

INTEGRATING MULTIPLE EVOLUTIONARY PROCESSES IN MODELS OF CAPTIVE
BREEDING

By

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Abstract

Models of captive breeding tend to come in two varieties: one type examines the potential for different strategies to preserve genetic diversity, while the other considers the impacts of many evolutionary processes but uses a simple mating system. This study presents an individual-based, stochastic simulation model that seeks to combine aspects of drift, mutation, selection, and gene flow with a breeding protocol similar to that used by captive population managers. It also tests a model of genetic adaptation to captivity, in which alleles are selected for in captivity that are deleterious in the wild. Both captive and wild fitness values are calculated. Results show that adaptation occurs rapidly and can lead to significant decline in wild fitness compared to the founding population. The most serious threats to captive fitness are the sensitivity of the species to inbreeding and the relative contribution of lethal and mildly deleterious alleles.

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I dedicate this work to my parents, Jim and Carol Sahrman, for all their love and support.

Table of Contents

| | |
|-------------------------|-----|
| Abstract | iii |
| Acknowledgements | iv |
| Table of Contents | v |
| List of Tables | vi |
| List of Figures | vii |
| INTRODUCTION | 1 |
| METHODS | 9 |
| RESULTS | 14 |
| DISCUSSION | 19 |
| References | 33 |

List of Tables

| | |
|-----------------------------------|----|
| Table 1. Base Parameter Set | 24 |
|-----------------------------------|----|

List of Figures

| | |
|--|----|
| Figure 1. The effect of varying the number of loci contributing to adaptation to captivity on initial captive fitness | 25 |
| Figure 2. The effect of varying the initial frequency of the tameness alleles on the final frequency of the tameness alleles | 26 |
| Figure 3. Genetic changes in simulated captive populations over 50 generations | 27 |
| Figure 4. The effect of varying the amount of migration between subpopulations | 28 |
| Figure 5. The effect of varying the duration of captive breeding | 29 |
| Figure 6. The effect of changing the sensitivity of the population to inbreeding depression | 30 |
| Figure 7. The effect of varying the proportion of inbreeding depression due to lethal alleles | 31 |
| Figure 8. The effect of varying carrying capacity of a single population | 32 |

INTRODUCTION

The 20th and 21st centuries are likely to witness a pace of extinction rivaled only by the mass extinction events in Earth's history (Butchart *et al.*, 2010). With the human population recently exceeding 7 billion and projected to rise significantly in the near future (Bloom, 2011), many other species will face growing challenges to their survival. Despite conservation efforts, it will likely be impossible to preserve many species in their natural habitats. Soule *et al.* (1986) estimated that as many as 2,000 terrestrial vertebrate species could be at risk of vanishing. When the prospects for a species in its natural habitat become particularly grim, *ex situ* conservation efforts can be utilized as a way of ensuring that the species persists in the future. A wide variety of animal species have been kept in zoos for hundreds of years, but in the last four or five decades, zoos have begun assuming responsibility for the long-term survival of the species they hold, including acknowledging threats to captive populations as well as taking part in conservation actions internationally.

Perhaps the first major threat to captive populations to gain attention was that posed by inbreeding depression. Bouman (1977) was among the first to identify a trend towards inbreeding depression in the captive population of Przewalski's horse (*Equus przewalskii*), which showed declining growth rates and higher mortality as the average inbreeding coefficient of the population increased. This was followed by surveys of captive ungulates by Ralls *et al.* (1979) and Ralls and Ballou (1983) that suggested that the condition was more the rule than the exception. As a result, there was a growing acceptance that careful management of captive populations was needed to ensure their long-term survival, with the primary goal being the preservation of genetic diversity (Lacy, 1994).

The genetic problems faced by managed populations are similar to those affecting small populations in the wild. In most cases, population sizes in zoos are not very large, and consequently neither are effective population sizes. Using the equation for the loss of average heterozygosity of neutral loci over time (Hartl and Clark, 2007),

$$H_t = \left(1 - \frac{1}{2N_e}\right)^t \times H_0,$$

we see that average heterozygosity is inversely related to effective population size, meaning that the cost of staying at low effective population size over many generations is a greater loss of heterozygosity. Since heterozygosity is associated with fitness traits in wild populations (Allendorf and Leary, 1986), this loss in average heterozygosity can itself be potentially detrimental. In addition, mating between relatives, which is inevitable in small, closed populations, leads to increased homozygosity, and inbreeding depression can result from the expression of recessive deleterious alleles in homozygous form (Charlesworth and Willis, 2009). Subtracting one from both sides of the above equation gives the equation for homozygosity, and if we invoke the infinite alleles model, in which each allele in the founding population is unique, it can also be interpreted as the average inbreeding coefficient of a population over time (Hartl and Clark, 2007),

$$F_t = 1 - \left(1 - \frac{1}{2N_e}\right)^t.$$

Thus, the dynamics of inbreeding and heterozygosity are related. As a result, the goal of maintaining genetic diversity is coupled with that of avoiding inbreeding. Drift can also lead to the loss of rare alleles due to sampling error as a result of Mendelian inheritance (Allendorf, 1986). Moreover, zoos can be viewed as fragmented populations, and if there is limited migration between zoos, these effects can be even more pronounced within each subpopulation

(Lacy, 1987). The consideration of all these factors led zoos and their regional governing bodies to more carefully collect demographic data and to design specific breeding programs (Foose and Ballou, 1988).

The most direct way to slow inbreeding and the loss of genetic diversity is to support larger populations. Unfortunately, zoos are limited by resources and space, and the decision to devote more resources to one species means that others will suffer (Conway, 1986). Every captive species has a defined carrying capacity based on available space, conservation need, and other considerations. A widely agreed upon goal of any breeding program is to reach the carrying capacity as quickly as possible to maximize the effective population size and preserve the variation in the founding population. One of the earliest models proposed for captive breeding was Flesness (1977), who used computer simulations based on the maximum avoidance of inbreeding to analyze the pedigree of the Przewalski's horse population studied by Bouman (1977). He found that, had such a protocol been followed in the past, the current population would have been considerably less inbred and possibly much healthier.

By contrast, the models of Chesser *et al.* (1980) and Chesser (1983) used subdivision as a tool to preserve variation between subpopulations, a strategy that has been proposed many times in the context of captive breeding (Lacy, 1987; Frankham, 2008). In theory, subdivision could maintain variation indefinitely as each subpopulation fixes different alleles. Inbreeding depression was dealt with by a complex network of migration to periodically restore heterozygosity; subpopulations would be organized into neighborhoods, with migration between subpopulations in the same neighborhood being more likely than with those in other neighborhoods. Simulations designed to more closely reflect the dynamics of wild populations, however, reveal that subdivision may be much riskier than these models suggest (Lindenmayer

and Lacy, 1995; Lacy and Lindenmayer, 1995). Processes like demographic stochasticity, ignored by Chesser *et al.*, (1980), Chesser (1983), and Lacy (1987), can lead to unexpected fluctuations in population size, with the probability of extinction growing dangerously high in small metapopulations.

