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Genetic signature of disease epizootic and reintroduction history in an endangered carnivore

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Emerging infectious diseases have recently increased in wildlife and can result in population declines and the loss of genetic diversity in susceptible populations. As populations of impacted species decline, genetic diversity can be lost, with ramifications including reduced effective population size and increased population structuring. For species of conservation concern, which may already have low genetic diversity, the loss of genetic diversity can be especially important. To investigate the impacts of a novel pathogen on genetic diversity in a genetically depauperate endangered species, we assessed the ramifications of a sylvatic plague-induced bottleneck in black-footed ferrets (*Mustela nigripes*). Following a plague epizootic, we genotyped 184 ferrets from Conata Basin and Badlands National Park, South Dakota, at seven microsatellite loci. We compared our results to pre-plague studies in the same population. We observed population substructuring into three genetic clusters. These clusters reflect founder effects from ferret reintroduction events followed by genetic drift. Compared to the pre-plague population, we observed losses of allelic diversity in all clusters, as well as significantly reduced heterozygosity in one cluster. These results indicate that disease epizootics may reduce population size and also genetic diversity. Our results suggest the importance of early and sustained management in mitigating disease epizootics in naïve populations for the maintenance of genetic diversity.

Key words: conservation, disease management, effective population size, inbreeding, prairie dog, translocation, vaccination

Emerging infectious diseases (EID) in wildlife can lead to severe population declines in naïve host populations, which may have low disease resistance. Taxonomically diverse EID have recently increased in incidence by 1) spreading to novel host species, 2) spreading to novel geographic ranges, or 3) newly evolving (Tompkins et al. 2015). These mechanisms are typically enabled by human activity (Daszak et al. 2000; Tompkins et al. 2015). Due to their global impact, EID have been increasingly identified as a threat to biodiversity; however, the impacts can be most profound for small, localized populations (George et al. 2015; Adams et al. 2017; Marino et al. 2017). Disease epizootics, that is, events sporadically affecting large numbers of individuals with high morbidity, may cause sharp population declines, followed by chronic population depression (Huijbregts et al. 2003; Lazenby et al. 2018) and potentially local extinction (Martin et al. 2018).

Disease-induced population declines also may have important ramifications in terms of population genetics (McKnight et al. 2017). For species of conservation concern, populations often already are small and may have low genetic diversity. Low genetic diversity is associated with inbreeding and may result in the loss of loci affecting disease resistance, making these smaller populations more susceptible to disease than larger, more outbred populations (Spielman et al. 2004; Marden et al. 2017). EID may further reduce genetic diversity by causing population declines, creating a genetic bottleneck, and thereby increasing the rate of genetic drift and inbreeding. However, studies have shown conflicting results when evaluating inbreeding and loss of genetic diversity following disease epizootics (McKnight et al. 2017). For instance, some studies have shown clear genetic bottlenecks and inbreeding following a disease outbreak (i.e., Serieys et al. 2015), while others have failed to find evidence of bottleneck (i.e., Lachish et al. 2011) or inbreeding (i.e., Le Gouar et al. 2009). The impacts of an EID on genetic diversity may vary depending on the number of survivors, the duration of the outbreak, subsequent population expansion, and the extent of gene flow (McKnight et al. 2017).

To investigate the impact of novel pathogen introduction in systems with low genetic diversity, we focused on a well-studied, free-ranging population of black-footed ferrets (*Mustela nigripes*; hereafter, ferrets) located in Conata Basin, a portion of the Buffalo Gap National Grassland, administered

by the U.S. Forest Service, and the adjacent Badlands National Park, South Dakota (CB/BADL). All ferrets descend from 18 individuals captured in Meeteetse, Wyoming between 1985 and 1987, following a disease-induced population crash (Williams et al. 1988; U.S. Fish and Wildlife Service 2013). These individuals represented the last wild ferrets, and due to early deaths, high relatedness of surviving individuals, and the failure of some individuals to breed, the captive population descends from only seven founder equivalents (Lockhart et al. 2006). Genetic diversity therefore is a major management concern (Wisely et al. 2002). Based on microsatellite genotyping, breeding strategies have thus far been successful in maintaining overall allelic diversity and heterozygosity in the ex situ population, despite low baseline genetic diversity (Wisely et al. 2002, 2003).

Although more than 4,000 ferrets have been released at 30 sites to reestablish in situ populations (Biggins 2012), CB/BADL is considered one of only four currently self-sustaining release sites (U.S. Fish and Wildlife Service 2013). One of the primary obstacles to the reestablishment of ferret populations is sylvatic plague (caused by the bacterium *Yersinia pestis*), a non-native disease introduced to the United States in 1900 through San Francisco ports (Link 1955; U.S. Fish and Wildlife Service 2013). While plague in human settlements is associated with commensal rats (*Rattus* spp.), especially the black rat (*R. rattus*), nearly every rodent species in western North America has tested positive for plague (Antolin et al. 2002). The broad host range of plague has resulted in its spread eastward to the 100th meridian, throughout the range of most prairie dog (*Cynomys* spp.) colonies in North America. This is significant for two reasons. First, ferrets are reliant on prairie dogs for most of their diet, making prairie dog colonies the only viable habitat for ferrets. Second, among small mammals, prairie dogs are particularly susceptible to plague, leading to explosive outbreaks with high mortality and rapid spread, including spillover to other species, such as ferrets (Antolin et al. 2002). Unlike most closely related mustelid species (e.g., domestic ferrets [*M. putorius furo*] and Siberian polecats [*M. eversmannii*]), ferrets show no immunity against plague infection (Williams et al. 1991), most likely as a result of historic bottlenecks and low genetic diversity (O'Brien and Evermann 1988; Williams et al. 1994). Godbey et al. (2006) found 90% mortality in ferrets that consumed infected prairie dogs. Ferret survival in plague-impacted regions therefore is dependent on management actions such as dusting prairie dog colonies with deltamethrin to reduce fleas (vector) and vaccinating ferrets against plague (Matchett et al. 2010).

