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# Gene flow after inbreeding leads to higher survival in deer mice

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## Abstract

We test the ability of gene flow to alleviate the deleterious effects of inbreeding in a small mammal, the deer mouse (*Peromyscus maniculatus*). After three generations of sib–sib mating, individuals from three lines of mice were either subject to further inbreeding or were mated with an outbred individual. Subsequently, these mice, plus a control line, which were first generation ($F_1$) mice from unrelated individuals kept in captivity for the same duration as the treatment lines, were released into isolated pens in a forest in western Montana. Survival of individual mice was recorded. Survival models that allowed variation in breeding treatments were well supported, whereas models explaining variation in line, or release location were not well supported. Survival was highest for offspring of the outcross group, intermediate for the inbred animals, and lowest for the control group. This suggests that the introduction of migrants can reduce inbreeding depression, as theory predicts. We also show limited evidence for purging of deleterious recessive alleles that can cause inbreeding depression. While purging may have occurred, the demographic cost was non-trivial as 5 of 8 of our inbred mouse lines went extinct during the inbreeding process.
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## 1. Introduction

Population genetic theory predicts that small, isolated populations will lose heterozygosity, leading to reductions in fitness or fitness surrogate measures (e.g., survival, litter size; Wright, 1922). Several empirical studies have demonstrated reductions in fitness surrogates in inbred populations (e.g. Charlesworth and Charlesworth, 1987; Keller, 1998; Ralls et al., 1988; Barrett and Charlesworth, 1991; Reed and Frankham, 2002). However more alarming are modeling efforts (Mills and Smouse, 1994; Lacy, 1987) and field studies that show inbreeding depression can increase probabilities of extinction, ultimately leading to higher extinction rates (Newman and Pilson, 1997; Saccheri et al., 1998).

For example, Newman and Pilson (1997) inbred lines of *Clarkia pulchella*, a Rocky Mountain plant, in a laboratory setting. Subsequent, plantings in *Clarkia*'s natural environment revealed that the extinction rates of the inbred lines were greater than that of the control lines. Jimenez et al. (1994) showed that after inbreeding a wild population of white-footed mice (*Peromyscus leucopus noveboracensis*) in the laboratory, survival in the wild was significantly reduced over a 10-week period after release when compared to non-inbred mice. Saccheri et al. (1998, 2001) examined genetic variability, demography, and extinction rates of 42 populations of the Glanville fritillary butterfly (*Meliaea cinxia*) while measuring environmental and ecological conditions surrounding these populations in southwestern Finland; inbreeding explained 26% of the variation in extinction rates.

To prevent the deleterious effects of inbreeding in endangered wild populations, enhancement of connectivity between populations is often recommended.

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Population genetic theory suggests that between one and ten migrants (dispersers that breed, thus leading to gene flow) per generation is sufficient to alleviate the negative effects of inbreeding, yet still allow for local adaptation (Wright, 1931; Allendorf and Phelps, 1981; Mills and Allendorf, 1996; Vucetich and Waite, 2000); this is regardless of the initial population size (Mills and Allendorf, 1996). However, recent theoretical work suggests that greater than ten migrants per generation may be needed to offset the loss of rare alleles in many wild populations (Vucetich and Waite, 2000).

Empirical evidence from laboratory experiments has shown that migration can increase fitness in inbred populations (Spielman and Frankham, 1992; Newman and Tallmon, 2001). Spielman and Frankham (1992) established that migration of one individual into inbred fruit fly populations (*Drosophila melanogaster*) significantly increased reproductive success. Similarly, Backus et al. (1995) and Bryant et al., 1999 demonstrated that fitness measures of bottlenecked housefly populations (*Musca domestica*) increased and population extinction rates decreased when one-migrant per generation was exchanged between bottlenecked populations.

Finally, several management actions have also provided evidence that migration is beneficial to both captive and wild populations. Ralls and Ballou (1983) reported that introducing one wildebeest (*Connochaetes taurinus*) each generation into a captive population increased the overall survival of the herd. More recently, the introduction of greater prairie chickens (*Tympanuchus cupido pinnatus*) into a rapidly declining population in Illinois increased egg viability, the demographic rate most likely to affect population growth rate (Soulé and Mills, 1998; Westemeier et al., 1998). Other examples of immigration in the wild relieving inbreeding include the natural introduction of a single immigrant gray wolf (*Canis lupus*) into a geographically isolated Scandinavian population increasing heterozygosity and rapidly increasing population growth (Vilà et al., 2002) and the introduction of male adders (*Vipera berus*) into a highly inbred isolated adder population increasing male recruitment and decreasing stillborns (Madsen et al., 1999).

