

Genetic rescue guidelines with examples from Mexican wolves and Florida panthers

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Abstract In populations or species with low fitness (high genetic load), a new management strategy called genetic rescue has been advocated to help avoid extinction. In this strategy, unrelated individuals from another population are introduced into the population with low fitness in an effort to reduce genetic load. Here we present ten guidelines that can be used to evaluate when genetic rescue is a good management option, the appropriate procedures for genetic rescue planning and management, and the potential negative genetic consequences of genetic rescue. These guidelines are then used to evaluate the genetic rescue aspects of the recovery programs for the Mexican wolf and the Florida panther.

Keywords Florida panthers · Gene flow · Genetic load · Genetic restoration · Inbreeding depression · Mexican wolves

Introduction

Although positive ecological factors are essential for the persistence of endangered species, genetic factors may also be significant in a number of situations. In general, genetic factors important in conservation can be divided into those with immediate effects, such as inbreeding depression, and

those with longer term effects, such as genetic load and loss of genetic variation for future adaptation. Perhaps the most significant early contribution of genetics to conservation was the recognition of the importance of inbreeding depression (for reviews, see Hedrick and Kalinowski 2000; Keller and Waller 2002). Inbreeding depression is an effect on fitness thought to be due to an increase in homozygosity of detrimental alleles from inbreeding (Charlesworth and Charlesworth 1999).

Several management approaches are generally accepted and commonly used in conservation genetics to manage these genetic impacts. First, in captive or controlled populations, inbreeding can be avoided as much as possible in order to minimize inbreeding depression (Hedrick and Kalinowski 2000). In captive pedigreed populations, avoiding inbreeding generally is managed by minimizing mean kinship between parents (Ballou and Lacy 1995). Second, in both captive and wild populations that can be managed, the effective population can be maximized to retain genetic variation (this strategy also minimizes fixation of detrimental variation). For example, the supplementation program in the endangered winter run Chinook salmon uses a breeding protocol that attempts to maximize the effective population size of released smolts (Hedrick et al. 1995, 2000). Third, the population can be divided into groups with limited gene flow between them; this both provides genetic connections among the subpopulations and can result in greater overall retention of variation than in an undivided population of the same total size (Fernández et al. 2008). Other management approaches that have been recommended in conservation genetics are more controversial, such as purging inbreeding depression (Boakes et al. 2007) or supplementation of natural populations with captive-raised individuals (Ryman and Laikre 1991). Here we will discuss genetic rescue, a relatively

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new management option that seeks to increase fitness in a population by introducing unrelated individuals.

Inbreeding depression, genetic load, and genetic rescue

Let us begin this discussion by differentiating between inbreeding depression and genetic load. Inbreeding depression can be defined as the reduction in fitness (or fitness-related traits) for progeny from matings between relatives, as compared to those from matings between unrelated individuals. This is in contrast to the lowered mean population fitness, called genetic load, that can occur over time when detrimental mutations with a small selective disadvantage in a small population become fixed or of high frequency by genetic drift much as if they were neutral (Wang et al. 1999; Kirkpatrick and Jarne 2000). More specifically, genetic load can be defined as the reduction in mean fitness in a population due to high frequencies or fixation of detrimental variants, as compared to a population without lowered fitness from detrimental variation.

In a large population at equilibrium, substantial standing detrimental genetic variation is expected and, consequently, a large reduction of fitness is expected if inbreeding occurs. However, there may be little genetic load, because, due to the efficacy of selection in large populations and the fact that most detrimental variants are recessive, detrimental variants are in low frequency. If the population declines in number, purging of detrimental variation should take place, especially for alleles of large detrimental effect, thereby reducing inbreeding depression. However, some detrimental variants might become fixed, particularly those of smaller effect, causing an increase in genetic load (Hedrick 1994; Wang et al. 1999). If the population remains small for an extended period, more detrimental variation could be purged, further reducing inbreeding depression, but more detrimental variants could be fixed, causing higher genetic load. Such a population might show no lowered fitness upon further inbreeding, that is, no inbreeding depression, but owing to fixation of detrimental variation, all individuals in the population might have a low fitness and the population might have a high genetic load.

Several caveats should be mentioned. First, if the population declines in numbers, some populations (or even species) might become extinct and the ones going extinct could be the ones with higher genetic load or lower population fitness. As a result, the remaining populations might not have as high a genetic load as would be expected from the initial amount of standing detrimental genetic variation before the population decline. Second, genetic load might be documented as a low estimate of fitness compared with

other populations, or by crossing with individuals from another population and observing an increase in the fitness of their progeny compared with progeny of within-population crosses. However, making such crosses experimentally might not be possible, or the groups might differ in other characteristics as well (Wang 2000).

In the fruit fly, *Drosophila melanogaster*, approximately half the effect of inbreeding depression is thought to be from nearly recessive lethals and half from detrimental variants of small effect but with higher dominance (Wang et al. 1999; Lynch et al. 1999). However, *D. melanogaster* generally has a very large effective population size ($>10^6$) and the genetic architecture of the detrimental genetic variation in this species probably reflects that of a large population near equilibrium. Alternatively, for many endangered species, genetic drift has been important, either because of a current small population size or a history of bottlenecks. As a result, endangered species might have a very different genetic architecture with fewer segregating variants of large detrimental effect (Hedrick 2002), lower inbreeding depression, and perhaps higher genetic load, than do species with histories of larger population size. With advances in identifying genes negatively influencing fitness and measuring their effect in other organisms, the genetic architecture of detrimental variation in endangered species should become known in the future (Charlesworth and Willis 2009).

