*Journal of Mammalogy,* 92(4):751–759, 2011

# Genetic evaluation of a reintroduced population of black-footed ferrets (*Mustela nigripes*)

Cynthia M. Cain, Travis M. Livieri, and Bradley J. Swanson*

*Department of Biology, Central Michigan University, Mount Pleasant, MI 48859, USA (CMC, BJS)*
*Prairie Wildlife Research, P.O. Box 308, Wellington, CO 80549-0308, USA (TML)*

** Correspondent: brad.swanson@cmich.edu*

The black-footed ferret (*Mustela nigripes*) went extinct in the wild when the last 18 known ferrets were captured for a captive-breeding program. Following the success of the captive-breeding program, 146 genetically nonessential ferrets were released at the Conata Basin, South Dakota, from 1996 to 1999. We conducted a genetic analysis of the Conata Basin black-footed ferret population from 2001 to 2003 to determine if genetic variation had been lost since the cessation of reintroductions and if demographic- and genetic-based estimates of effective population size ($N_e$) accurately predicted observed levels of heterozygosity. We used DNA from wild-born kits ($n = 254$) in the Conata Basin population (2001–2003) to calculate current genetic diversity levels. Both allelic diversity (A = 2, both subpopulations) and mean heterozygosity were low for both subpopulations—0.39 ± 0.12 *SE* in Agate-Sage Creek and 0.39 ± 0.16 *SE* in Heck Table—but not significantly different from estimates made in 1999. We found no significant difference between observed and expected heterozygosity levels. Demographic-based estimates of $N_e$ were an order of magnitude higher than genetic-based estimates of $N_e$, but the 2 estimates provide a range of $N_e$ values for the population. This study shows that the Conata Basin ferret population is able to maintain its genetic diversity over time despite its population history.

Key words: black-footed ferret, bottleneck, effective population size, heterozygosity, microsatellites, *Mustela nigripes*, reintroduction, translocation


DOI: 10.1644/10-MAMM-S-104.1

Reintroductions and translocations of threatened, endangered, and extirpated species are increasingly common as more species experience declining populations. Reduced population sizes most commonly result from the loss and fragmentation of habitat (Bouzat et al. 1998), overharvesting (Larson et al. 2002), disease (Trudeau et al. 2004), introduction of exotic species (Kim 2005), or a combination of these factors (Uphyrkina et al. 2002). These population reductions often result in a significant loss of genetic variation (Table 1) leading to the need for reintroductions, translocations, or altered management plans (Van Houtan et al. 2009).

Despite the continuation of many of the above threats, reintroductions and translocations of species are often successful (Bremner-Harrison et al. 2004; Richards and Short 2003; Van Houtan et al. 2009). One of the most important factors of a successful reintroduction is the size of the founding population; larger founding populations capture more of the genetic diversity of the source population and reduce the loss of genetic variation over time (Allendorf 1986). Genetic variation impacts long-term population persistence by allowing populations to evolve with changing environmental conditions. However, because reintroduced populations are often small, they experience significant genetic drift and inbreeding, which quickly reduces variation (Saccheri et al. 1998; Taylor et al. 1999). The consequences of the loss of genetic variation are manifested in a variety of ways, including increased extinction risk (Frankham 1995b), increased expression of deleterious genetic traits (Wang et al. 1999), decreased fecundity in females (Taylor et al. 1999) and males (Roelke et al. 1993), and increased disease susceptibility (O'Brien and Evermann 1988). The effects of genetic drift in wild populations are exacerbated further by environmental stress, an especially important concern when reintroducing captive individuals into the wild (Reed et al. 2002).

The amount of genetic drift a population experiences is controlled by the effective population size ($N_e$—Wright 1969),

**Table 1.**—Comparison of genetic diversity in bottlenecked species shown in increasing order of average allelic diversity (A). Loci = number of loci used in the study; $H_O$ = average observed heterozygosity; Cause = reported reason for the small population size (1 = hunting; 2 = disease; 3 = habitat disturbance, loss, or fragmentation; 4 = island population; 5 = reintroduction; 6 = unknown); $n$ = number of individuals sampled for the genetic analysis.

| Species | Common name | Loci | A | $H_O$ | Cause | $n$ | Source |
|---|---|---|---|---|---|---|---|
| *Petrogale lateralis* | Black-footed rock wallaby | 10 | 1.2 | 0.05 | 3, 4 | 28.1[a] | Eldridge et al. (1999) |
| *Panthera leo persica* | Asian lion | 25 | 1.4 | 0.09 | 1, 3 | 10 | Uphyrkina et al. (2002) |
| *Puma concolor coryi* | Florida puma | 25 | 1.9 | 0.24 | 1, 3 | 10 | Uphyrkina et al. (2002) |
| *Ursus arctos* | Brown bear | 8 | 2.1 | 0.30 | 3, 4 | 34 | Paetkau et al. (1998) |
| *Capra ibex* | Alpine ibex | 19 | 2.4 | 0.40 | 1, 5 | 20–36 | Maudet et al. (2002) |
| *Petrogale penicillata* | Brush-tailed rock-wallaby | 11 | 2.5 | 0.45 | 6 | 10 | Eldridge et al. (2004) |
| *Panthera pardus orientalis* | Far Eastern leopard | 25 | 2.6 | 0.37 | 1, 2, 3 | 22 | Uphyrkina et al. (2002) |
| *Glaucomys sabrinus* | Northern flying squirrel | 6 | 3.1 | 0.31 | 3, 4 | 17–30 | Bidlack and Cook (2002) |
| *Hippotragus equinus* | Roan antelope | 8 | 3.1 | 0.46 | 1, 2, 3 | 2.4–15.5[a] | Alpers et al. (2004) |
| *Bison bison* | Bison | 54 | 3.8 | 0.53 | 1, 3 | 30–100 | Halbert et al. (2004) |
| *Ursus arctos* | Brown bear | 8 | 4.4 | 0.55 | 3 | 57 | Paetkau et al. (1998) |
| *Macropus rufogriseus rufogriseus* | Bennett's wallaby | 5 | 4.6 | 0.57 | 5 | 44.2[a] | Le Page et al. (2000) |
| *Lynx lynx* | European lynx | 11 | 5.1 | 0.58 | 1 | 32–196 | Hellborg et al. (2002) |
| *Acinonyx jubatus raineyi* | African cheetah | 25 | 5.2 | 0.57 | 1, 7 | 20 | Uphyrkina et al. (2002) |

[a] Mean number of samples per locus.

which is often significantly smaller than the census population size ($N_c$), making it a crucial parameter to estimate in conservation (Frankham 1995a). $N_e$ is the size of an ideal population that experiences the same amount of genetic drift as the focal population; an ideal population has a constant size, an equal sex ratio, a Poisson distribution of reproductive success, and nonoverlapping generations (Wright 1969). $N_e$ can be estimated indirectly using demographic methods introduced by Wright (1931, 1969) and later adapted by others (Caballero and Toro 2002; Harris and Allendorf 1989), or by other novel methods (Chesser 1991; Tallmon et al. 2004). Demographic estimation of $N_e$ might not always be practical or feasible in natural populations because of the cost involved (Frankham 1995a) and can produce inflated $N_e$ values (Harris and Allendorf 1989).