In response to the theory and experimental evidence to date, some Federal Recovery Plans have implemented into management plans the "rule of thumb" that one-migrant per generation can reduce the negative effects of inbreeding (US Fish and Wildlife Service, 1988). For example, the Grizzly Bear (*Ursus arctos horribilis*) recovery plan in the contiguous United States (US Fish and Wildlife Service, 1993) recommended "…that one animal should enter the breeding population each generation." (p. 27). The efficacy of such a strategy has not yet been experimentally tested on vertebrates exposed to natural conditions. Because the one-migrant-per generation rule cannot be tested on endangered or threatened species for ethical and logistical reasons we tested the basis of this theory in an inbred laboratory populations of mice (*Peromyscus maniculatus*). Specifically, we tested whether introducing migrants (that is, creating an outcross) can mitigate inbreeding depression at the level of the individual, as theory would predict; whether this alleviates inbreeding at the population level is certainly dependent on minimizing inbreeding on the individual level. To conduct our test, we examined fitness consequences by releasing mice subject to four generations of sib–sib mating and mice subject to three generations of sib–sib mating, then mated with an outbred individual. Our index of fitness was the mean survival of each treatment, the vital rate most likely to affect population growth in deer mice in the wild (Citta, 1999). Mice are a good model system because of their short generation time, abundance, and the wealth of information on mouse handling and husbandry.

## 2. Methods

### 2.1. Laboratory

On August 1, 1997 we founded a small breeding colony of 20 mice (*Peromyscus maniculatus*) purchased from the *Peromyscus Stock Center* (University of South Carolina). Wild mice were not used because of the logistical constraints of housing deer mice born in Montana with known high prevalence of *Sin Nombre* virus, the primary etiologic agent of Hantavirus pulmonary syndrome in North America (Douglass et al., 1996, 2001). The South Carolina mice were descended from 19 individuals 50–70 generations ago, but the colony has remained large since the initial bottleneck and sib matings is avoided in the colony. Furthermore, a study of microsatellite variability in the parent colony at the *Peromyscus Stock Center* suggests that severe inbreeding has been avoided (Prince et al., 2002; M. Dewey, *Peromyscus Stock Center*, pers. comm.).

Captive mice were housed in one room maintained at 20 °C with a photoperiod of 18 h – light, 6 h – dark. For general mouse colony maintenance, we adopted the husbandry techniques recommended by the *Peromyscus Stock Center*. This included weaning mice 21 days after birth, and separating the sexes a few days afterwards. The diet consisted of standard mouse chow and water provided *ad libitum*. We uniquely identified each mouse with a small ear-punch (*Peromyscus Stock Center* pers. comm.; Jimenez et al., 1994).

Initially, we formed eight random pairs of mice, ensuring that none of the individuals in the pairs shared any parents or grandparents (*Peromyscus Stock Center* pers. comm.). After three generations of sib-sib matings, offspring from each line (8 lines total) had an inbreeding coefficient of 0.375 (Fig. 1). During this

Line 1 (of 8)
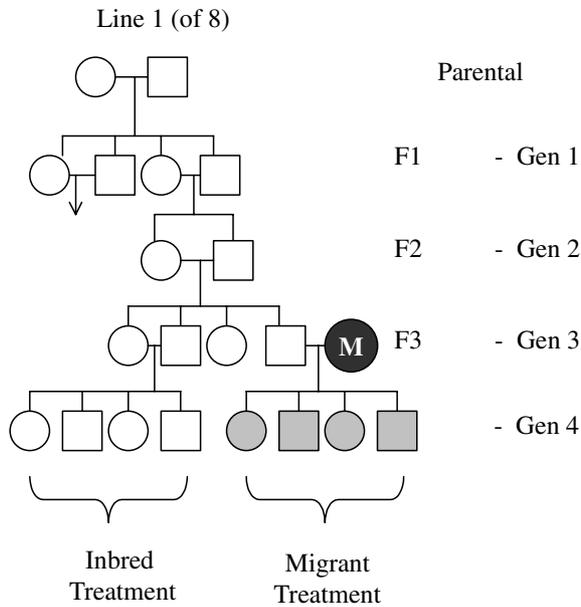


Fig. 1. Diagram of the breeding scheme that used for each line. The black circle with the "M" is an outcross (migrant) individual, defined as an individual from a control line not used in the inbreeding portion of the experiment.

inbreeding procedure five lines stopped producing offspring.

In generation four of our experiment we initiated our treatments: (1) inbred: randomly chose sibs to continue inbreeding (providing an inbreeding coefficient of 0.5); and (2) outcross (migrant): bred individuals of inbred lines with a random individual from a different line for one generation (providing an inbreeding coefficient of 0.0). The third treatment was a control: bred unrelated individuals not used in the inbred or outcross treatment.

### 2.2. Field

The inbred, outcross, and control mice were kept with their parents for 21 days and housed separately until we had maximized our sample size for the field. Once all mice were reared we transported them to our field site at the University of Montana's Lubrecht Experimental Forest. The area of the Lubrecht Experimental Forest used is a dry forest composed of mostly Ponderosa pine (*Pinus ponderosa*) with some Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) in the overstory and elk sedge (*Carex geyeri*), snowberry (*Symphoricarpos albus*), and arrowleaf balsamroot (*Balsamorhiza sagittata*) in the understory.