In some populations or species with low fitness (high genetic load), as indicated by low numbers caused by low reproduction and/or high mortality, a new management strategy called genetic rescue has been advocated recently to help avoid extinction. In this strategy, unrelated individuals from another population are introduced into the population with low fitness in an effort to reduce genetic load caused by high frequency detrimental variants that have accumulated in the population. Note that genetic rescue is intended to reduce the amount of genetic load, not the amount of inbreeding depression. Two useful general reviews of the introduction of genetic variation to promote genetic rescue are by Tallmon et al. (2004) and Edmands (2007). More specifically, genetic rescue has been examined theoretically (Wang et al. 1999; Whitlock et al. 2000) and experimentally (Newman and Tallmon 2001; Saccheri and Brakefield 2002; Swindell and Bouzat 2005, 2006), has occurred naturally in a few situations (Vilà et al. 2003), and has been the result of management actions in several others (Madsen et al. 1999, 2004; Westemeier et al. 1998; Bouzat et al. 2009; Land and Lacy 2000). In addition, genetic rescue has seen widespread application in plant species, see Tallmon et al. (2004), Bossuyt (2007), and Willi et al. (2007).

Here we will focus on planning and carrying out genetic rescue as a management action and provide an ideal set of ten guidelines for implementation of genetic rescue. Then

we will examine these guidelines in two case studies, Mexican wolves and Florida panthers, in which management for genetic rescue (or genetic restoration, see below) has been carried out.

Guidelines for genetic rescue

(a) When genetic rescue may be a good management option?

Before genetic rescue as a management strategy is begun, the overall situation and other management, including doing nothing, should be carefully evaluated. In other words, the potential benefits of genetic rescue to help avoid extinction should be documented to be greater than the risks from translocation of individuals into the endangered population. In reality, some aspects of these suggested guidelines may not be possible to carry out before the action is begun but they should be part of the context for examining genetic rescue management. The following guidelines present three conditions under which genetic rescue may be a recommended option.

(1) There should be *evidence of low fitness* in the endangered population (or high inbreeding from either pedigree or molecular data) so that making an introduction is likely to increase fitness, that is, reduce genetic load in the endangered population. It may be possible to compare fitness parameters to either historical data from the population or species or to compare fitness measures to populations of a closely related organism. Low numbers in the population for a long time may potentially be of ecological causation but if the possibility of extinction appears to be high, then experimental examination of fitness in crosses may be informative as to whether the low fitness has a genetic basis.

(2) For successful genetic rescue, a *closely related donor population* should be available so that likelihood of outbreeding depression (low fitness in offspring, or future generations, from crosses) is low (Tallmon et al. 2004; Edmands 2007). In general, the donor population should be from the same species as the endangered population and either from a nearby area and/or from a similar habitat.

(3) Before the actual genetic rescue is attempted in the wild, endangered population, there should be *experimental data* from a captive population to support validity of genetic rescue. In other words, there should be experimental demonstration of successful mating, good reproduction, and good survival in crossed progeny and no evidence of outbreeding depression between these populations. This is particularly important if molecular data suggest the donor and endangered populations are not closely related. However, in species with a longer generation time or ones that are difficult to breed in captivity, it

may not be possible to carry out a complete experimental evaluation in captivity before initiation of genetic rescue management. Given evidence of low fitness and the presence of a closely related donor population, managers could then consider the tradeoff between the time, money, and effort needed for this evaluation and the risk of extinction and argue for immediate genetic rescue management.

(b) Genetic rescue planning and management

Once a decision is made to carry out genetic rescue, then the basic protocol for introduction and procedures in the subsequent generations should be established. These procedures should be as well thought out as possible but they also need to have some flexibility if there are unpredicted outcomes. The following represent three recommended conditions for genetic rescue and management.

(4) There should be an established and cautionary *translocation protocol* so that the introduction of outside individuals makes negative non-genetic effects unlikely. For example, the risk of the introduction of disease from the donor population or detrimental changes in the behavior of the endangered population should be minimal. Further, detrimental behavioral effects might be minimized by only introducing females, introducing groups of animals, or only introducing animals to unoccupied territories or locations.

(5) There should be a detailed *monitoring plan* for the introduction and its consequences in succeeding generations. This would entail identification of individuals by some established means and monitoring of their movement, mating behavior, survival, and reproduction as thoroughly as possible. In addition, the relationships between individuals, such as maternity, paternity, and other close genetic relationships, should be determined and verified by genetic means in a timely manner so that an accurate and complete pedigree of the rescued population can be constructed and potentially used in management.

For some populations or types of organisms, however, construction of complete pedigrees may not be possible. In these cases, the change in genetic composition of the population over time could be estimated based on classes of admixed (e.g., F₁ and backcross individuals) and non-admixed individuals (pure individuals from the donor and endangered populations). Ideally measures of individual fitness indexing demographic rates having the greatest effects on population growth would be quantified for classes of admixed and non-admixed individuals. However, if this is not possible, estimates of the population growth rate over time combined with information on the change in proportion of admixed and non-admixed classes could be used to gauge success of management efforts.

