Genetic Diversity and Fitness in Black-Footed Ferrets Before and During a Bottleneck

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The black-footed ferret (*Mustela nigripes*) is an endangered North American carnivore that underwent a well-documented population bottleneck in the mid-1980s. To better understand the effects of a bottleneck on a free-ranging carnivore population, we used 24 microsatellite loci to compare genetic diversity before versus during the bottleneck, and compare the last wild population to two historical populations. We also compared genetic diversity in black-footed ferrets to that of two sibling species, the steppe polecat (*Mustela eversmanni*) and the European polecat (*Mustela putorius*). Black-footed ferrets during the bottleneck had less genetic diversity than steppe polecats. The three black-footed ferret populations were well differentiated (F_{ST} = 0.57 ± 0.15; mean ± SE). We attributed the decrease in genetic diversity in black-footed ferrets to localized extinction of these genetically distinct subpopulations and to the bottleneck in the surviving subpopulation. Although genetic diversity decreased, female fecundity and juvenile survival were not affected by the population bottleneck.

The black-footed ferret (*Mustela nigripes*) is a highly endangered North American mammal; population decline was attributed to habitat loss and land conversion during the 20th century (IUCN 1988). In 1985 the last wild population (N = 40 adults) experienced simultaneous epizootics of canine distemper and sylvatic plague (*Yersinia pestis*). Eighteen individuals were captured for breeding; by 1986 no animals were known in the wild and only seven adults bred in captivity to contribute to the present gene pool. Although the black-footed ferret passed through a well-documented population bottleneck, the genetic and phenotypic effects of this bottleneck have not been previously described. Previous investigations of genetic diversity in black-footed ferrets found very little or no allozyme variation (Kilpatrick et al. 1986; O'Brien et al. 1989).

The genetic and fitness consequences of bottlenecks are unclear (Amos and Balmford 2001). Only a few studies have documented genetic diversity for an endangered taxon before and after such an event (Bouzat et al. 1998; Groombridge et al. 2000; Matocq and Villablanca 2001), and studies that document accompanying changes in fitness are equally scarce (but see Bouzat et al. 1998; Saccheri et al. 1998). Population genetic theory predicts and some empirical data suggest that genetic variability will decline (Leberg 1992), and individual fitness and adaptability will be lowered (Frankel and Soulé 1981), yet documenting the decrease in genetic diversity is difficult.

Conservation biologists often study populations only after they become small and threatened, identify low genetic variability, and attribute the low level to the population bottleneck (reviewed in Lacy 1997). Such inferences about population dynamics based on these comparisons have been criticized because low levels of genetic diversity are not necessarily due to reductions over brief time periods. Alternatively researchers may infer historical levels of genetic diversity in a species from current levels in closely related taxa that have not experienced bottlenecks (e.g. Hoelzel et al. 1993; Houlden et al. 1996). However, such comparisons may be invalid because different taxa have independent demographic histories and life-history strategies that differentially affect genetic diversity (Amos and Harwood 1998; Matocq and Villablanca 2001).

When available, museum specimens offer a solution to the problems associated with identifying bottlenecks and inferring historical levels of genetic diversity (Culver et al. 2000; Paxinos et al. 2002). By directly measuring past levels of diversity and comparing them with current levels, a

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more accurate assessment of loss or maintenance of diversity can be obtained. Drawbacks to using museum specimens include finite collections with small sample sizes and incomplete representation of populations across a species' distribution and through time. Even with these limitations, however, museum specimens contain valuable information otherwise unavailable with only modern samples.

To better understand how genetic and phenotypic attributes respond to a severe bottleneck, we studied genetic variability in black-footed ferrets using museum specimens collected before and during events that led this species to the brink of extinction. We hypothesized that extirpation of subpopulations decreased genetic diversity of this formerly widely distributed, small-bodied carnivore and that a population bottleneck in the only remaining subpopulation further decreased genetic diversity. We also estimated genetic diversity in two sibling taxa with different population histories: an undisturbed population of the steppe polecat (*Mustela eversmanni*) and a bottlenecked population of the European polecat (*Mustela putorius*) to determine whether interspecific comparisons of genetic diversity would accurately reflect the bottleneck in black-footed ferrets. To determine whether fitness changes accompanied population changes, we compared an estimator of fitness—litter size—between pre- and postbottleneck wild populations of black-footed ferrets. These data document the decline of genetic variability in a wild mammal population due to a bottleneck and localized extinctions of subpopulations and provide a test of a fundamental tenet of conservation biology, that severe bottlenecks lead to fitness losses that increase the likelihood of extinction.

