987), e.g. 80% persistence over more than 20 years (Shaffer, 1981) or 99% persistence over 40 generations (Frankham *et al.*, 2014). The inference obtained by the review of population viability analyses carried out by Traill *et al.* (2007) suggests a median of the MVP size of about 4,200 individuals (95% CI of 3,600 - 5,100). Ratios of N_e/N are of the order of 0.1-0.2 (Frankham, 1995; Palstra and Fraser, 2012), so considering a conservative value of 0.1 this would imply a minimum effective size (*Ne*) around 400. These inferences, however, cannot be taken as general rules, because of the huge variation in the estimates of N_e/N across species and other factors (Flather *et al.*, 2011; Jamieson and Allendorf, 2012).

Our results are not directly addressing the issue of the long-term survival and minimum viable population sizes because they ignore environmental stochastic factors and only consider genetic issues. We assumed a ceiling model with a maximum population size (N_{max}), so that populations cannot increase over that ceiling, and this would be a factor increasing the chances of extinction of the population. Therefore, any inference on long-term survival arising from our results should be taken with caution. Even so, our results suggest that, unless the reproductive rate of the species is very low, the chances of extinction for genetic reasons after 50 generations are low when maximum population sizes are around N_{max} = 130 (N_e = 70) (Fig. 3). Assuming again a ratio N_e/N = 0.1 this would imply census sizes of N = 700 individuals. However, for low reproductive rate species (K = 1.5) we found that the probability of extinction at generation 50 was nearly 100% even for maximum census sizes of N_{max} = 200 (Fig. 3). Therefore, genetic extinction for low reproductive rate species could be avoidable only with very large population sizes, perhaps of the order of thousands, in agreement with Traill *et al.* (2007) meta-analysis results.

We found that the average times until extinction for species with reproductive rates of K = 1.5, 2.5, 3.5 and 10 were about 0.3N_e, 0.8N_e, 1.2N_e and 2.9N_e generations. The averages obtained for intermediate values of K are therefore consistent with those estimated by Soulé (1980) based on the experience of animal breeders (1.5N_e generations), and by Reed and Bryant (2000) for populations in captivity (N_e generations). In addition, the median values of time to extinction estimated by

simulation by O´Grady *et al*. (2006) for 30 species were 5.7, 11.9 and 21.8 generations for population sizes of N = 50, 250 and 1000, respectively (averages obtained from Table 2 of O´Grady *et al*., 2006). Assuming N_e/N = 0.1, this implies times to extinction of 1.1N_e, 0.5N_e and 0.2N_e generations, which are similar to our results for K values of 1.5-3.5.

Forced excessive inbreeding as a management method in captive breeding populations

We have compared the performance of a conservation method restraining inbreeding, equalisation of parental contributions (EC), and a method forcing it, circular mating (CM). Theodorou and Couvet (2010, 2015) compared CM with minimum coancestry contributions and matings (Fernández and Caballero, 2001), a method which makes use of pedigree or molecular marker information to minimize genetic drift and inbreeding in conservation management. This method is more powerful than EC in the initial generations, but we used EC instead as a more simple method that can be applied in all circumstances, thus giving conservative results in terms of amount of variation maintained.

Theodorou and Couvet (2010, 2015) showed that CM maintains higher levels of gene diversity than EC in the long-term and, because it is more effective in removing the inbreeding load, it leads to higher population fitness. They also acknowledged that the main drawback of CM is the possibly high short-term inbreeding depression and high extinction risk. However, they concluded that, with the mutational model they investigated, this would be only a relevant issue for small populations (N_{max} < 30) and low reproductive ability ($F < 3$, where F is analogous to our K value). We cannot make a direct comparison between their simulations and ours because the models followed by Theodorou and Couvet (2010, 2015) are not exactly the same as those used here. First, as mentioned above, Theodorou and Couvet (2010, 2015) used minimum coancestry contributions with minimum coancestry matings whereas we just applied equalisation of contributions with only avoidance of full-sib mating. Second, Theodorou and Couvet (2010) used a model of mutations of fixed effects, rather than a model with variable mutational effects. Furthermore, whereas Theodorou and Couvet (2015) considered such variable mutational effects, they not only considered deleterious mutations for fitness but also stabilising selection on a quantitative trait, so that overall fitness is a