Some of the earliest captive breeding programs were designed to equalize the contribution of founders (Ballou and Lacy, 1995). Such a plan, advocated by Foose (1983), could maintain the genetic composition of the founding population by compensating for skews in founder representation that occur during periods of mismanagement. It also included elements like equalizing family sizes and avoiding mating between close relatives. As Ballou and Lacy (1995) point out, though, this method also has flaws. Those founders that have bred less are also likely to have passed on fewer of their alleles than more successful founders. By then equalizing contribution, the remaining alleles from the less successful founders end up becoming overrepresented in the captive population, reducing genetic diversity.

Ballou and Lacy (1995) used a simulation model to compare the effectiveness of different captive breeding designs, including maximum avoidance of inbreeding and equalization of founder contribution. The model consisted of a population maintained at a constant size and 50/50 sex ratio for 20 discrete generations, with genetic drift as the only evolutionary process. A key feature was an initial period of unmanaged breeding to create a variety of complex pedigrees. Each management strategy was judged based on its ability to maintain gene diversity, allelic diversity, and avoid inbreeding given each type of pedigree. A program based on minimizing the mean kinship of the captive population was the most effective across all pedigrees. The basis of this protocol is measuring the kinship value of each individual to every other individual including itself. By averaging all the kinship values for an individual, we arrive

at its mean kinship, and individuals with the lowest mean kinship have the highest breeding priority. Furthermore, the average of the mean kinships of all individuals is equal to the expected average inbreeding coefficient of the population if it was to mate randomly. By this argument, a program that minimizes the average mean kinship also maximizes the effective population size and minimizes the rate of decline of heterozygosity (Ballou and Lacy, 1995). It also avoids the problem of the Foose (1983) plan by equalizing the contribution of founders in proportion to the expected amount of their alleles that should remain after taking drift into account, and although it may call for more inbreeding in the short term than the maximum avoidance of inbreeding method, on a longer time scale, it performs better.

Since this study, the consensus view in the zoo community appears to be that minimizing mean kinship provides the best opportunity to achieve the genetic goals of captive breeding programs (Ballou *et al.*, 2010). While each species is unique in terms of its demographic and logistical considerations, this genetic model seems to unify those species that will form breeding pairs. The general procedure seems to start with a demographic model used to determine the number of offspring required to keep the population near its carrying capacity. Then males and females are ranked in terms of their genetic importance using minimization of mean kinship; pairs are formed based on these rankings, with other factors like avoiding pairs of close relatives also taken into account.

Despite the wide acceptance of this plan, its underlying model makes numerous simplifying assumptions, in particular the exclusion of evolutionary forces besides genetic drift. Other studies have revealed additional genetic threats to captive populations. Theory suggests that small population size leads to weakened selection relative to genetic drift, which could allow for the accumulation of deleterious mutations, culminating in a 'mutational meltdown' (Lynch

and Gabriel, 1990; Lynch *et al.*, 1995). Species in captivity could be ripe for such a meltdown, especially those facing many generations at small population size.

Selection of various forms can also lead to undesirable change. Lacy (2009) distinguished between artificial selection imposed by managers for or against particular traits and natural selection favoring adaptations to the captive environment. There is some debate centered around whether artificial selection should be used in an attempt to eliminate harmful alleles generated by mutation or promote heterozygosity at particular loci (e.g., MHC loci), but the general consensus appears to be that genetic change of any kind should be avoided (Lacy, 2000).

Selection for adaptations to captivity is unintentional but may have a significant impact on the genetic composition of zoo populations. There are likely strong selective pressures exerted on threatened species in their transition from the wild to captivity; Lacy (2009) noted that for some species in zoos, as many as 50% of breeding pairs fail to reproduce. If variation in reproductive success is due to heritable variation in some traits favored by the captive environment, then adaptation is bound to occur over time. In his computer simulations, Lacy (1987) found that selection was not strong enough to counteract drift unless the selection intensity was large, perhaps 10%. This was not unexpected, and Lacy cited a theoretical result from Kimura (1955) that selection is more powerful than drift when $N_e \times s \gg 1$.

Experimental studies by Frankham and colleagues using *Drosophila* populations provided evidence that adaptation to a captive environment is possible. Woodworth *et al.* (2002) found that populations maintained at large effective sizes had higher captive fitness than control populations, while populations with intermediate effective sizes were more similar to the controls. They also included a simulated reintroduction component in which flies that had adapted to a benign captive environment were exposed to a more competitive, crowded

environment meant to represent the wild; they found a marked reduction in fitness upon reintroduction to the wild. In addition, populations with large effective sizes performed worse than populations with intermediate effective sizes, further indicating that the large effective size populations had adapted to captivity to a greater extent. This is to be expected since selection should have been more powerful in the populations with higher effective size. Based on their results, they hypothesized that the adaptation they observed was due to rare, recessive alleles maintained in the natural population by mutation-selection balance (Woodworth *et al.*, 2002). These empirical results contribute to a wealth of literature concerning the failure of reintroduction programs of a variety of captive species (reviewed by Frankham, 2008), including endangered species, fish raised in hatcheries, and biocontrol agents. Thus, genetic adaptation to captivity represents a threat to our ultimate goal of reintroduction for most captive species, and it is comparatively much less understood than the loss of genetic variation.

A few models have been proposed over the years that sought to look at the effects of selection and mutation in captivity. Hedrick (1994) tested the ability of purging to reduce a population's genetic load by mating close relatives; the load consisted of two types of deleterious alleles: highly recessive, lethal alleles and slightly recessive, mildly detrimental alleles. His simulation results showed that purging is effective only in eliminating the lethal alleles, whereas the mildly deleterious alleles persist. Inbreeding also increases the chances of fixing these mild alleles, leading to an irreparable loss of fitness and greater risk of extinction. Robert (2009) created a detailed model of different evolutionary processes using the genetic and demographic parameters typical of small mammals or birds. He included mutation as well as the relaxation of selection that might result from a benign captive environment. Simulations consisted of a period of captive breeding with random mating of individuals followed by a reintroduction effort into a

wild environment with stronger selection. The analysis identified time in captivity as the most important factor influencing the probability of successful reintroduction, with the structure of the captive population also having a strong influence.

Still, no model provides a comprehensive look at the genetic changes that occur in captivity along with a breeding protocol that reflects current practices. It is unclear what breeding strategies designed to minimize loss of genetic diversity will do to the dynamics of mutation, for instance. There is also no model that looks at the potential for adaptation to improve fitness in captivity while jeopardizing future reintroduction efforts and how this could impact population management. The purpose of this project was twofold. One goal was to write a computer simulation program that samples from aspects of the aforementioned models; in particular, it integrates the effects of different evolutionary forces on a population managed by a controlled breeding program. The second goal was to test the feasibility of a model of adaptation to captivity as hypothesized by Woodworth *et al.* (2002) using the simulation program. The hope was that by combining a more comprehensive treatment of the evolutionary processes occurring in captivity with a realistic breeding protocol, we might arrive at a more complete understanding of the threats to the long term viability of captive populations.