Alternatively, $N_e$ can be estimated directly using genetic methods (Leberg 2005). A variety of techniques have been developed to estimate $N_e$ from genetic information (Leberg 2005; Wang 2005), such as temporal variance in allelic frequencies (Waples 1989), and are used commonly (Husband and Barrett 1992; Jorde and Ryman 1996; Kaeuffer et al. 2004). Both demographic and genetic methods generate $N_e$ estimates that will provide better determination of the changes in genetic variation of a population than $N_c$, but the accuracy of $N_e$ is important. This is particularly true for small or declining populations such as the black-footed ferret (*Mustela nigripes*).

The black-footed ferret was one of the initial species to be listed by the United States government under the 1966 Endangered Species Preservation Act, the precursor to the current Endangered Species Act (http://www.fws.gov/laws/lawsdigest/esact.html, accessed 13 June 2010). Much of what is known about black-footed ferrets prior to their extirpation from the wild comes from studies done in South Dakota between 1964 and 1974 (Forrest et al. 1988). In 1971, as population numbers continued to decline, 9 ferrets were removed to start the 1st captive-breeding program. Four of the 9 captured ferrets died after contracting canine distemper from a live vaccine previously tested in Siberian polecats (*Mustela eversmanii*), the closest relative to the black-footed ferret (Carpenter 1985). The program struggled with reproductive complications until the last captive ferret died in 1979, at which time black-footed ferrets were thought to be extinct.

The discovery of a previously unknown population near Meeteetse, Wyoming, in 1981 gave black-footed ferret conservation efforts a 2nd chance and provided an opportunity to collect additional natural history data (Biggins et al. 1985; Campbell et al. 1985; Clark et al. 1984; Hammer and Anderson 1985). The populations at Meeteetse, Wyoming, increased between 1982 and 1984 when a plan was made to trap 3 males and 3 females to establish a captive breeding population in 1985 (Thorne and Belitsky 1989). Concurrent with the planning, an outbreak of canine distemper and sylvatic plague led to a rapid decline in the Meeteetse population (Forrest et al. 1988; Lockhart et al. 2006). After the first 6 ferrets trapped in 1985 succumbed to canine distemper (Thorne and Williams 1988), a decision was made to capture all remaining ferrets. Trapping efforts from 1985 to 1987 produced 18 ferrets, assumed to be the total population, and acted as the founding population for the captive-breeding program (Thorne and Belitsky 1989).

Although 18 individuals were captured and 15 were bred in captivity, the genetic basis of the founding captive population is considered to be 10 ferrets because of the degree of relatedness between some ferrets and the death of others before they reproduced (Russell et al. 1994). The extremely small number of founders made rapid population growth the 1st goal of the captive-breeding program. Subsequent goals of the program were establishing a self-sustaining captive population, reintroduction of ferrets into the wild, and maintenance of genetic diversity in captive and future wild populations (Ballou and Oakleaf 1989; Lacy and Clark 1989).

The maintenance of genetic diversity was fostered by constructing breeding pairs of individuals with the lowest mean kinship values, the average degree of relatedness between an individual and all ferrets in the population (Ballou and Oakleaf 1989; Foose 1989).

By 1991 the population had grown to 180 ferrets, achieving the 1st goal of the captive-breeding program. The 2nd goal was accomplished with the release of 49 ferrets in Shirley Basin, Wyoming, in 1991 (Biggins et al. 1998). Since 1991 ferrets have been released at 19 sites ranging from southern Canada to northern Mexico. Initially, all populations experienced high mortality, requiring continual additions of ferrets each year. Mortality was reduced significantly following the development of prerelease conditioning methods that expose the ferrets to habitat and prey prior to release (Biggins et al. 1998, 1999).

Prerelease conditioning ferrets to prey and habitat was crucial, because black-footed ferrets are extreme specialists, subsisting almost entirely on prairie dogs (*Cynomys*); this dependency is key to 2 factors limiting the success of reintroduced populations. First, black-footed ferrets use prairie dogs as their main food source and subsequently usurp the prairie dog burrows as their dens. However, both species have suffered a significant loss of habitat with only about 2% of the original prairie dog habitat remaining (Miller et al. 1996). Second, outbreaks of sylvatic plague (*Yersinia pestis*) in prairie dog towns drastically reduced prey base for black-footed ferrets and directly killed ferrets in many of the reintroduction locations (Cully and Williams 2001; Daley 1992; Lockhart et al. 2006; Trudeau et al. 2004).

Sylvatic plague has prevented many reintroduced populations from becoming self-sustaining; however, black-footed ferret populations in the Conata Basin (Fig. 1) have been self-sustaining since 1999. Conata Basin has more than 8,100 ha of prairie dog habitat, and approximately 7,100 ha of these are occupied by ferrets (Livieri 2006). Between 1996 and 1999 Conata Basin received 146 genetically surplus ferrets from the captive-breeding facility for release. The population at the Heck Table site was established in 1999 with 18 ferrets from the captive breeding population and 18 animals translocated from the population at the Agate site (Livieri 2006). Additional translocations within Conata Basin occurred from 2000 to 2003, with 16 ferrets being moved from the Heck Table site to the Agate and Sage Creek sites and 1 ferret being translocated from the Sage Creek site to Heck Table (Livieri 2006). Since the original reintroductions the Conata Basin population has continued to grow and now acts as a source of ferrets to augment other reintroduction sites; 68 ferrets were removed from Conata Basin for other reintroduction sites from 2000 to 2005 (Livieri 2006).