Materials and Methods

Samples

We received tissue from a total of 20 black-footed ferrets collected between 1891 and 1985. Eighteen samples came from catalogued museum specimens. From the Museum of Southwestern Biology we sampled MSB 39933, MSB 39932, MSB 39515, MSB 39386, MSB 39385, MSB 39384, MSB 39383, MSB 39382, MSB 39381, MSB 39378, MSB 39379, MSB 39822, MSB 39373, and MSB 39377, and from the National Museum of Natural History we sampled USNM A35011, USNM 15470, USNM 188458, and USNM 34977. Some prebottleneck samples came from museum specimens collected in Trego County, Kansas in 1891 (n = 4, dried skeletal muscle) and Mellette County, South Dakota in 1972 (n = 4, frozen skeletal muscle). Although data concerning the size and fate of the Kansas population were scarce, we assumed that the samples were representative of the relatively large Great Plains population prior to severe human disturbance. Museum records indicated that black-footed ferrets were collected in that county 23 years later in 1914 and were collected in other Kansas counties as long as 66 years later (Anderson et al. 1986), suggesting that black-footed ferrets persisted in the area long after our samples were collected. Historically the South Dakota population was also part of the large and continuous Great Plains prairie ecosystem. By 1968 it was estimated that 96% of black-footed ferret habitat (prairie dog towns) had been eliminated from South Dakota (Rose 1973) and the species was extirpated from South Dakota by 1974 (Clark 1994). Thus samples collected in 1972 represent a small, recently isolated, and declining population.

We also received tissue (frozen kidney, heart, or skeletal muscle) from 12 individuals from Meeteetse, Wyoming. This population was discovered in 1981, seven years after it was thought that black-footed ferrets went extinct. Meeteetse, Wyoming was considered outside the historical distribution of black-footed ferrets because the area was dominated by sagesteppe with only isolated patches of grassland habitat, therefore this population was likely small and isolated since the late Pleistocene or early Holocene. Between 1981 and 1985 this population was studied extensively; the population appeared stable until the fall of 1985, when simultaneous epizootics rapidly reduced population size (Biggins et al. 1998). The 12 samples were collected between 1983 and 1985; 7 of the sampled individuals became the genetic founders of the captively propagated population. We considered all 12 animals to represent the prebottleneck population for Wyoming (sampled prior to and during the sudden population decline). The 7 genetic founders of the captive population were a subset of those 12 individuals and were the only animals to contribute to the gene pool of extant black-footed ferrets. We considered these individuals to be the genetic and demographic representatives of the population bottleneck.

For interspecific comparisons of genetic diversity we acquired tissue (frozen skeletal muscle) from five steppe polecats (subspecies *M. e. dauricus*) from a single locality near Ba Daer Hu, Inner Mongolia. This population underwent no known bottleneck (Biggins D, personal communication). We also received tissue (skeletal muscle in ethanol) from six European polecats from a single contemporary population in west-central England. Polecat populations in Great Britain declined in the 1850s and continued to decline until their near extinction in the 1920s. They existed as relict populations until the 1950s, when their numbers increased and their range expanded; introgression with domestic ferrets (*M. p. furo*) was likely (Davison et al. 1999). We considered European polecat samples to represent a population that had undergone a known historic bottleneck.

DNA Analysis

We extracted DNA by incubating 20 μg of frozen or desiccated tissue with 700 μl lysis buffer, 20 μl of 20 μg/μl proteinase K, and 10 μl of 10 μg/μl RNase. Samples were placed in a 62°C water bath for 40 h. Midway through the incubation we added 10 μl of proteinase K, 5 μl of RNase, and for desiccated samples, 20 μl of DTT. We extracted DNA with two phenol/chloroform/isoamyl alcohol extractions. We precipitated DNA with 70% ethanol and washed it with 95% isopropyl alcohol. All DNA was stored in a 1× Tris EDTA solution at −20°C. DNA concentrations varied (17–70 ng/μl) among samples.