METHODS

I wrote a forward simulation program modeling one or more captive populations through a number of discrete generations that allows for genetic drift, migration, selection, and mutation. Individuals are diploid organisms with separate sexes. The model is specified by the following parameters: the number of populations (*num_pops*), the number of founding individuals in each population (*N_found*), the carrying capacity for each population (*K_per_zoo*), the rate of migration between populations (*migration_rate*), the number of generations of captive breeding (*gens*), the rate of decline in fitness due to inbreeding (β), the proportion of inbreeding depression due to lethal versus mildly deleterious alleles (*k*), the number of loci contributing to adaptation in captivity (*num_ga_loci*), the starting frequency of the tameness allele (*q*), and selection and dominance coefficients for mildly deleterious (*sm*, *hm*), lethal (*sl*, *hl*), and adaptation loci (*sg*, *hg*). The values for the base parameter set are given in Table 1, along with a short description of each one. Each unique set of conditions was tested with 500 iterations. The simulation program was written in Python version 2.7 (Python Software Foundation), with additional statistical functionality provided by the numpy package (Oliphant, 2007).

Inbreeding depression

The sensitivity of the captive population to the effects of inbreeding depression is determined by β , the slope of the regression line of the logarithm of viability onto inbreeding coefficient. Morton *et al.* (1956) showed that twice β is equal to the number of lethal equivalents in a diploid genome. Ralls *et al.* (1988) used this model to estimate the cost of inbreeding in a

variety of captive species and found a median value of 3.1 lethal equivalents, although the variation among species was high. A number of authors have questioned the validity of this method because of bias introduced by a small sample size correction (e.g. Lacy, 1997), but Kalinowski and Hedrick (1998), using a maximum likelihood approach, arrived at a similar estimate on the same data set.

Following Hedrick (1994), I model two types of deleterious loci: slightly recessive, mildly deleterious alleles and highly recessive, lethal alleles. Instead of using a fixed number of loci, I assume that each allele is unique, including those in the founding population as well as subsequent mutations. Starting frequencies and mutation rates for mild and lethal alleles are derived from β , k , and the selection and dominance coefficients of each type of allele according to Kelly (2003). Values for k and the selection and dominance coefficients in the base parameter set are taken from Hedrick (1994).

Adaptation to captivity

The characteristics of these loci were inspired by Woodworth *et al.* (2002). I model genetic adaptation as a fixed number of loci containing two types of alleles: a wild-type allele that incurs a fitness cost in captivity but is advantageous in the wild, and a tameness allele that is advantageous in captivity but costly in the wild.

Life cycle

I first create the founding population(s). Each individual consists of two lists of deleterious alleles (one inherited from the individual's mother and one inherited from the father),

another set of lists of adaptation loci (again, one maternal in origin and one paternal), a sex, a location, and a counter representing the number of offspring that individual has produced. To assign deleterious alleles, I draw from Poisson distributions based on the starting frequency of mild and lethal alleles. Each deleterious allele is given a unique integer ID, with mildly deleterious alleles being positive integers and lethal alleles having negative integer IDs.

Homozygotes are recognized by having the same integer ID in both maternal and paternal lists.

In addition to the information unique to each organism, I track kinship values for each pair of individuals in a matrix. The matrix is built using the additive relationship method of Ballou (1983); values on the diagonal = $1 +$ the inbreeding coefficient of the individual, while off-diagonal values = $2 * \text{the inbreeding coefficient of an offspring produced by the individuals represented by the row and column}$. All founders are assumed to be unrelated. As such, the initial matrix contains 1s on the diagonal and 0s on all off-diagonal spaces.

With the founders initialized, I start the first generation of captive mating. Working one population at a time, the program chooses each female in the population one at a time and finds the best male in the local population and the best male in all the other populations. 'Best' refers to a measure of genetic importance. For the results presented here, the best male is simply the male with the lowest kinship to the particular female. A version of minimizing mean kinship was also implemented, but because it significantly increased runtime of the program, it was not used. Ties in genetic importance are broken by comparing the number of offspring that each male has produced, and if this is not decisive, by random choice. I draw a random uniform number between 0 and 1 and pair the female with the best local male if it is greater than the user-specified migration rate or pair it with the best nonlocal male otherwise.

Each pair produces one offspring. Alleles are inherited from both parents based on Mendelian segregation, and all loci are independent (assumed to be unlinked). I assign new mutations by drawing from Poisson distributions based on the mutation rates for mild and lethal alleles. Adaptation loci are inherited by randomly choosing alleles from the mother and father with no mutation. I calculate two fitness values for each offspring: fitness in captivity and fitness in the wild. The only difference between these values arises from the adaptation loci. When calculating captive fitness, wild-type alleles negatively impact fitness (i.e., they represent poor adaptation to the captive environment), and for wild fitness, tameness alleles negatively impact fitness. Selection acts on viability, which is equivalent to the captive fitness of the individual. To test if the offspring survives, I draw a random uniform number between 0 and 1, and if this number is less than the captive fitness, the offspring survives; otherwise, I discard the offspring. Because the program uses discrete generations, I add the offspring to a new list that will be filled during the current generation and used as the source of parents in the next generation. I also recalculate the relationship matrix to include the new offspring.

The program creates a number of offspring for each population equal to the carrying capacity. If there is no selection, each population will be at the carrying capacity after the first generation of breeding. The addition of selection on deleterious alleles and the adaptation loci means that the size of each population is routinely much less than the carrying capacity. After all offspring are produced each generation, I discard the adults of the current generation and replace them with the surviving offspring in preparation for the start of the next generation. Similarly, I simplify the relationship matrix such that the parents of the current generation are removed, leaving the section of the matrix describing the relationships between the offspring of the current generation. This provides continuity of the genetic makeup of the populations and their history

of breeding. There is also a mechanism built into the program to transfer individuals to populations that are empty or lack a particular sex at the end of each generation; this becomes important for simulation runs with many small populations or when the selection parameters are high. At the end of each generation, I calculate averages of captive fitness, wild fitness, number of mildly deleterious alleles, number of lethal alleles, and frequency of the tameness alleles, as well as the total size of the metapopulation.

RESULTS

Number of adaptation loci

The first test varied the number of loci used in adaptation; the initial frequency of the tameness alleles was 0.25 for all tests. Figure 1 shows how changing the number of loci affects the initial captive fitness of individuals. With 12 or more loci, the average initial captive fitness falls below 0.05, and the probability of extinction approaches 100%. This decline in fitness is a result of the model's use of hard selection; when the tameness alleles start at low frequency, initial captive fitness is also low, and fecundity is not high enough to overcome the low initial fitness. A complex trait like tameness is likely due to many loci (Woodworth *et al.*, 2002), so 4 loci was chosen as a compromise.

Starting frequency of tameness alleles

Results for different starting frequencies of the tameness alleles are given in Figure 2. At high starting frequencies, nearly all loci become fixed for the tame alleles by the end of 50 generations. At the lowest frequency tested (0.125), the frequency of the tame alleles had not exceeded 50% after 50 generations. In many of these simulations, the tameness alleles were simply lost at some loci because of drift. A frequency of 0.25 was chosen as a compromise between realism and maintaining the potential for effective adaptation over 50 generations.

