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Authors: Trudeau, Kristie M., Britten, Hugh B., and Restani, Marco

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SYLVATIC PLAGUE REDUCES GENETIC VARIABILITY IN BLACK-TAILED PRAIRIE DOGS

Kristie M. Trudeau,[1] Hugh B. Britten,[1,3] and Marco Restani[2]

[1] Department of Biology, University of South Dakota, Vermillion, South Dakota 57069, USA
[2] Department of Biological Sciences, St. Cloud State University, St. Cloud, Minnesota 56301, USA
[3] Corresponding author (email: hbritten@usd.edu)

ABSTRACT: Small, isolated populations are vulnerable to loss of genetic diversity through inbreeding and genetic drift. Sylvatic plague due to infection by the bacterium *Yersinia pestis* caused an epizootic in the early 1990s resulting in declines and extirpations of many black-tailed prairie dog (*Cynomys ludovicianus*) colonies in north-central Montana, USA. Plague-induced population bottlenecks may contribute to significant reductions in genetic variability. In contrast, gene flow maintains genetic variability within colonies. We investigated the impacts of the plague epizootic and distance to nearest colony on levels of genetic variability in six prairie dog colonies sampled between June 1999 and July 2001 using 24 variable randomly amplified polymorphic DNA (RAPD) markers. Number of effective alleles per locus (n_e) and gene diversity (h) were significantly decreased in the three colonies affected by plague that were recovering from the resulting bottlenecks compared with the three colonies that did not experience plague. Genetic variability was not significantly affected by geographic distance between colonies. The majority of variance in gene frequencies was found within prairie dog colonies. Conservation of genetic variability in black-tailed prairie dogs will require the preservation of both large and small colony complexes and the gene flow among them.

Key words: Black-tailed prairie dog, gene diversity, heterozygosity, plague, population genetic structure, RAPD-PCR, *Yersinia pestis*.

INTRODUCTION

The role of genetic diversity in the persistence of populations and species is well understood on theoretical grounds (e.g., Frankel and Soulé, 1981; Frankham et al., 2002). Genetic diversity at loci that are currently or potentially subject to natural selection provides genetic capital for future adaptation. Prominent examples of species or populations in decline, at least in part as the result of low levels of genetic diversity, include several felids (O'Brien et al., 1996) and the Illinois population of the greater prairie chicken (*Tympanuchus cupido pinnatus*; Westemeier et al., 1998). These taxa expressed numerous deleterious effects, including deformed sperm, increased susceptibility to disease, and reduced reproductive output, which resulted in declining fitness with concomitant reductions in population performance. The main drivers of the loss of genetic variability are inbreeding and genetic drift. There is a direct negative relationship between these two population genetic phenomena and population size. Disease may play a role in decreasing population size to the point where genetic diversity is lost (O'Brien and Evermann, 1988; Seddon and Baverstock, 1999).

Sylvatic plague, caused by the bacterium *Yersinia pestis*, was introduced to North America from Asia approximately 100 years ago. Plague is a generalist bacterium spread by fleas or direct contact and is now found in over 200 species of mammals (Pollitzer and Meyer, 1961; Poland and Barnes, 1979; Biggens and Kosoy, 2001; Parkhill et al., 2001). In North America, the significant and widespread declines of black-tailed prairie dogs (*Cynomys ludovicianus*), which prompted candidate listing under the Endangered Species Act, are attributed, in part, to the devastating effects of plague (Gober, 2000). Sylvatic plague is the only disease known to cause widespread fatalities of black-tailed prairie dogs, and no evidence has been found confirming resistance (Barnes, 1993; Cully and Williams, 2001). High-density communal living and clumped distributions of prairie dog colonies favor the spread of

plague. Plague epizootics lead to substantial reductions in colony size (often 99%), local extirpations, and increased isolation among surviving colonies (Biggens and Kosoy, 2001; Cully and Williams, 2001; Lomolino and Smith, 2001).

Population bottlenecks caused by sylvatic plague are expected to decrease heterozygosity and increase the loss of alleles in affected prairie dog colonies (Hartl and Clark, 1997; Frankham et al., 2002). Moreover, recovering colonies, particularly those that are small and isolated, are subject to genetic drift and inbreeding, processes that contribute further to the loss of variability. However, gene flow via migration among recovering colonies can potentially restore genetic variability lost during bottlenecks. Sylvatic plague epizootics provide a unique opportunity to test the relative importance of genetic drift, inbreeding, and migration in prairie dogs.