We used three 0.81 ha pens (90 m × 90 m) as an arena for testing mouse survival. Each pen was built of high-density polyethylene plastic sheeting buried at least 10 cm below ground and extending 1 m above ground (Citta, 1999). To prevent mice from accessing the forest canopy and moving between or leaving pens, we wrapped

Table 1
Numbers of mice introduced into pens by treatment

|       | Inbred | Migrant | Control | Total |
|-------|--------|---------|---------|-------|
| Pen 1 | 8      | 12      | 9       | 29    |
| Pen 2 | 10     | 11      | 10      | 31    |
| Pen 3 | 8      | 15      | 9       | 32    |
| Total | 26     | 38      | 28      | 92    |

trees near enclosure walls with a 1-m strip of sheet metal and coated all fence posts with *Tanglefoot* (Grand Rapids, MI), a chemical-free sticky residue. Lastly, we installed a three-wire solar electric fence around the pens to prevent bears and other carnivores from entering the area and destroying the pens and associated equipment. Over the duration of the study no mice were found to move between pens.

The mouse cages were placed outside the pen structures for 24-h to provide a brief "acclimation period". We specifically chose to release mice during the mildest time of the year, when rainfall is unlikely and nighttime temperatures are favorable. Prior to introducing mice to the pens we removed all wild mice using 49 Sherman traps per pen. The traps were placed on a 7 × 7 grid with 12.8 m spacing between traps. We considered the pens void of native mice after no mice were trapped for three consecutive days.

On July 14, 1999 each of the 92 captive mice (26 in bred, 38 outcross individuals, and 28 control animals; See Table 1) were placed equidistant between two trapping stations in a 1-litre plastic-coated, paper milk carton with polyester batting, three mouse-chow pellets (lab food), and a small handful of mixed seed. Each pen received equal numbers of mice from each line, sex, and treatment (inbred, outcross, control). The densities used in each pen were close to the natural densities found in local populations (Citta, 1999). The exact placement of each mouse carton within a pen was determined by a random number generated prior to arrival in the field.

The first of five trapping sessions began five days after initial release, following the same scheme used to remove native mice. Each trapping session consisted of pre-baiting traps for a day then trapping for four days. Consecutive trapping sessions were separated by a mean interval of 9.25 days.

### 2.3. Statistical measures

We estimated survival rates ($\Phi$) by treatment in Program MARK (White and Burnham, 1999) using Cormack–Jolly–Seber models (Seber, 1982; Pollock et al., 1990; Lebreton et al., 1992). Typically Cormack–Jolly–Seber models estimate apparent survival, which is the probability of survival and site fidelity. By using pens we could eliminate the possibility that animals dispersed, thereby obtaining estimates of true survival (s). We used

Program MARK's bootstrapping procedure to test the goodness-of-fit for our most parameterized model (global model), which included time ($t$), breeding treatment, pen, and their interaction in both survival ($\Phi$) and capture probability ($p$). Out of 1000 bootstrap simulations our global model ranked 170th; therefore, we assumed the model fit the data relatively well. Next, we followed the strategy advocated by Burnham and Anderson (2001, 2002) and derived a candidate set of models to be tested based on our biological understanding of deer mice and population genetics (King, 1968; Crow and Kimura, 1970; Metzgar, 1979; Millar et al., 1979; Teferi and Millar, 1993). Our candidate models explained the variation in the data based on differences in breeding treatments, pens, pens and breeding treatments combined (pb), time ($t$), and statistical interactions with time and other grouping variables (e.g., pen$*t$ and breeding$*t$; Table 2).

We used a two-stage procedure for optimizing our parameters. First, we tested the sources of variation in capture probability ($p$) while keeping survival constant (i.e., $\Phi(pb*t)$). The best approximating model for capture probability was selected based on Akaike's Information Criteria, which identifies the model that best explains the significant variation in the data, while excluding all unnecessary parameters (AICc, Harvich and Tsai, 1995; Burnham and Anderson, 2001, 2002). We only accepted models within approximately 4 AICc values of the best approximating model. Next, we used the best fitting model for capture probability and optimized survival. We then tested some additional models which appeared logical after examining our initial results.

Lacy et al. (1996) found that the genetic load of deleterious alleles was unequally apportioned among founding pairs of mice (*Peromyscus polionotus*). We

tested for differences in survival between the lines of mice, but sample size prevents us from including both line and breeding treatments as grouping variables. Therefore, we repeated our analysis, using line instead of breeding treatment to explain variation in our data.