compound of deleterious mutations and selection on the trait. Thus, our results for extinction rates are rather different from those of Theodorou and Couvet (2015). For example, the probability of extinction with N_{max} = 20 and F = 2.4 from Table 1 of Theodorou and Couvet (2015) was 66% and 10% for CM and minimum coancestry contributions and matings at generation 25, whereas our corresponding probabilities for K = 2.5 were 0%. Our simulations give, therefore, lower extinction rates.

Our simulations using the mutational parameters of Theodorou and Couvet (2015) (Model TC in Suppl. Table S1), which account for deleterious inbreeding loads of the order of 2 lethal equivalents, show that extinction risk under CM is negligible for K > 2.5 and N_{max} > 20 even at generation 50 (Suppl. Table S1). However, assuming inbreeding loads of the order of those observed experimentally, CM may imply an extinction risk too high to be an advisable management method in captive breeding programmes.

Circular mating can be considered a type of population subdivision where each individual is considered a subpopulation and each subpopulation contributes equally to the next generation. Without selection, this mating scheme is expected to produce a higher rate of decrease in expected heterozygosity in the early generations but a final lower rate than under random mating (Kimura and Crow, 1963; Robertson, 1964; Wang and Caballero, 1999). Thus, it is expected that CM would produce a larger effective population size and higher gene diversity than EC after a number of generations if a model with no selection is assumed (see Suppl. Fig. S5). When selection is taken into account, this may still be the case if mutational models with low inbreeding load are assumed, as the model considered by Theodorou and Couvet (2015) (model TC). Under these parameters, expected heterozygosity (at later generations) is sometimes larger than that obtained with EC (Suppl. Table S1). However, for models with an inbreeding load of the order of 6 lethal equivalents, this is no longer the case; both the effective size and the maintained neutral heterozygosity for CM are lower than those for EC. Hence, the possible advantage of CM in further increasing the effective size and maintaining larger neutral diversity than EC clearly disappears when models involving high inbreeding loads are assumed.

Overall, we can therefore conclude that circular mating should not be generally advised as a management method in captive breeding conservation programmes. This is in accordance with other studies which suggest that purging the detrimental variation through forced inbreeding is a risky strategy in the genetic management of endangered

species (Miller and Hedrick, 2001) and the common practice of avoiding inbreeding should be followed (Boakes et al., 2007; Leberg and Firmin, 2008).

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CONFLICT OF INTEREST

We declare no conflict of interests.

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Table 1. Deleterious mutation models considered in simulations. λ: rate of mutations per haploid genome and generation. $\bar{s}$: average homozygous effect of non-lethal mutations. β: shape parameter of the gamma distribution for mutational effects of non-lethal mutations. $\bar{h}$: average coefficient of dominance for non-lethal mutations. λ_L: rate of lethal mutations per haploid genome and generation, with dominance coefficient h_L = 0.02. B: inbreeding load in the base population, measured as the number of recessive lethal equivalents.

Model	$\boldsymbol{\lambda}$	$\bar{\boldsymbol{s}}$	β	$\bar{\boldsymbol{h}}$	λ_L	B
A	0.5	0.05	0.45	0.20	0.015	6.00
B	1.0	0.02	0.11	0.20	0.015	6.07
C	0.5	0.05	1.0	0.20	0.070	6.18
TC	0.5	0.05	1.0	0.35	0.015	2.12

Figure legends

Figure 1. Evolution over generations of different parameters for populations of maximum size N_{max} = 50 and maximum reproductive rate K (2K is the maximum number of progeny per individual) under mutational model A (see Table 1). A case with no selection (no purging) is shown as a reference. (A) Probability of extinction. (B) N: average population size (excluding extinct populations). (C) W_f: average population fecundity. (D) W_v: average population viability. (E) B_f: inbreeding load for fecundity. (F) B_v: inbreeding load for viability.