Plague greatly reduced the range of black-tailed prairie dogs in Phillips County, Montana, USA, during the early 1990s (FaunaWest Wildlife Consultants, 1999; Gober, 2000; Potera, 2000). Occupied range of prairie dogs decreased by 45%, from approximately 10,600 to 5,800 ha. The smallest occupied range was recorded in 1996, but since then the black-tailed prairie dog population has slowly increased. Our goal was to identify the effects of sylvatic plague and geographic isolation on genetic variability in prairie dog colonies. We hypothesized that colonies recovering from plague lost genetic variability by both the initial reduction in the number of individuals and subsequent drift and inbreeding. Furthermore, we expected to detect higher levels of genetic variability in clumped colonies as they should have received more gene flow, which would have ameliorated the effects of drift regardless of plague history. The combination of plague and isolation was expected to contribute to substantial decreases in genetic variability within colonies and genetic differentiation among colonies.

MATERIALS AND METHODS

Between June 1999 and July 2001, tissue samples were collected from black-tailed prairie dogs occupying colonies UL Bend, P-002, B-1, B-49, B-98, and B-105 through a collaboration with the US Fish and Wildlife Service (FWS) as part of a black-footed ferret (*Mustela nigripes*) reintroduction project in Phillips County, Montana, USA (48°N15′N, 107°N 50′W). We sampled prairie dogs from the six colonies based on their sylvatic plague histories (plague, no plague) and proximity to neighboring colonies (clumped, peripheral). We used long-term data from the US Bureau of Land Management (BLM) and FWS to assign plague histories. In addition, colonies were deemed "clumped" if they were within 0.3 and 1.4 km of their nearest neighboring colony, or "peripheral" if more isolated (within 1.6–14.5 km of nearest neighbor). These designations were assigned with the aim of having an equal number of clumped and peripheral colonies. Additionally, the colonies sampled were selected based on the needs of the FWS, rather than data on dispersal. We collected approximately 1 g each of muscle and liver from each prairie dog, placed samples in individually labeled cryotubes, and shipped samples on dry ice. Tissue samples were stored at −80 C until processed.

We extracted DNA from 0.1 g of liver tissue using the Gen Elute™ Mammalian Genomic DNA Kit (Sigma, St. Louis, Missouri, USA). Randomly chosen oligonucleotide 10-mer primers (Operon Technologies, Alemeda, California, USA) were used in randomly amplified polymorphic DNA (RAPD) analysis (Palumbi, 1996) following initial screening confirming polymorphism. Ready-To-Go™ RAPD Analysis Beads (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) were used in PCR reactions following manufacturer's protocols. To ensure consistent results, we used negative controls in all RAPD-PCR reactions and gel runs, and we reanalyzed samples for correspondence (Silva and Russo, 2000). We electrophoresed amplified samples on 1% agarose gels in 1× TBE buffer for approximately 2 hr at 100 V using a Power-Pac 300 power supply (Bio-Rad, Hercules, California, USA). AmpliSize™ Molecular Rulers were included as size standards (Bio-Rad). Gels were stained with ethidium bromide, visualized with ultraviolet light, and recorded digitally. Only primers exhibiting polymorphism upon screening were chosen for amplification, and all loci consistently reproduced were scored. We scored bands as 1 or 0 indicating band presence and absence, respectively. Loci were assumed to be biallelic and

representative of only one chromosomal location.

Because RAPD markers are dominant, the presence of a band on a gel can represent either a homozygous or heterozygous genotype at any given locus. Therefore, it is necessary to estimate the frequencies of recessive alleles at any locus using the Hardy-Weinberg equilibrium equations and their attendant assumptions. A previous study using allozymes (codominant markers for which all heterozygotes can be directly identified) at four of the current study colonies (UL Bend, B01, B49, and B98) showed overall conformance to Hardy-Weinberg expectations (Britten, unpublished data), which suggested that this approach was appropriate. However, comparisons across studies using RAPDs and other markers that provide multilocus genotypes (e.g., isozymes and microsatellites) should be interpreted with caution because the same set of multilocus markers is rarely used in more than one study and because of the need to estimate frequencies of recessive alleles when using dominant markers (Sunnucks, 2000).

Number of alleles per locus, number of effective alleles per locus, allelic diversity, percent polymorphism, and Nei's (1987) gene diversity (h, an analog of Hardy-Weinberg expected heterozygosity) were estimated for all six study colonies using POPGENE (version 1.31; Yeh et al., 1999). Mean per locus and per colony gene diversities were determined for all six colonies. We pooled per locus mean gene diversities for colonies that had experienced plague versus colonies without plague and clumped versus peripheral colonies. We used Wilcoxon paired-sample tests to determine differences in average per locus effective number of alleles and gene diversity between both colonies with and without plague and clumped and peripheral groups.