The reintroduction sites at CB/BADL were plague-free at the time of initial reintroduction in 1996, allowing the population to experience rapid growth and become self-sustaining; there have been no subsequent reintroductions from the ex situ colony since 1999 (Wisely et al. 2008). The absence of plague in this region prior to 2008 provides a unique opportunity to evaluate the impacts of a newly introduced pathogen on genetic diversity in an already genetically depauperate population. Following the detection of plague in the region, the total ferret population rapidly declined from over 300 individuals in 2007 to below 50 individuals in 2013. The population has since slowly increased to over 100 individuals.

Two studies have examined genetic diversity in this population, both prior to the plague-induced population crash. Wisely et al. (2003) genotyped 194 ferrets sampled in 1997–1999, during which time the population was still receiving reinforcements from the ex situ colony. Cain et al. (2011) genotyped 254 ferrets sampled in 2001–2003, following cessation of captive reinforcement. Both studies relied on data and samples collected during the course of yearly population monitoring and management and found that allelic diversity and heterozygosity were maintained in the wild at levels comparable to the ex situ colonies. These studies provide a baseline of genetic diversity prior to the disease outbreak, a comparative point that has been lacking in many studies of the impacts of disease on genetic diversity (McKnight et al. 2017). Yearly management continued after the last study, including population monitoring and genetic sampling. Management also has included, since 2008, dusting prairie dog colonies with deltamethrin to control flea populations and vaccinating ferrets against plague to improve ferret survival during plague epizootics (Matchett et al. 2010; Livieri 2011).

The long-term monitoring and management of reintroduced ferrets in CB/BADL provides the opportunity to examine the impacts of a plague-induced population decline on genetic diversity. Here, we used seven microsatellite loci to genotype post-plague ferrets and compared our findings to pre-plague genetic samples to evaluate the impacts of disease-associated population decline on effective population size and genetic diversity. We also evaluated how the population decline and reintroduction history of these ferrets may have influenced population structure, isolation, and differentiation. Because ferrets in this population already had a low effective population size prior to the outbreak of plague (Cain et al. 2011), we expected that plague-induced mortality would lead to increased declines in genetic diversity in terms of both number of alleles and heterozygosity.

Materials and Methods

Sample collection.—Samples were collected at CB/BADL in 2014–2016 (Fig. 1). Ferrets in the area are found on six prairie dog subcomplexes (small, compact areas of prairie dog colonies within a larger group of colonies—Biggins et al. 2006a): Heck Table, Sage Creek, Agate, Prairie Wind, Steer Pasture, and Roberts (Fig. 1; Table 1). Ferrets are not distributed evenly in the complexes, but we sampled almost all known ferrets in the population as part of annual monitoring and vaccination programs (Table 1). The ferret population is monitored annually using spotlighting surveys to assess demography, determine spatial distribution, collect data, gather samples for research, vaccinate against disease, and provide animals for translocation (Black-Footed Ferret Recovery Implementation Team 2016). Surveys begin in August to coincide with the time at which young ferrets become sufficiently active above ground

