

Final Report

30 September 2011 – 30 September 2012

**Patterns and Processes of Dispersal of Black-Tailed Prairie Dogs in a Heavily
Managed Landscape of the Great Plains Landscape Conservation Cooperative**

USFWS Agreement # F11AP00113

Dr. Samantha M. Wisely, Associate Professor, Division of Biology, 116 Ackert Hall, Kansas State University, Manhattan, Kansas, 66506. Phone: 785.532.0978, FAX: 785.532.6653, Email: wisely@ksu.edu

Dr. Jack Cully, Associate Professor and Asst. Unit Leader, USGS Kansas Cooperative Fish and Wildlife Research Unit, 204 Leasure Hall, Kansas State University, Manhattan, Kansas, 66506. Phone: 785.532.6534, FAX: 785.532.6653, Email: bcully@ksu.edu

Charles Lee, Extension Wildlife Specialist, K-State Research and Extension, 131 Call Hall, Kansas State University, Manhattan, Kansas, 66506. Phone: 785.532.5734, FAX: 785.532.6653, Email: clee@ksu.edu

Prepared by:

**Samantha M. Wisely, P.I.
Rachel Pigg, Graduate Research Assistant**

8 October 2012

Abstract

The black-tailed prairie dog (*Cynomys ludovicianus*) is considered an indicator species for the short grass prairie of North America; however, this species currently occupies an estimated 2% of its original distribution. Persistent and pervasive poisoning, and sylvatic plague have fragmented the remaining populations. It is not well understood how these population fragments are connected in a heterogeneous landscape of land use practices and land cover types, but quantifying population isolation and individual measures of dispersal across the landscape are essential to predicting both the vulnerability of extinction due to stochastic processes and the probability of disease emergence. To better understand how land use practices and grassland productivity affect individual dispersal and population connectivity, we conducted a population genetic analysis of black-tailed prairie dogs across the longitudinal breadth of the Great Plains Landscape Conservation Cooperative (GPLCC), from the core of their distribution in the short grass prairie of Colorado to the eastern periphery of their distribution in the mixed grass prairie of Kansas. Our experimental design was hierarchical in nature in order to assess the relative importance of migration among colonies, complexes, and regions. Estimates of gene flow, effective number of migrants, and spatial autocorrelation indicated that colonies throughout the GPLCC are highly connected to one another, although colonies on the western periphery of the distribution of black-tailed prairie dogs were less connected to one another and to the core than colonies within core. We provide an estimate of the appropriate size for a prairie dog management unit based on results from spatial autocorrelation (40-60 km), and demonstrate that, while isolation by distance predicts genetic distance at broad spatial scales, at hierarchically sampled regional locations, distance alone did not perform as well, nor did models incorporating habitat features that were implicated as complete or semipermeable barriers in a previous study. Overall, our results suggest that prairie dogs are not as sensitive to the effects of fragmentation as other grassland species.

Introduction

Black-tailed prairie dogs are simultaneously considered a keystone species of the prairie ecosystem and an agricultural pest (Kotliar et al. 1999, Miller et al. 2007). These conflicting viewpoints make management of prairie dogs inherently difficult. Persistent and pervasive poisoning, intermittent outbreaks of the exotic disease sylvatic plague, and widespread fragmentation within the heavily managed landscape of the central Great Plains threaten the long-term survival of remnant populations (Cully and Williams 2001, Lomolino et al. 2003). The persistence of isolated, local populations depends on the ability of prairie dogs to successfully migrate between those populations (Hanski 1998). Successful migrations lead to the recolonization of areas that have experienced a local extinction event and maintain the demographic and genetic health of remnant populations. Ultimately, as regional climate patterns change over time, these patterns of fragmentation and isolation may be exacerbated. Thus, identifying patterns of dispersal and the underlying processes that enhance or inhibit population connectivity is essential to the long term conservation and management of this species (Antolin et al. 2006).