Testing the base parameter set

Figure 3 shows the average trajectory of a captive population over 50 generations using the base parameter set (see Table 1). Because individuals start poorly adapted to the captive environment, initial captive fitness is low, and population size decreases sharply from the starting generation. The low size of the population means that selection is less effective in removing the recessive, mildly deleterious alleles, and as a result, they begin to increase above their starting frequency through mutation; the increase stops after about 35 generations with around 20% more mild alleles than at the start. On the other hand, lethal alleles are effectively purged and only exist at low frequencies by the end of 50 generations. Adaptation to the captive environment progresses steadily over the course of the breeding program: the final frequency of the tameness alleles is almost triple the starting frequency, and the average wild fitness is half of what it was at the start. The overall probability of extinction of the total population during the breeding program was 0.002.

Migration

Figure 4 shows the effects of different levels of migration between subpopulations. The no migration case has smaller total population sizes, lower captive and wild fitness, fewer mild and lethal alleles, and less adaptation to captivity compared to the cases with migration, which are all quite similar. Higher inbreeding in the closed subpopulations could explain the lower population sizes and fitness values paired with the greater degree of purging of the mild and lethal alleles. The overall probability of extinction for all conditions was less than 1%.

Duration of captive breeding

Other tests look at the dynamics of variables over a 50 generation breeding program, but time in captivity will also vary greatly among species. Figure 5 suggests that the majority of genetic change seems to occur within the first 100 generations in captivity. In particular, the frequency of the tame alleles starts at 0.25 and increases to 0.57 after 25 generations, 0.73 after 50 generations, 0.83 after 100 generations, and then levels off, staying nearly constant after another hundred generations in captivity. Total population size also levels off to between 55-60 after 100 generations. Both captive and wild fitness stabilize as well, with captive fitness approximately 50% higher than the average initial captive fitness, and wild fitness approximately 50% lower than the average initial value. Figure 5d shows that lethal alleles are purged to low levels in as little as 25 generations and that selection is strong enough to prevent the accumulation of mildly deleterious alleles to dangerous levels, even as the amount of time in captivity increases. Once again, the probability of extinction was less than 1% for all tests except for a breeding program of 200 generations, in which the rate was 1.4%.

Sensitivity to inbreeding depression

Figure 6 suggests that slight differences in β can have profound implications on the fate of the captive population. Most alarming is the probability of extinction of the entire captive species, which is 0 for $\beta = 1.5$ or lower (and less than 1% for other iterations of the base parameter set) but balloons to nearly 50% at $\beta = 2.5$. Captive and wild fitness decrease steadily

with more inbreeding depression, and both converge on about 0.13 with $\beta = 2.5$. The final frequency of tame alleles also decreases, which could account for the faster decline in captive than wild fitness. Figure 6d hints at the underlying cause of the loss of fitness; while the average number of lethal alleles per individual at the end of captive breeding stays below 1.0 (despite the linear increase in the starting number of lethal alleles and the higher mutation rate for lethal alleles), the final average number of mild alleles slightly exceeds the starting number in all cases. Thus, the populations are incapable of purging these slightly recessive but deleterious alleles.

Underlying genetics of inbreeding depression

Figure 7 shows what happens when adjusting the proportion of inbreeding depression due to lethal alleles. These results further emphasize the serious differences between the fates of mild and lethal alleles. With the same level of β (1.5), the probability of extinction ranges from 0% with half or more of the inbreeding depression due to lethal alleles to about 80% with all the inbreeding depression due to mild alleles. Because the lethal alleles are rare, they should exist mostly in the heterozygous form, and because they are highly recessive, they do little to reduce fitness. As a result, the average final captive fitness of the populations increases rapidly as the influence of lethal alleles increases. Another impact of the greater contribution of lethal alleles is the higher degree of adaptation to captivity, with the tame alleles reaching a frequency of 0.80 after 50 generations when $k = 1.0$. This leads to a significant decline in wild fitness after 50 generations in captivity, with the most extreme case being a fall from 0.83 to 0.48 when $k = 1.0$. Figure 7d shows that, as with the test on different levels of β , selection effectively keeps lethal alleles at manageable levels but cannot purge mild alleles.

Carrying capacity

A motivating idea for this study was the finding by Woodworth *et al.* (2002) that there is not a simple relationship between the effective population size at which a captive population is maintained and that population's wild fitness when later reintroduced into wild conditions; their results suggested a parabolic relationship, with intermediate effective sizes leading to highest wild fitness. They hypothesized that for low effective sizes, inbreeding depression was the cause of lowered fitness, and for high effective sizes, they pointed to adaptation in captivity. Figure 8 seeks to recreate this result. As the simulated populations were not maintained at a constant effective population size, an exact comparison cannot be made. Instead, single populations were simulated with varying carrying capacities; initial population sizes were half the carrying capacities.

Figure 8 shows the results of these simulations fitted with a polynomial curve. They appear to agree with the finding of Woodworth *et al.* (2002), with carrying capacities of 150-300 having higher wild fitness after 50 generations of captive breeding than higher or lower values. However, the range of wild fitness across these simulations is not very wide.

DISCUSSION

Inbreeding depression

The results presented here support the idea that inbreeding depression is the most serious threat to the survival of captive populations. Changing the inbreeding load, β , from 1.5 to 2.5 caused a decrease in captive fitness after 50 generations from 0.31 to 0.13. Nearly half of the simulations with $\beta = 2.5$ ended in extinction of the entire species. In their survey of captive populations, Ralls *et al.* (1988) found the median estimate of β at about 1.5 but with substantial variation among species. Part of this variation was due to small sample sizes in the analysis, but it should be expected that species will differ in their tolerance of inbreeding. Furthermore, Lacy (1992) found no correlation between β and history of inbreeding in natural populations of mice. This suggests that it may be impossible to predict *a priori* how sensitive a species will be to inbreeding depression.

Perhaps even more important than the absolute value of β is the underlying cause of the inbreeding depression. Figure 7 shows that with a constant β , the proportion of inbreeding depression due to lethal alleles has a significant impact on the fate of a captive population. As the influence of mild alleles increases, fitness decays rapidly, and the risk of extinction reaches dangerous levels. While purging can eliminate lethal alleles, it cannot do the same for mildly detrimental mutations. Figures 6 and 7 show that the final frequency of mild alleles is always slightly higher than the initial frequency, likely because low captive population sizes weaken selection relative to drift. These results are in agreement with Hedrick (1994), whose model used much closer inbreeding. This trend towards higher average number of mutations per

individual suggests a similarity between populations in captive breeding and mutation accumulation studies. Both involve high inbreeding and a relaxation of selection and result in the increased frequency of mutations, though the potential similarities are not often considered in breeding programs.

Because inbreeding depression was one of the earliest concerns of captive population managers, there is already considerable effort to combat it. Frankham (2008) notes that most captive species are already managed as essentially one panmictic population. Minimizing mean kinship limits inbreeding more effectively than other strategies in the long-term, and it can be adjusted to more strictly avoid inbreeding if necessary (Ballou and Lacy, 1995).