Body mass has been shown to vary proportionally with survival in some small mammals (Sauer and Slade, 1985, 1987), thus we also examined differences in mean body mass between treatments of mice. We did not consider body mass as a covariate in our initial model, again because of small sample sizes. One-way analysis of variance (ANOVA) was conducted on the release weights of all mice with breeding treatment as a factor.

## 3. Results

The mark-recapture survival models which best fit variation in capture probability were ones which either constrained all variation in capture probability (absolute best fit) or had differences among breeding treatments such that the outcross/migrant treatment (m) and the control treatment (c) were the same, but the inbred treatment (i) was different [$\Phi(pb*t)p(.)$ and $\Phi(pb*t)p(c = m, i)$, second best fit, with a difference in AICc of 2.10; Table 3]. This top model suggests that probability of capture did not vary by pen, breeding treatment, time, or any interaction between these variables. The estimates of capture probability derived from these two best fitting models were 0.84 (SE = 0.04) for the first model and 0.78 for inbred (SE = 0.097) and 0.85 (SE = 0.04) for outcross and control treatments in the second model. After determining which capture probability model had the best support given the data we optimized the survival parameters.

Table 2
Candidate set of models

| Model | Model description |
| --- | --- |
| $\Phi(pb*t)p(pb*t)$ | Survival and capture probability varies by the interaction between group and time |
| $\Phi(pb*t)p(t)$ | Survival varies by the interaction between group and time, capture probability varies by time |
| $\Phi(pb*t)p(breeding)$ | Survival varies by the interaction between group and time, capture probability varies by breeding |
| $\Phi(pb*t)p(pen)$ | Survival varies by the interaction between group and time, capture probability varies by pen |
| $\Phi(pb*t)p(pb)$ | Survival varies by the interaction between group and time, capture probability varies by groups |
| $\Phi(pb*t)p(.)$ | Survival varies by pen and breeding treatment interacting with time, capture probability does not vary |
| $\Phi(pb*t)p(c = m)$ | Survival varies by pen and breeding interacting with time, capture probability is equal between migrant and control |
| $\Phi(.)p(.)$ | Survival and capture probability do not vary |
| $\Phi(pen*time)p(.)$ | Survival varies by the interaction between pen and time, capture probability does not vary |
| $\Phi(pen)p(.)$ | Survival varies by pen, capture probability does not vary |
| $\Phi(t)p(.)$ | Survival varies by time, capture probability does not vary |
| $\Phi(c = m, i)p(.)$ | Survival varies by breeding, but is the same between control & migrant groups, capture probability does not vary |
| $\Phi(c = m, i*t)p(.)$ | Survival varies by breeding treatment interacting with time, but control and migrant are equal |
| $\Phi(breeding) p(.)$ | Survival varies by breeding treatment, capture probability does not vary |
| $\Phi(breeding*t)p(.)$ | Survival varies by breeding treatment interacting with time, capture probability does not vary |

$\Phi$ = survival, $p$ = capture probability, $t$ = time, pb = group (both breeding treatment and pen) c = control, m = migrant/outcross treatment, and i = inbreeding treatment.

Table 3
Optimization of capture probability (*p*)

| Model | AICc | Delta AICc | AICc Weights | Parameters |
|---|---|---|---|---|
| *Φ(pb∗t)p(.)* | *356.93* | *0.00* | *0.74* | *26* |
| *Φ(pb∗t)p(c = m, i)* | *359.03* | *2.10* | *0.26* | *27* |
| Φ(pb∗t)p(pb) | 641.92 | 284.99 | 0 | 28 |
| Φ(pb∗t)p(pb∗t) | 645.37 | 288.44 | 0 | 31 |
| Φ(pb∗t)p(t) | 647.83 | 290.90 | 0 | 27 |
| Φ(pb∗t)p(breeding) | 647.83 | 290.89 | 0 | 27 |
| Φ(pb∗t)p(pen) | 647.99 | 291.06 | 0 | 27 |

Symbols are the same as in Table 2. Models in italics fit the data and were considered. AICc weights are a way to transform the AIC values to conditional probabilities for each model, recommended by Akaike (1978, 1979). Parameters refer to the number of parameters in each model.

Our mark-recapture data indicate that over the sampling period mice exposed to migration had highest survival, followed by inbred mice, and control mice. This was true regardless of whether we modeled capture probability constraining all variation in capture probability (absolute best fit) or had differences among breeding treatments such that the outcross/migrant treatment (m) and the control treatment (c) were the same, but the inbred treatment (i) was different (difference in AICc of 0.84; data not shown). The model where survival varied by breeding treatment had the best support, much stronger than the next best-supported model where survival varied by the interaction of breeding treatment and time (Table 4). The best approximating model indicates that outcross mouse survival was 0.98 (SE = 0.005), inbred mouse survival was 0.93 (SE = 0.014) and the control mouse survival was 0.89 (SE = 0.021; Fig. 2).