Figure 2. (A) Drop in fitness (fecundity × viability) after five generations for populations of maximum size N_{max} under mutational model A (see Table 1) for different reproductive rates (K; where 2K is the maximum number of progeny per individual) and for a model without selection (no purging). (B) Ratio of the effective population size (N_e, estimated from the change in pedigree inbreeding between generations 5 and 10) to the maximum population size (N_{max}).

Figure 3. Time to extinction (average generation number when populations become extinct) and probability of extinction at generations 10, 25 or 50 (percentage of replicates extinct at a given generation), for populations of maximum size N_{max} under mutational model A (see Table 1) and different reproductive rates (K).

Figure 4. Evolution over generations of different parameters for populations of maximum size N_{max} = 40 and maximum reproductive rate K = 2.5 (2K is the maximum number of progeny per individual) under mutational model A (see Table 1) in a conservation management scenario. Selection coefficients of deleterious non-lethal mutations were halved. CM: circular mating. EC: equalisation of contributions. (A) Probability of extinction. (B) N: average population size (excluding extinct populations). (C) W_f: average population fecundity. (D) W_v: average population viability. (E) B_f: inbreeding load for fecundity. (F) B_v: inbreeding load for viability. (G) F: average pedigree inbreeding coefficient. (H) H: average expected heterozygosity for neutral genes.

Figure 5. Probability of extinction at generations 10, 25 or 50 (percentage of replicates extinct at that generation), for populations of maximum size N_{max} and two reproductive rates (K; where $2K$ is the maximum number of progeny per individual) under mutational model A (see Table 1) in a conservation management scenario. Selection coefficients of deleterious non-lethal mutations were halved. CM: circular mating. EC: equalisation of contributions.