(6) There should be the potential for *management continuation* over time, even several generations, and the

possibility to modify management when necessary. For example, if the initial success is less than expected, then further management, including further introductions in future generations should be possible. Or if the initial amount of ancestry from the donor population is more than expected or wanted, then further introductions in future generations should be restricted. In other words, there should be flexibility if there are unpredicted outcomes but changes should be implemented only when they are based on scientifically credible information.

(c) Potential negative genetic consequences

Although genetic rescue should have positive genetic effects by increasing the fitness and population persistence of the rescued endangered population, it may have genetic effects that could be detrimental. Some of these potentially negative effects may be difficult to document initially and may be of less significance than the positive impact of genetic rescue. The following are four negative genetic consequences that may be potentially associated with genetic rescue.

(7) If the introduction from the donor population results in a very high donor population ancestry, then there may be *swamping of local genetic variation* and traits (some of which may be adaptive). To counteract this effect, the level of introduction should be kept at a level low enough so that the genetic rescue effect will be successful (elimination of detrimental variation at high frequency) but not too much to completely change allele frequencies and eliminate locally adaptive variation (Hedrick 1995). Further, if the level of the introduction is too large or the introduced animals are very reproductively successful, then the ancestry of the endangered population may actually be replaced by ancestry from the donor population. Obviously, this would result in the genetic replacement of the population (maybe an appropriate outcome in some cases), not in genetic rescue, and all local variation may be eliminated (e.g., Hogg et al. 2006).

(8) If a high proportion of ancestry after genetic rescue is from a few individuals from the donor population (and/or from a few individuals from the endangered population), then in the next few generations there could again be a *small effective population size* (N_e). This effect could be minimized if more migrants from the donor population are included and they contribute fairly equally to the genetically rescued population. Also inclusion of as much of the endangered population as possible in the initial crosses from the donor population should reduce this effect. If the initial F_1 and backcross individuals have a high fitness, it may be difficult to keep the effective population size high because a large part of the population may be descended from these few individuals.

(9) Bringing in individuals from the donor population may result in a relatively *short-lived fitness increase*. For example, the first generation may have a higher fitness because recessive, detrimental variants will be covered up. However, in second generation and succeeding generation crosses, there may be segregation that again results in recessive homozygotes and lowered fitness (e.g., Liberg et al. 2005). In fact, inbreeding depression may be increased in the rescued population (Wang et al. 1999) relative to that of the endangered population before rescue. If the endangered population is considered unique (e.g., the last remaining population of a subspecies), following initial genetic rescue there may be a desire to increase the ancestry of the endangered subspecies in the rescued population by creating backcrosses with individuals that have ancestry only from endangered population. This however runs the risk of reconstituting the genetic load that contributed to the endangerment of the subspecies and may lead to failure of the rescue program.

(10) The possibility of genetic rescue in a given endangered population from a given donor population may be a one-time option and/or have the *most effect in the first attempt*. If genetic rescue is attempted more than once using the same pair of endangered and donor populations, then the impact could be substantially less in successive attempts because detrimental variants at particular loci that caused the genetic load would have been reduced in frequency in the endangered population by the introduction of non-detrimental variants from the donor population. For the detrimental variants remaining in the endangered population, there may not be non-detrimental variants in the donor population or the donor population itself may have some detrimental variants that could reduce the fitness of the endangered population.

Genetic rescue in Mexican wolves and Florida panthers

Mexican wolves

The Mexican wolf (*Canis lupus baileyi*), an endangered subspecies of the gray wolf, is the most genetically distinct wolf subspecies in North America (Leonard et al. 2005). Human activities and killing throughout its range reduced and isolated Mexican wolf populations so that by 1925 they were rare in the United States (and probably extinct by the 1970s) and by the 1950s, their range and numbers in Mexico were greatly reduced. As a result, the Mexican wolf subspecies was listed as endangered in 1976. Only a few Mexican wolves remained in isolated groups in Mexico by 1980 and surveys since then have not detected any wild Mexican wolves in Mexico.

All Mexican wolves alive today descend from three captive lineages founded between 1960 and 1980 from a total of seven wolves (Hedrick et al. 1997). In 1995, after genetic evaluation of the existing captive Mexican wolves, the Aragón and Ghost Ranch lineages were merged into the larger captive McBride lineage to increase founder number, heterozygosity, and fitness, and to reduce inbreeding. (The McBride lineage was originally named the certified lineage but was renamed after Roy McBride, who caught the founders of this lineage and whose work on Florida panthers is discussed below.) The McBride, Ghost Ranch, and Aragón lineages were founded by 3, 2, and 2 different, unrelated individuals, respectively. The estimated average inbreeding coefficients within the McBride, Ghost Ranch, and Aragón lineages were 0.18, 0.61, and 0.26, respectively, before they were merged. The initial goal, based on a number of factors, was for the merged population to have 10% of its ancestry from each of Aragón and Ghost Ranch lineages and 80% from the McBride lineage, and upon review of the fitness effects of the merger, ancestry from the Ghost Ranch and Aragón lineages could be increased to a maximum of 25% each with McBride ancestry reduced to 50% (Hedrick et al. 1997).