After our initial analyses we added four tests (not presented in the tables), two where survival was highest in the outcross treatment, and lower, but equal between the inbred and control treatment (with capture probability modeled both under the best two models; $Φ(m > i = c)p(.)$ and $Φ(m > i = c)p(c = m, i)$), and a second set of models with survival equal between the out-

Table 4
Ranking of the candidate set of models for estimating survival

| Model | AICc | Delta AICc | AICc weights | Parameters |
|---|---|---|---|---|
| *Φ(breeding)p(.)* | *339.48* | *0* | *1.0* | *4* |
| Φ(breeding∗t)p(.) | 354.50 | 15.02 | 0 | 13 |
| Φ(pb∗t)p(.) | 356.93 | 17.45 | 0 | 26 |
| Φ(pb∗t)p(c = m,i) | 359.03 | 19.55 | 0 | 27 |
| Φ(c = m, i)p(.) | 365.78 | 26.29 | 0 | 3 |
| Φ(.) p(.) | 367.25 | 27.77 | 0 | 2 |
| Φ(t)p(.) | 367.27 | 27.79 | 0 | 5 |
| Φ(pen∗time)p(.) | 369.87 | 30.39 | 0 | 12 |
| Φ(c = m, i∗t) p(.) | 371.06 | 31.58 | 0 | 9 |
| Φ(pen)p(.) | 371.34 | 31.86 | 0 | 4 |

Model symbols are described in Table 2 and in the text. Only models within 4 AICc of the best approximating model were considered (in italics).
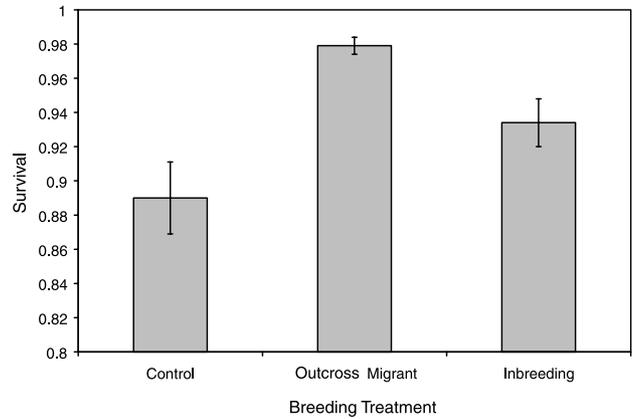


Fig. 2. Survival estimates of the three breeding treatments. Error bars are one standard error from the mean.

cross and inbred treatments and lower for the control group (again using the top two models for capture probability; $Φ(m = i > c)p(.)$ and $Φ(m = i > c)p(c = m, i)$). The two models with survival highest in the migrant treatment and equal between the inbred and control treatment were well supported (difference in AICc = 0.99 and 2.58, respectively). The models with survival equal between the inbred and migrant line, but higher than the control treatment were not supported (difference in AICc = 7.03 and 10.58, respectively).

Using the candidate set of models to test for differences in survival between the three extant lines, we found that the model with the best support was the one where survival did not vary by line (Table 5), however the global model with line was poorly supported (bootstrap goodness of fit test *p* = 0.94). Survival varying by time was also well supported, but again there was no support for survival varying by line. Survival under the two most supported models was estimated to be 0.96 (SE = 0.01) and 0.95 (SE = 0.01), respectively.

Table 5
Ranking of the candidate set of models for testing for differences between lines

| Model | AICc | Delta AICc | AICc weights | Parameters |
|---|---|---|---|---|
| *Φ(.)p(line)* | *365.18* | *0.00* | *0.46* | *5* |
| *Φ(.)p(.)* | *367.25* | *2.07* | *0.16* | *2* |
| *Φ(t)p(.)* | *367.27* | *2.09* | *0.16* | *5* |
| *Φ(.)p(t)* | *368.06* | *2.88* | *0.11* | *4* |
| *Φ(t)p(line)* | *369.12* | *3.95* | *0.06* | *8* |
| Φ(t)p(t) | 370.63 | 5.45 | 0.03 | 7 |
| Φ(.)p(line∗t) | 372.40 | 7.22 | 0.01 | 14 |
| Φ(t)p(line∗t) | 374.97 | 9.79 | 0.00 | 17 |
| Φ(line∗t)p(.) | 797.21 | 432.02 | 0.00 | 10 |
| Φ(line∗t)p(line) | 802.98 | 437.80 | 0.00 | 13 |
| Φ(line∗t)p(line∗t) | 811.33 | 446.14 | 0.00 | 17 |
| Φ(line)p(.) | 854.14 | 488.96 | 0.00 | 2 |