Figure 1

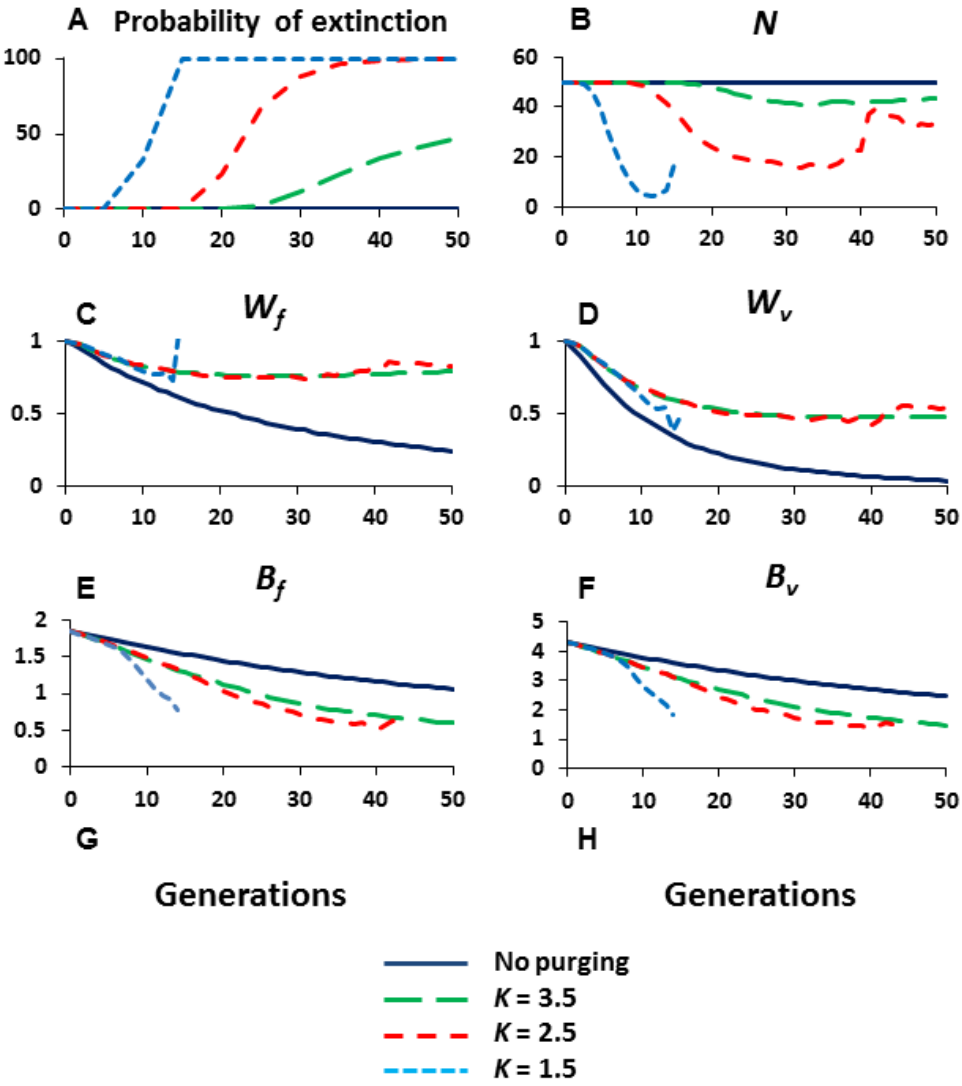

A Probability of extinction
B N
C W_f
D W_v
E B_f
F B_v
G
H
Generations
Generations
No purging
K = 3.5
K = 2.5
K = 1.5