We used POPGENE to estimate G_{ST}, a measure of genetic differentiation between colonies. Arlequin (version 2.000; Schneider et al., 2000) was used to perform analyses of molecular variance (AMOVA) to examine population genetic structuring in sampled prairie dog colonies. Analysis of molecular variance is a hierarchical analysis of population substructure that partitions allele frequency variance components among all colonies and within colonies (Excoffier et al., 1992; Schneider et al., 2000). We then generated genetic differentiation statistics (Φ-statistics). Analysis of molecular variance and the F-statistic variants (G_{ST} and Φ-statistics) all estimate levels of variance in allele frequencies within vs. among colonies. Finally, we used POPGENE to calculate the number of genetically effective migrants among colonies per generation (Nm) from Φ_{ST} and G_{ST}.

RESULTS

A total of 127 prairie dogs were analyzed with seven oligonucleotide primers, which generated 24 reproducibly amplified loci ranging in size from 200 to 1,800 base pairs. The RAPD marker data are available (H.B.B.).

The percentage of polymorphic loci per colony ranged from 58.3% to 95.8% (Table 1). Colonies that experienced plague ($h=0.27\pm0.18$) had significantly lower average per locus pooled gene diversity than colonies without plague ($h=0.36\pm0.14$, $T=40$, $P<0.001$, $n=24$; Table 1). Similarly, colonies that experienced plague had a lower average effective number of alleles per locus ($n_e=1.44\pm0.35$) than colonies without plague ($n_e=1.62\pm0.31$; $T=56$, $P<0.01$, $n=24$; Table 1). The difference in pooled gene diversity between clumped ($h=0.29\pm0.17$) and peripheral colonies ($h=0.32\pm0.17$) was not statistically significant ($T=113$, $P>0.20$, $n=24$; Table 1). Likewise, peripheral colonies exhibited a slight but nonsignificantly higher average effective number of alleles per locus ($n_e=1.55\pm0.35$) than clumped colonies ($n_e=1.50\pm0.35$, $T=117$, $P>0.05$, $n=24$; Table 1).

An AMOVA revealed that 0.223 of the total genetic variation was attributed to differences among colonies ($P<0.01$) and 0.777 due to differences within colonies ($P<0.01$). A significant level of genetic differentiation existed among the six black-tailed prairie dog colonies ($\Phi_{ST}=0.232$, $P<0.0001$; $G_{ST}=0.194$, $P<0.0001$). Numbers of genetically effective migrants per generation (Nm) were 0.873 and 1.039 estimated from Φ_{ST} and G_{ST}, respectively.

DISCUSSION

Black-tailed prairie dog colonies that experienced population bottlenecks from sylvatic plague exhibited significantly lower mean pooled per locus effective number of alleles and gene diversity than did those

TABLE 1. Plague histories, level of geographic isolation, preplague colony sizes, and levels of genetic variability in black-tailed prairie dog colonies sampled in southern Phillips County, Montana, USA.[a]

Classification of colony	Colony	Number of prairie dogs sampled	Distance to nearest neighbor (km)	Preplague colony size (ha)	n_a	n_e	Percent P	h
Peripheral, plague	P002	26	1.6	140	1.67±0.48	1.38±0.38	67	0.22±0.20
Peripheral, no plague	B01	20	14.5	61	1.96±0.20	1.64±0.31	96	0.36±0.15
Peripheral, plague	B98	20	2.1	83	1.71±0.46	1.40±0.36	71	0.24±0.20
Clumped, no plague	UL Bend	21	0.3	188	1.71±0.46	1.49±0.39	71	0.28±0.21
Clumped, plague	B49	20	0.3	306	1.71±0.46	1.45±0.38	71	0.26±0.20
Clumped, no plague	B105	20	1.4	77	1.58±0.50	1.36±0.40	58	0.20±0.21

[a] n_a = Observed number of alleles; n_e = effective number of alleles; Percent P = percent polymorphism; h = Nei's gene diversity.

