

Evaluation of the Genetic Management of the Endangered Black-Footed Ferret (*Mustela nigripes*)

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Empirical support for the genetic management strategies employed by captive breeding and reintroduction programs is scarce. We evaluated the genetic management plan for the highly endangered black-footed ferret (*Mustela nigripes*) developed by the American Zoo and Aquarium Associations (AZA) as a part of the species survival plan (SSP). We contrasted data collected from five microsatellite loci to predictions from a pedigree-based kinship matrix analysis of the captive black-footed ferret population. We compared genetic diversity among captive populations managed for continued captive breeding or reintroduction, and among wild-born individuals from two reintroduced populations. Microsatellite data gave an accurate but only moderately precise estimate of heterozygosity. Genetic diversity was similar in captive populations maintained for breeding and release, and it appears that the recovery program will achieve its goal of maintaining 80% of the genetic diversity of the founder population over 25 years. Wild-born individuals from reintroduced populations maintained genetic diversity and avoided close inbreeding. We detected small but measurable genetic differentiation between the reintroduced populations. The model of random mating predicted only slightly lower levels of heterozygosity retention compared to the SSP strategy. The random mating strategy may be a viable alternative for managing large, stable, captive populations such as that of the black-footed ferret. Zoo Biol 22:287–298, 2003. © 2003 Wiley-Liss, Inc.

Key words: endangered species; captive propagation; reintroductions; mean kinship; microsatellite DNA; population genetics

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INTRODUCTION

The conservation of genetic variability in endangered species is crucial to their recovery and long-term survival [Frankel and Soulé, 1981]. Although the genetic management of captive populations of endangered species has been the subject of much theoretical work [Ballou and Lacy, 1995], few empirical studies have documented the outcome of these genetic management strategies [Rodriguez-Clark, 1999]. Genetic management has tended to focus on captive rather than reintroduced populations, although ultimately these reintroduced populations are the founders of new, free-ranging populations of endangered species. In this study we use microsatellite DNA analyses in conjunction with pedigree-based analyses to evaluate the success of genetic management strategies for the captive breeding and reintroduction of the highly endangered black-footed ferret (*Mustela nigripes*).

The success of reintroduction programs depends on the success of their respective captive breeding programs. Captive populations, in turn, tend to suffer from problems expected with small numbers: inbreeding, inbreeding depression, and genetic drift [de Boer, 1994]. Various approaches have been used to maximize the retention of genetic variability, and the availability of pedigrees provides unique opportunities for such management. The mean kinship strategy (MKS) is commonly used to maximize the retention of genetic variability in pedigreed captive populations [Ballou and Lacy, 1995], particularly of endangered species. MKS selects mating pairs by minimizing mean kinship (*mk*) scores among hypothesized matings, and has been shown by simulation modeling [Ballou and Lacy, 1995] and in empirical studies in *Drosophila melanogaster* [Montgomery et al., 1997] to outperform other strategies. Still, its efficacy in captive vertebrate populations is largely untested.

Once captive-born animals are released into the wild, further challenges face the recovery program. These include a possible underrepresentation of genetic variability in the captive population [Earnhardt, 1999], poor fidelity of transplants to release sites [Reading and Clark, 1996], and adaptations to captive living conditions that would decrease the chances of success in the wild [Frankham et al., 1986]. Thus, the demographic and genetic attributes of the captive population affect the attributes and success of reintroduced populations [Jiménez et al., 1994; FitzSimmons et al., 1997]. Reciprocally, the selection of animals for release removes them and their potentially unique genes from the captive breeding pool. This sets up a potential conflict between the goals of captive breeding and those of reintroduction [Earnhardt, 1999]. Thus, a successful captive-breeding–reintroduction program can involve substantive but reconcilable trade-offs, the outcomes of which are poorly documented.