We amplified 24 microsatellite loci (Table 1) with a 10 μl polymerase chain reaction (PCR). We chose loci if they amplified and gave consistent scores from replicate amplifications. We developed two loci, *Mer049* and *Mvis9700,* using the protocol of Fleming et al. (1999). Each genotype was verified with a second amplification; if genotypes did not match in a given individual at a particular locus, the sample was reamplified two more times. If the genotype in question was not consistent for at least three amplifications, it was not included in the analysis. Each reaction contained 1 μl of sample DNA, 2.5–3.7 mM $MgCl_2$, 2 nmol dNTPs, 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 1× Sigma or Fisher PCR buffer without $MgCl_2$. All forward primers were constructed with a 19 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).

Table 1. Allele length (bp) and number of alleles (in parentheses) of 24 microsatellite markers for three species of *Mustela* including three populations of *M. nigripes*

Primer name	Source	*M. nigripes* (n = 20)	*M. eversmanni* (n = 5)	*M. putorius* (n = 6)
G10B[a]	*Ursus americanus*	131 (1)	131 (1)	131 (1)
G10C[a]	*U. americanus*	105 (1)	105 (1)	105 (1)
G1A[a]	*U. americanus*	165–167 (2)	161–167 (4)	163 (1)
Gg4[b]	*Gulo gulo*	109–115 (2)	112–114 (2)	113–115 (2)
Gg7[b]	*G. gulo*	145 (1)	143–145 (2)	145 (1)
Ma1[b]	*Martes americana*	191 (1)	191–193 (2)	180 (1)
Ma19[b]	*M. americana*	207 (1)	203–205 (2)	205 (1)
Mvi057[c]	*Mustela vison*	112–114 (3)	100–104 (3)	100–108 (4)
Mvi087[c]	*M. vison*	102–108 (3)	90–94 (2)	80–86 (3)
Mvi232[c]	*M. vison*	143–149 (3)	147–149 (2)	155–159 (3)
Mvi39[c]	*M. vison*	101 (1)	101 (1)	101 (1)
Mer005[d]	*Mustela erminea*	300 (1)	294–302 (2)	292–298 (3)
Mer009[d]	*M. erminea*	204–206 (2)	206–210 (3)	200 (1)
Mer022[d]	*M. erminea*	250–254 (3)	248–254 (4)	242–244 (2)
Mer041[d]	*M. erminea*	151 (1)	145 (1)	153–157 (2)
Mer095[d]	*M. erminea*	148–160 (3)	148–152 (3)	148–152 (3)
Mvis002[d]	*M. vison*	218–220 (2)	197–219 (3)	193 (1)
Mvis022[d]	*M. vison*	274–280 (4)	276–282 (4)	272–284 (2)
Mvis027[d]	*M. vison*	192 (1)	190–192 (2)	192 (1)
Mvis072[d]	*M. vison*	276–280 (3)	258–278 (3)	271 (1)
Mvis075[d]	*M. vison*	125–152 (4)	147–149 (2)	139 (1)
Mvis099[d]	*M. vison*	339 (1)	339–341 (2)	337 (1)
Mvis9700[e]	*M. vison*	307–311 (2)	307 (1)	303 (1)
Mer049[f]	*M. erminea*	191–199 (3)	177–187 (4)	185 (1)

[a] Paetkau et al. 1995.
[b] Davis and Strobeck 1998.
[c] O'Connell et al. 1994.
[d] Fleming et al. 1999.
[e] GenBank no. AF474150.
[f] GenBank no. AF474149.

We initially denatured samples at 95°C for 2 min, then for 39 cycles samples were denatured for 10 s and annealed for 40 s, with a 30 s extension at 72°C. The reaction ended with 10 min of extension. Annealing temperatures varied from 53°C to 61°C. We ran each set of reactions with a negative control (no DNA) to ensure 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 a LI-COR Model 4200 IR^2 Series DNA Sequencer (LI-COR Inc., Lincoln, NE).

Statistical Analysis

We tested for deviations from Hardy–Weinberg equilibrium (HWE) in the prebottleneck Wyoming population (n = 12) of black-footed ferrets with an exact test of HWE (GENEPOP version 2.0; Raymond and Rousset 1995). We tested for linkage disequilibrium between pairs of loci in the Wyoming population using an exact test of P (Arlequin version 2.0; Excoffier et al. 1996–2002). We calculated inbreeding (F_{IS}) within subpopulations of black-footed ferrets and genetic structure among subpopulations (F_{ST}, θ in Weir and Cockerham 1984) using TFGPA (version 1.3; Miller 1997) and FSTAT (version 2.9.1; Goudet 2001). We calculated 95% CI estimates for F statistics by jackknifing over loci.