The level to which fragmentation of the landscape and proximity to source populations affects the extinction probability of a colony also depends on the productivity of the grassland (Sala et al. 2000, Harrell et al. 2002, Thomas et al. 2004). Across the longitudinal range of the Great Plains Landscape Conservation Cooperative (GPLCC), there exists a steep gradient of annual precipitation which in turn creates a steep gradient in grassland productivity (Sala et al. 1988). Prairie dog densities are highest at an intermediate level of productivity and decrease as forage quantity and quality decrease (Lomolino and Smith 2001, Knowles et al. 2002). In highly productive landscapes, tall grasses create visual barriers that impede dispersal behavior and prevent colonization. We used genetic tools to investigate the effects of geographic distance and land cover/land use on dispersal patterns within and among prairie dog complexes, from the core of their distribution in the short-grass steppe of Colorado to the periphery of their distribution in the mixed grass prairie of eastern Kansas.

Our study provides a more comprehensive understanding of the movement behavior and population connectivity of black-tailed prairie dogs at multiple spatial scales in a heavily managed landscape. Prairie dogs rely on dispersal to maintain metapopulation dynamics across a landscape, particularly in landscapes where localized extinction is a reflection of both plague epizootics and/or poisoning (Cully and Williams 2001, Roach et al. 2001, Antolin et al. 2006). A regional study of the landscape ecology of black-tailed prairie dogs from the core of their distribution in Colorado to the eastern periphery of their distribution in eastern Kansas provides a continuum of population isolation to provide a deeper understanding of the dispersal and movement characteristics that allow prairie dogs to persist despite >100 years of eradication efforts.

Specifically, we used population genetic methods, in the form of multi-locus genotyping at 19 microsatellite loci, to describe the patterns of connectivity (Objective 1) and understand the underlying ecological processes of dispersal (Objective 2) in black-tailed prairie dogs in the heavily managed landscape of the GPLCC.

Integration

Our conservation and landscape genetics approach provides managers with information to predict the likelihood of connectivity between populations at multiple spatial scales, as well as provides a quantitative assessment of how variation in population proximity, range productivity, and landscape fragmentation affects the connectivity and isolation of populations (in accordance with Performance Measure 11). Our analysis was hierarchical in nature, addressing both dispersal among colonies within a prairie dog complex, and connectivity among complexes across the longitudinal breadth of their distribution. Thus, our approach addresses both the landscape scale conservation strategies that need to be developed for this species (Performance Measure 5) and provides the baseline data needed to forecast how ecological and climate change will affect black-tailed prairie dogs (Performance Measure 14).

Black-tailed prairie dogs are considered a top priority species in grassland habitats in the GPLCC. The FWS Spotlight Action Plan and the GPLCC Priority List identify both population

monitoring and elucidating patterns and processes of prairie dog movement as high priority research activities. Our research directly addresses these information needs at multiple scales: across the longitudinal breadth of the GPLCC, regionally within ecoregions, and locally within prairie dog complexes.

Project Objectives

Objective 1. Patterns of connectivity.

We sampled black-tailed prairie dogs within and among 14 regional locations (Figure 1) from core short grass prairie in the west to the easternmost populations at the periphery of this species distribution to estimate population isolation and connectivity at multiple spatial scales.

- ***Objective 1a. Determine the frequency of long distance dispersal.*** We compared the relative rate of dispersal in a hierarchical fashion: among colonies within a complex, among complexes within region, and among regions. This was accomplished with an analysis of molecular variance (Excoffier et al. 1992).
- ***Objective 1b. Estimate the average dispersal distance for male vs. female migrants of each colony and complex.*** Spatial autocorrelation of genetic similarity of males vs. females was used to infer the dispersing sex's average dispersal distance using the relationship between genetic similarity and geographic distance (Spong and Creel 2001).
- ***Objective 1c. Estimate the connectivity of regions over multiple generations.*** The effective number of migrants among complexes provided an estimate of connectivity and isolation among complexes and across ecoregions (Beerli et al. 2010).