Despite the success of the Conata Basin ferret population, the reintroduction effort faced several obstacles. First, high levels of inbreeding existed in the ferrets used to initiate the captive-breeding program. Second, to retain the maximum amount of genetic diversity in the captive population only ferrets with the highest mean kinship values were removed from the captive-breeding facility and used for reintroduction (Lacy and Clark 1989; Russell et al. 1994; Wisely et al. 2003), resulting in closely related ferrets being released into the same population. Finally, a potential existed for loss of genetic diversity at the founding event because as few as 30 ferrets initially were released at some sites and initial mortality rates were $\leq$80% (Livieri 2006).

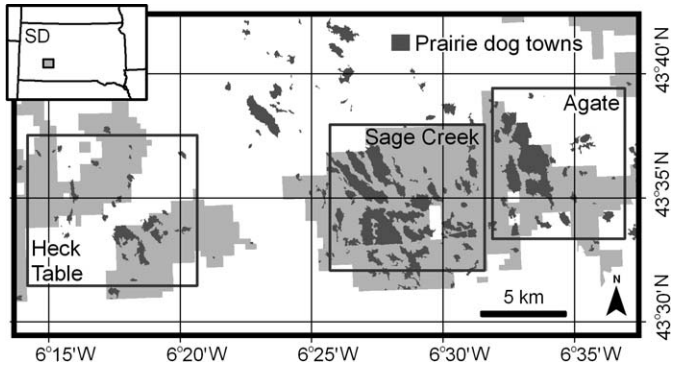


**Fig. 1.**—Location of the 3 reintroduced subpopulations of black-footed ferrets (*Mustela nigripes*) in Conata Basin, southwestern South Dakota. The numerous prairie dog (*Cynomys ludovicianus*) towns are shown in dark gray. Squares indicate the 3 study sites—Heck Table, Sage Creek, and Agate. Buffalo Gap National Grassland, which contains the Conata Basin, is shown in light gray.

Challenges associated with the black-footed ferret captive-breeding program also led to a major strength of the program. Individuals and populations have been closely managed and monitored, and the genetic impact of decisions has been considered since the start (Ballou and Oakleaf 1989; Conservation Breeding Specialist Group 2004). Since their reintroduction at Conata Basin the black-footed ferret population has been censused yearly through an intensive tagging and recapture program that tags all individuals in the population. This provided us with an opportunity to examine closely a reintroduced population that experienced a severe bottleneck, inbreeding in captivity, and the release of closely related individuals (high mean kinship values). Specifically, monitoring provided the necessary demographic and genetic data to determine whether the change in genetic diversity over time was different than that expected based on $N_e$ theory of demographic parameters and to evaluate accuracy of genetic and demographic estimates of $N_e$. Our study evaluated the specific hypothesis of no difference between the expected levels of genetic variation within our populations, based on the demographic parameters of the population, and the observed levels. We also compare our results to the 1999 data of Wisely et al. (2003) to extend the period of our comparisons. Finally, we determined whether a difference existed in the estimates of $N_e$ based on genetic and demographic methods and, if so, which estimate was closer to the observed value. Understanding how genetic variation is lost at Conata Basin will provide insights for future black-footed ferret reintroductions and aid in the planning, execution, and monitoring of reintroductions for other species.

## Materials and Methods

Our study was done in 2001–2003 on black-footed ferrets in the Conata Basin, a portion of the Buffalo Gap National Grasslands in southwestern South Dakota (Livieri 2006). The Conata Basin is composed of 3 large prairie dog subcomplexes, each with black-footed ferrets (Biggins et al. 1993), the Agate, Sage Creek, and Heck Table sites (Fig. 1). The Agate site is separated from the Sage Creek site by a gravel road on the western side, and the Heck Table site is 10 km west of the Sage Creek site with drainages, sharply eroded buttes, and open prairie terrain separating them.

Spotlighting surveys have been used to monitor and locate black-footed ferrets since the initial reintroduction in 1996 (Biggins et al. 2006; Clark et al. 1984). Spotlighting began annually in August when ferret young were old enough and sufficiently active above ground to be captured for marking and identification (Biggins et al. 2006). All captive-bred ferrets were implanted with passive integrated transponders (PIT tags; AVID, Norco, California—Fagerstone and Johns 1987) in their hip and neck scruff prior to release for future identification. Tagged ferrets were identified using a PIT tag reader (AVID, Norco, California) that fit over the entrance to the burrow (Biggins et al. 2006; Stoneberg 1996). Any untagged ferrets we captured were anesthetized using the inhalant isoflurane (Halocarbon; River Edge, New Jersey), and implanted with PIT tags (Biggins et al. 2006; Kreeger et al. 1998) prior to release. We used 3 min of 4% isoflurane for induction and then maintained the ferrets on 2–3% isoflurane for the duration of the procedure. For our genetic analysis 3 tufts of hair (approximately 30 total hairs) were removed from each ferret born from 2001 to 2003 and stored in desiccators at room temperature in a small envelope; additionally, a buccal swab was taken using a nylon swab and stored in 70–80% ethanol at 4°C. After recovery from anesthesia ferrets were released at the capture location. Handling of all animals followed guidelines approved by the American Society of Mammalogists that were published later (Gannon et al. 2007).

The DNA was extracted from 264 buccal swabs and 8 hair samples using QIAGEN QIAamp DNA mini kits following published protocols (QIAGEN, Valencia, California). The workspace was a cubicle enclosed in acrylic sheets that was thoroughly cleaned, along with all utensils, using a with a 10% bleach solution followed by a water rinse and finally a wash with 95% ethanol between samples to reduce contamination.

We were able to obtain complete genotypes from a total of 254 ferrets from the 274 samples using 5 microsatellite loci: Mvis002 and Mvis072 (Fleming et al. 1999), and Mvis9700, Mer049, and Mer095 (Wisely et al. 2002), all of which were found to be polymorphic in the black-footed ferret population of the Conata Basin in 1999 (Wisely et al. 2003). Additionally, 13 primers were screened for polymorphism: Mvis022, Mvis020, and Mer082 (Fleming et al. 1999), and Ma-2, Ma-9, Ma-10, Ma-14, Ma-15, Gg-3, Gg-9, Gg-14, Tt-1, and Tt-4 (Davis and Strobeck 1998). All primers were monomorphic and had no amplification, or nonspecific amplification, except Mvis022 and Gg-14, which were included in the analysis.