We tested for differences in genetic diversity among five groups of ferrets (steppe polecat, European polecat, and three groups of black-footed ferrets from Kansas, South Dakota, and Wyoming). We used three measures of genetic diversity: the number of alleles per locus averaged across loci (A), the percent of polymorphic loci (P_o), and expected heterozygosity (H_e). We tested for differences in A and H_e among groups using a Kruskal–Wallis nonparametric analysis of variance by ranks. We conducted post hoc tests among mean ranks using the nonparametric analog of Fisher's least significant difference (Dowdy and Wearden 1985). Because equal ranks diminish the power of the test, we only used loci that were polymorphic in the black-footed ferret. Using the same treatment groups we determined which species and which black-footed ferret group had the greatest P_o using chi-square contingency table analysis. Each locus was categorized as polymorphic or monomorphic for each group. We included all 24 loci in this analysis. We tested for pairwise differences in P_o between groups with post hoc chi-square tests. All tests for differences in H_e, A, and P_o were calculated using the statistical software SPSS version 10.0.5.

In order to compare the bottlenecked population of black-footed ferrets to steppe polecats and European polecats, we reanalyzed genetic diversity in ferret groups substituting the 7 individuals representing the bottleneck for the 12 prebottleneck animals. Because the animals representing the bottleneck were a subset of the prebottleneck population, these two groups were not independent and precluded simultaneous analysis. Multiple species comparisons were further complicated by the possibility of ascertainment and mutational bias. Because microsatellite libraries are developed to maximize the length and number of polymorphic loci, they tend to be the most heterozygous and polymorphic in the target species (Ellegren et al. 1995). We used loci that were not developed for any of the species we genotyped in order to avoid such ascertainment bias in our interspecific comparisons. In addition, because longer microsatellites tend to mutate at a faster rate and be more heterozygous (mutational bias; Amos et al. 1996), we tested for differences in range midpoint allele lengths among species and we tested for a correlation between range midpoint allele length and number of alleles per locus.

Although the three groups of black-footed ferrets were temporally and spatially distinct, we pooled data from them to approximate historical levels of genetic diversity in the species and to investigate the possible effects of different historical events on this diversity. We estimated species-wide genetic diversity at four time periods. We estimated genetic diversity in 1891 by combining all individuals from the three subpopulations. The estimate of genetic diversity in 1972 included South Dakota and Wyoming, but we assumed that the Kansas subpopulation was extinct. The two estimates of genetic diversity in 1985 included only Wyoming individuals: all 12 individuals in the prebottleneck estimate and 7 individuals for the estimate of genetic diversity during the bottleneck. Because temporal data were not independent, statistical testing for differences in measures of genetic diversity through time was not possible.

Fitness

To evaluate how fitness was affected by loss of genetic diversity, we used previously published estimates of litter size from free-ranging populations surveyed before and after the bottleneck. Because

Table 2. Observed (H_o) and expected (H_e) heterozygosities for 14 microsatellite loci in black-footed ferrets from Wyoming, South Dakota, and Kansas combined (representing the species prior to disturbance; $n = 20$), and from Wyoming only ($n = 12$), and HWE P, the probability associated with an exact test of Hardy–Weinberg equilibrium[a]

	WY, SD, KS		WY only		
Primer	H_o	H_e	H_o	H_e	HWE P
Mvis002	0.65	0.48	0.50	0.39	0.53
Mvis9700	0.33	0.36	0.33	0.39	1
Mvis072	0.47	0.65	0.67	0.58	0.77
Mer095	0.20	0.27	0.17	0.16	1
Mer049	0.35	0.69	0.50	0.51	1
Mvi57	0.10	0.54	0.17	0.16	1
Mvis022	0.11	0.63	0.08	0.23	0.13
Gg4	0.05	0.14	—	—	—
Mvis075	0.17	0.53	—	—	—
Mvi87	0.00	0.43	—	—	—
G1A	0.10	0.19	—	—	—
Mvi232	0.20	0.19	—	—	—
Mer022	0.11	0.45	—	—	—
Mer009	0.06	0.06	—	—	—

[a] HWE was tested only in polymorphic loci from the Wyoming population.