Fortunately, the simulation results do not suggest that deleterious mutations will continue to accumulate in captive populations until they go extinct. Figure 5 indicates that the increase in the average number of mild alleles per individual stops after about 100 generations, while the same happens to lethal alleles in as little as 50 generations. These represent the formation of a new mutation-selection balance for these alleles, but despite the smaller sizes of captive populations, selection may still be effective in combating the proliferation of deleterious alleles.

Genetic adaptation

The results of this study show that a model of genetic adaptation to captivity similar to that proposed by Woodworth *et al.* (2002) can influence the evolution of a captive species. Using the base parameter set, the frequency of the tameness alleles almost triples in 50 generations, coinciding with a decrease in wild fitness of approximately one-half the average initial value. Moreover, Figure 8 shows that single populations kept in captivity at varying sizes differ in their wild fitness after 50 generations in a way predicted by Woodworth *et al.* (2002).

Interestingly, tests changing the duration of captive breeding suggest that adaptation happens rapidly, with the greatest increase in the frequency of tameness alleles occurring in the first 25-50 generations, after which the rate of increase levels off. Beyond 100 generations, the variation for tameness has been exhausted, and no further adaptation can take place. However, these results are contingent on the parameters of the adaptation loci; with a greater number of loci, the period of adaptation could proceed longer. Mutation was also not simulated for these loci, which could represent an important source of additional genetic variation.

In documenting the effects of adaptation to captivity in experimental *Drosophila* populations, Frankham and Loebel (1992) also explored possible solutions using a modified version of the breeder's equation

$$R = \frac{(1 - m) \times S \times h^2}{L}$$

where R is the response to selection, m is the rate of immigration of wild animals into the captive population, S is the selection differential, h^2 is heritability, and L is generation length. Any management actions that could reduce R would slow genetic adaptation. The possibility of immigration has traditionally not been considered in captive breeding programs because of the stress it might place on the remnant wild population. Attempting to recreate aspects of the wild environment in captivity should reduce S , and increasing L is possible through breeding choices that favor older individuals.

Frankham (2008) proposed the use of population subdivision to reduce the extent of adaptation to captivity. By dividing a species into small, isolated units, genetic drift could overpower selection and prevent adaptation. It would also reduce costs by virtually eliminating long distance transfer of animals and buffer a species against the spread of disease among zoos

(Frankham, 2008). However, such a plan could add undo risks to the long-term survival of captive populations. Figure 4 shows that a configuration with isolated populations has a lower total population size than the same number of populations that are connected by some amount of migration. The isolated populations also have lower average captive fitness, and although the frequency of the tameness alleles is reduced compared to the cases with migration, the lower degree of adaptation does not translate into a higher wild fitness than the other conditions. This is likely because of a slightly more severe inbreeding depression in the isolated zoos, as suggested by the lower average number of mild and lethal alleles in these populations. That coupled with the risks due to demographic stochasticity means that complete population fragmentation is not a viable option for the future.

The future of zoos

Soule et al. (1986), in an attempt to develop a universal strategy to combat the loss of genetic variation, proposed that the goal of captive breeding programs should be to preserve 90% of the genetic diversity present in the founding population for 200 years, a timeline that has subsequently been reduced to 100 years (Lacy, 2013). There seems to be a growing recognition that such a goal is arbitrary and does not necessarily reflect the best way to maintain species in captivity. A more radical and potentially sustainable idea is the recent call by some leaders in the zoo community for a closer integration between *ex situ* and *in situ* conservation efforts (Conway, 1995; Conway, 2011; Lacy, 2013). This would include both material support and expertise from zoos but also the possibility of exchange of individuals between captive and wild populations. Immigration from the wild promises a variety of benefits, including alleviation of inbreeding depression, restoration of genetic diversity, and reduction of adaptation to captivity

(Lacy, 1987; Frankham and Loebel, 1992). Supplementation from captivity could also help wild populations remain viable, though it would be important to ensure that genetic changes resulting from captivity do not harm the wild population (Ford, 2002). Such a union of captive breeding and conservation biology in the field could help save many endangered species, and it would ensure the place of zoos as conservation leaders for the future.

Table 1. Base Parameter Set

| Parameter | Value | Description |
|----------------|--------|--|
| num_pops | 5 | number of subpopulations |
| N_found | 20 | number of founding individuals in each subpopulation |
| beta | 1.5 | a measure of the rate of decline of fitness with increasing inbreeding ¹ |
| k | 0.5 | proportion of inbreeding depression due to lethal alleles ² |
| num_ga_loci | 4 | number of loci contributing to adaptation to the captive environment |
| q | 0.25 | starting frequency of the ‘tame’ alleles |
| gens | 50 | number of generations of captive breeding |
| K_per_zoo | 40 | carrying capacity of each subpopulation |
| migration_rate | 0.0125 | probability that each mating event is with a nonlocal male; along with the K_per_zoo parameter, equivalent to 0.5 migrants per population per generation |
| hm | 0.25 | dominance coefficient for mildly deleterious alleles ² |
| sm | 0.25 | selection coefficient for mildly deleterious alleles ² |
| hl | 0.02 | dominance coefficient for lethal alleles ² |
| sl | 1.0 | selection coefficient for lethal alleles ² |
| hg | 0.25 | dominance coefficient for ‘tame’ alleles |
| sg | 0.20 | selection coefficient for ‘wild’ & ‘tame’ alleles; a locus that is homozygous for the ‘wild’ allele decreases captive fitness by 20%, and likewise, a locus that is homozygous for the ‘tame’ allele decreases wild fitness by 20% |

¹ - Ralls, *et al.* (1988)² - Hedrick (1994)

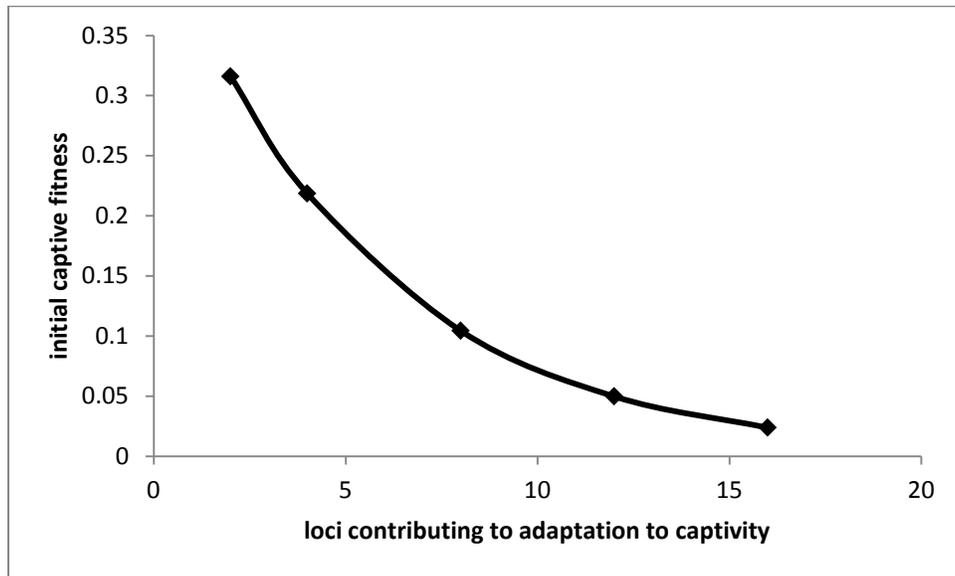


Figure 1. The effect of varying the number of loci contributing to adaptation to captivity on initial captive fitness. The starting frequency of the tameness alleles was 0.25 for all cases.