To understand how a design for matings and allocation of animals to release sites affected the genetic attributes of a critically endangered, captive-bred, reintroduced vertebrate species, we studied the genetic attributes of captive and reintroduced black-footed ferrets, and wild-born descendants of reintroduced black-footed ferrets. Ferrets were once widespread throughout the short- and mixed-grass prairies of North America; land conversion and the eradication of prairie dogs (*Cynomys* spp.), their obligate prey species, eventually brought this species to the brink of extinction. Eighteen individuals taken from the wild in 1985 and 1986 were the founders of the captive breeding program, and seven of the eighteen individuals

became the genetic founders of all extant black-footed ferrets [Garell et al., 1998]. Three years before captive breeding was initiated for black-footed ferrets, the American Zoo and Aquarium Association (AZA) developed the Species Survival Plan (SSP) to coordinate the recovery efforts for highly endangered species [Ralls and Ballou, 1986]. An SSP master plan was developed to breed black-footed ferrets in captivity using MKS augmented with line breeding of underrepresented founders [Garell et al., 1998]. In 1991 the first black-footed ferrets were reintroduced to the wild. Only animals considered nonessential to the success of the captive breeding program were released. As a result, litter mates descended from overrepresented founders were released together. Although this strategy should have increased inbreeding among reintroduced animals, it should have also maximized genetic variability in the captive population, a more important consideration at the time [Russell et al., 1994]. The success of the reintroduction program today is mixed: some populations are self-sustaining, while others apparently are not. Disease and a lack of suitable habitat are the primary reasons for the poor success rates, although genetic factors may also contribute. With more reintroductions planned, it is important to understand how the genetic management of black-footed ferrets affects relationships among animals maintained in captivity and released, and among their wild-born descendants.

METHODS

We used data from five microsatellite DNA markers to compare genetic diversity in the seven founders, in captive animals managed for breeding ($mk < 0.125$), in those managed for reintroduction ($mk \geq 0.125$), and in wild-born animals at two reintroduction sites. We compared molecular measures of genetic diversity to pedigree-based predictors, and used those predictors to establish the effectiveness of microsatellite markers to measure genetic diversity in black-footed ferrets. Because both methods assume the absence of selective pressures operating on the alleles being managed, we predicted that molecular genetic analyses and pedigree analyses of genetic diversity would be concordant. Because the reintroduced populations were smaller than the captive ones, and from year to year were composed of litter mates, we predicted that the released animals would be more closely related to one another compared to the animals retained in the captive population. We predicted that inbreeding coefficients would be greater in the wild-born animals than in their reintroduced ancestors, because of the presumed higher inbreeding in released animals. Finally, because two reintroduced populations (in Montana and South Dakota) came from the same fairly homogeneous captive stock, were augmented with captive animals yearly, and had been established for only 5–7 years at the time of our sampling, we predicted that founder effects and post-release genetic drift would be minimal, and consequently there would be little genetic divergence.

Sampling and DNA Extraction

We received kidney tissue from S.J. O'Brien ($n=2$), skeletal muscle tissue from the Museum of Southwestern Biology ($n=5$ (NK39373, NK39378, NK39379,

NK39380, NK39822)), and hair samples ($n=187$) from the U.S. Fish and Wildlife Service (USFWS), National Park Service (NPS), and U.S. Forest Service (USFS). These tissues represented five groups of black-footed ferrets:

1. Founders of the current population established in 1986 ($n=7$, founders).
2. Captive animals that were released into the wild in 1998 ($n=36$, releases).
3. Animals that were maintained and bred in the captive breeding program, and were alive in 1999 ($n=29$, breeders).
4. Wild-born animals from a reintroduced population in the Charles M. Russell National Wildlife Refuge, Montana, sampled in 1997–1999 ($n=81$, wild MT).
5. Wild-born animals from a reintroduced population in Badlands National Park and Buffalo Gap National Grasslands, South Dakota, sampled in 1999 ($n=41$, wild SD).

We extracted DNA by incubating 20 μg of skeletal or kidney tissue or 20 hair follicles with 700 μl lysis buffer, 20 μl of 20 $\mu\text{g}/\mu\text{l}$ proteinase K, and 10 μl of 10 $\mu\text{g}/\mu\text{l}$ RNase. For hair samples we also added 20 μl of 1M DTT. The samples were placed in a 62°C water bath for 40 hr. Midway through the incubation we added 10 μl of Proteinase K, 5 μl of RNase, and for hair samples 20 μl of DTT. We extracted DNA with two phenol/chloroform/isoamyl alcohol extractions. We precipitated the DNA with 70% ethanol and washed it with 95% isopropyl alcohol. All of the DNA was put into a 1 X Tris EDTA solution and stored at -20°C . DNA concentrations varied (17–70 $\text{ng}/\mu\text{l}$) among samples.