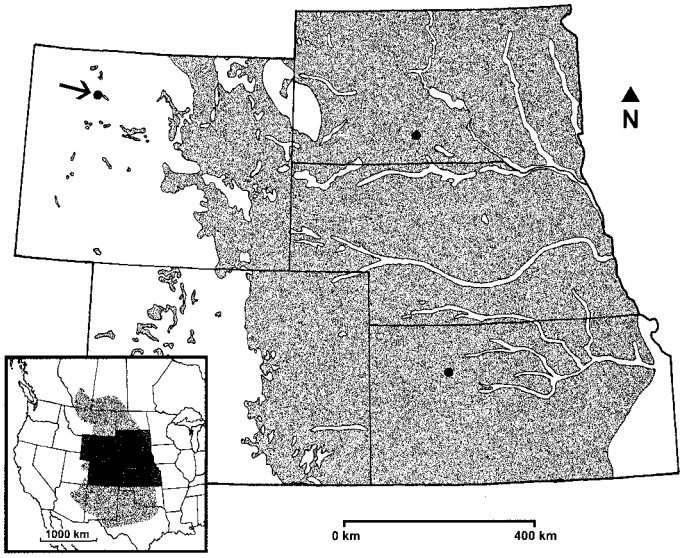

Figure 1. Historical populations of black-footed ferrets fit a core-satellite metapopulation model. Closed circles indicate locations from which samples were collected; the arrow points to the location in Wyoming (WY). Shaded areas indicate grassland habitat. The shaded area of the insert indicates the historical distribution of the black-footed ferret (Owen et al. 2000). Kansas (KS) and South Dakota (SD) populations were more similar to each other ($F_{ST} = 0.40$) than to WY ($F_{ST} = 0.56$ for KS and 0.66 for SD).

kits attained 95% of their adult weight by the end of the survey period (Vargas and Anderson 1996), litter size not only represented the fecundity of the mother, but also survival of the kits through most of their development. We therefore considered litter size to be a robust component of fitness. We compared litter size between a reintroduced population in South Dakota and the prebottleneck Wyoming population. The same methodology was used in both studies to determine litter size. Spotlight surveys were conducted during July and August 1982–1985 in Meeteetse, Wyoming. Litter size data were collected prior to the epizootics in 1985 and litter size did not significantly differ among years (Forrest et al. 1988). Postbottleneck data were collected during July and August 1997–2000 at a black-footed ferret reintroduction site in Buffalo Gap National Grassland, South Dakota (USDA 2000). Litter size was considered to be the number of kits observed above ground with an adult female and kits were 75–145 days old during the survey period. We compared the yearly average number of kits per litter between populations using a Student's t-test, assuming equal variance. We tested for variance in litter size among years between populations using Levene's test for equal variance.

Results

DNA Analysis

We amplified DNA from 27 samples at 24 loci (648 genotypes) that we considered to be of high quality. Tissues from South Dakota and Wyoming were preserved for DNA analysis soon after they were collected and yielded large quantities of high molecular weight DNA. Ten percent of the replicate amplifications did not match initial genotypes and were amplified an additional two times. Seven single-locus genotypes remained irresolvable and were dropped from the analysis. The DNA extracted from the desiccated tissue of museum specimens was of lower quality than DNA from modern samples or frozen tissue of museum specimens. Of the 96 single-locus genotypes, 20% of the replicate amplifications did not match initial amplifications. Nine genotypes remained irresolvable after additional amplifications.

Fourteen of 24 loci were polymorphic in black-footed ferrets and 21 loci were polymorphic in at least one species (Table 1). We found no deviation from HWE (Table 2) and we found no significant linkage disequilibrium. Although seven loci were polymorphic in all three species (Table 1); only five loci were polymorphic in all three species when we included only samples from the least-disturbed black-footed ferret subpopulation (Kansas). Using those five loci we found no significant difference in allele size among species ($F_{2,12} < 0.01$, $P = .99$) and no correlation between the number of alleles and range midpoint allele length among loci and species ($F_{1,13} = 0.87$, $P = .37$).