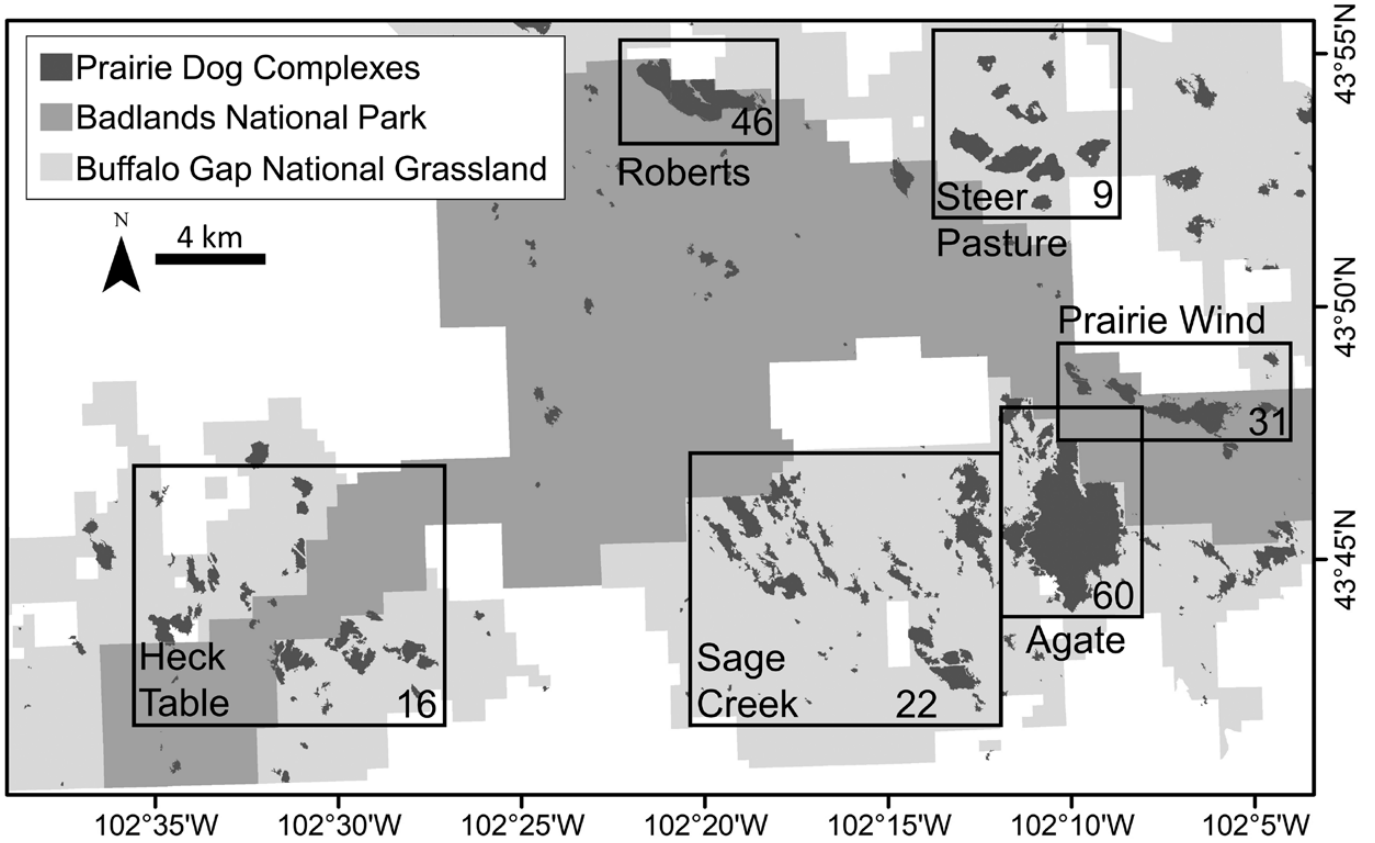

Fig. 1.—The 2017 borders of six prairie dog complexes within Badlands National Park and Conata Basin (part of the Buffalo Gap National Grassland), South Dakota where black-footed ferret sampling occurred. Complexes reflect management units assigned based on grazing allotments. Numbers in each box indicate the number of ferrets sampled at each location.

Table 1.—Establishment history of black-footed ferret populations at prairie dog subcomplexes in Badlands National Park and Conata Basin, South Dakota. The first year of population introduction and the source population of ferrets is noted for each subcomplex, where known. The number of ferrets sampled from each complex is noted for the pre-plague period (Cain et al. 2011) and for the post-plague period.

Subcomplex	Established	Source	Pre-plague	Post-plague
Agate	1996	Captive	207 (AG + SC)	60
Sage Creek	1997	Captive		22
Heck Table	1998	Captive/Sage Creek	47	16
Roberts	2004	Agate		46
Prairie Wind		Natural movement		31
Steer Pasture		Natural movement		9

for capture and marking (Biggins et al. 2006b). Spotlighting occurs between sunset and sunrise and continues in the same area for three to four consecutive nights with most areas surveyed twice each year.

Managers livetrap and anesthetize ferrets annually for microchip implantation to aid population counts, vaccination, and sampling (Black-Footed Ferret Recovery Implementation Team 2016). While ferrets were anesthetized, researchers collected hair and buccal samples. Buccal swabs were stored in 70–80% alcohol at 4°C; hair samples were stored in envelopes at −20°C or dried. After recovery from anesthesia, ferrets were released at the capture location. All animal handling protocols followed the guidelines of the American Society of Mammologists (Sikes et al. 2016) and the U.S. Fish and Wildlife Service (Black-Footed Ferret Recovery Implementation Team 2016). DNA was extracted from hair and buccal swabs using the Macherey-Nagel Nucleospin Tissue Kit, following published protocols (Machery-Nagel, Düren, Germany).

Microsatellite genotyping.—We genotyped 184 ferrets using seven microsatellite loci: Mvis002, Mvis022, Mvis072, and Mer095 (Fleming et al. 1999); Mvis9700 and Mer049 (Wisely et al. 2002); and Gg-14 (Davis and Strobeck 1998). These loci are the only previously tested, polymorphic loci available for ferrets (Wisely et al. 2003; Cain et al. 2011). We followed established polymerase chain reaction (PCR) protocols (Cain et al. 2011), although for reactions using primer pairs for locus Mer095, we replaced H_2O with 0.5 mg/ml of bovine serum albumin (BSA) to improve amplification. PCR products were sized on an ABI model 3730 DNA Analyzer. Individual genotypes were assigned using Genemapper Software version 4.0

(Applied Biosystems, Foster City, California). For quality control purposes, we independently reamplified 20% of all samples to ensure that genotyping error rates were below 1%. We used the online population genetics software package Genepop (web v. 4.2—Raymond and Rousset 1995; Rousset 2008) to test for deviations from Hardy–Weinberg equilibrium (HWE) and genotypic linkage. In both cases, we applied a Bonferroni-corrected alpha for multiple tests.