The captive population has been examined for the effects of inbreeding level and crosses between lineages for several fitness-related traits (Fredrickson et al. 2007). For example, Fig. 1 gives the mean number of pups for wolves in the McBride lineage with low and high levels of inbreeding and cross-lineage wolves. There is a decline in the number of pups in the McBride lineage between those with low and high inbreeding levels but this effect on inbreeding depression was not statistically significant. However, the cross-lineage wolves had a significantly higher number of pups than the contemporaneous high inbreeding group (similar results were found for survival). This recovery in fitness in crosses between lineages indicates genetic rescue of the captive Mexican wolf population (for a similar finding for sperm quality, see Asa et al. 2007).

In 1998, a population of Mexican wolves was reintroduced into eastern Arizona and western New Mexico. Initially these wolves had only ancestry from the McBride lineage, but starting in 2000 wolves with ancestry from more than one lineage have been released (Hedrick and Fredrickson 2008). As of January, 2009, there were 52 wolves in this reintroduced population, a number that had not changed significantly for several years. It is more difficult to obtain data on reproduction in wild wolves than in captive wolves because the first measurement of reproduction is usually some time after the pups emerge from the den at around 10 weeks of age. However, Fig. 2 gives the data for reproduction in the reintroduced population as a function of the inbreeding coefficient of the progeny. The

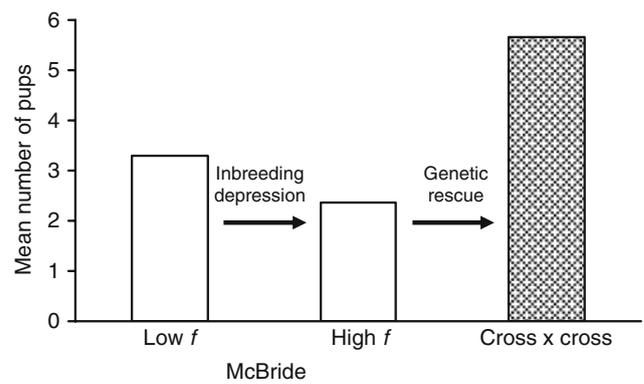


Fig. 1 The mean number of Mexican wolf pups in litters from the McBride lineage with either a low or high inbreeding coefficient and in litters from cross-lineage by cross-lineage wolves (Hedrick and Fredrickson 2008)

cross-lineage wolves (indicated by closed circles) have a lower inbreeding coefficient and higher number of pups than the highly inbred McBride wolves. In other words, as in the captive population, there appears to be genetic rescue for reproduction in the reintroduced population.

Florida panthers

Pumas (*Puma concolor*) were aggressively hunted by Europeans in North America and bounties were offered for hides. In the eastern United States by the late 1920s, pumas were present only in central and south Florida and possibly along some river drainages in Louisiana (Young and Goldman 1946). The numbers in Florida continued to decline because of continued persecution (Tinsley 1970) and in 1967, the puma subspecies called the Florida panther (*P. concolor coryi*) was federally listed as endangered.

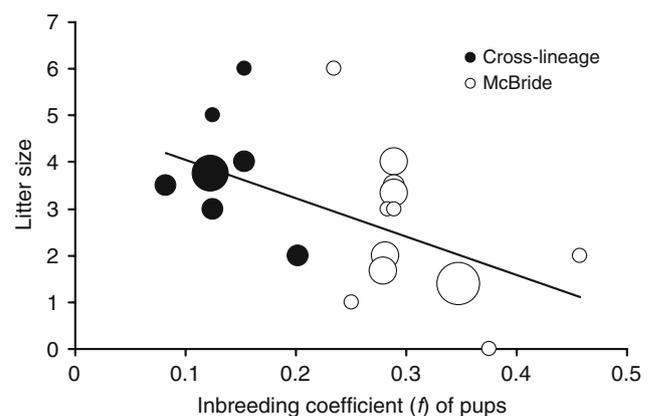


Fig. 2 The maximum number of progeny observed in litters in the reintroduced Mexican wolf population as a function of the inbreeding coefficient of the progeny. Open and closed circles indicate McBride and cross-lineage wolves, circle size indicates the number of litters with a given number of progeny, and the line indicates the linear regression between these points (Fredrickson et al. 2007)

In the early 1970s, the Florida panther was believed extinct and no breeding population was known (Nowak 1993). However, several animals were treed by dogs in 1973 and 1974 in southern Florida (Nowak 1993). The numbers found over the next few years were small but for the 1980s and early 1990s, the general claim was that the census population number was between 30 and 50 (Seal 1994; Maehr et al. 2002) although detailed monitoring suggests that the numbers were lower (McBride et al. 2008). In addition to this low population size, a suite of previously rare and potentially deleterious traits were observed in the population that suggested that genetic drift had nearly fixed detrimental variants. These traits, which are found in high frequency only in the Florida panther and are unusual in other puma subspecies, include high frequencies of cryptorchidism (unilateral or bilateral undescended testicles), kinked tail for the last five vertebra, cowlick on the back (this is not assumed to be deleterious), atrial septal defects, and the poorest semen quality recorded in any felid (Roelke et al. 1993; Table 1). In addition, a large survey of microsatellite loci has shown that Florida panthers have much lower molecular variation than other North American populations of pumas (Culver et al. 2000; see also Driscoll et al. 2002).

A program to release females from the closest natural population from Texas was initiated in 1995 to genetically restore fitness in the Florida panther with an initial goal of 20% Texas ancestry (Seal 1994; Hedrick 1995). Five of the eight introduced Texas females produced offspring with resident Florida panther males and subsequently F_2 and backcross offspring were produced (Land and Lacy 2000). Although little has been published about Florida panther genetics in recent years, even a decade ago Land and Lacy (2000) estimated that approximately 20% of the overall ancestry was from the introduced Texas cougars.