Genetic Typing

We chose loci if they amplified, gave consistent scores in replicate amplifications, and were polymorphic in the 7 founders. We amplified microsatellite loci (Table 1) in 194 black-footed ferret samples with a 10- μl polymerase chain reaction (PCR). Each genotype was verified with a second amplification; if the genotypes did not match in a given individual at a particular locus, the sample was reamplified an additional two times. If the genotype in question was not consistent for at least three amplifications, it was not included in the analysis. We added 1 μl DNA, 2.5–3.7 mM

TABLE 1. Characteristics of 5 microsatellite loci amplified in 194 black-footed ferrets*

Locus	Allele size (bp)	Number of alleles	H_o	H_{em}	HWD P
Mvis002 ^a	218/220	2	0.39	0.44	0.37
Mvis9700 ^b	308/312	2	0.40	0.47	0.05
Mvis072 ^a	276/278	2	0.44	0.42	0.82
Mer095 ^a	148/150	2	0.14	0.16	0.13
Mer049 ^b	197/199	2	0.37	0.41	0.25

* H_o and H_{em} is the observed and expected heterozygosity, respectively. HWD P is the probability value associated with the hypothesis of heterozygote deficiency.

^aFleming et al. [1999].

^bWisely et al. [2002].

MgCl₂, 2 nmol dNTP's, 100 ng/μl BSA, 0.8–1.0 M betaine, 0.5–1.4 pmol forward and reverse primers, 0.6 units Taq polymerase, and 1X Sigma (St. Louis, MO) or Fisher (Pittsburgh, PA) PCR buffer without MgCl₂. All forward primers were constructed with a 19-base-pair (bp) tail on the 5' end so that we could attach a complementary third primer with a fluorescing molecule. We ran reactions in a PTC-200 Peltier thermal cycler (MJ Research, Waltham, MA). We initially denatured samples at 95°C for 2 min, then for 39 cycles the reaction was denatured for 10 sec and annealed for 40 sec, with a 30-sec extension at 72°C. The reaction ended with 10 min of extension. Annealing temperatures varied from 53°C to 58°C. To ensure that different tissues gave identical genotypes, we compared DNA from hair and muscle tissues from seven animals. We also ran each set of reactions with a negative control (no DNA) to ensure that the PCR reagents and the laboratory environment were not contaminated with either ferret DNA or foreign DNA. We resolved individual profiles electrophoretically on 7% polyacrylamide gels using an LI-COR model 4200 IR² Series DNA Sequencer (LI-COR Inc., Lincoln, NE). The resulting digital images characterized the genotype for each individual, including the size of alleles (number of nucleotide bp), and whether individuals were heterozygous or homozygous at a locus.

Statistical Methods

We tested for deviations from the Hardy-Weinberg equilibrium (HWE) using a Markov chain estimation of probability with GENEPOP, version 1.2 [Raymond and Rousset, 1995]. The use of the Markov chain procedure allowed us to test the separate hypotheses of heterozygote excess and heterozygote deficiency. We calculated the probability of excess and deficiency across loci and across populations. We calculated variance estimates for F_{IS} by jack-knifing over loci. To test for genetic structure in the wild-born groups, we generated 95% CI (confidence interval) for F_{ST} (θ in Weir and Cockerham [1984]) by bootstrapping over loci with 1,000 iterations using FSTAT [Goudet, 2001]. Genetic diversity was expressed as the number of alleles averaged across loci (A_m), observed heterozygosity (H_o), and expected molecular heterozygosity (H_{em}). We considered among-group differences in H_{em} significant when 2 standard errors (SEs) did not overlap.

We compared the predictions derived from the pedigrees with the molecular data. The pedigrees were generated from the studbook of captive black-footed ferrets [Marinari, 1999] and analyzed with the computer program SPARKS V.1.0 [International Species Information System, Apple Valley, MN]. This program calculated individual inbreeding coefficients (F) from known relatedness of animals. We used an additive kinship matrix to calculate founder genome equivalent, f_{ge} [Lacy, 1989], mk , percent heterozygote retention, and percent allelic retention using the program GENES [Lacy, 1994a]. The studbook for the black-footed ferret listed three animals with unknown sires, but those three and their descendants constituted only 4% of the total studbook. As recommended by Ballou and Foose [1996] when the proportion of individuals with unknown lineage is small compared to the total pedigreed population, we eliminated those individuals from analysis.