Population structure.—We used the Bayesian program Structure (Pritchard et al. 2000) to identify genetic clusters of ferrets located in different prairie dog complexes, with all years combined. We conducted five runs of K = 1–12, allowing for population structure within each colony. The model ran with 250,000 Markov Chain Monte Carlo (MCMC) repetitions following a burn-in period of 100,000 under the admixture model with correlated allele frequencies. The model was assumed to have reached convergence as the alpha values ranged within 0.2 (Pritchard et al. 2000; Gilbert et al. 2012). We determined the optimal number of clusters in Structure Harvester (Earl and vonHoldt 2012) using both maximum likelihood (Pritchard et al. 2010) and Evanno's ΔK (Evanno et al. 2005). We visualized Q-plots of all clusters of K using CLUMPAK (Kopelman et al. 2015).

Individuals for which Structure assigned less than 75% of their genotype to any given cluster were assigned or excluded from clusters using GeneClass2 (Piry et al. 2004). We included all loci and used the Bayesian computation method of Rannala and Mountain (1997), as this method has been shown to outperform other frequency-based methods and distance-based methods (Cornuet et al. 1999). We enabled Monte Carlo resampling, also under the Rannala and Mountain (1997) algorithm, with 10,000 simulated individuals and an alpha of 0.05 and used the default assignment threshold score of 0.05.

We calculated the degree of population differentiation between colonies using F_{ST} (Wright 1969) and Jost's D (Jost 2008). While F_{ST} has been most commonly used in past research, using multiple combined statistics provides a more robust analysis of population structure (Meirmans and Hedrick 2011). Differentiation statistics were calculated for the overall population and for pairwise clusters in the R environment (v 3.4.0—R Development Core Team 2017) using the "fastDivPart" function in the diveRsity package (Keenan et al. 2013). We calculated significance using 2,000 bootstraps to calculate 95% confidence intervals.

Genetic diversity.—For each cluster identified by Structure, we calculated 1) allelic richness, 2) inbreeding coefficients (F_{IS}), and 3) multilocus heterozygosity (MLH). We calculated allelic richness and F_{IS} using the "basicStats" function in diveRsity. We calculated MLH for each individual in the population using the "MLH" function in the inbreedR package (Stoffel et al. 2016) in R (v. 3.5.2). Because Cain et al. (2011) calculated heterozygosity per locus, we obtained the raw genotypes from their study to calculate MLH. Raw genotypes for locus Mer059 were not available, so we compared heterozygosity using only the six matching loci (Gg-14, Mer049, Mvis002, Mvis022, Mvis072, and Mvis9700). Cain et al. (2011) also only collected samples at Agate, Sage Creek, and Heck Table, the only existing populations at the time, treating them as two population groups: Agate and Sage Creek were grouped together (hereafter, AGSC-03) separately from Heck Table (hereafter, HT-03). These were the only prairie dog subcomplexes with ferret populations at the time of their study (Table 1). We compared individual MLH scores across the clusters identified by Structure and the population groups used by Cain et al. (2011) using a Kruskal–Wallis one-way analysis of variance, executed in R. We followed the Kruskal–Wallis test with a pairwise Wilcoxon signed-rank test in order to identify which populations differed specifically from others.

Effective population size.—We calculated the effective population size, N_e, for each genetic cluster using the LDNe method (Waples 2006), as implemented in the program NeEstimator v. 2.1 (Do et al. 2014). The LDNe method calculates N_e based on linkage disequilibrium (Hill 1981) and has been shown to perform well and consistently in both isolated populations and those experiencing low levels of migration (Gilbert and Whitlock 2015). We applied the model for random mating and used the lowest allele frequency of 0.02. This accounts for all present alleles given that all loci used in this study had only two alleles. We used jackknife 95% confidence intervals.

Results

Microsatellite data.—After Bonferroni correction, there was evidence of linkage disequilibrium between four pairs of loci (Supplementary Data SD1). In addition, after Bonferroni correction, three loci were out of HWE (see Supplementary Data SD2). Despite this, we included all loci in the analyses because the deviations from linkage equilibrium and HWE were not consistent with previous studies (Wisely et al. 2003; Cain et al. 2011). In addition, the decline in population size over the last decade, accompanied by the relatively low effective population sizes calculated by Cain et al. (2011), indicates that genetic drift and inbreeding may play a strong role in this population, leading to expected deviations from HWE (Kimura 1983).

Population structure.—Our results from Structure, according to both Pritchard's suggested method and Evanno's ΔK, supported K = 3 populations (Fig. 2). Cluster 1 primarily includes individuals from Agate (hereafter referred to as A-16); cluster 2 includes individuals from Heck Table, Prairie Wind, Sage Creek, and Steer Pasture (hereafter Mix-16); and cluster 3 includes most individuals from Roberts, as well as some individuals from Heck Table, Prairie Wind, Sage Creek, and Steer Pasture (hereafter R-16). All three clusters included individuals from all three sampled years.

Of 184 individuals, 60 ferrets (33.1%) had less than 75% of their genotype assigned to a single cluster by Structure. We evaluated the probability that each of the 60 ferrets belonged to each of the three genetic clusters using GeneClass2 (Supplementary Data SD3). Twelve individuals were assigned exclusively to A-16, 17 to Mix-16, and three to R-16. All ferrets that were assigned uniquely to a specific cluster were