One of the initial findings was that the frequency of the rare and potentially deleterious traits dramatically declined in animals with Texas ancestry (Mansfield and Land 2002; Land et al. 2002; Table 1). For example, of the animals with Texas ancestry, only 7% had a kinked tail (compared to 77% without Texas ancestry) and the animals with

kinked tail were all progeny from backcrosses to Florida cats. Similarly, but not as dramatic, only 24% of the animals with Texas ancestry had a cowlick (compared to 80% without Texas ancestry). Only seven males with Texas ancestry had been evaluated for cryptorchidism and all had two descended testicles, in other words, a reduction from 49 to 0% cryptorchidism because of Texas ancestry. In other words, the introduction of Texas cougars initially resulted in a substantial reduction of the frequency of the rare and/or detrimental traits that had accumulated in the Florida panther but a current analysis remains to be published.

Recently, McBride et al. (2008) published the number of Florida panthers documented by physical evidence from 1985 to 2007 (Fig. 3; 2008 number, R. McBride, pers. comm.). Notice that the numbers from 1985 to 1995 fluctuated between 19 and 30 animals before mortalities (15–27 after mortalities were removed) and did not increase. However, after the introduction of the Texas pumas in 1995, the numbers grew very quickly to 104 in 2008 (84 after mortalities were removed). Using a simple logistic model, it is estimated that the population grew at a rate of approximately 13% per year over this period (12% per year using the after mortality data). In other words, it is compelling that the substantial increase in numbers after the introduction of the Texas pumas demonstrates genetic rescue. In addition, McBride et al. (2008) documented an increase in the number of dispersing individuals and road kills over this period, both indirect indicators of an increase in population density. Pimm et al. (2006) also observed an increase in survival after the introduction of the Texas animals although a study of survival based on genetically determined ancestry remains to be published.

Using the guidelines to evaluate genetic rescue in Mexican wolves and Florida panthers

Now let us evaluate both the Mexican wolf and Florida panther genetic rescue actions in light of the guidelines given above (Table 2 gives a summary). Although both

Table 1 The frequencies of three traits found in high frequency in Florida panthers with no Texas puma ancestry and data from F_1 , F_2 , and backcrosses to Texas and Florida with Texas ancestry (Mansfield and Land 2002; Land et al. 2002; sample size in parentheses)

	No Texas ancestry	Texas ancestry				Mean
		F_1	F_2	BC-TX	BC-FL	
Cryptorchidism	0.49 (49)	0.00 (2)	0.00 (2)	–	0.00 (3)	0.00 (7)
Kinked tail	0.77 (176)	0.00 (17)	0.00 (7)	0.00 (3)	0.20 (15)	0.07 (42)
Cowlick	0.80 (115)	0.20 (10)	0.00 (5)	0.00 (1)	0.60 (5)	0.24 (21)

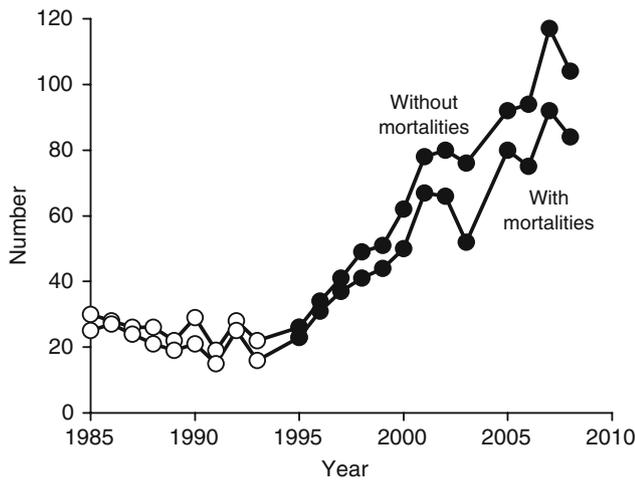


Fig. 3 The number of Florida panthers documented by physical evidence annually from 1985 to 2008, both without including mortalities and after including all known mortalities for the year (McBride et al. 2008; R. McBride pers. comm.; D. Land pers. comm.). Data for the years before the translocation are indicated by open circles and data for those after the translocation by closed circles

actions were started only 15 years ago, or around 4–5 generations in both species, there is enough information to give general evaluations.

Mexican wolves

(a) *Was it a good option to plan genetic rescue for the Mexican wolf?* First, in the mid-1990s, the inbreeding coefficient of the McBride captive lineage was around 0.18

Table 2 An evaluation of the genetic rescue programs for the Mexican wolf and Florida panther and whether they met the guidelines discussed

	Mexican wolf	Florida panther
(a) Good option?		
(1) Low fitness	Yes	Yes
(2) Donor population	Yes	Yes
(3) Experimental data	Yes	No
(b) Management		
(4) Introduction protocol	Yes	Yes
(5) Monitoring plan	Yes (genetic?)	Yes (genetic?)
(6) Continued management	No	No
(c) Negative genetic effect		
(7) Swamping local	No	No
(8) Small N_e	Yes	Maybe
(9) Short-lived fitness increase	Yes?	Not known
(10) One time or most effect in first attempt	Yes?	No

Evaluations in *boldface* did not meet the guidelines or are questionable

and was predicted to increase quickly, even though there was good captive management to keep the inbreeding level as low as possible. Although the data were published after the crossing of the lines were initiated, there did not appear to be inbreeding depression for viability or fecundity (Kalinowski et al. 1999) but there did appear to be inbreeding depression for body size (Fredrickson and Hedrick 2002). In particular, the body size of captive animals appeared to be smaller than that of wild animals caught before the captive program was initiated, suggesting genetic load.