Subpopulations of black-footed ferrets were genetically distinct, as indicated by high values of F_{ST} (0.57 ± 0.15, mean ± SE). Sixteen alleles in 10 loci were unique to the Great Plains subpopulations. Pairwise F_{ST} values suggested greater differentiation between Wyoming and either Great Plains populations than between South Dakota and Kansas (Figure 1). Although sample sizes were small for Kansas and South Dakota, we considered our samples from Wyoming to be representative of the population ($n = 12$, $N = 40$). While small sample sizes can bias estimates of F_{ST}, we used them in this study simply to illustrate differentiation among populations. Because the Great Plains subpopulations contained many alleles that were absent in the well-sampled Wyoming subpopulation, we inferred genetic differentiation between these locations rather than as an artifact of sample size.

We found a significant difference in H_e among the five groups of ferrets ($H_{14} = 20.6$, $P < .001$; Table 3). Within black-footed ferret subpopulations we measured greater H_e in individuals from Kansas than in the 12 Wyoming individuals ($Z = 2.00$, $P = .04$), but no other comparisons of H_e were significant among black-footed ferret subpopulations. Although we found a significant difference in the number of alleles per locus among all ferret groups ($H_{14} = 16.3$, $P = .003$; Table 3), we found no significant difference among black-footed ferret subpopulations. P_o differed significantly among all groups of ferrets ($\chi^2 = 16.8$, df = 4, $P = .002$; Table 3), but not among black-footed ferret subpopulations.

Table 3. Proportion polymorphic loci (P_o), observed heterozygosity (H_o), expected heterozygosity (H_e), and average number of alleles per locus (A) using microsatellite loci for three species of *Mustela* and three populations of *M. nigripes* (we calculated P_o using all 24 loci, but A, H_o, and H_e were calculated using only the 14 loci polymorphic in black-footed ferrets)

Species/population	n	P_o	A	H_o	H_e
M. eversmanni	5	0.79	2.3	0.30	0.39
M. putorius	6	0.38	1.6	0.13	0.17
M. nigripes					
Kansas	4	0.42	1.5	0.20	0.20
South Dakota	4	0.29	1.3	0.11	0.11
Wyoming					
Prebottleneck	12	0.29	1.4	0.10	0.10
During bottleneck	7	0.21	1.2	0.08	0.07

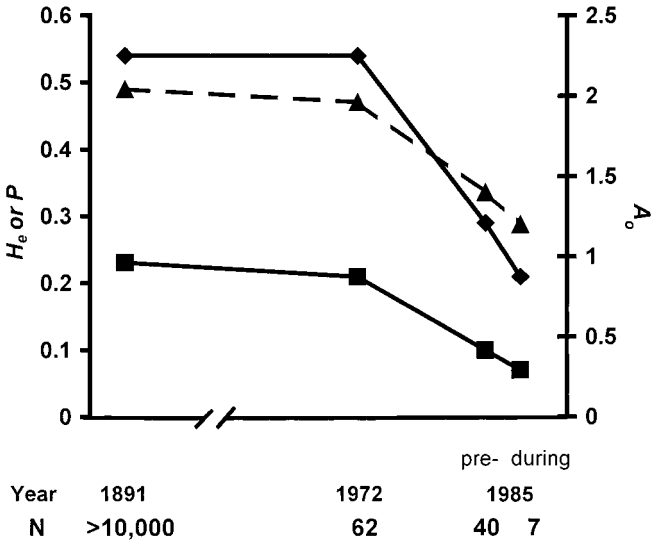

Figure 2. Genetic diversity in black-footed ferrets (*M. nigripes*) declined as populations were extirpated. The 1891 values were derived by combining samples from Kansas, South Dakota, and Wyoming and represented the estimated diversity of the species before disturbance. The 1972 values represented populations remaining (South Dakota and Wyoming) after the extirpation of most Great Plains populations. The remaining values were from the Wyoming population before and during (1985) the bottleneck. Genetic diversity was estimated using the proportion of polymorphic loci (P_o, diamonds), expected heterozygosity (H_e, squares), and number of alleles per locus (A, triangles) averaged over 14 microsatellite loci.

We estimated the loss of genetic diversity in black-footed ferrets at four time periods. All measures of genetic diversity decreased as subpopulations (South Dakota and Kansas) went extinct and when the last population experienced a bottleneck (Figure 2). Genetic diversity dramatically decreased in black-footed ferrets between 1972 and 1985 due to the extinction of the last Great Plains subpopulation. The subpopulation from Wyoming had less genetic diversity during the bottleneck than before the bottleneck. Overall population decline corresponded with a decrease in P_o, H_e, and A.