We tested the precision and accuracy of the molecular data with expected pedigree heterozygosity (H_{ep}) and average number of alleles (A_p) predicted by

pedigree analysis. We obtained the predicted estimates of genetic diversity using the same kinship matrix as GENES, with one exception. In order to calculate the percent retention of heterozygosity or allelic diversity, GENES seeds the kinship matrix with founders that have all unique alleles. We seeded the matrix by assigning the allele frequencies found in the molecular analyses of the founders in order to compare pedigree and molecular data in the 1999 population. We ran 1,000 simulations for each locus to estimate the distribution of possible values of H_{ep} and A of animals alive in 1999. We then compared H_o and A_m from the combined molecular data of captive breeders and releases to the distribution of values obtained from the kinship matrix analysis using a z-test.

To determine whether the SSP strategy of combining MKS with line breeding (hereafter called the SSP strategy) conserved more genetic diversity than did random mating, we compared pedigree-generated estimates of heterozygote retention after 14 generations of captive breeding to estimates modeled under the assumption of random mating, using the equation [Wright, 1931]:

$$H_{er} = H_e(1 - 1/2N_e)^t \quad [1]$$

where t =number of generations, and assuming (as does the kinship matrix analysis) that $H_e=0.99$ in the founding generation. We averaged the male and female generation length [Keyfitz and Fleiger, 1971] to estimate the total generation length of the species in captivity. We divided the total generation length by the number of years in captivity (14) to estimate the number of generations. We estimated N_e demographically using the approximation:

$$N_e = y/(1/N + \dots + 1/N_y) \quad [2]$$

where N =the number of breeding-age adults (1-4 years old), and y =each year animals bred in captivity. We thus had two measures of heterozygosity: H_{em} , an estimate based on five microsatellite markers, and H_{ep} , a prediction based on analysis of the pedigree. In addition, we had two estimates of the amount of heterozygosity lost during captivity: one based on the observed kinship matrix, and one based on Wright's model of random mating.

RESULTS

Molecular Genetic Analyses

We screened 42 previously published microsatellite loci, none of which had been developed for black-footed ferrets. Twenty-four amplified with repeatable and interpretable results, but only five loci were polymorphic in the founders. We found no significant probability of heterozygote excess among loci or among groups (global $P=0.87$). We found one marginally significant heterozygote deficiency at locus Mvis 9700 (Table 1). Using the Markov chain estimation of probability, we found heterozygote deficiency in both captive groups (releases and breeders; Table 2). However, we found no global heterozygote deficiency ($P=0.09$), and SEs estimated across loci were large (Table 2). All loci were polymorphic in all groups, and all alleles were present in all groups. H_{em} did not differ significantly among groups (Table 2). For all animals genotyped at all loci, $H_o=0.35$ and

TABLE 2. Measures of genetic diversity, A , the average number of alleles per locus, H_o , the observed heterozygosity, and H_{em} , the expected heterozygosity for five groups of black-footed ferrets, and HWD P , the probabilities of heterozygote deficiencies within groups*

Group	A	H_o	$H_e + 2SE$	HWE P
Founders	2	0.40	0.33 ± 0.14	0.76
Captive breeders	2	0.32	0.41 ± 0.12	0.03
Captive releases	2	0.31	0.38 ± 0.14	0.01
Wild Montana	2	0.33	0.33 ± 0.14	0.47
Wild South Dakota	2	0.43	0.38 ± 0.12	0.87

*The probability of a heterozygote deficiency over all loci and groups was 0.09.

TABLE 3. How well molecular measurements of heterozygosity (H_o) fit the predicted distribution of heterozygosity (H_{ep}) generated by a 1000 simulations of the pedigree*

Locus	$H_{ep} \pm SD$	Molecular H_o	Z	P
Mvis002	0.28 ± 0.11	0.35	0.64	0.26
Mvis9700	0.19 ± 0.08	0.37	2.25	0.01
Mvis072	0.47 ± 0.03	0.32	-5.33	<0.001
Mer095	0.21 ± 0.12	0.14	0.58	0.28
Mer049	0.46 ± 0.04	0.40	-1.5	0.07

*A Z test and the associated P value were used to determine how many standard deviations from the mean the molecular estimates were.

$H_{em}=0.38$. We detected a small but significant difference in allele frequencies between wild MT and wild SD populations (pairwise $F_{ST}=0.09$, lower 95% CI=0.04, upper 95% CI=0.13).