Only models within 4 AICc of the best approximating model were considered (italicized). Nomenclature for each model follows Table 2.
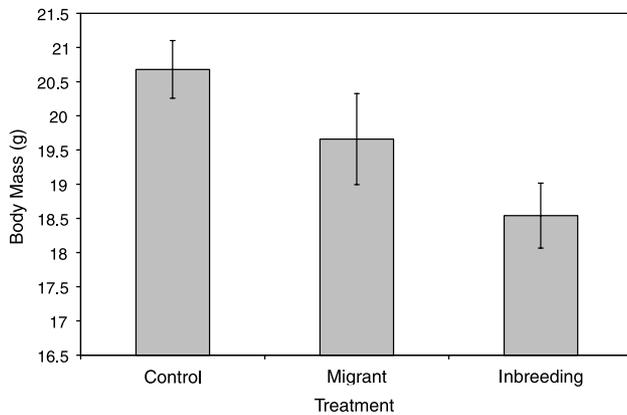
Fig. 3. Differences in mass between the three breeding treatments. Error bars are one standard error from the mean.

Mass varied across treatments (ANOVA $F_{2,89} = 3.03$, $p = 0.053$) with control animals being the largest, followed by animals in outcross treatments; inbred animals were smallest (Fig. 3). This leaves the possibility that maternal effects caused the observed pattern among treatments in body mass because the outcross treatment sometimes had inbred females, whereas other times the outcross was between the control female and the inbred male.

## 4. Discussion

We found that the introduction of an outcross individual (migrant) to an inbred line can have positive effects on survival in a free-living mammal. Thus, our study supports the theory and experiments with plants and captive animals showing that the effects of inbreeding can be mitigated by outcrossing, a result of critical conservation importance (Tallmon et al., 2004). Newman and Tallmon (2001) clearly show that the addition of one-migrant per generation increases fitness in the plant *Brassica campestris*, but they did not find increases in fitness as more migrants were added to the population. Others have recommended that an initial pulse of several migrants be introduced into a small, isolated population to abate inbreeding depression (Hedrick, 1995). There are many reasons why more than one migrant may be desired. Migrants may have reduced survival or breeding success compared to residents, or genes from the migrant may already be represented in the isolated population (i.e., the source of the migrants is genetically similar to the population where migrants are introduced). Vucetich and Waite (2000) model genetic data from 44 species and demonstrate that because of fluctuating population size, more than ten migrants per generation (sometimes >20) are likely necessary to prevent losses of genetic variation.

Unfortunately, if too many migrants are added to an isolated population, genetic "swamping" can occur, obliterating any uniqueness of once isolated populations, and potentially causing outbreeding depression (Templeton, 1986; Edmands, 1999, 2002). Although our experiment could not address the relative contribution and benefits of higher migration rates, we caution that the specific level of connectivity for any given species should be based on the genetic, ecological, and evolutionary history of the species (Mills and Allendorf, 1996).

Our inbreeding treatment also had higher survival than the control group (Fig. 2), although this was not as well supported using AICc criteria. This may suggest that limited amounts of purging, or the removal of deleterious genes through the inbreeding process, occurred. Few studies have convincingly demonstrated purging. Ballou (1997) compared fitness traits in ancestrally inbred offspring to those of recently inbred offspring for 25 species of captive mammals and found only one case of significant purging (although there was a trend towards neonatal survival being lower in offspring of recently inbred animals compared to ancestrally inbred animals for 15 of 17 species). Lacy and Ballou (1998) found mixed evidence for purging in different subspecies of *Peromyscus polionotus* in the laboratory. Similarly, Reed et al. (2003) found the purging of deleterious alleles and slower rate of extinction in *Drosophila melanogaster* populations bottlenecked for 60 generations. Overall, there is mixed evidence for purging existing in wild and captive populations; in a review of purging in plants, Byers and Waller (1999) found only 14 of 34 studies that demonstrate purging. Byers and Waller (1999) suggest that deliberate attempts to reduce the genetic load via inbreeding are liable to fail. It appears that, at best, inbreeding with the hopes of genetic purging can produce a small positive effect on fitness (e.g., Ballou, 1997), but more likely inbreeding will lead to negative fitness consequences (Hedrick, 1994). In our study 62.5% of the initial lines went extinct. Therefore, based on our results a population would most likely face extinction before purging improves its fitness via inbreeding. Similar extinction rates have been observed with *Drosophila* and house fly research (Reed and Bryant, 2001; Reed et al., 2003).

Lacy and Ballou (1998) suggest that the genetic load is not equally divided among founders; different founders have dissimilar genetic loads that affect various fitness measures. We had no support for survival models that varied by line, suggesting no detectable fitness differences between our three lines.