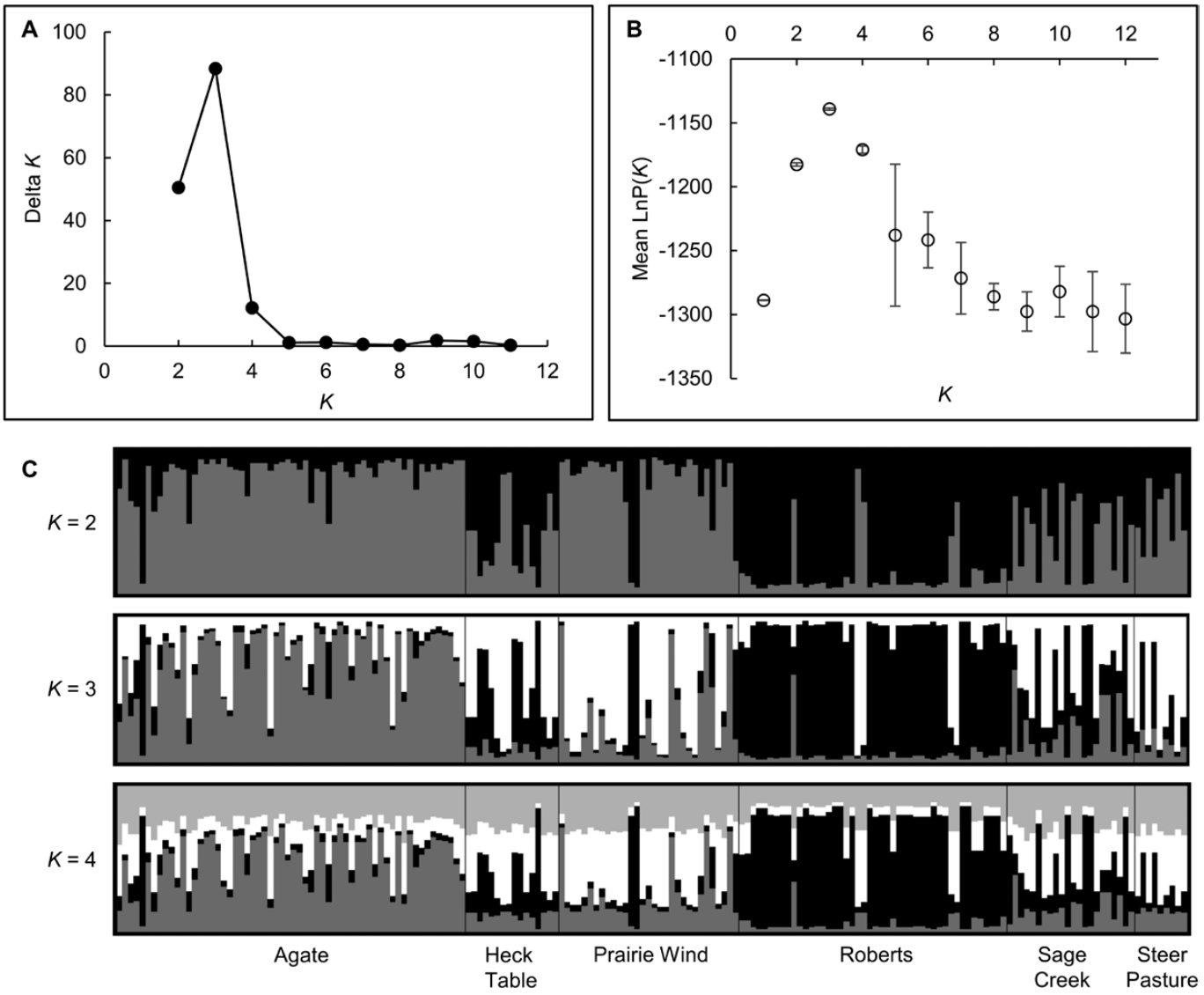

Fig. 2.—Results of Bayesian genetic cluster analysis from program Structure for black-footed ferrets (*Mustela nigripes*) at six prairie dog complexes in Conata Basin and Badlands National Park, South Dakota. A) Graph of Evanno's Δ*K* for each value of *K* = 1–12. B) Maximum likelihood for each value of *K* = 1–12. Error bars represent standard deviation around the mean. C) *Q*-plots of genetic clusters assigned by structure for *K* = 2–4. Grayscale represents unique genetic clusters, and vertical bars represent the proportion of each cluster represented in the genotype of an individual ferret. Sampling locations are noted below the *Q*-plot for *K* = 4 and are separated in each *Q*-plot by the thin vertical lines.

sampled from locations associated with those specific clusters. Seventeen individuals could not be excluded from two of the three genetic clusters. We assigned these individuals to the cluster to which they had a higher probability of belonging, the same cluster from which they were sampled for all individuals, for subsequent analyses of population differentiation and inbreeding. Eleven additional ferrets were excluded from all three genetic clusters. These individuals were excluded from subsequent analyses of the genetic clusters identified by Structure.

Across the three genetic clusters, overall F_{ST} = 0.329. For the overall population, Jost's *D* = 0.072. Pairwise F_{ST} and Jost's *D* values suggest contradictory patterns of pairwise differentiation between colonies (Table 2). With F_{ST}, A-16 and R-16 are the most strongly differentiated clusters; however, Jost's *D* suggests that these two colonies are the least strongly differentiated. Instead, A-16 and Mix-16 are more strongly differentiated.

Table 2.—Genetic differentiation measures, with 95% confidence intervals, for the three genetic clusters of black-footed ferrets at Badlands National Park and Conata Basin. Clusters were identified by Structure and are described in Table 3 and Fig. 2. F_{ST} is shown below the diagonal, while Jost's *D* is shown above the diagonal.