Pedigree Analysis

The observed heterozygosity of captive animals (breeders + releases) estimated from the molecular data ($H_o=0.32 \pm 0.10$, SD) did not differ from the pedigree-based data ($H_{ep}=0.32 \pm 0.13$), although we found variation in estimates among loci (Table 3). Simulations suggested that one locus, Mer095, had a small but measurable probability (1.2%) of losing alleles after 14 years of captive breeding. In simulations for the four other loci, alleles were never lost.

Results of the kinship matrix analysis generated by GENES indicated that in 1999 the captive population had an f_{ge} of 4.10. The captive population retained 88% of its heterozygosity and 77% of its alleles. The genes of each founder were more evenly represented in the gene pool of the captive population in 1999 than in the gene pool of the first generation of captive-born black-footed ferrets (Fig. 1). The average ± 2 SE of individual inbreeding coefficients for breeders was 0.12 ± 0.00 , and 0.11 ± 0.01 for releases. For the model of random breeding during 14 generations, we estimated generation length to be 2.3 years, $t=6.1$ generations, $N_e=18$, and $H_{er}=0.83$.

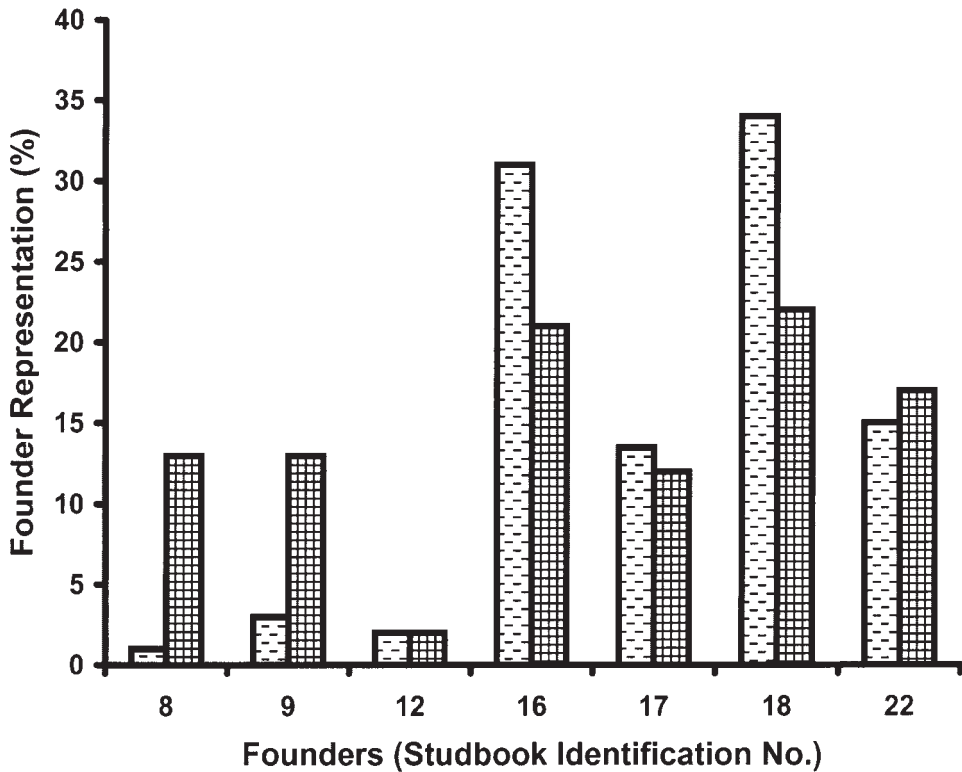


Fig. 1. Founder representation in the first generation of captive breeding (i.e., all direct descendants) (hatched bars), and in 1999, after 14 years of captive breeding (cross-hatched bars).

DISCUSSION

Comparison of Molecular Genetic Analysis and Kinship Matrix Analysis

A comparison of heterozygosity and allelic diversity estimated with kinship matrix analysis vs. molecular analysis enabled us to determine how well the five microsatellites represented genome-wide genetic diversity in the captive population. Allele frequencies were similar between analyses. We found no loss of alleles in the molecular data as predicted by the pedigree data. We found no significant difference between average H_o measured by the molecular data and average H_{ep} , although the molecular data for two loci deviated significantly from the mean pedigree-based heterozygosity, indicating only moderate precision (Table 3). We found no bias for over- or underestimating genetic diversity among loci. We therefore concluded that the molecular data accurately estimated genetic diversity for wild-born animals.