Steppe polecats had significantly higher H_e's than European polecats ($Z = 3.08$, $P = .002$), black-footed ferrets during the bottleneck from Wyoming ($Z = 4.21$, $P < .001$) and South Dakota ($Z = 3.40$, $P < .001$), but not Kansas ($Z = 1.91$, $P = .06$; overall Kruskal–Wallis test $H_{14} = 22.7$, $P < .001$). We found no significant pairwise differences in H_e or A between European polecats and any subpopulation of black-footed ferret. We found significantly more alleles per locus (A) in steppe polecats than in European polecats ($Z = 2.60$, $P = .009$) or black-footed ferrets (for Wyoming, $Z = 3.06$, $P = .002$; for South Dakota, $Z = 3.40$, $P < .001$; for Kansas, $Z = 2.32$, $P = .02$). Steppe polecats exhibited significantly more polymorphic loci than the European polecat ($\chi^2 = 8.6$, $P = .003$) or the three subpopulations of black-footed ferret (for Wyoming, $\chi^2 = 12.1$, $P = .001$; for South Dakota, $\chi^2 = 12.1$, $P = .001$; for Kansas, $\chi^2 = 7.1$, $P = .008$). We found a similar percentage of polymorphic loci in European polecats and the three subpopulations of black-footed ferrets.

Fitness

Litter size did not differ before and after the bottleneck ($t = 2.45$, df = 6, $P = .73$). At the reintroduction site, 115 litters were observed over 4 years with a mean of 3.1 ± 0.2 (SE) kits per litter (USDA 2000) between 1997 and 2000. In the last prebottleneck population, 68 litters were observed from 1982 to 1985 with an average of 3.2 ± 0.1 kits per litter per year (Forrest et al. 1988). We found no difference in variance among years in mean litter size before and after the bottleneck ($F_{3,3} = 4.3$, $P = .08$).

Discussion

Historical Population Genetics

Comparisons of the 1891 Kansas subpopulation to the 1972 South Dakota and 1985 Wyoming subpopulations (Table 3) suggest that genetic diversity in black-footed ferrets was greatest in the large undisturbed grassland habitat in Kansas. The Kansas samples showed significantly higher H_e values, a greater percentage (although not statistically) of polymorphic loci, and a slightly elevated average number of alleles per locus. In South Dakota and Wyoming, we speculate that genetic diversity was low due to the small, isolated nature of these populations. In summary, genetic diversity was positively correlated with the large population size of Kansas and the small isolated populations of South Dakota and Wyoming, a pattern previously found with microsatellites in brown bears (*Ursus arctos*; Paetkau et al. 1998).

Genetic differentiation was great among subpopulations, which can be partially explained by genetic drift incurred from the large temporal separation of Kansas from Wyoming and South Dakota (a minimum of 81 years). We observed, however, the greatest differentiation between the temporally closest subpopulations (Wyoming and South Dakota), and the least differentiation between the two subpopulations with the highest continuity of habitat, Kansas and South Dakota. Historically black-footed ferrets in South Dakota and Kansas were within the uninterrupted expanse of short and mixed grass prairie that characterized the Great Plains. In contrast, animals from Wyoming were historically isolated by unsuitable shrub-steppe and alpine habitat separating them from the Great Plains. Under this scenario, the pattern of genetic differentiation from the Great Plains to Wyoming could reflect a core-satellite metapopulation structure.

Conservation Genetics

Extirpation of the Kansas subpopulation had a relatively minor effect on genetic diversity in black-footed ferrets (Figure 2), while the subsequent extirpation of the South Dakota population substantially reduced heterozygosity, the average numbers of alleles, and the percentage of polymorphic loci. This result further supports a core-satellite model of population structure: because the Great Plains subpopulations were not as differentiated from one another as they were from the Wyoming

subpopulation (Figure 1), the loss of one Great Plains subpopulation did not result in a dramatic loss of alleles species wide. The extirpation of the last Great Plains subpopulation, however, caused a much larger decrease in genetic diversity: H_e and P_o decreasing by 52% and 46%, respectively, and A decreased by 32% (Figure 2). Because sample sizes were small for Kansas and South Dakota, our estimate is likely a conservative estimate of the amount of genetic diversity lost. Because genetic differentiation between populations in the Great Plains and the peripheral Wyoming population was likely considerable, we infer that the loss of subpopulations facilitated a loss of genetic diversity.