Our experiments demonstrated increased survival for offspring whose parents were outbred (compared to the control group and inbreeding treatment). However, it is important to consider how the consequences of gene flow would manifest in an inbred population, where

immigrants mate with just one or a few individuals per generation. In the short-run (or at least until equilibrium is reached), the effects of introducing a migrant into a small, isolated population each generation will not increase the overall fitness of the population. In the generation when an outcross individual is introduced to an isolated population, any increases in fitness (or fitness measure) are either due to an Allee effect or the superior performance of the introduced individual. The generation after the introduction of one outcross individual, the average inbreeding coefficient may decline, because the outcross individual breeds, producing offspring with a pedigree inbreeding coefficient of zero. However, all other pairs in the small population are still driving the inbreeding coefficient higher; that is, only one mating pair has the maximal possible level of outcrossing. Therefore, we expect the variance in inbreeding coefficients to increase. Only through the continual introduction of one-migrant per generation for many generations is the inbreeding coefficient reduced for the entire population.

Because of the large number of generations required to show the impacts of introducing one-migrant per generation, field studies are likely to be constrained to species which can temporarily be brought into the laboratory setting, have small effective population sizes, or short generation times. While we did not show changes in population growth rates, we showed that a migrant/outcross-individual mating with an inbred individual produces more fit offspring – the mechanism behind population level changes. It should be noted that we did not observe breeding in the pens after introduction, thus our inferences for population growth are limited. Likely, the mortality we observed was due to inability to adapt to the release situation, or the inability to cope with predation. Aerial predators were common in and around the pens with observations of great horned owls (*Bubo viginianus*), goshawks (*Accipiter gentiles*) and other raptors being frequent.

Overall, we recommend that the obvious first step for conservation efforts be on preserving large populations. If connectivity needs to be mitigated, we recommend at least one migrant per generation into isolated populations, although the life history traits of the species must be considered (Tallmon et al., 2004). Thus, we commend efforts like the Federal Grizzly bear Recovery Plan (US Fish and Wildlife Service, 1993) and the Black-footed ferret Recovery Plan (US Fish and Wildlife Service, 1988) that recommended migration of at least one migrant per generation into isolated populations. The next major questions are where should these migrants be taken from (i.e., how different should these donor populations be), and what are the negative effects of introducing too many migrants?

## References

Akaike, H., 1978. On the likelihood of a time series model. The Statistician 27, 217–235.

Akaike, H., 1979. A Bayesian extension of the minimum AIC procedure of autoregression model fitting. Biometrika 66, 237–242.

Allendorf, F.W., Phelps, R.S., 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences 38, 1507–1514.

Backus, V.L., Bryant, E.H., Hughes, C.R., Meffert, L.M., 1995. Effect of migration or inbreeding followed by selection on low founder-number populations: implications for captive breeding programs. Conservation Biology 9, 1216–1224.

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 genetic load. Nature 352, 522–524.

Bryant, E.H., Backus, V.L., Clark, M.E., Reed, D.H., 1999. Experimental tests of captive breeding for endangered species. Conservation Biology 13, 1487–1496.

Burnham, K.P., Anderson, D.R., 2001. Kullback-Leibler information as a basis for strong inference in ecological studies. Wildlife Research 28, 111–119.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multiple-Model Inference: a Practical Information-Theoretic Approach. Springer-Verlag, New York.

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.

Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44, 887–888.

Citta, J., 1999. How to Change the Growth of Wildlife Populations: Applications and Experimental Tests of Demographic Sensitivity Analysis. MS Thesis, University of Montana, Missoula.

Crow, J.F., Kimura, M., 1970. An Introduction to Population Genetics Theory. Burgess, Minneapolis, Minnesota.

Douglass, R.J., Van Horn, R., Coffin, R., Zanto, S.N., 1996. Hanta virus in Montana deer mouse populations: preliminary results. Journal of Wildlife Diseases 32, 527–530.

Douglass, R.J., Wilson, T., Semmens, W.J., Zanto, S.N., Bond, C.W., Van Horn, R.C., Mills, J.N., 2001. Longitudinal studies of Sin Nombre virus in deer mouse-dominated ecosystems of Montana. American Journal of Tropical Medicine and Hygiene 65, 33–41.

Edmands, S., 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53, 1757–1768.

Edmands, S., 2002. Does parental divergence predict reproductive compatibility?. Trends in Ecology and Evolution 17, 520–527.

Harvich, C.M., Tsai, C., 1995. Model selection for extended quasi-likelihood models in small samples. Biometrics 51, 1077–1084.

Hedrick, P.W., 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. Heredity 73, 363–372.

Hedrick, P.W., 1995. Gene flow and genetic restoration: the Florida panther as a case study. Conservation Biology 9, 996–1007.

Jimenez, J.A., Hughes, K.A., Alaks, G., Graham, L., Lacy, R.C., 1994. An experimental study of inbreeding depression in a natural habitat. Science 266, 271–273.

Keller, L.F., 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52, 240–250.

King, J.A., 1968. Biology of *Peromyscus* (Rodentia). The American Society of Mammalogists, Stillwater, Oklahoma.

Lacy, R.C., 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1, 143–158.

Lacy, R.C., Alaks, G., Walsh, A., 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. Evolution 50, 2187–2200.