Objective 2. Ecological processes of dispersal

We determined habitat suitable for dispersal among colonies but within complexes, and determined the ecological processes responsible for maintaining connectivity or isolation among complexes and ecoregions using molecular methods.

- ***Objective 2a. Determine habitat suitable for dispersal among colonies but within regions.*** We used landscape genetic metrics to analyze how local habitat management impedes or facilitates dispersal in the heavily managed landscape of northeastern Kansas.
- ***Objective 2b. Determine the ecological processes responsible for maintaining connectivity or isolation among ecoregions.*** Both geographic distance and the matrix of habitat types among complexes serve to impede or enhance dispersal. Using circuit theory and isolation by resistance modeling, we determined the relative strengths that habitat type and distance have on impeding or enhancing dispersal (McRae 2006).

Methods

Sample Collection

We collected 1127 samples that represent the east to west distribution of black-tailed prairie dogs as described in the research proposal (Figure 1, Table 1). We collected representative samples within 14 locations which were necessary to meet Objectives 1 and 2. Seven locations were at the core of the distribution of black-tailed prairie dogs. Within the core, four locations were large prairie dog complexes containing multiple colonies. Three of the locations were isolated colonies in State or Federal parks surrounded by areas with prairie dog eradication campaigns and were thus devoid of any other prairie dog activity. The remaining 7 locations were on the eastern periphery of black-tailed prairie dog distribution where large complexes of prairie dogs were absent. These seven locations were located in the mixed prairie ecoregion; the rest were located in short grass prairie.

We collected systematically from the 4 locations with large complexes of prairie dogs in order to understand the between colony dispersal dynamics. Within each location, we randomly placed 3-4 10-km circles. As colonies separated by ≤ 10 -km likely exchange migrants, we referred to these circles as complexes (Antolin et al. 2006). Each complex contained at least 3 colonies and was separated from neighboring, sampled complexes in the same regional location by 10 and 30 km. Within each complex, we selected 3 colonies and sampled ~ 30 individuals from each colony (Figure 2, Table 2).

Samples were collected in 2 ways: 1) opportunistically when a USDA APHIS Wildlife Biologist or Kansas Wildlife Extension Specialist was called upon to eradicate animals via shooting, or 2) during live trapping sessions conducted by the Kansas Cooperative Fish and Wildlife Research Unit. When live animals were handled, a small portion of the ear was clipped. Otherwise, for dead animals, a small piece of muscle tissue or tail was collected from the remains. Tissues were preserved in ethanol and frozen until DNA extraction occurred.

Laboratory Procedures

DNA extractions were completed using a Qiagen DNeasy Blood and Tissue Kit (Cat. No. 69506). Of the collected samples, DNA was successfully extracted from 1099 individuals, representing all 14 regional locations (Table 2). All extractions were diluted to a concentration of 0.25 ng/uL prior to primer optimization of microsatellite loci.

For multilocus microsatellite genotyping, we selected 30 microsatellite loci from previously published literature (Sackett et al. 2009, Jones et al. 2005, Stevens et al. 1997, May et al. 1997). Of those 30, we optimized primers for and genotyped individuals at 19 loci (Table 3). Individuals were genotyped using program GeneMarker (Holland and Parson 2011).

Statistical Analyses and Results

Objective 1a. Determine the frequency of long distance dispersal.

To identify patterns of connectivity within and among our 14 regional locations, we first conducted analyses of molecular variance (AMOVAs) using program Arlequin 3.5 (Excoffier

et al. 2005). To test the hypothesis that among complex variation was greater than among colony variation and thus that among colony dispersal occurred more regularly than long distance dispersal (Table 4A), we used multilocus genotypes from individuals in the 4 locations with multiple colonies (Figure 2). To test the hypothesis that gene flow occurred on the landscape scale, we conducted a second AMOVA using data from all individuals and all regions in which more than 3 individuals were genotyped (Table 4B).