	A-16	R-16	Mix-16
A-16	—	0.043 (0.031–0.058)	0.055 (0.039–0.077)
R-16	0.391 (0.349–0.435)	—	0.049 (0.032–0.068)
Mix-16	0.274 (0.230–0.321)	0.328 (0.277–0.382)	—

Genetic diversity and effective population size.—Each locus we examined only had two alleles, consistent with previous

Table 3.—Genetic diversity of black-footed ferrets. Groups consist of black-footed ferret populations residing at prairie dog subcomplexes in Badlands National Park and Conata Basin, South Dakota. AGSC-03 and HT-03 are pre-plague groupings used by Cain et al. (2011), while the remaining groups are genetic clusters assigned by Structure in the post-plague population. Measures included are N = samples size, A_r = average allelic richness, mean MLH = average multilocus heterozygosity calculated with six and seven loci, N_e = effective population size, and F_{IS} = inbreeding. MLH is presented with standard deviation, while N_e and F_{IS} are presented with 95% confidence intervals. The asterisk indicates a significant difference in MLH at R-16, according to a pairwise Wilcoxon signed-rank test.

Group	Subcomplexes	N	A_r	MLH mean (six loci)	MLH mean (seven loci)	N_e	F_{IS}
AGSC-03	Agate, Sage Creek	207	2	0.38 (0.199)		10.6 (2.2–18.9)	
HT-03	Heck Table	47	2	0.386 (0.194)		2 (0–3.6)	
A-16	Agate	55	1.86	0.321 (0.143)	0.278 (0.127)	8.9 (2.2–31.8)	–0.003 (–0.103 to 0.104)
R-16	Roberts	60	1.71	0.158 (0.117)*	0.163 (0.126)	17.7 (4.4–65)	–0.05 (–0.115 to 0.018)
Mix-16	Sage Creek, Heck Table, Prairie Wind, Steer Pasture	58	1.86	0.341 (0.170)	0.321 (0.158)	14.6 (3.3–56.1)	–0.086 (–0.174 to –0.012)

studies in this population (Wisely et al. 2003; Cain et al. 2011). However, not all alleles were present in each of the genetic clusters identified by Structure (Table 3; see Supplementary Data SD4). Observed heterozygosity values for the overall population had generally declined at each locus compared with pre-plague levels (Supplementary Data SD2). MLH for the three genetic clusters assigned by Structure ranged from 0.163 to 0.321 (Table 3). A Kruskal–Wallis exact test indicated differences in the means across genetic clusters and pre-plague populations (χ^2_{10}= 29.245, P = 0.001). Pairwise comparisons of each group indicated that the mean MLH of the R-16 cluster differed from every other group (Supplementary Data SD5). Inbreeding levels (F_{IS}) overlapped zero for the AG-16 and R-16 clusters. However, the Mix-16 colony showed evidence of slight outbreeding (Table 3). N_e was between 8.9 and 17.7 for the three genetic clusters (Table 3).

Discussion

Population structure.—Our results indicated the presence of three genetic clusters, one consisting primarily of individuals from the Agate subcomplex in Conata Basin, another consisting primarily of individuals from the Roberts subcomplex in Badlands National Park, and a final cluster made up of the four remaining smaller subcomplexes. These genetic clusters are likely more the result of the complex reintroduction history rather than the disease history of black-footed ferrets in Conata Basin and Badlands National Park. Because of the nature of reintroduction from the ex situ colonies, which prioritizes the release of genetically "expendable" or overrepresented individuals, the genetic makeup of released kits may shift from year to year (Russell et al. 1994). This can result in founder effects in which each colony consists of individuals with only a subset of the available genetic material. Reintroduction occurred in Conata Basin first in Agate and then in Sage Creek and Heck Table in subsequent years from 1996 to 1999. The Heck Table subcomplex was initiated using in situ animals via translocation from Sage Creek (Biggins et al. 2011) and animals from the ex situ population. Additional translocations occurred between the three colonies from 2000 to 2003 (Cain et al. 2011). The common shared lineage of ferrets in Heck Table with those in Sage Creek helps explain their placement within the same genetic cluster, separate from those in Agate and Roberts colonies. In addition, despite their close proximity, Sage Creek experienced a more severe population decline than that at Agate during the 2008 plague outbreak. This likely created a founder event in the Sage Creek complex, which contributed to its differentiation from Agate through genetic drift.

The population in Roberts was established with ferrets sourced from Agate in 2004, giving them a shared lineage. However, the two colonies have become differentiated into two unique genetic clusters over time. Roberts colony has lower average allelic richness and significantly lower MLH than either of the other two genetic clusters. This suggests that the limited genetic diversity in the small founder population used to establish Roberts, followed by random genetic drift, has resulted in differentiation from the Agate cluster. The population structuring patterns observed here are somewhat similar to patterns of differentiation for three ferret reintroduction sites that began to genetically differentiate from the ex situ colony and from each other relatively quickly (Wisely et al. 2008). While patterns of differentiation previously have not been studied within ferret reintroduction sites, local differentiation has been observed for American martens (*Martes americana*) in reintroduced populations with low connectivity (Grauer et al. 2017).

The separation of ferrets into three genetic clusters is supported by high F_{ST} values among all three genetic clusters. On the other hand, values for Jost's D show significant, but low, differentiation. The discrepancy between Jost's D and F_{ST} is not unexpected as Jost's D is highly sensitive to mutation rates, which are apparently quite low for these biallelic loci (Kronholm et al. 2010). Jost's D also is biased by very low migration rates, and both our calculated values for F_{ST} and Jost's D are in line with simulated values for populations with low migration rates (Kronholm et al. 2010). While both measures confirm differentiation between the three genetic clusters, they do not show identical patterns of differentiation in pairwise comparisons among clusters. These differences may be explained by the differences in how the differentiation statistics are calculated. While F_{ST} relies on expected heterozygosity, Jost's D is based on effective number of alleles (Meirmans and Hedrick 2011). The presence of alleles at different frequencies in each

cluster, accompanied by the complete absence of alleles from some clusters, therefore could yield differing results for the two statistics.