Amplification of microsatellite DNA was performed in a 20-μl polymerase chain reaction using an Eppendorf Mastergradient thermocycler (Brinkman Instruments, Inc., Westbury, New York). Each reaction contained 45–100 ng of DNA, 250 μM of deoxynucleoside triphosphates, 0.16 μM of forward and reverse primers, 1x Eppendorf HotMaster Taq buffer containing 2.5 mM $MgCl_2$ (Brinkman Instruments Inc.), and 1.25 units of Eppendorf HotMaster Taq polymerase (Brinkman Instruments, Inc.). Fluorescent markers (FAM, HEX, and TET; Eurofins MWG Operon, Huntsville, Alabama) were attached to the 5′ end of the reverse primer for all primer pairs.

All polymerase chain reactions had an initial denaturation of 2 min at 95°C, followed by 39 cycles of 95°C for 10 s, primer-specific annealing temperatures of 53°C (Mvis022), 56°C (Gg-14), or 54°C (all other primers) for 40 s, and a 30-s extension period at 72°C. A final extension time of 5 min at 70°C ended the reaction. Polymerase chain reaction products from all samples were analyzed on an ABI 310 Genetic Analyzer using GeneScan Analysis 3.1.2, and individual genotypes were determined using Genotyper 2.0 software (all from Applied Biosystems, Foster City, California). To control for potential allelic dropout or variation between amplification methods 10% of our samples went through 3 rounds of polymerase chain reaction and analysis to evaluate their initial allelic assignment.

We assumed that any untagged ferrets classified as adults were alive prior to the year of their capture, and we included them with individuals from the previous year for analysis. We lumped ferrets from the Agate and Sage Creek sites into a single population (Agate–Sage Creek) based on the number of ferrets recorded as moving between these sites (Livieri 2006) and their geographic proximity (Fig. 1). The more isolated Heck Table site was considered disjunct and analyzed as a separate population. We used the population genetics software package Genepop (Raymond and Rousset 1995; http://genepop.curtin.edu.au, accessed April 2009) to test for deviations from Hardy–Weinberg equilibrium and also for genotypic linkage (Bonferroni corrections applied for multiple tests in both cases) and genic differentiation. We calculated the degree of population structure using $Rho_{ST}$, which assumes a stepwise mutation model (Rousset 1996), and $F_{ST}$, which assumes an infinite allele model of mutation (Wright 1969), following the method of Michalakis and Excoffier (1996) and Weir and Cockerham (1984), respectively.

We used the computer software package GENECLASS2 (Piry et al. 2004) to estimate the amount of dispersal between subpopulations (Paetkau et al. 1995). Thirty-eight ferrets were translocated between the sites by Bureau of Land Management personnel from 1999 to 2003. To determine if any bias would arise in our results from including these ferrets, which did not represent natural dispersal, we performed the GENECLASS2 analysis with and without these animals included. The inclusion of these animals did not qualitatively change our results, because the same individuals were identified as dispersers regardless of the inclusion of the translocated

animals. We used a resampling algorithm with 1,000 as the minimum number of simulated individuals with a type I error value of $\alpha = 0.01$. To determine any disproportionate impacts on our results we reanalyzed our data in GENECLASS2 after sequentially removing each locus in turn.

In our comparison to the 1999 heterozygosity value (H = 0.43) reported by Wisely et al. (2003) for this population, we followed those authors in treating all of the Conata Basin as a single population. Similarly, we recalculated heterozygosity using only the 5 loci (Mer049, Mer095, Mvis002, Mvis072, and Mvis9700) used by Wisely et al. (2003) in their analysis of the 1999 data for this population. A Mann–Whitney *U*-test was performed to test for significant differences in heterozygosity between the time periods.

We measured the observed heterozygosity ($H_O$) for all ferrets in the 2003 population ($n_{\text{Agate–Sage Creek}} = 207$; $n_{\text{Heck Table}} = 47$). Next, $N_e$ was calculated using sex ratio data and population counts of adults from 2001 ($n_{\text{Agate–Sage Creek}} = 68$; $n_{\text{Heck Table}} = 18$), 2002 ($n_{\text{Agate–Sage Creek}} = 76$; $n_{\text{Heck Table}} = 13$), and 2003 ($n_{\text{Agate–Sage Creek}} = 121$; $n_{\text{Heck Table}} = 21$) using the equation of Barrowclough and Coats (1985):

$$\frac{1}{N_e} = \frac{1}{t}\left[\sum_{i=1}^{t} \frac{1}{\left(\frac{4N_mN_f}{N_m+N_f}\right)}\right], \quad (1)$$

where $N_m$ is the number of males, $N_f$ is the number of females in a generation, and $t$ is the number of generations. We use $t = 2$ because the generation time at Conata Basin is 1.67 years (McDonald et al. 2006). Equation 1 is a multiplicative combination of equations from Wright (1969) that corrects for sex ratio, $N_e = (4N_mN_f)/(N_m N_f)$, and fluctuating population size, $1/N_e = (1/t)\left[\sum_{i=1}^{t}(1/N_i)\right]$.

We used the $N_e$ estimate calculated from the demographic data ($N_{e\text{-dem}}$) to determine an expected heterozygosity estimate ($H_{dem}$) using the following equation from Wright (1931): $H_{t+1} = H_t\{1 - [1/(2N_e)]\}$, where $H_t$ is the heterozygosity in the 1st generation, $H_{t+1}$ is the heterozygosity in the next generation, and $N_e$ is the effective population size. The heterozygosity value measured in 1999 by Wisely et al. (2003) was used as the initial heterozygosity ($H_t$). We then compared the $H_{dem}$ estimate to the observed heterozygosity ($H_O$) we measured in 2003, using a Mann–Whitney *U*-test.

Additionally, demographic $N_e$ values were compared to $N_e$ calculated from temporal changes in allele frequencies ($N_{e\text{-gen}}$) between ferrets from 1999 data (Wisely et al. 2003) and our 2003 data using the following formula from Waples (1989) for plan 1 (after reproduction):

$$N_e = \frac{t}{2\left(F_k - \frac{1}{2S_0} - \frac{1}{2S_t} + \frac{1}{N}\right)}, \quad (2)$$

where $t$ is the number of generations between sampling periods, $S_0$ and $S_t$ are sample sizes from the 1st (0) and 2nd ($t$) sampling periods, and $N$ is the harmonic mean of the population sizes for time 0 and $t$. The standardized variance in change of allele frequency over time ($F_k$), averaged across all loci, was calculated as in Pollak (1983): $F_k = [1/(A-1)]\sum_i^A \left\{(x_i - y_i)^2 \Big/ [(x_i + y_i)/2]\right\}$, where $A$ is the number of alleles at the locus and $x_i$ and $y_i$ are the allelic frequencies of the $i$th allele at time $t$ and 0, respectively. We then calculated $N_e$ using a correction to equation 2, which reduces $N_e$ bias in nonselfing species: $\hat{N}_e = \{1/[2(1 - e^a)]\} - 0.5$, where $a = \{\ln[1 - F_k - 1/(2S_0) - 1/(2S_i) - 1/N]\}/t$ (Luikart et al. 1999).