from colonies without plague. Colonies that experienced plague also had lower mean numbers of observed alleles per locus. Genetic differences between colonies with and without plague existed nearly 10 years after the plague epizootic began in the early 1990s. Drift is expected to reduce genetic variability in small colonies at a greater rate than in larger colonies and in colonies that have experienced repeated population bottlenecks compared with demographically stable ones (Vucetich et al., 1997; Kalinowski and Waples, 2001). For example, Travis et al. (1997) noted a possible correlation between low levels of DNA fingerprint diversity and repeated epizootics of plague in two colonies of Gunnison's prairie dog (*Cynomys gunnisoni*) in Colorado. In our study, mean preplague colony size (taken as an indicator of colony effective size) was not a factor. Mean (SE) preplague colony sizes (in 1988) for those colonies that experienced plague (176.7±66.85 ha) and those that did not have plague (85.77±53.74 ha) were similar (t=0.778, df=2, P=0.52), although the larger average size of colonies that experienced plague suggests greater buffering against loss of genetic diversity (Table 1). We know of no plague epizootic or other phenomenon that would drive population size fluctuations in our study colonies prior to 1988. Long-term consequences of reduced genetic diversity in prairie dogs remain unknown, yet are of conservation concern because reduced genetic diversity may decrease fitness and lower resilience to changing environmental conditions (Daley, 1992; Roach et al., 2001).

We predicted that degree of geographic isolation would result in a negative correlation with genetic variability. However, peripheral prairie dog colonies exhibited higher, yet statistically nonsignificant, average gene diversity than clumped colonies. There are two possible explanations for this result. First, designation of peripheral colonies in this study may not have captured the scale of isolation necessary to

distinguish clumped from peripheral colonies because black-tailed prairie dogs migrate up to 10 km (Knowles, 1985; Garrett and Franklin, 1988; Table 1). Thus, gene flow may account for the maintenance of variability in peripheral colonies. Second, Fennell (2002) found that colonies farther than 7 km from their nearest neighbor with plague persisted during an epizootic on the Northern Cheyenne Indian Reservation in central Montana, whereas colonies with closer neighbors with plague were unlikely to persist. The peripheral colony that did not experience plague had the highest genetic variability of colonies we sampled and was located approximately 50 km from the nearest colony with plague. Thus, geographic isolation appears to protect colonies from contracting plague, allowing them to remain large and to retain genetic variability.

A large proportion of total genetic differentiation (22.3%) was attributed to variation among colonies. Estimates of fixation indices support significant genetic structuring among the six sampled colonies, and our indices (Φ_{ST}=0.223; G_{ST}=0.194) are higher than reported in the literature for other black-tailed prairie dog colonies (Chesser, 1983, F_{ST}=0.103; Daley, 1992, F_{ST}=0.115; Foltz and Hoogland, 1983, F_{ST}=0.028; Roach et al., 2001, Θ=0.118). Relatively high fixation indices observed for prairie dog colonies in Montana may represent lower levels of gene flow among colonies than observed in other studies, although comparisons across studies should be viewed with caution because of the variety of molecular markers (isozymes and microsatellites) used in the previous studies.

Number of genetically effective prairie dog migrants per generation ranged from 0.87 to 1.04 and are low enough that population genetic differentiation may be expected (Mills and Allendorf, 1996). Estimates of genetically effective numbers of migrants per generation are derived from *F*-statistics and their analogs as a means of comparing gene flow levels among populations, not as a means of comparing the actual number of dispersing individuals. Mills and Allendorf (1996) and Frankham et al. (2002) discuss the assumptions used to estimate gene flow. Intermediate levels of gene flow will not prevent divergence in allele frequencies among colonies, but may maintain levels of genetic variability within each disjunctive colony (Stenseth and Lidicker, 1992; Frankham et al., 2002). Although we observed a significant reduction in genetic diversity within colonies that experience plague, gene flow may ameliorate the effects of the sylvatic plague by reintroducing variability to genetically depauperate postplague colonies. Given enough time, gene flow will erase the effects of plague on genetic variability, assuming that colony size is stable or increases following recovery.

Finally, our results suggest that management of clumped black-tailed prairie dog colonies in north-central Montana should focus on preserving gene flow among colonies to maintain genetic variability within the system following plague-induced bottlenecks. This form of metapopulation structure would also facilitate demographic rescue of colonies that are affected by plague. Implementation of this strategy, however, must be balanced by the threat of increased mobility of plague-infected fleas during intercolony movement of prairie dogs. Peripheral colonies benefit from demographic and genetic input from dispersers. However, peripheral colonies may also act as relatively persistent reservoirs of genetic variability during plague epizootics because of their isolation. As such, care in managing peripheral colonies may be critical to the persistence of black-tailed prairie dogs in a given area during a plague epizootic.

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