Figure 2

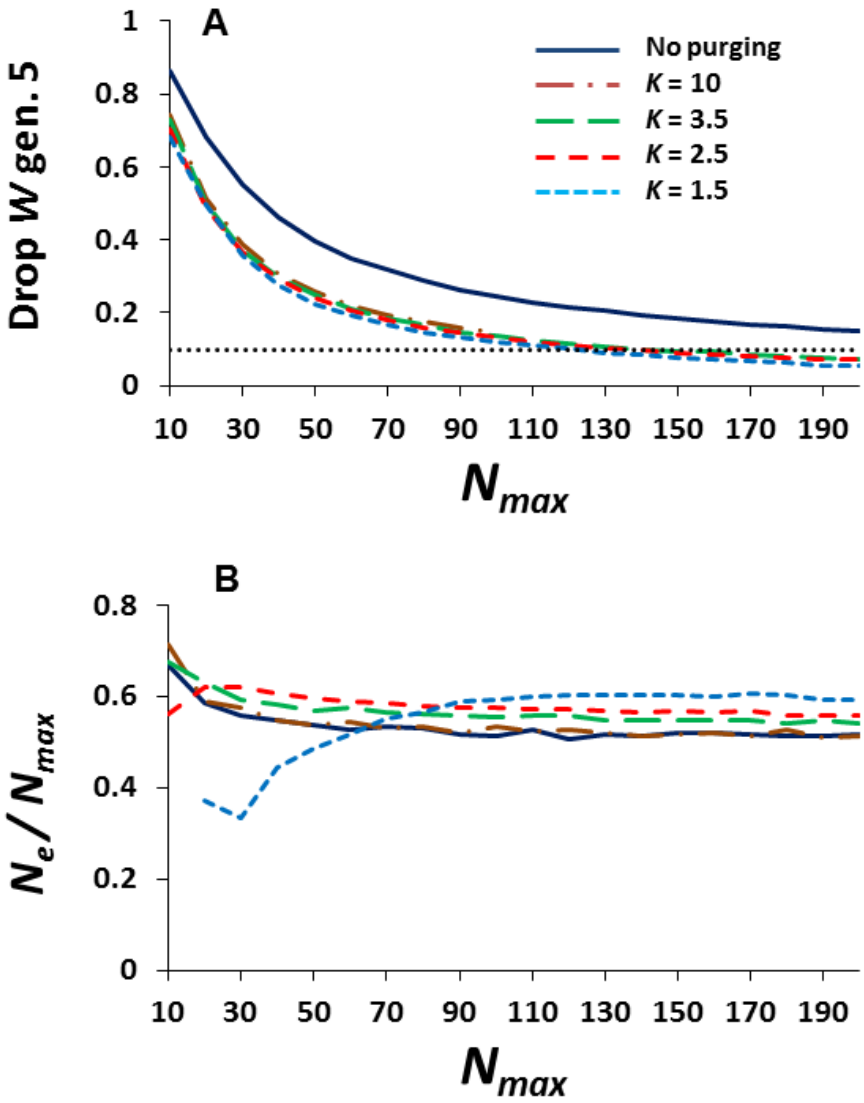

A
Drop W gen. 5
1
0.8
0.6
0.4
0.2
0
No purging
K = 10
K = 3.5
K = 2.5
K = 1.5
10 30 50 70 90 110 130 150 170 190
Nmax
B
Ne / Nmax
0.8
0.6
0.4
0.2
0
10 30 50 70 90 110 130 150 170 190
Nmax

Figure 3

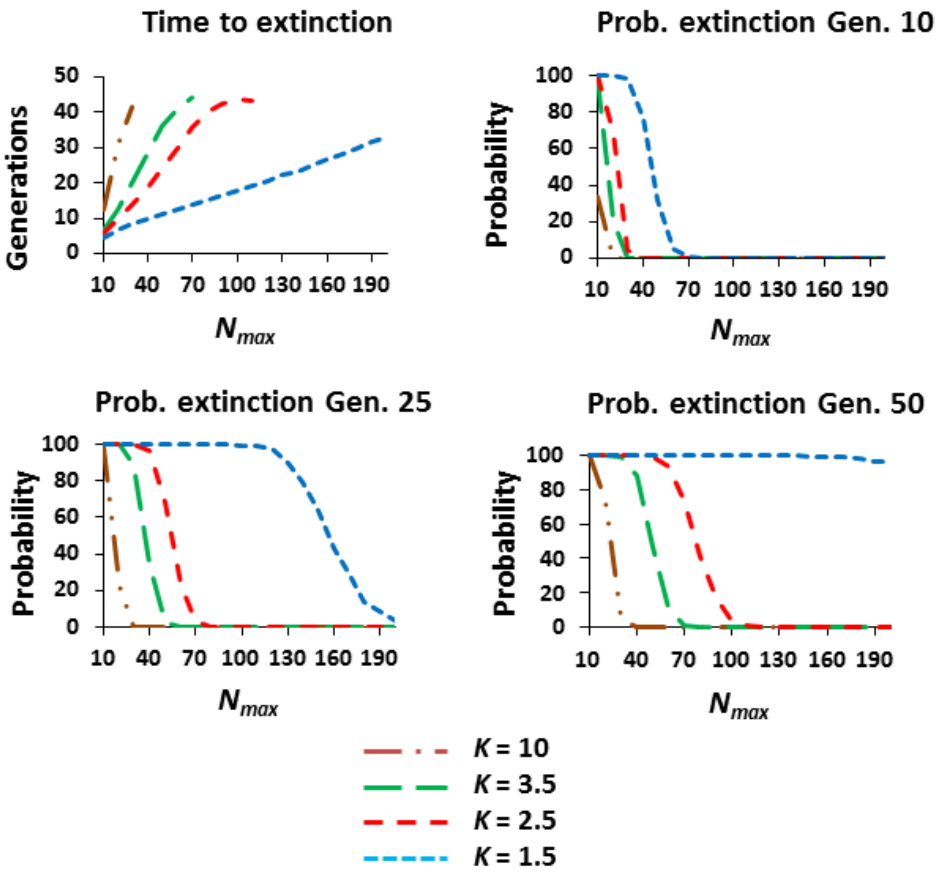

Time to extinction
Generations
50
40
30
20
10
0
10 40 70 100 130 160 190
N_{max}
Prob. extinction Gen. 10
Probability
100
80
60
40
20
0
10 40 70 100 130 160 190
N_{max}
Prob. extinction Gen. 25
Probability
100
80
60
40
20
0
10 40 70 100 130 160 190
N_{max}
Prob. extinction Gen. 50
Probability
100
80
60
40
20
0
10 40 70 100 130 160 190
N_{max}
K = 10
K = 3.5
K = 2.5
K = 1.5