Second, there were only two other known captive lineages, Ghost Ranch and Aragón, of putative Mexican wolves (no wild animals were known), making these the only donor populations of the same subspecies. These two lineages were evaluated using genetic markers, specifically mtDNA and microsatellite loci (García-Moreno et al. 1996; Hedrick et al. 1997). Both lineages appeared to be of Mexican wolf ancestry (and without dog or coyote ancestry) and to have been founded from animals unrelated to the founders of the McBride lineage. Finally, crosses between the McBride and Ghost Ranch lineages, and McBride and Aragón lineages were made in captivity to evaluate their potential. Both types of crosses were successful and the progeny survived, grew, and reproduced well.

(b) *Was the management adequate and appropriate?* First, because the initial crosses were made in captivity, progeny with ancestry from more than one lineage were available to release into the reintroduced population. In other words, a separate protocol for introduction was not necessary beyond the protocol already in place for releasing animals from captivity. Second, there was already a program in place to physically monitor animals released into the reintroduced population and this program was used to monitor reproduction and survival of animals of cross-lineage ancestry as well as pure McBride ancestry. In addition, the program includes annual counts that can be used as an index of population size. Although genetic analysis of paternity and relatedness has been implemented for monitoring in the reintroduced Mexican wolf program, genetic analysis has not been done in a timely manner by the National Forensics Laboratory. Finally, management to continue the genetic rescue program after the initial releases was in place and the ancestry from the Ghost Ranch lineage averaged around 13% in alpha wolves from 2006 to early 2009 but the ancestry from the Aragon lineage averaged only 6% over the same period—below the suggested minimal level of 10%. However, mainly because of non-scientific considerations, further releases were greatly reduced in the 4-year period 2005–2008 to only a total of five wolves. At this point, both the reintroduced population and the program of genetic rescue are presently

at great risk because of the low growth rate of the wild population (see “Discussion”).

(c) *Have there been negative genetic effects of the management?* First, since the level of ancestry of Ghost Ranch and Aragón lineages are still very low, any particular alleles in the McBride lineage could not have been greatly influenced, and certainly not swamped. Further, from both genetic and morphological evaluation, there do not appear to be characteristics only found in the McBride lineage. Second, because of the continued low numbers of packs and breeding individuals in recent years, it appears that there is a low effective population size and a very high ancestry of the inbred McBride lineage. Although there has been no formal estimation of the effective size of the introduced population, it appears to be at most 10 but may be less because the average number of pairs with at least one pup surviving to 8 months of age for the period 2006–2008 was only 5.3 and over the last two breeding seasons at least half of the packs had an alpha wolf descended from a single highly successful pack. Already in backcrosses the inbreeding coefficient is around 0.13 (see Fig. 2) and the average expected inbreeding coefficient of progeny from known pairs is 0.2. Nearly all of this inbreeding is because of identity by descent from the McBride lineage but also somewhat from the Ghost Ranch or Aragón lineages when, for example, both parents shared ancestry from either of these lineages.

Finally, there may not be a second chance for genetic rescue here because none of lineages are still maintained separately in captivity. Although semen was collected from one Aragón and six Ghost Ranch wolves from 1996 to 2000, the efficacy of artificial insemination using frozen semen from strongly inbred wolves is uncertain. It was assumed that the increase in fitness from lineage crossing would be used expeditiously to enhance the numbers of wild wolves and that a second round of crosses would not be necessary. However, mainly because of non-scientific reasons, the cross-lineage wolves were not incorporated into the reintroduced population in a timely manner and this opportunity may have been lost. If the reintroduced population does not increase soon, it may be necessary to consider extraordinary measures, such as introducing northern gray wolves, a closely related subspecies (Leonard et al. 2005), into the reintroduced Mexican wolf population.

Florida panther

(a) *Was it a good option to plan genetic rescue for the Florida panther?* First, as we discussed above there was a suite of traits, including several that appear to have strong negative effects on fitness, that suggested that Florida panthers had experienced genetic drift and had been

isolated from other puma populations. In addition, the very low genetic variation, compared to other puma populations, suggested that the Florida panther population had been through an extreme bottleneck (Culver et al. 2008).

Second, although the nearest donor population in the United States was relatively far away in west Texas, as recently as the late nineteenth century there probably was genetic exchange between these populations through intermediate populations (Seal 1994). In addition, Culver et al. (2000) found that although the Florida panther (*P. concolor coryi*) and the Texas puma (*P. concolor stanleyana*) are classified as different subspecies, it appears that the genetic variation found in the Florida panther is a subset of that found in other North American pumas (they also suggested that all North American pumas be subsumed into one subspecies). Although the arid, west Texas desert habitat is quite different from the humid, tropical habitat of south Florida, there is no United States puma population presently living in a tropical habitat. Finally, it was recommended that experimental crosses between Texas cougars and Florida panthers be carried out in captivity before the translocation of Texas animals into the wild population (Seal 1994) to check for the potential success of genetic rescue and the lack of outbreeding depression. However, for non-scientific reasons, this was never done.