## Results

Based on analysis of recapture data, dispersal between subpopulations was male biased (69% of all dispersers were male) and directionally biased. Nine ferrets dispersed from the Agate site to the Sage Creek site, and 3 ferrets dispersed in the opposite direction, supporting the lumping of the 2 sites as a single population. Only 1 ferret dispersed from the Heck Table site to the Sage Creek site, so Heck Table was assessed as a separate population. The average male : female sex ratio for adults in 2000–2003 was 1:1.2 for the Agate–Sage Creek population and 1:1.9 in the Heck Table population.

We found no discrepancies in our reanalysis of the 10% ($n = 154$) of our total genotypes and failed to find any evidence of linkage disequilibrium at any of the loci in any of the populations after Bonferroni correction ($\alpha = 0.0016$). We did find that locus Mer049 was out of Hardy–Weinberg equilibrium ($P = 0.0004$) in the Agate–Sage Creek population following Bonferroni correction ($\alpha = 0.0042$). However, we included Mer049 in all analyses because the deviation from Hardy–Weinberg equilibrium was neither consistent across populations (Kyle et al. 2000; Wilson and Strobeck 1999) nor consistent with previous studies (Wisely et al. 2003). Each locus we examined had only 2 alleles in each population, and the 2 alleles were identical in each population. The highest-frequency allele at each locus was the same for both subpopulations except for Mvis002.

Genic distributions were significantly different between the 2 subpopulations ($P < 0.0001$), and $F_{ST}$ and $\text{Rho}_{ST}$ values were 0.084 and 0.087, respectively. GENECLASS 2 estimated that 5.5% of the ferrets moved between the 2 populations (all $P < 0.05$) between 2001 and 2003. Specifically, 6% of the Agate–Sage Creek population was assigned to the Heck Table population and 4% of the Heck Table ferrets were assigned to the Agate–Sage Creek population.

Observed heterozygosity ($H_O$) values for loci were similar in each population and ranged from 0.15 to 0.53 in Agate–Sage Creek and 0.11 to 0.51 in Heck Table. Heterozygosity calculated across all loci for the 2 subpopulations was not significantly different from the expected heterozygosities based on demographic measures ($H_{dem}$; Agate–Sage Creek, $U_{7,7} = 28.0$, $P = 0.71$; Heck Table, $U_{7,7} = 31.5$, $P = 0.38$; Table 2). Using the same 5 loci as Wisely et al. (2003), we found no significant difference ($U_{5,5} = 6.0$, $P = 0.22$) between the heterozygosity we calculated across the 2 subpopulations for 2003 ($H_O = 0.44 \pm 0.04$ *SE*) compared to the estimate for 1999 ($H_O = 0.43$) of Wisely et al. (2003).

**Table 2.**—Summary table of genetic diversity measures for black-footed ferrets (*Mustela nigripes*), by subpopulation, sampled from 2001 to 2003 in Conata Basin, South Dakota. Abbreviations: $H_O$ = observed level of heterozygosity; $H_{dem}$ = expected heterozygosity based on the effective population size estimated from demographic data; A = allelic diversity; $N_c$ = population census size calculated by taking harmonic mean of adult census sizes from 2001 to 2003; $N_{e\text{-dem}}$ = effective population size based on demographic data; $N_{e\text{-gen}}$ = effective population size based on the change in allelic frequencies over time.

| | *n* | $H_O$ (± *SD*) | $H_{dem}$ | A | $N_c$ | $N_{e\text{-dem}}$ | $N_{e\text{-gen}}$ (95% *CI*[a]) |
|---|---|---|---|---|---|---|---|
| Agate–Sage Creek | 207 | 0.39 ± 0.12 | 0.43 | 2.0 | 81 | 75 | 10.6 (2.2–18.9) |
| Heck Table | 47 | 0.39 ± 0.16 | 0.41 | 2.0 | 17 | 14 | 2.0 (0.0–3.6) |
| Combined population, 5 loci[b] | 254 | 0.44 ± 0.04 | 0.43 | 2.0 | | | |

[a] Confidence intervals calculated using equation 16 of Waples (1989).

[b] Agate–Sage Creek and Heck Table were combined into 1 population and analyzed to match analysis conditions of 1999 estimates (Wisely et al. 2003) of the founding population for the Conata Basin site.

The $N_e$ estimates calculated using demographic data in equation 1 were close to the census size ($N_c$) of the Conata Basin population (Table 2). Estimates of $N_e$ based on the demographic parameters ($N_{e\text{-dem}}$) fell outside of (above) the 95% confidence interval of $N_e$ estimates based on temporal change in allelic frequencies ($N_{e\text{-gen}}$; Table 2).

## Discussion

The bottleneck experienced by the black-footed ferret has reduced dramatically the amount of genetic variation found in the species (Wisely et al. 2008), even compared to other species with small populations (Table 1). During our study period the allele frequencies within each population continued to change as evidenced by the low genetic-based estimates of $N_e$. However, with traditional estimates of genetic variation such as average heterozygosity and allelic diversity, we failed to find any significant change in either population from 1999 (Wisely et al. 2003) to 2003 (our data). This indicates that the allelic frequencies at each site are changing, but all of the genetic variation found in the captive-breeding program has been retained at the Conata Basin reintroduction site. Our results show that in the short term the goal of the recovery plan of maintaining genetic variation in a wild populations of ferrets is possible and has been met.

The retention of genetic variation we found could be due to multiple factors. Wisely et al. (2003) suggested that black-footed ferrets exhibit inbreeding avoidance (Fisher 2005; Kamler et al. 2004; Pillay 2002; Stow and Sunnucks 2004). The amount of dispersal we observed between the Agate and Sage Creek sites supports the hypothesis of inbreeding avoidance. The male-biased dispersal we found also supports inbreeding avoidance, because male-biased dispersal has long been considered a mechanism of inbreeding avoidance (Greenwood 1980). However, it appears that ferrets were less likely to disperse between ferret populations separated by areas lacking ferret populations, with only 1 ferret moving between the Agate–Sage Creek and Heck Table sites compared to the 12 animals dispersing between the essentially contiguous Agate and Sage Creek sites.