Figure 4

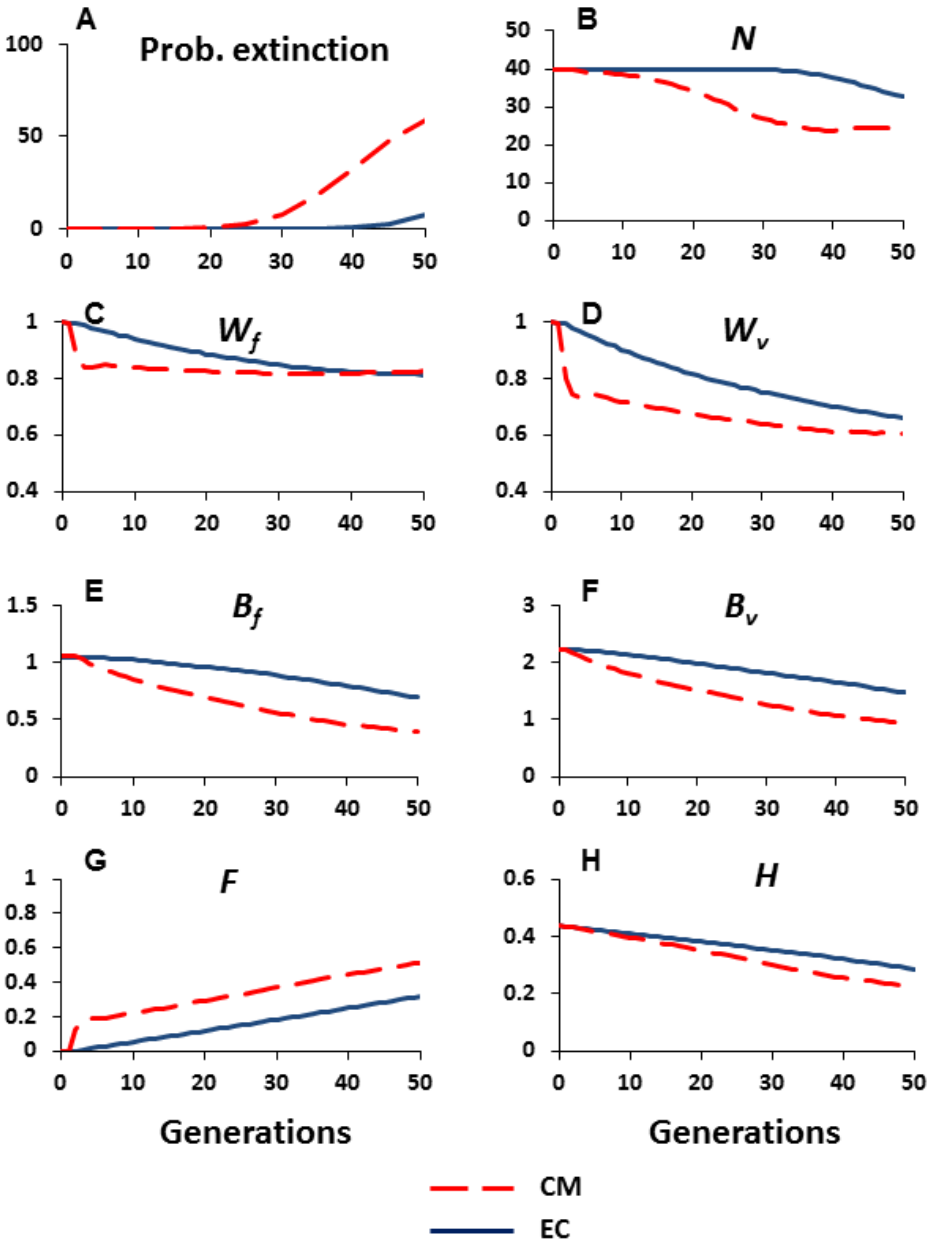
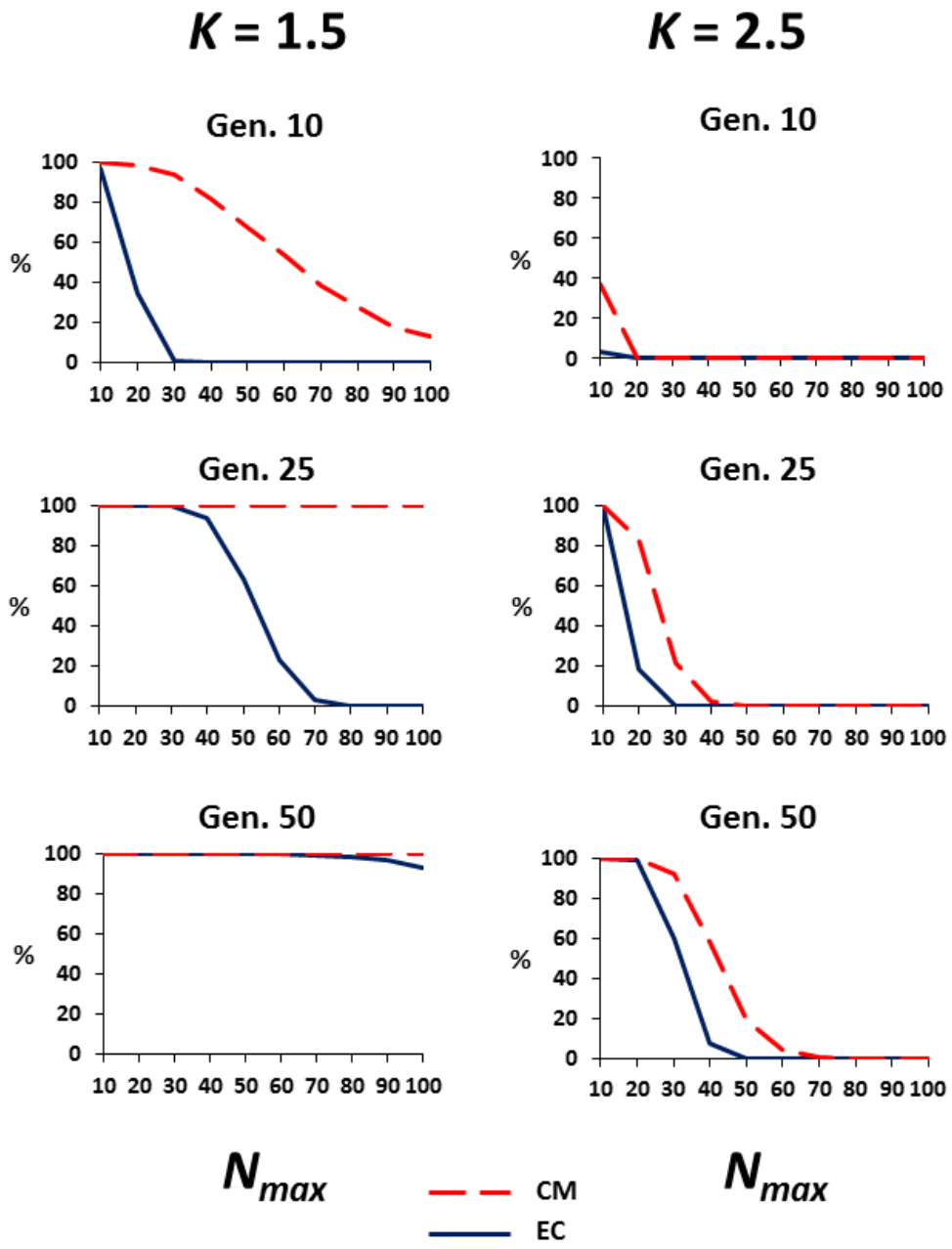

A
Prob. extinction
B
N
C
W_f
D
W_v
E
B_f
F
B_v
G
F
H
H
Generations
Generations
CM
EC

Figure 5


K = 1.5
K = 2.5
Gen. 10
Gen. 25
Gen. 50
%
100
80
60
40
20
0
10 20 30 40 50 60 70 80 90 100
Nmax
CM
EC