(b) *Was the management adequate and appropriate?* First, the protocol for translocation to prevent introduction of disease was thorough and effective and no known diseases were introduced into Florida. Further, all eight animals (all females) were successfully transferred although three died before they could reproduce. Second, the monitoring of the introduced animals was carried out as for the existing wild Florida panther population (McBride et al. 2008). Samples for genetic analysis have been taken for all the animals that have been captured and sent to Steve O’Brien and his colleagues at the National Cancer Institute. Although it appears that these samples have been analyzed, and paternity and relatedness will be assessed (D. Onorato, pers. comm.), these data have not been published and are not available.

Finally, the recommendation by Seal (1994) was to introduce eight animals in the first generation so that gene flow would be about 20%, and then introduce a single new animal each generation thereafter. The first generation release was generally successful (however, only 5 of the 8 animals produced two or more offspring, rather than the 8 recommended by Seal 1994) but there have been no further introductions and there are no plans that have been made public for further introductions.

(c) *Have there been negative genetic effects of the management?* First, were some adaptive traits lost by swamping the ancestry (for this concern, see Maehr and Caddick 1995)? No such adaptive genetic traits have ever

been identified, either before or after the translocation of Texas pumas. Furthermore, as discussed above the overall fitness of the Florida panther population has greatly improved because of the introduction of the Texas animals.

Second, two of the five Texas females that had offspring contributed approximately 40 and 30% of the Texas ancestry in the present population. As a result, Land and Lacy (2000) suggested that the Texas ancestry was represented in an amount reflecting only three effective founders. In other words, although there was a genetic rescue effect, the limited contributions of the different female Texas pumas may result in a “bottleneck” for this part of the Florida panther population. However, the potentially low effective population size from the Texas pumas (and the Florida panthers to which they mated) has not yet been evaluated because genetic analysis is still in preparation.

Third, it is not yet clear how long the increase in fitness from genetic rescue will endure. Although there have been several generations since the introduction of Texas pumas, genetic pedigree analysis and Texas ancestry in different individuals is still in preparation. Combining this genetic information with survival and reproduction data would provide an evaluation of the extent of the genetic rescue effect.

Finally, there are many more unrelated animals from Texas (or other areas) that could be introduced if necessary. In other words, it is possible if the fitness of the Florida panther does begin to decline because of small population size that more unrelated individuals could be introduced for an additional program of genetic rescue. Although it appears that the Florida panther population may be reaching carrying capacity in its present environment (McBride et al. 2008), a genetically healthy population with a higher intrinsic rate of increase would better be able to rebound in response to a future catastrophe.

General discussion

Although genetic rescue has become an important management option in some cases, and probably will become more important as endangered populations become smaller and more isolated, it is essential to realize that it is not the ultimate solution for the recovery of endangered species. What genetic rescue may do is temporarily increase the population size, which may in turn temporarily reduce the probability of extinction and increase the probability of establishment of an introduced or endangered population. This may give more time to fix environmental and other problems that have caused endangerment. For the long term genetic health of endangered species, recovery of populations to an effective population size resembling that before endangerment is necessary, a goal that is dependent

upon eliminating, or greatly reducing, the factors that caused endangerment.

As discussed specifically above, the implementation of genetic rescue management should be undertaken only when it is clear that the benefits outweigh the risks. For example, when there is a demonstrated low population fitness making the risk of population extinction high in the near future, and risks from disease and outbreeding depression appear small, then genetic rescue management is a reasonable option. In choosing management options, Edmands (2007) stated that, “managers should strive to do no harm.” However, doing no harm should not be used as a reason to do nothing, often the option chosen by managers that are seeking to avoid decisions that could result in criticism, lawsuits, or other negative reactions.

The 10 guidelines given above provide a framework for consideration of genetic rescue management in other situations besides that of the Mexican wolf and Florida panther. Species such as fish, amphibians, and plants may differ enough in various life history attributes, such as number of offspring and/or dispersal, or the ability to monitor nearly every individual, that the guidelines may have to be significantly modified. However, the evaluation of these two programs shows that many of the factors contained in these guidelines were utilized in the planning and implementation of genetic rescue. Unfortunately, this evaluation also suggests that some aspects of genetic rescue should have been carried out in a more timely manner and that for Mexican wolves, the recovery of the reintroduced population is now in jeopardy, partly because of poor implementation of genetic rescue management.

A similar but more comprehensive approach to genetic rescue has been called genetic restoration. This approach seeks to restore neutral genetic variation and maintain adaptive genetic variants, as well as eliminate the effects of detrimental variants (Seal 1994; Hedrick 2005; Bouzat et al. 2009). The more comprehensive view of genetic restoration, which includes maintaining and/or recovering both adaptive and neutral variation, may result in a longer term improvement in population persistence and the potential to adapt to future environmental changes than genetic rescue.

The potential positive and negative genetic effects of introducing individuals from genetically diverse but geographically isolated populations into apparently inbred population was theoretically evaluated before the introduction of Texas pumas into Florida (Hedrick 1995) and this analysis, or an expansion of it, could be used to examine other potential genetic rescue (or restoration) situations. Hedrick (1995) found that 20% gene flow from outside in the first generation and 2.5% every generation thereafter, as recommended in Seal (1994), resulted in quickly improved fitness or elimination of genetic load.