Lacy, R.C., Ballou, J.D., 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. Genome 41, 900–909.

Lebreton, J.-D., Burnham, K.P., Clobert, J., Anderson, D.R., 1992. Modeling survival and testing biological hypotheses using marked animals: case studies and recent advances. Ecological Monographs 62, 67–118.

Madsen, T., Shine, R., Olsson, M., Wittzell, H., 1999. Restoration of an inbred adder population. Nature 402, 34.

Metzgar, L.H., 1979. Dispersion patterns in a *Peromyscus* population. Journal of Mammology 60, 129–145.

Millar, J.S., Willie, F.B., Iverson, S.L., 1979. Breeding by *Peromyscus* in seasonal environments. Canadian Journal of Zoology 57, 719–727.

Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10, 1509–1518.

Mills, L.S., Smouse, P.E., 1994. Demographic consequences of inbreeding in remnant populations. American Naturalist 144, 412–431.

Newman, D., Tallmon, D.A., 2001. Experimental evidence for beneficial fitness effects of gene flow in recently isolated populations. Conservation Biology 15, 1054–1063.

Newman, D., Pilson, D., 1997. Increased probability of extinction due to decreased genetic effective population size, experimental populations of *Clarkia pulchella*. Evolution 51, 354–362.

Pollock, K., Nichols, J.D., Brownie, C., Hines, J.E., 1990. Statistical inference for capture–recapture experiments. Wildlife Monographs 107, 1–97.

Prince, K.L., Glenn, T.C., Dewey, M.J., 2002. Cross-species amplification among peromyscines of new microsatellite DNA loci from the oldfield mouse (*Peromyscus polionotus subgriseus*). Molecuar Ecology Notes 2, 133–136.

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., Frankham, R., 2002. Inbreeding and extinction: the effect of environmental stress and lineage. Conservation Genetics 3, 301–307.

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.

Ralls, K., Ballou, J., 1983. Extinctions: lessons from zoos. In: Schonewald-Cox, C.M., Chambers, S.M., MacBryde, B., Thomas, L. (Eds.), Genetics and Conservation: a Reference for Managing Wild Animals and Plant Populations. Benjamin/Cummings, Menlo Park California, pp. 164–184.

Ralls, K., Ballou, J.D., Templeton, A., 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2, 185–193.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., Hanski, I., 1998. Inbreeding and extinction in a butterfly meta-population. Nature 392, 491–494.

Saccheri, I.J., Nichols, R.A., Brakefield, P.M., 2001. Effects of bottlenecks on quantitative genetic variation in the butterfly *Bicyclus anynana*. Genetical Research 77, 167–181.

Sauer, J.R., Slade, N.A., 1985. Mass-based demography of a hispid cotton rat (*Sigmodon hispidus*) population. Journal of Mammalogy 66, 316–328.

Sauer, J.R., Slade, N.A., 1987. Uinta ground squirrel demography: is body mass a better categorical value than age?. Ecology 68, 642–650.

Seber, G.A.F., 1982. The Estimation of Animal Abundance and Related Parameters, second ed. Macmillan, New York.

Soulé, M., Mills, L.S., 1998. No need to isolate genetics. Science 282, 1658–1659.

Spielman, D., Frankham, R., 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. Zoo Biology 11, 343–351.

Tallmon, D.T., Luikart, G., Waples, R.S, 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19, 489–496.

Teferi, T., Millar, J.S., 1993. Early maturation by northern *Peromyscus maniculatus*. Canadian Journal of Zoology 71, 1743–1747.

Templeton, A.R., 1986. Coadaptation and outbreeding depression. In: Soule, M.E. (Ed.), Conservation Biology: the Science of Scarcity and Diversity. Sinauer Associates, Sunderland, Massachusetts, pp. 105–116.

US Fish and Wildlife Service, 1988. Black-footed Ferret Recovery Plan. In: Forrest, S.C., Biggins, D.E. (Eds.), US Fish and Wildlife Service, Denver, Colorado.

US Fish and Wildlife Service, 1993. Grizzly Bear Recovery Plan. US Fish and Wildlife Service, Missoula, Montana.

Vilà, C., Sundqvist, A.K., Flagstad, O., Seddon, J., Bjornerfeldt, S., Kojola, I., Casulli, A., Sand, H., Wabakken, P., Ellegren, H., 2002. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proceedings of the Royal Society of London Series B 270, 91–97.

Vucetich, J.A., Waite, T.A., 2000. Is one migrant per generation sufficient for the genetic management of fluctuating populations?. Animal Conservation 3, 261–266.

Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L., Paige, K.N., 1998. Tracking the long-term decline and recovery of an isolated population. Science 282, 1695–1698.

White, G.C., Burnham, K.P., 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46, 120–138 (Supplement).

Wright, S., 1922. Coefficients of inbreeding and relationship. American Naturalist 56, 31–38.

Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–259.