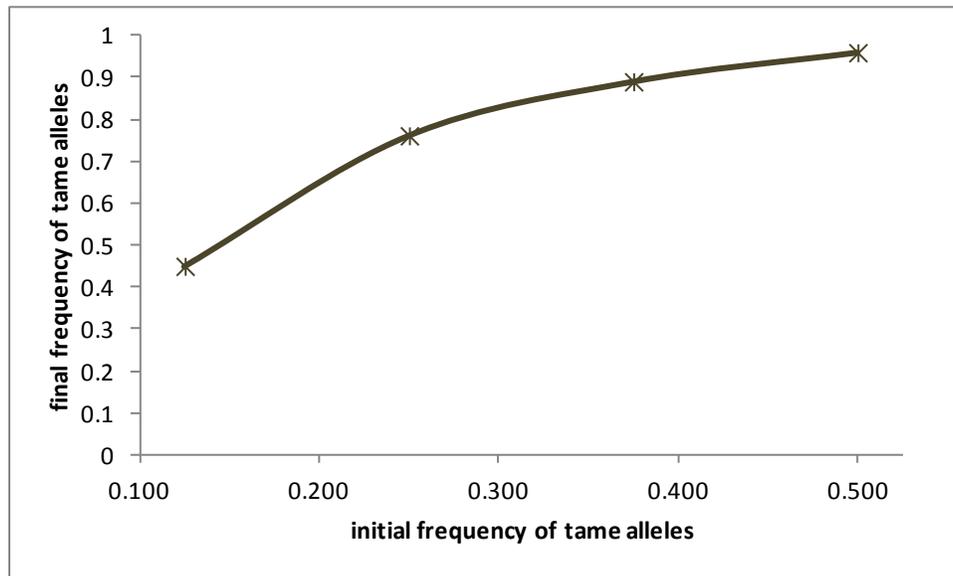


Figure 2. The effect of varying the initial frequency of the tameness alleles on the final frequency of the tameness alleles after 50 generations. For all cases, the number of loci contributing to adaptation was 4.

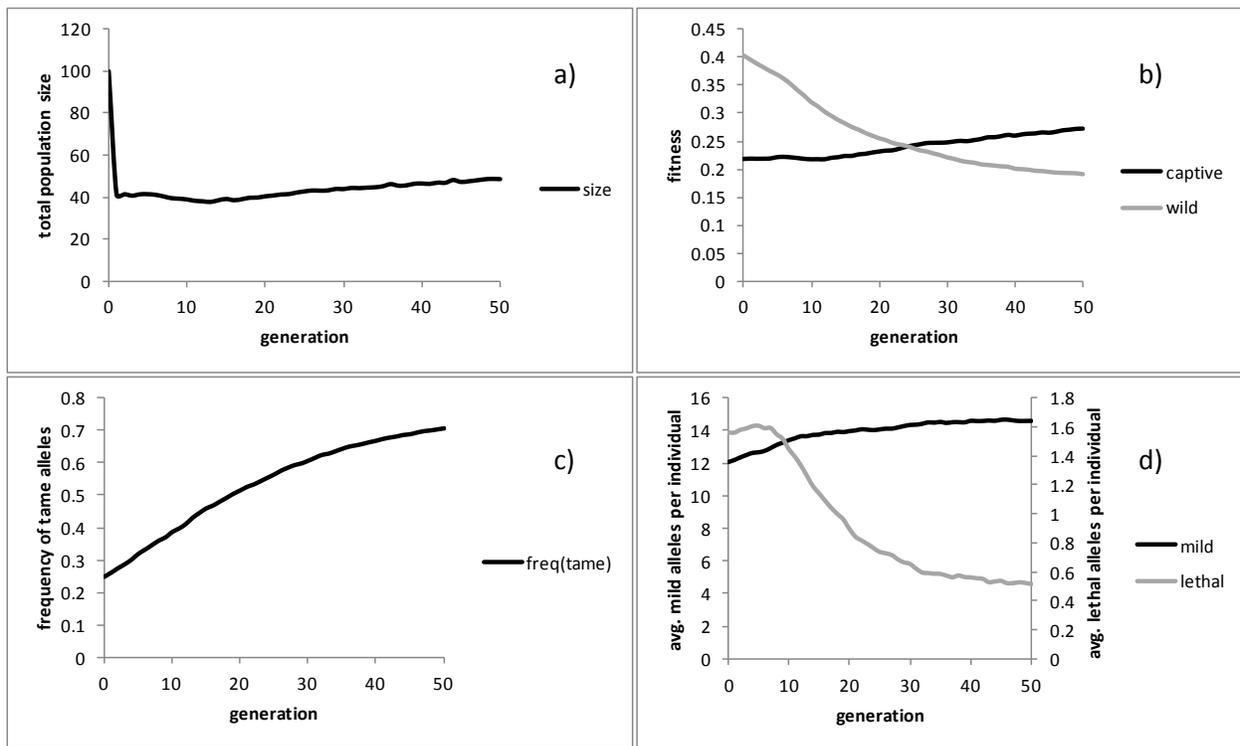


Figure 3. Genetic changes in simulated captive populations over 50 generations. a) population size; b) average captive and wild fitness; c) frequency of tameness alleles; d) average number of mild and lethal alleles per individual. Data are averages over 500 simulations. Parameter values are given in Table 1.