We found a further, although less dramatic, decrease in genetic diversity due to a population bottleneck associated with a founder event (establishment of the captive population; Figure 2). When the Wyoming subpopulation was reduced to the seven genetic founders of the captive population, H_e decreased by 30% and A and P_o declined by 14% and 28%, respectively. The genetic diversity of the founders of the captive population, and ultimately of the extant black-footed ferret population, was decreased below historic subpopulation levels and greatly diminished when considering the species as a whole.

We compared genetic diversity among subpopulations of black-footed ferrets, a large undisturbed population of steppe polecats, and a population of European polecats that had passed through a bottleneck (Table 3). The interspecific comparison of genetic diversity in ferret species also supports the hypothesis that genetic diversity in black-footed ferrets decreased due to a population bottleneck. H_e was greater in the undisturbed steppe polecats than in the bottlenecked European polecats and two subpopulations of black-footed ferret that had suffered from rapid population decline (Wyoming and South Dakota). Furthermore, we found no difference in H_e between the undisturbed Kansas black-footed ferret subpopulation and the undisturbed steppe polecat population. P_o and A were less informative; both were greatest in steppe polecats, while we found similar levels in all subpopulations of black-footed ferrets and European polecats.

Our results contrast with those of Matocq and Villablanca (2001), who found that historical populations of the endangered Morro Bay kangaroo rat (*Dipodomys heermani morroensis*) exhibited low genetic diversity even prior to a population bottleneck that endangered the subspecies. In that study, a closely related species exhibited higher levels of genetic diversity than the postbottleneck endangered population, casting doubts on the utility of cross-species comparisons to uncover patterns of temporal changes in genetic diversity. In contrast, our interspecific comparison corroborated results of our intraspecific comparison, although we concur with Matocq and Villablanca: when possible, sampling from the same population prior to a population bottleneck provides a more accurate representation of changes in genetic diversity. However, cross-specific comparisons using multiple unbiased nuclear loci may be useful when historical specimens are not available.

Fitness

Litter size did not differ between prebottleneck Wyoming animals and postbottleneck reintroduced animals, suggesting that at least some components of fitness were not correlated with the observed reduction in genetic diversity. Furthermore, the similar litter sizes found in pre- and postbottleneck populations indicate that 11 years of captive breeding did not induce changes in fecundity that can result from unintentional selection (Frankham 1994). Our results contrast with those for the greater prairie chicken (*Tympanuchus cupido*), which exhibited reduced hatching rates associated with a loss of genetic variability (Bouzat et al. 1998).

Alternative explanations for unchanged fitness are possible. Because the Wyoming subpopulation was likely small and isolated through the Holocene, the relatively homozygous population may have been able to purge deleterious recessives, accounting for the lack of inbreeding depression in the postbottleneck population (Lande 1988). Alternatively, under this same scenario of continuous isolation and small population size, fitness could have been lowered by the fixation of deleterious alleles, decreasing litter size prior the population's discovery in 1981. The paucity of field studies of black-footed ferrets makes comparisons with other historical populations impossible. Nonetheless, the loss of genetic diversity associated with the founder event (establishment of the captive population) and 11 years of subsequent captive breeding did not appear to affect female fecundity or juvenile survival.

Conclusion

Our comparison of genetic diversity among subpopulations of black-footed ferrets over the last 110 years suggests greater genetic variability prior to the extinction of the Great Plains subpopulations. Population differences were consistent with higher gene flow among populations on the Great Plains than between the Great Plains and populations at the westernmost limit of the species' distribution. With the extirpation of Great Plains populations (represented in this study by the successive loss of subpopulations in Kansas and South Dakota), the estimated genetic diversity of the species declined. The bottleneck of the Wyoming population in 1985 induced further losses. In spite of these losses, however, litter sizes in wild-born animals did not differ before versus after the bottleneck, providing no evidence for drastic and comprehensive fitness consequences of these genetic losses. Genetic diversity of bottlenecked black-footed ferrets was lower than the undisturbed steppe polecat population, and similar to the bottlenecked European polecat population. Such cross-taxon comparisons may be useful when historical specimens are not available. Longitudinal studies of black-footed ferrets incorporating museum DNA show that the pattern of declining genetic diversity was due to population losses, ultimately attributable to habitat loss and land conversion over the last 110 years.

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