The results from the GENES kinship matrix analysis predicted that 12% of heterozygosity and 23% of alleles were lost between 1986 and 1999. This estimate reflected the genome-wide loss of genetic diversity in a randomly mating, large population more accurately than our molecular data. The molecular data, however, incorporated the history of the population prior to captivity. The black-footed ferret

population from Meeteetse, Wyoming, represented only a portion of the genetic diversity of the species, and this population was likely small and had been isolated since the early Holocene [Wisely et al., 2002]. Thirteen of 24 loci were polymorphic in the species and seven were polymorphic in the pre-captive population; however, only 5 of 24 loci were polymorphic in captive animals. The low level of molecular diversity in captive animals reflected the effects of metapopulation collapse in the pre-captive population, and a founder effect in the captive population [Wisely et al., 2002]. Used together, molecular and pedigree data can elucidate both the current and historic effects of population dynamics in a captive population.

Evaluation of Genetic Management of the Captive Population

Small captive populations are subject to a loss of genetic diversity [Falconer, 1981], which genetic management tries to minimize. We found that heterozygosity in the captive black-footed ferret decreased by 12% over 14 years. We hypothesize that this loss occurred during the first three years of captive breeding, when production was low (Fig. 2). These years are the most crucial for maintaining genetic diversity [Lacy, 1994b], and they are also the most challenging for animal husbandry. Demographic concerns can also outweigh genetic concerns early in captive breeding programs, particularly when only a small number of founders exist. Still, when possible, efforts should be made to equalize founder contributions early in the program.

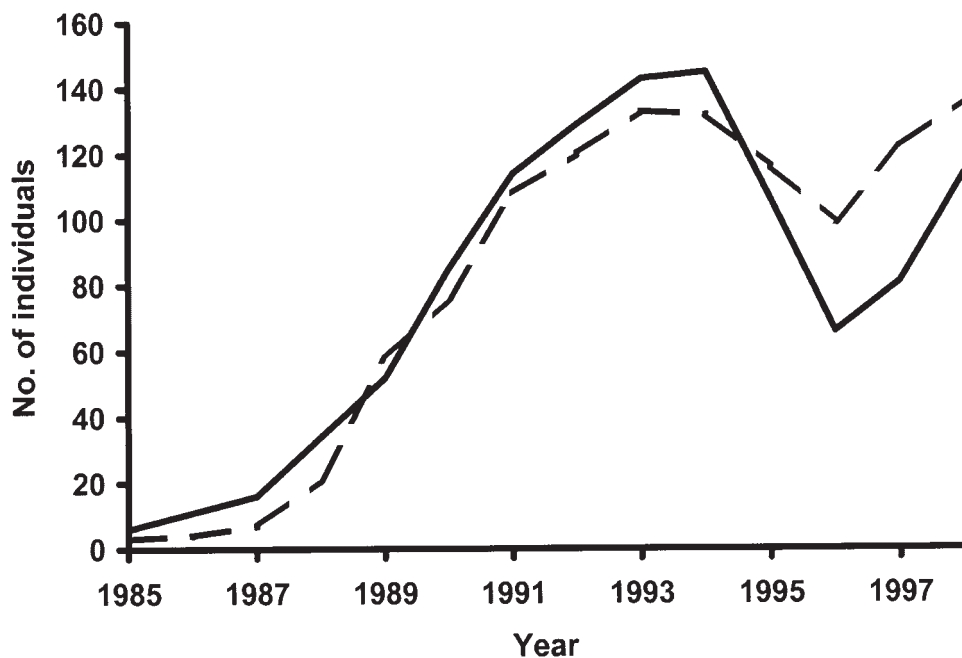


Fig. 2. Number of individuals in the captive population from 1985 through 1998. Males are represented by dashed lines, and females by solid lines. The large decline in the population between 1994 and 1996 was largely due to nonlethal removal of senescing animals, and increased releases to reintroduction sites [Garell et al., 1998].