This was part of the basis for the recommendation that 20% ancestry level (10% each from the Ghost Ranch and Aragon lineages) was an appropriate initial goal for genetic rescue in Mexican wolves. As we mentioned above, one concern about introducing outside animals was that any locally adapted alleles would be swamped by outside gene flow. However, as evaluated theoretically by Hedrick (1995), an initial level of 20% gene flow only resulted in a slightly reduced in fitness by lowering the frequency of locally advantageous alleles and restored the variation for neutral variants.

Both the Mexican wolf and Florida panther nearly became extinct because of killing by humans. Both have been protected species for many years and efforts have been made to increase their population numbers. However, human-caused mortality is still a major factor limiting the recovery of these two species. Since the release of Mexican wolves in 1998, 31 have been illegally shot and 12 have been killed by vehicles (Table 3a). In addition, 144 Mexican wolves have been removed from the population because of rules that require removal for depredation of livestock and straying beyond designated boundaries. On the other hand, only two Florida panthers have been shot since the translocation of Texas pumas but 105 have been killed by vehicles (Table 3b). In other words, both species continue to have high human-caused mortality and Mexican wolves have the high added cost to the population of management removals which have removed many wolves and packs of high genetic value. The high cost of human-caused failures (mortality and removals) raises serious doubts about the success of recovery and population persistence, as well as the ultimate success of genetic rescue in both species.

For two other wolf populations, genetic rescue has or could have a substantial impact. First, the contemporary Scandinavian wolf population in Sweden and Norway appears to have been established by a pair of migrants from the large Finland–Russia population in the early 1980s. The number remained at less than ten individuals in a single pack during the 1980s. A third founder naturally migrated from the Finland–Russia population around 1990, resulted in genetic (or behavioral) rescue, and the population increased to around 100 individuals (Vilà et al. 2003). Subsequently, the amount of inbreeding greatly increased, as determined by a near-complete pedigree, and there was a significant decline in the number of surviving pups per litter (Liberg et al. 2005). As a result of this subsequent decline in fitness and other factors (for example, the Norwegian government has killed a number of wolves in the part of the population residing nearby in Norway), the annual population increase has declined and inbreeding has continued to rise. Several other migrants from Finland have reached northern Sweden (Seddon et al. 2006) but they

Table 3 Cause of mortality and management removals by year in the (a) reintroduced Mexican wolf population (USFWS at <http://www.fws.gov/southwest/es/mexicanwolf/>) and cause of mortality in the (b) Florida panther population (Florida Fish and Wildlife Conservation Commission, unpubl. data, pers. comm. E. D. Land 2009)

Year	Shooting	Vehicle	Natural	Unknown	Total	Removals
(a) Mexican wolf						
1998	4	–	–	–	5	6
1999	–	1	2	–	3	12
2000	1	2	1	–	4	23
2001	4	1	2	1	9	10
2002	3	–	–	–	3	7
2003	7	4	–	1	12	15
2004	1	1	1	–	3	7
2005	3	–	–	1	4	21
2006	1	1	1	2	6	18
2007	1	–	1	2	4	23
2008	5	2	2	2	13	2
2009	1	–	–	–	1	–
Total	31	12	10	9	67	144
(b) Florida panther						
1995	–	2	–	1	3	
1996	–	2	–	1	3	
1997	–	1	2	1	4	
1998	1	4	3	1	9	
1999	–	3	4	–	7	
2000	–	7	1	5	13	
2001	–	7	2	2	11	
2002	–	6	7	1	14	
2003	–	10	8	6	24	
2004	–	11	5	4	20	
2005	–	9	2	1	12	
2006	–	11	3	5	19	
2007	–	15	7	3	25	
2008	–	10	6	6	22	
2009	1	7	2	–	10	
Total	2	105	52	37	196	

have either been killed or disappeared before they became part of the breeding population except for one recent migrant (O. Liberg, pers. comm.) To an outside observer, this situation suggests that some artificial gene flow of several animals for several generations would increase the founder number, result in a second episode of genetic rescue (or even genetic restoration), and potentially increase the number of wolves in this population to a self-sustaining number.

Second, the population of wolves on Isle Royale, a United States national park in Lake Superior, has long been offered as an example of a small population that has existed

for many decades without detrimental genetic effects. However, a recent study by Rääkkönen et al. (2009) has shown that 58% of the Isle Royale wolves exhibited some kind of congenital abnormality in the vertebral column compared to around 1% in outbred populations. Although the clinical significance of these defects is not known in wolves, similar abnormalities in dogs are associated with debilitating effects (Rääkkönen et al. 2009). The Isle Royale population is thought to have been founded by one female and one or a few males in the late 1940s and its longterm effective population size has been estimated as around 3.8 (Peterson et al. 1998). Because of global climate change, the likelihood of new natural founders coming across winter ice to the island is very unlikely. Again, this situation suggests that artificial gene flow from only a few successful animals could result in genetic rescue or restoration. However, before the extreme deterioration was documented by Rääkkönen et al. (2009), it had been suggested that it would be inappropriate to introduce unrelated wolves for esthetic, ecological, and political reasons (Peterson 1995; Rääkkönen et al. 2009), an opinion that deserves reconsideration at this point.

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