The low migration rates suggested by genetic differentiation statistics reflect known movement data for the CB/BADL ferrets. Known natural movement is very low, with only four observed instances of a ferret independently relocating to another prairie dog subcomplex occurring during the study period (Supplementary Data SD6). Even between Agate and Sage Creek, which are adjacent on the landscape (Fig. 1), movement is rare. This may be explained by the high habitat quality in Agate, as well as the presence of a dirt road separating the two areas from each other. In 2015, 12 additional ferrets were translocated by population managers (Supplementary Data SD6). Many of these movements occurred between Heck Table, Sage Creek, and Steer Pasture, which may explain further their grouping within the same genetic cluster. Other translocations, such as those between Roberts and Prairie Wind, can be observed in our Structure analysis as individuals from one genetic cluster appearing at a different location, and in the signature of admixed individuals. In other systems, translocations have been shown to reduce genetic differentiation between populations (Zimmerman et al. 2019), suggesting that increasing the frequency and number of translocations in CB/BADL might reduce some of the observed population structuring.

Genetic diversity.—Despite population declines resulting from sylvatic plague, ferrets within the CB/BADL metapopulation have retained all alleles present at the time of reintroduction. However, all alleles are not found in all genetic clusters. A-16 and Mix-16 are missing an allele at a single locus, while R-16 is missing alleles at two loci. Cain et al. (2011) suggested that translocations and natural movement between populations could be partially responsible for the maintenance of alleles within CB/BADL following reintroduction. Translocations essentially create a metapopulation, in which genetic diversity is shared among previously disconnected locations. Human-mediated translocations have been shown to counteract genetic drift by significantly increasing both allelic richness and heterozygosity (Tensen et al. 2019; Zimmerman et al. 2019).

The loss of two alleles in cluster R-16 has contributed to the significantly lower MLH in this cluster. This was the only cluster that experienced a significant loss of genetic diversity when compared to the two pre-plague population groups studied by Cain et al. (2011). As discussed above, the low genetic diversity in the Roberts cluster may result from the colony's reintroduction history, rather than from population decline following the sylvatic plague outbreak. However, the loss of alleles within all three clusters likely is the result of genetic drift in the small populations following the plague epizootic. Other studies of wildlife populations pre- and post-disease outbreak have observed varying effects, with some studies reporting a loss of allelic diversity, as well as significant declines in heterozygosity (Serieys et al. 2015; Hudson et al. 2016), and others suggesting conservation of genetic diversity (Le Gouar et al. 2009).

While microsatellites, such as those used in this study, generally are selectively neutral, they still can serve as a proxy for genome-wide diversity. Inbred individuals are likely to have higher genome-wide homozygosity relative to less inbred individuals (Coltman and Slate 2003). Correlations between heterozygosity and fitness therefore exist that can be informative about inbreeding depression (Hedrick and Garcia-Dorado 2016). Previous studies of black-footed ferrets found a 30% reduction in heterozygosity, with accompanying loss of allelic richness, during the establishment of the ex situ colony from the last wild population (Wisely et al. 2002). In that case, there was no apparent reduction of fitness in terms of litter size or juvenile survival (Wisely et al. 2002). Whether the absence of apparent negative effects of increased homozygosity was due to already lowered fitness following historic population isolation and bottleneck, or resulted from the purging of deleterious alleles, is unknown. Given the relationship between heterozygosity and population fitness, decreased heterozygosity that we observed in the Roberts cluster, as well as the general loss of alleles, could be a concern for future genetic management of this population.

Declines in heterozygosity generally can be expected in small, inbred populations. While our results do not currently show significant inbreeding in any of the genetic clusters, pedigree-based estimates showed inbreeding for both released (0.09) and nonreleased (0.05) individuals during the early phases of the ferret reintroduction program (Russell et al. 1994) and in the current ex situ population (0.13—Santymire et al. 2019). The absence of an inbreeding signature here therefore may be the result of genotyping based on a small number of biallelic loci.

Effective population size.—Future declines in heterozygosity and allelic diversity are likely to occur in all three genetic clusters as the current effective population sizes are between 8.9 and 17.7 individuals. These values are below the suggested $N_e \geq 100$ required to limit the loss of total fitness to < 10% for five generations, and are well below the level of $N_e \geq 1{,}000$ required to preserve evolutionary potential for fitness in perpetuity (Frankham et al. 2014). While the current species recovery plan recognizes the importance of maintaining genetic diversity, it does not consider effective population size recommendations (U.S. Fish and Wildlife Service 2013). Because N_e is below recommended levels, the goal of 80% maintenance of genetic diversity over time may be difficult to achieve in wild ferret populations without continued introductions from the ex situ program. These results reinforce the importance of maintaining the ex situ colonies of ferrets, and other species threatened by disease (i.e., Tasmanian devils—Hogg et al. 2017) as a reservoir for genetic diversity in the face of future declines.