The rapid population growth seen in some reintroduced ferret populations (Grenier et al. 2007) also would minimize the loss of genetic variation. Additionally, the management strategy of repeated reintroductions and occasional translocations, in addition to natural dispersal, could allow the populations to mix without homogenizing their genetics (Swanson et al. 2006). The interpopulation dispersal rate of 5.5% equates to an exchange of 13 ferrets between the 2 populations at the 2003 population sizes. This amount of dispersal prevents genetic drift from removing alleles from either population. However, the low estimates of $N_e$ show that genetic drift was a stronger evolutionary force than dispersal, allowing the allelic frequencies to change rapidly between the 2 populations and resulting in the moderately high $F_{ST}$ values we found (Wright 1969). Further, dispersal appears to be biased, with more ferrets moving from the smaller Heck Table population into the relatively large Agate–Sage Creek population than in the reverse direction. The impact of these few individuals on the gene pool of the Agate–Sage Creek population will be reduced given the relatively larger size of the Agate–Sage Creek population. During the early stages of this reintroduction the addition of new ferrets from the captive-breeding facility likely played a similar role in retarding genetic drift and increasing the allelic frequencies if they started to drift toward 0. Regardless of the mechanism, examination of our data suggests that reintroduction of black-footed ferrets at Conata Basin has been genetically successful.

This study also allowed us to compare demographic and genetic estimates of $N_e$ in a natural population, and we found the demographic $N_e$ estimates to be significantly higher than the genetic-based estimates of $N_e$. However, the underground mating system of the ferrets and the low number of alleles per locus prevented us from incorporating the variation in reproductive success into our estimates of $N_e$. We suspect that the mating system is polygynous, as in other mustelids (Powell 1979), so our inability to account for this factor inflated our demographic-based estimates of $N_e$ (Ardren and Kapuscinski 2003; Nunney 1996; Storz et al. 2001; but see Frankham 1995a).

Our genetic-based estimates of $N_e$ could be biased because of our small sample size in 2001 and the number of generations ($t = 2$) separating our sampling periods (Waples 1989). Although sample sizes were small in 2001, sampling was much more complete in 2003, and despite the small sample sizes in 2001, we sampled a relatively high percentage of the populations in each year of the study (14–100% for

Heck Table and 25–79% for Agate-Sage Creek). This should reduce the impact of sample size on our estimates. The impact of the number of generations separating our samples is unlikely to be a concern, however, given the low $N_e$ estimates we found. The major concern regarding the number of generations between samples is when insufficient time prevents allelic frequencies from diverging and biasing the $N_e$ estimate upward. In contrast, however, we found low estimates of $N_e$ showing that the allelic frequencies change quickly in this system.

Although the demographic and genetic $N_e$ estimates are significantly different, they are within an order of magnitude; therefore, the difference, at these values, might not be biologically significant. Only a 2% difference in heterozygosity estimates is realized when $N_e$ is 10 compared to when it is 100 over the short term. Thus, our genetic and demographic methods, together, provide a range of $N_e$ estimates that can be used for management of the black-footed ferret. When direct genetic estimation of $N_e$ is unfeasible, our results suggest that the use of demographic data to estimate heterozygosity can be used to measure the rate of loss of genetic variation. Additionally, we suggest that natural and anthropogenic movement of ferrets between populations was a major factor in maintaining the level of genetic variation in the black-footed ferret populations at Conata Basin as found in the captive program, and this strategy is likely to facilitate the preservation of genetic variation in other populations and species as well.

## Acknowledgments

We thank the United States Forest Service—Wall Ranger District, particularly W. Perry and D. Sargent, for the extensive data and sample collection and contribution of the geographic information system data layer of prairie dog distributions in 2004. Personnel from the United States Fish and Wildlife Service, National Park Service, Bureau of Land Management, Utah Division of Wildlife Resources, and World Wildlife Fund assisted with field data collection. We thank S. Wisely for her contributions to this project, including discussions with her regarding earlier work on black-footed ferrets, J. Willoughby for creating Fig. 1, and D. Biggins and S. Breck for comments on earlier drafts. Funding for this project was contributed by Prairie Wildlife Research, American Museum of Natural History—Theodore Roosevelt Memorial Fund, Sigma Xi Grants-in-Aid, and the College of Graduate Studies, College of Science and Technology, and Biology Department at Central Michigan University.

## Literature Cited

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5:181–190.

Alpers, D. L., B. J. Van Vuuren, P. Arctander, and T. J. Robinson. 2004. Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. Molecular Ecology 13:1771–1784.

Ardren, W. R., and A. R. Kapuscinski. 2003. Demographic and genetic estimates of effective population size ($N_e$) reveals genetic compensation in steelhead trout. Molecular Ecology 12:35–49.

Ballou, J. D., and R. Oakleaf. 1989. Demographic and genetic captive-breeding recommendations for black-footed ferrets. Pp. 247–267 in Conservation biology and the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut.

Barrowclough, G. F., and S. L. Coats. 1985. The demography and population genetics of owls, with special reference to the conservation of the spotted owl (*Strix occidentalis*). Pp. 74–85 in Ecology and management of the spotted owl in the Pacific Northwest (R. J. Gutierrez and A. B. Carey, eds.). United States Department of Agriculture Forest Service, Portland, Oregon.

Bidlack, A. L., and J. A. Cook. 2002. A nuclear perspective on endemism in northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska. Conservation Genetics 3:247–259.

Biggins, D. E., J. L. Godbey, L. R. Hanebury, B. Luce, M. R. Matchett, and A. Vargas. 1998. The effect of rearing methods on survival of reintroduced black-footed ferrets. Journal of Wildlife Management 62:643–653.

Biggins, D. E., J. L. Godbey, M. R. Matchett, L. R. Hanebury, T. M. Livieri, and P. E. Marinari. 2006. Monitoring of black-footed ferrets during reestablishment of free-ranging populations: discussion of alternative methods and prescription of minimum standards. Pp. 155–174 in Recovery of the black-footed ferret—progress and continuing challenges (J. E. Roelle, B. J. Miller, J. L. Godbey, and D. E. Biggins, eds.). United States Geological Survey Scientific Investigations Report 2005—5293.

Biggins, D. E., et al. 1993. A technique for evaluating black-footed ferret habitat. Pp. 73–87 in Management of prairie dog complexes for the reintroduction of the black-footed ferret (J. L. Oldenmeyer, D. E. Biggins, B. J. Miller, and R. Crete, eds.). United States Fish and Wildlife Service Biological Report 13.