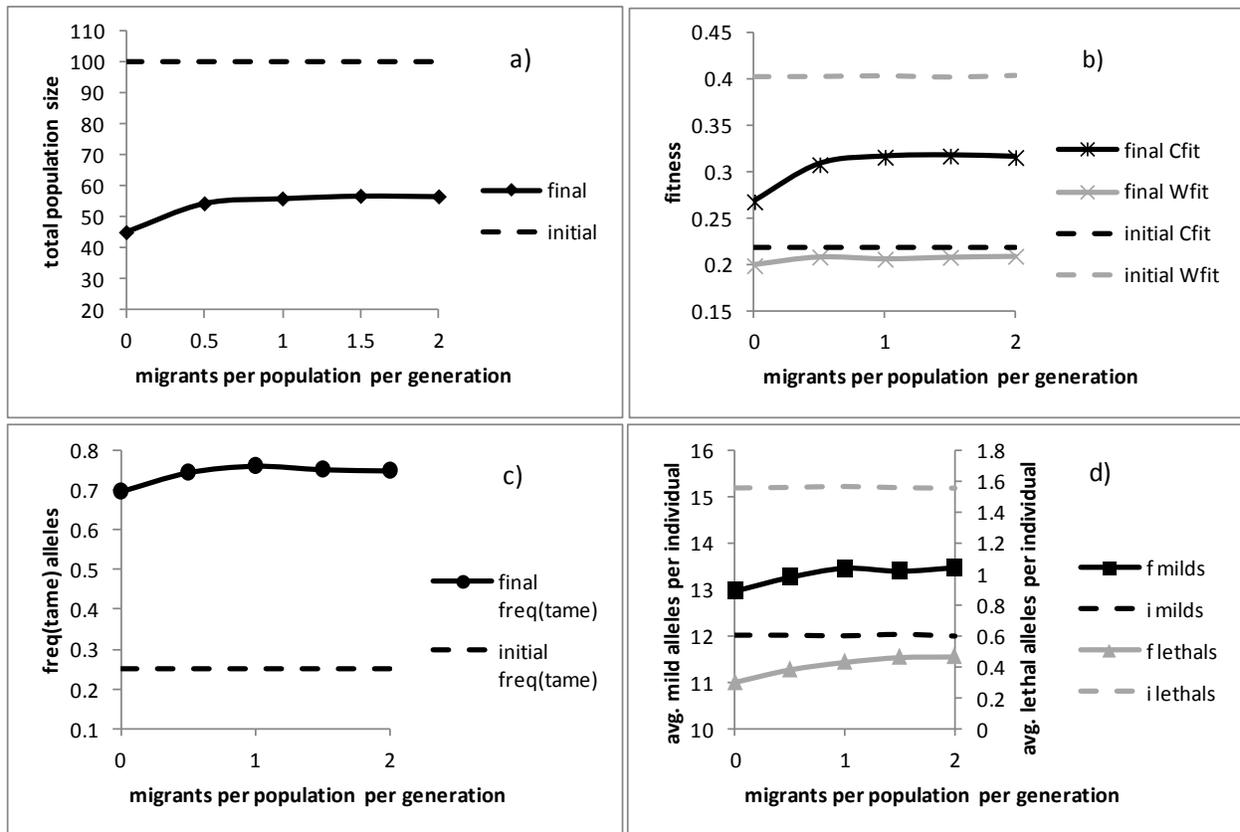


Figure 4. The effect of varying the amount of migration between subpopulations on a) total size of the metapopulation; b) average captive and wild fitness; c) frequency of tameness alleles; and d) average number of mild and lethal alleles per individual after 50 generations of captive breeding.

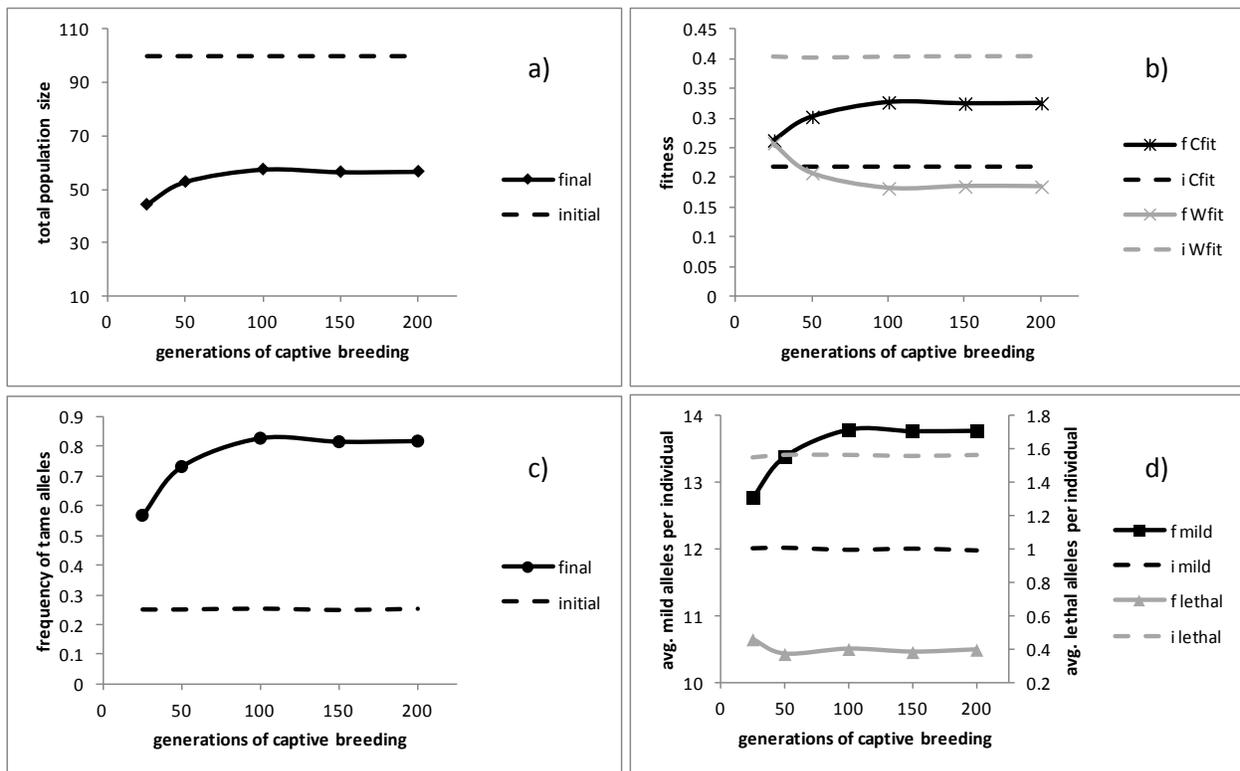


Figure 5. The effect of varying the duration of captive breeding on a) total size of the metapopulation; b) average captive and wild fitness; c) frequency of tameness alleles; and d) average number of mild and lethal alleles per individual after 50 generations of captive breeding.