Limitations of this study.—We employed only seven microsatellites for this study. At the time of this study, these were the only genetic evaluation tools available for black-footed ferrets. Microsatellite markers often are used for population genetic studies (Putman and Carbone 2014), even with the increasing utility and advantages of single-nucleotide

polymorphisms (SNPs). Importantly, microsatellite-based genetic diversity measures such as heterozygosity and allelic richness do correlate with genome-wide diversity (Väli et al. 2008; Ljungqvist et al. 2010; Fischer et al. 2017; Lemopoulos et al. 2019). In addition, microsatellites can differentiate similar population structure patterns as those detected by SNPs (Lemopoulos et al. 2019). An important caveat is that the use of large numbers of SNPs (thousands) can provide higher resolution of population structure while more accurately reflecting whole-genome diversity. Even so, we believe that the use of previously developed microsatellites is most appropriate for this study because it provides the opportunity for comparisons to previously collected data from the same population prior to the disease outbreak. Such comparisons are lacking in the disease ecology literature (McKnight et al. 2017).

Conservation implications.—In general, bottlenecks caused by disease epizootics may lead to declines in genetic diversity (e.g., Serieys et al. 2015), which may reduce the capacity of the population to respond to environmental change. In a plague-impacted population of reintroduced ferrets, we observed the loss of alleles across all genetic clusters. The survival of this population, and therefore the preservation of any level of genetic diversity, likely is attributed to the intervention of population managers to begin dusting plague-infected prairie dog colonies with deltamethrin. This early intervention reduced the spread of plague to adjacent prairie dog colonies and has been maintained and augmented with vaccination of ferrets against plague (Livieri 2011). Our study shows that early and sustained intervention can be pivotal in maintaining genetic diversity through disease events. Vaccination against EID has been utilized for other endangered carnivores with limited range sizes (e.g., island foxes—Coonan et al. 2014), and has been suggested as a strategy for other species with high disease risk (e.g., Amur tigers—Seimon et al. 2013; Tasmanian devils—Tovar et al. 2018). The loss of alleles from the genetic clusters in this study highlights the importance of maintaining population levels through disease outbreaks. For highly susceptible populations similar to black-footed ferrets, vaccination programs could be useful not only in preserving populations, but also in preserving genetic diversity in these small populations.

Genetic diversity also may be preserved, especially in populations with significant structuring, by increasing connectivity and gene flow through translocation. This tactic commonly is used by conservation practitioners and has been shown to successfully help maintain genetic diversity (Tensen et al. 2019; Zimmerman et al. 2019). However, the need for genetic connectivity must be balanced with the risk of disease spread (Perzanowski et al. 2020). For black-footed ferrets, the high levels of population management and vaccination make translocation a safe prospect. An increased understanding of the impacts of disease in endangered populations has substantial conservation implications given the increase in EID in wildlife populations and the potential impacts on population genetics (Daszak et al. 2000; McKnight et al. 2017).

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Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Tests for linkage disequilibrium between seven black-footed ferret loci. An asterisk indicates significant deviation from linkage equilibrium after Bonferroni correction ($\alpha = 0.002$).

Supplementary Data SD2.—Locus parameters for seven microsatellite loci used to genotype black-footed ferrets (*Mustela nigripes*) from Conata Basin and Badlands National Park, South Dakota. Parameters include number of alleles (*A*), observed heterozygosity (H_o), and expected heterozygosity (H_e). HWE*P* indicates the *P*-value of test for Hardy–Weinberg equilibrium (HWE) at each locus. An asterisk indicates significant deviation from HWE after Bonferroni correction ($\alpha = 0.007$). Reinforcement period measurements are from 194 ferrets sampled in 1997–1999 by Wisely et al. (2003); pre-plague estimates are from 254 ferrets sampled in 2001–2003 by Cain et al. (2011); and post-plague estimates are from the 184 ferrets sampled in 2014–2016 genotyped in this study. Data for Mer095 during the pre-plague period is unavailable.

Supplementary Data SD3.—Assignment probabilities calculated in GeneClass2 for black-footed ferrets (*Mustela nigripes*) that had less than 75% of their genotype assigned to any single genetic cluster identified in STRUCTURE. Numbers shown in bold are above the assignment threshold for the indicated cluster. Where a ferret could be assigned to two clusters, the cluster with the greater assignment probability was utilized for subsequent analysis and is indicated with italics. Eleven ferrets were excluded from all three genetic clusters and are shaded in light gray. An asterisk indicates that the ferret moved during the period of study via either natural movement or translocation.

Supplementary Data SD4.—Allelic richness of each locus for genetic clusters assigned by Structure.

Supplementary Data SD5.—*P*-values for pairwise comparisons of mean multilocus heterozygosity (MLH) for genetic clusters identified in Structure and pre-plague population groups. A Kruskal–Wallis test indicated significant differences

in the means across the five groups (χ^2_{10} = 29.245, P = 0.001). Pairwise comparisons were completed using a Wilcoxon signed-rank test. An asterisk indicates significant difference of the mean MLH between two groups.

Supplementary Data SD6.—Known movements of black-footed ferrets between prairie dog complexes during the 2014–2016 study period. A natural movement is defined as an animal moving itself from one subcomplex to another subcomplex. These movements are based on fall spotlighting surveys; animals may move additionally throughout the year. Translocations are undertaken by managers to move an individual from one subcomplex to another for population augmentation purposes. Animals indicated with an asterisk were known to have survived the move to a new colony, thus potentially having offspring and contributing to the genetic mixing observed in this study. Ferret 15-016, indicated by a carrot, was translocated from STP to SC, but then naturally moved to AG.

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