Biggins, D. E., M. H. Schroeder, S. C. Forrest, and L. Richardson. 1985. Movements and habitat relationships of radio-tagged black-footed ferrets. Pp. 11.1–11.17 in Proceedings of the black-footed ferret workshop (S. H. Anderson and D. B. Inkley, eds), Wyoming Department of Game and Fish, Cheyenne.

Biggins, D. E., A. Vargas, J. L. Godbey, and S. H. Anderson 1999. Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). Biological Conservation 89:121–129.

Bouzat, J. L., L. A. Lewin, and K. N. Paige. 1998. The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. American Naturalist 152:1–6.

Bremner-Harrison, S., P. A. Prodohl, and R. W. Wood. 2004. Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). Animal Conservation 7:313–320.

Caballero, A., and M. A. Toro. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. Conservation Genetics 3:289–299.

Campbell, T. M., III, D. Biggins, S. Forrest, and T. W. Clark. 1985. Spotlighting as a method to locate and study black-footed ferrets. Pp. 24.1–24.7 in Black-footed ferret workshop proceedings, 18–19 September 1984 (S. H. Anderson and D. B. Inkley, eds.). Wyoming Department of Game and Fish, Cheyenne.

Carpenter, J. W. 1985. Captive breeding and management of black-footed ferrets. Pp. 12.11–12.13 in Proceedings of the black-footed ferret workshop (S. H. Anderson and D. B. Inkley, eds.). Wyoming Department of Game and Fish, Cheyenne.

Chesser, R. K. 1991. Influence of gene flow and breeding tactics on gene diversity within populations. Genetics 129:573–583.

CLARK, T. W., T. M. CAMPBELL, M. H. SCHROEDER, AND L. RICHARDSON. 1984. Handbook of methods for locating black-footed ferrets. Wyoming Bureau of Land Management Wildlife Technical Bulletin.

CONSERVATION BREEDING SPECIALIST GROUP. 2004. Black-footed ferret population management planning workshop. Final report. International Union for Conservation of Nature Conservation Breeding Specialist Group, Apple Valley, Minnesota.

CULLY, J. F., AND E. S. WILLIAMS 2001. Interspecific comparisons of sylvatic plague in prairie dogs. Journal of Mammalogy 82:894–905.

DALEY, J. G. 1992. Population reductions and genetic-variability in black-tailed prairie dogs. Journal of Wildlife Management 56:212–220.

DAVIS, C. S., AND C. STROBECK. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7:1776–1778.

ELDRIDGE, M. D. B., ET AL. 1999. Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conservation Biology 13:531–541.

ELDRIDGE, M. D. B., C. RUMMERY, C. BRAY, K. R. ZENGER, T. L. BROWNING, AND R. L. CLOSE. 2004. Genetic analysis of a population crash in brush-tailed rock-wallabies (*Petrogale penicillata*) from Jenolan caves, south-eastern Australia. Wildlife Research 31:229–240.

FAGERSTONE, K. A., AND B. E. JOHNS. 1987. Transponders as permanent identification markers for domestic ferrets, black-footed ferrets, and other wildlife. Journal of Wildlife Management 51:294–297.

FISHER, D. O. 2005. Population density and presence of the mother are related to natal dispersal in male and female *Antechinus stuartii*. Australian Journal of Zoology 53:103–110.

FLEMING, M. A., E. A. OSTRANDER, AND J. A. COOK. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology 8:1352–1354.

FOOSE, T. J. 1989. Species survival plans: the role of captive propagation in conservation strategies. Pp. 210–222 in Conservation biology and the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut.

FORREST, S. C., ET AL. 1988. Population attributes for the black-footed ferret (*Mustela nigripes*) at Meeteetse, Wyoming, 1981–1985. Journal of Mammalogy 69:261–273.

FRANKHAM, R. 1995a. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66:95–107.

FRANKHAM, R. 1995b. Inbreeding and extinction: a threshold effect. Conservation Biology 9:792–799.

GANNON, W. L., R. S. SIKES, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

GREENWOOD, P. J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. Animal Behavior 28:1140–1162.

GRENIER, M. B., D. B. MCDONALD, AND S. W. BUSKIRK. 2007. Rapid population growth of a critically endangered carnivore. Science 317:779.

HALBERT, N. D., T. RAUDSEPP, B. P. CHOWDHARY, AND J. N. DERR. 2004. Conservation genetic analysis of the Texas state bison herd. Journal of Mammalogy 85:924–931.

HAMMER, D. A., AND S. H. ANDERSON. 1985. Using scent attractants as a technique to locate black-footed ferrets. Pp. 26.1–26.12 in Proceedings of the black-footed ferret workshop (S. H. Anderson and D. B. Inkley, eds.). Wyoming Department of Game and Fish, Cheyenne.

HARRIS, R. B., AND F. W. ALLENDORF. 1989. Genetically effective population size of large mammals: an assessment of estimators. Conservation Biology 3:181–191.

HELLBORG, L., ET AL. 2002. Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. Conservation Genetics 3:97–111.

HUSBAND, B. C., AND S. C. H. BARRETT. 1992. Effective population size and genetic drift in *Tristylous eichhornia-paniculata* (Pontederiaceae). Evolution 46:1875–1890.

JORDE, P. E., AND N. RYMAN. 1996. Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. Genetics 143:1369–1381.

KAEUFFER, R., D. PONTIER, AND S. DEVILLARD. 2004. Effective size of two feral domestic cat populations (*Felis catus* L.): effect of the mating system. Molecular Ecology 13:483–490.

KAMLER, J. F., W. B. BALLARD, E. M. GESE, R. L. HARRISON, AND S. M KARKI. 2004. Dispersal characteristics of swift foxes. Canadian Journal of Zoology 82:1837–1842.

KIM, K. D. 2005. Invasive plants on disturbed Korean sand dunes. Estuarine Coastal Shelf Science 62:353–364.

KREEGER, T. J., A. VARGAS, G. E. PLUMB, AND E. T. THORNE. 1998. Ketamine–medetomidine or isoflurane immobilization of black-footed ferrets. Journal of Wildlife Management 62:654–662.

KYLE, C. J., C. S. DAVIS, AND C. STROBECK. 2000. Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest territories. Canadian Journal of Zoology 78:1150–1157.

LACY, R. C., AND T. W. CLARK. 1989. Genetic variability in black-footed ferret populations: past, present, and future. Pp. 83–103 in Conservation biology and the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut.