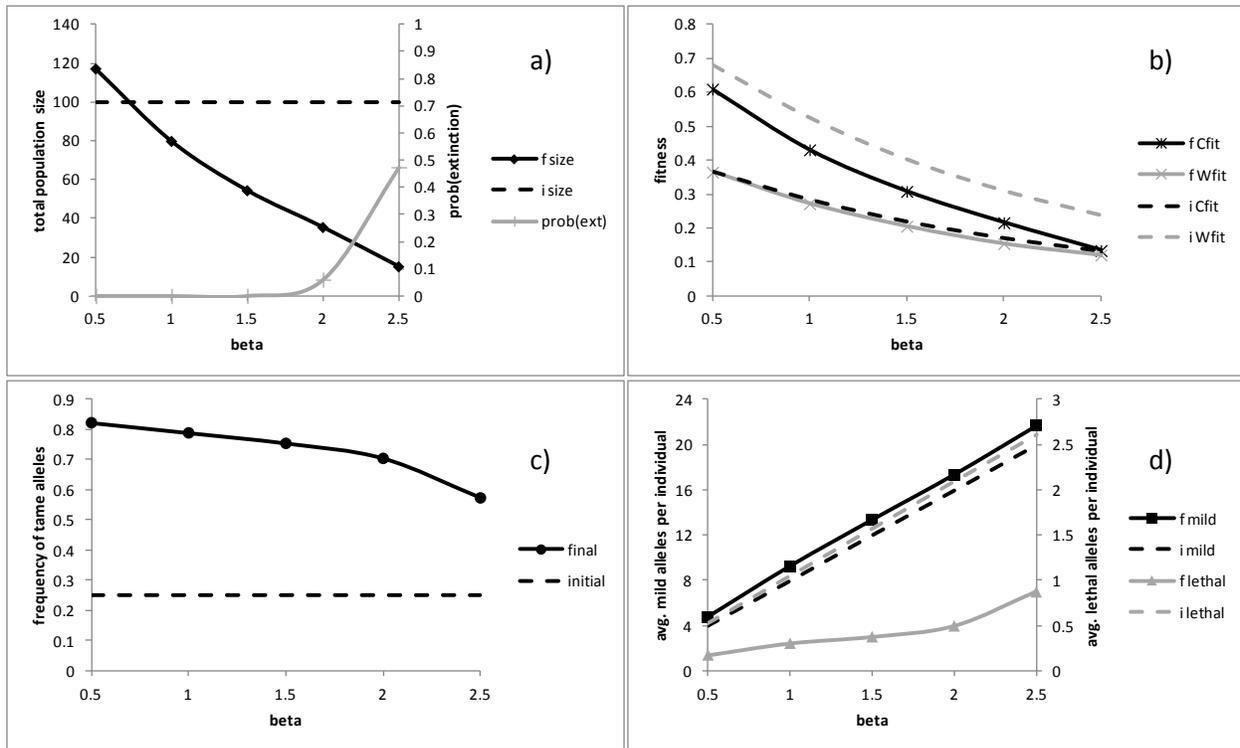


Figure 6. The effect of changing the sensitivity of the population to inbreeding depression on a) total size of the metapopulation and probability of extinction of the metapopulation; b) average captive and wild fitness; c) frequency of tameness alleles; and d) average number of mild and lethal alleles per individual after 50 generations of captive breeding.

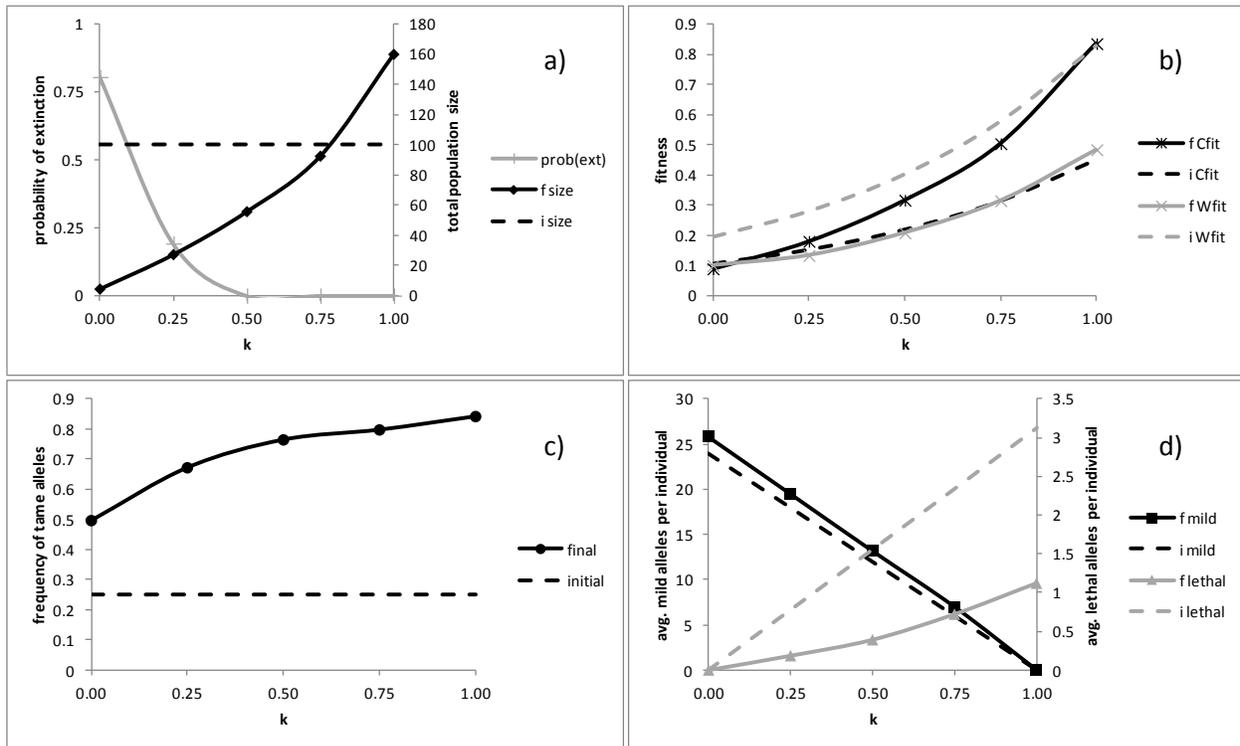


Figure 7. The effect of varying the proportion of inbreeding depression due to lethal alleles on a) total size of the metapopulation and probability of extinction of the metapopulation; b) average captive and wild fitness; c) frequency of tameness alleles; and d) average number of mild and lethal alleles per individual after 50 generations of captive breeding.

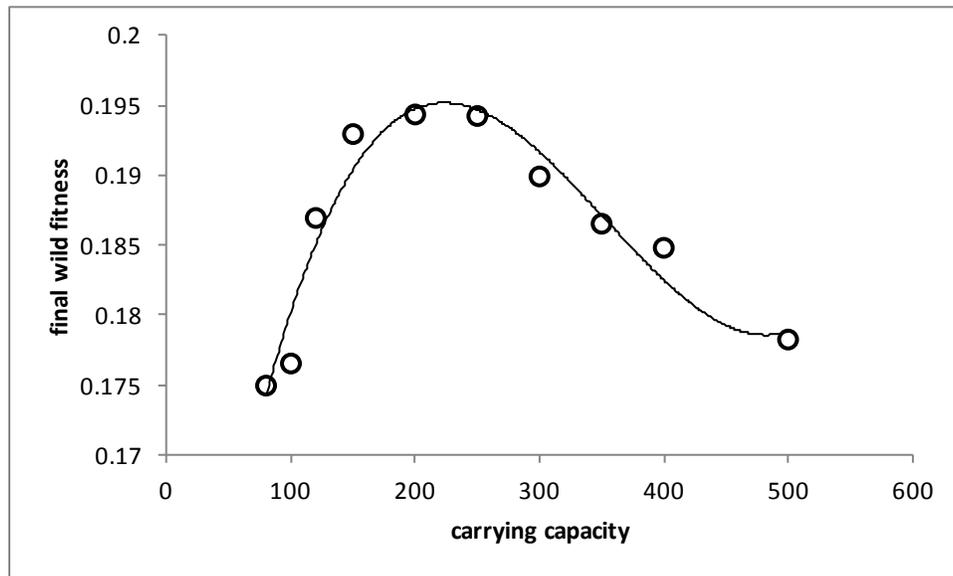


Figure 8. The effect of varying carrying capacity of a single population held in captivity for 50 generations on wild fitness. The data are fitted with an order 3 polynomial curve. The initial population size was one-half the carrying capacity in all cases.

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