Not surprisingly, the random breeding model estimated more heterozygosity lost (17%) than the loss we predicted using the pedigree (12%). Nonetheless, both estimates are currently within the SSP goal of 20% loss in 25 years [Garell et al., 1998]. Line-breeding and MKS minimized over- and under-representation of the founders in the living population. Evidence for the success of the SSP strategy could also be seen in the distribution of the founders' genes through time. The founders' genes were more evenly represented in the captive population in 1999 than in the first generation of captive black-footed ferrets (Fig. 1). Our data were consistent with the prediction that MKS should minimize the loss of rare alleles through time [Ballou and Foose, 1996]; none of the 10 alleles present in the founders were lost in descendant captive or reintroduced populations. Contributing to the genetic conservation of black-footed ferrets was the rapid population growth that occurred after the first three years of captive breeding (Fig. 2), which minimized losses due to the population bottleneck at the onset of captivity [Lacy, 1994b].

Although MKS minimizes inbreeding for each generation, inbreeding cannot be avoided in small, closed populations. Without input from genetically unique individuals (which is not possible with black-footed ferrets), the relatedness among all individuals in the captive population increases. Pedigree analysis indicated moderate inbreeding ($F=0.12$) in the captive population.

Inbreeding in 1991, as estimated from pedigree analysis, was higher in releases ($F=0.092$) than in breeders ($F=0.052$) [Russell et al., 1994]. Russell et al. [1994] described this outcome as a result of a management strategy that segregated individuals with low *mk* (breeders) from those with high *mk* (releases). By 1999, however, the difference between breeders and releases in inbreeding, heterozygosity, and allelic diversity was negligible. We suspect that the difference between breeders and releases decreased because founder genes were more evenly represented in the 1999 captive population than in the 1991 captive population. Equal founder representation reduced the variance in *mk* of individuals, which in turn decreased the difference in genetic composition between breeders and releases.

Evaluation of Genetic Management of Reintroduced Populations

We found no difference in H_{em} or A between releases and their wild descendants, which indicates that sufficient numbers of animals were released to halt drift-induced losses in diversity. The augmentation of the reintroduced populations with captive animals likely also helped maintain genetic diversity. The small but significant F_{ST} between the wild MT and wild SD populations suggests that even with augmentation of captive animals to the reintroduced population, these two populations experienced measurable founder effects. Whether this statistically significant difference is also biologically significant is difficult to say [Hedrick, 1999]. We predict, however, that as populations grow and augmentation ceases, continuing genetic drift will decrease genetic diversity within populations and increase genetic distance among populations.

The wild-born animals were not inbred, as was predicted. This result is surprising inasmuch as the free-ranging populations were smaller than the captive population, they were the descendants of overrepresented individuals, and litter mates often were released together. A possible explanation for the lack of inbreeding in the wild populations is a behavioral response of inbreeding avoidance. Several researchers have proposed that closely related individuals avoid mating in such

species as the black-tailed prairie dog (*Cynomys ludovicianus*) [Hoogland, 1982] and olive baboon (*Papio anubis*) [Packer, 1979], and in the marsupial genus (*Antechinus*) [Cockburn et al., 1985]. Given that the reintroduced founding populations were small, and that the black-footed ferrets avoided close kin matings, the survival and reproduction of the founding populations may have been less than optimal because reintroduced individuals avoided breeding with one another and dispersed to find unrelated mates.

CONCLUSIONS

1. We showed that line breeding combined with MKS can reduce variance in founder contributions through time. Combining these two approaches had the effect of equalizing inbreeding between captive-raised breeders and captive-raised releases. By 1999, animals managed for release had inbreeding coefficients equal to those of animals managed for captive breeding. In 1991, when founder contributions were more uneven in the captive population, inbreeding was greater in releases. It appears that efforts to equalize founder contributions have succeeded and benefited the relatively less inbred reintroduced populations.

2. For captive populations of black-footed ferrets, the SSP strategy appears to be able to meet its goal of maintaining 80% of the genetic diversity of the founder population over 25 years. However, the strategy conserved genetic diversity only slightly better than would random mating. While we do not suggest that a random mating plan should be used to implement a captive breeding program, it may be a useful alternative strategy for large captive populations with no new potential founders, in which production is maximized due to reintroduction efforts.

3. The small but significant difference in genetic composition among reintroduced populations suggested a founder effect. To maintain genetic diversity, Brussard and Gilpin [1989] recommended that 20 black-footed ferret reintroduction sites, each with 100 animals, should be established. The state of ferret recovery is far from meeting that goal. In order to maintain genetic diversity throughout the current reintroduction sites, it may be prudent to exchange a few animals among the sites to decrease genetic drift and increase genetic diversity. However, extreme caution, must be used when translocating animals, because of the likelihood of transmitting disease.

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