LARSON, S., R. JAMESON, J. BODKIN, M. STAEDLER, AND P. BENTZEN. 2002. Microsatellite DNA and mitochondrial DNA variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy 83:893–906.

LEBERG, P. 2005. Genetic approaches for estimating the effective size of populations. Journal of Wildlife Management 69:1385–1399.

LE PAGE, S. L., R. A. LIVERMORE, D. W. COOPER, AND A. C. TAYLOR. 2000. Genetic analysis of a documented population bottleneck: introduced Bennett's wallabies (*Macropus rufogriseus rufogriseus*) in New Zealand. Molecular Ecology 9:753–763.

LIVIERI, T. M. 2006. Ten-year history of the Conata Basin black-footed ferret population: 1996–2005. Prairie Wildlife Research, Wall, South Dakota.

LOCKHART, J. M., E. T. THORNE, AND D. R. GOBER. 2006. A historical perspective on recovery of the black-footed ferret and the biological and political challenges affecting its future. Pp. 6–19 in Recovery of the black-footed ferret: progress and continuing challenges (J. E. Roelle, B. J. Miller, J. L. Godbey, and D. E. Biggins, eds.). United States Geological Survey Scientific Investigations Report 2005—5293.

LUIKART, G., J.-M. CORNUET, AND F. W. ALLENDORF. 1999. Temporal changes in allele frequencies provide estimates of population bottleneck size. Conservation Biology 13:523–530.

MAUDET, C., ET AL. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in alpine ibex (*Capra ibex*). Molecular Ecology 11:421–436.

McDonald, D. B., D. Albertson, T. Livieri, and S. W. Buskirk. 2006. Demographic analysis of the black-footed ferret in the Conata Basin–Badlands National Park, South Dakota. Great Plains Cooperative Ecosystem Studies Unit, Report J1300040027/0001:1–15.

Michalakis, Y., and L. Excoffier. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142:1061–1064.

Miller, B., R. P. Reading, and S. Forrest. 1996. Prairie night black-footed ferrets and recovery of endangered species. Smithsonian Institution Press, Washington, D.C.

Nunney, L. 1996. The influence of variation in female fecundity on effective population size. Biological Journal of the Linnean Society 59:411–425.

O'Brien, S. J., and J. F. Evermann. 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends in Ecology & Evolution 3:254–259.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347–354.

Paetkau, D., et al. 1998. Variation in genetic diversity across the range of North American brown bears. Conservation Biology 12:418–429.

Pillay, N. 2002. Father–daughter recognition and inbreeding avoidance in the striped mouse, *Rhabdomys pumilio*. Mammalian Biology 67:212–218.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536–539.

Pollak, E. 1983. A new method for estimating the effective population size from allele frequency changes. Genetics 104:531–548.

Powell, R. A. 1979. Mustelid spacing patterns: variations on a theme by *Mustela*. Zeitschrift für Tierpsychologie 50:153–165.

Raymond, M., and F. Rousset. 1995. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

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

Richards, J. D., and J. Short. 2003. Reintroduction and establishment of the western barred bandicoot *Perameles bougainville* (Marupialia: Peramlidae) at Shark Bay, Western Australia. Biological Conservation 109:181–195.

Roelke, M. E., J. S. Martenson, and S. J. O'Brien. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology 3:340–350.

Rousset, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142:1357–1362.

Russell, W. C., E. T. Thorne, R. Oakleaf, and J. D. Ballou. 1994. The genetic basis of black-footed ferret reintroduction. Conservation Biology 8:263–266.

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

Stoneberg, R. 1996. Implanted microchips used to individually identify black-footed ferrets in Montana. Prairie Naturalist 28:163–168.

Storz, J. F., H. R. Bhat, and T. H. Kunz. 2001. Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. I. Inbreeding, outbreeding, and population subdivision. Evolution 55:1215–1223.

Stow, A. J., and P. Sunnucks. 2004. Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. Molecular Ecology 13:443–447.

Swanson, B. J., L. R. Peters, and C. J. Kyle. 2006. Demographic and genetic evaluation of an American marten reintroduction. Journal of Mammalogy 87:272–280.

Tallmon, D. A., G. Luikart, and M. A. Beaumont. 2004. Comparative evaluation of a new effective population size estimator based on approximate Bayesian computation. Genetics 167:977–988.

Taylor, A. C., et al. 1999. Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conservation Biology 13:531–541.

Thorne, E. T., and D. Belitsky. 1989. Captive propagation and the current status of free-ranging black-footed ferrets in Wyoming. Pp. 223–234 in Conservation biology and the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut.

Thorne, E. T., and E. S. Williams. 1988. Disease and endangered species: the black-footed ferret as a recent example. Conservation Biology 2:66–74.

Trudeau, K. M., H. B. Britten, and M. Restani. 2004. Sylvatic plague reduces genetic variability in black-tailed prairie dogs. Journal of Wildlife Diseases 40:205–211.

Uphyrkina, O., D. Miquelle, H. Quigley, C. Driscoll, and S. J. O'Brien. 2002. Conservation genetics of the Far Eastern leopard (*Panthera pardus orientalis*). Journal of Heredity 93:303–311.

Van Houtan, K. S., J. M. Halley, R. van Aarde, and S. L. Pimm. 2009. Achieving success with small, translocated mammal populations. Conservation Letters 2:254–262.

Wang, J. L. 2005. Estimation of effective population sizes from data on genetic markers. Philosophical Transactions of the Royal Society of London, B. Biological Sciences 360:1395–1409.

Wang, J. L., W. G. Hill, D. Charlesworth, and B. Charlesworth. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. Genetical Research 74:165–178.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379–391.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wilson, G. A., and C. Strobeck. 1999. Genetic variation within and relatedness among wood and plains bison populations. Genome 42:483–496.

Wisely, S. M., S. W. Buskirk, M. A. Fleming, D. B. McDonald, and E. A. Ostrander. 2002. Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. Journal of Heredity 93:231–237.

Wisely, S. M., D. B. McDonald, and S. W. Buskirk. 2003. Evaluation of the genetic management of the endangered black-footed ferret (*Mustela nigripes*). Zoo Biology 22:287–298.

Wisely, S. M., R. M. Santymire, T. M. Livieri, S. A. Mueting, and J. Howard. 2008. Genotypic and phenotypic consequences of reintroduction history: case study of the black-footed ferret. Conservation Genetics 9:389–399.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Wright, S. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago, Illinois.

*Special Feature Editor was Barbara H. Blake.*