Results from our AMOVAs suggested that limited spatial structuring occurs because high dispersal rates and high levels of gene flow among populations within the core keep the genetic structure among colonies admixed. In eastern peripheral populations where grassland productivity is higher, and the distance between colonies is great due to poisoning, we expected to find indications of isolation and gene flow. Indeed, there was evidence that colonies separated by long distances were experiencing some genetic drift due to reduced gene flow, but the effect was smaller than anticipated and clearly indicated that gene flow occurred within peripheral populations and among core and peripheral populations. Measures of variance in our global AMOVA suggested that differences in genetic composition among regional locations explained only a small portion of the genetic variance observed (~6% of the total variation). Most variation was within individuals without regard to location, suggesting that gene flow occurred at a rate high enough to maintain genetic admixture via connectivity even in isolated, peripheral colonies.

Objective 1b. Estimate the average dispersal distance for emigrants of each colony and complex.

Spatial autocorrelation analysis revealed that related individuals were found up to 60 km apart from one another. While related individuals could take more than one generation to disperse 60 km, this radius can be thought of as the basis for the genetic neighborhood within which gene flow and connectivity is great.

Using both spatial autocorrelation separated by sex (Figure 3) or using a single sex assignment test (Figure 4), we found no significant difference in dispersal capability between males and females. This finding agrees with previous research (Roach et al. 2001).

Objective 1c. Estimate the connectivity of regions over multiple generations.

To visualize connectivity among and within our regional locations in graphical space, we ran a principal coordinates analysis (PCoA) in Genalex 6.5 based on observed allele frequencies. Populations that shared high levels of gene flow are expected to share similar principal coordinate space, while those which are isolated and undergoing genetic drift, will have differentiated principal component space. Results of this PCoA (Figure 5) clustered Comanche, Cimarron, Kiowa-Rita Blanca National Grasslands, Colony F and Colony 9 together with no clearly definable landscape phenomena to explain the partitioning of genetic variation. This complex pattern of genetic variation likely reflects the complex history of extirpation and recolonization that has occurred over the last 100 years due to poisoning and epizootics of sylvatic plague.

To estimate the connectivity among our regional locations, we used Bayesian inference to determine migration rates ($M = m/\mu$, where m is the immigration rate per generation

among populations and μ is the mutation rate per generation per locus) among 13 of our regional locations, in which >2 individuals were trapped and genotyped. We also calculated a relative measure of the effective population size, theta (θ), for each region (Table 5). These calculations were performed in program Migrate-N 3.2.2, which estimates the mutation-scaled effective population size (θ), as well as mutation-scaled migration rates $M = m/\mu$, where m is the immigration rate per generation among populations and μ is the mutation rate per generation per locus (Beerli et al. 2010).

Our results suggest high rates of migration occur within the core region; however, those migration rates are asymmetrical. For instance, while migration from Cimarron National Grassland to Kiowa-Rita Blanca National Grassland occurs frequently, the migration rate from Kiowa-Rita Blanca to Cimarron is quite low. This result might be tied to the sylvatic plague history of the core region (Johnson et al. 2012). Plague began extirpating colonies in Kiowa-Rita Blanca 2+ years before the colonies in Cimarron were affected. It's possible that migrants from Cimarron participated in the recolonization of Kiowa-Rita Blanca prior to the emergence of plague in Cimarron itself.

Compared to migration within the core region ($M = 79.4$), migration within the periphery ($M = 11.1$), from the periphery to the core ($M = 11.7$), and into the periphery from the core ($M = 35.4$) occurs at a lower rate (Table 5). These findings further reinforce that despite the relative isolation of peripheral populations compared to colonies in the core, regions within the periphery still exchange migrants regularly with other regions in our study. Our results suggest that colonies within mixed grass prairie are not as isolated as previously believed.

At the broadest spatial scale, migrants from core regions to the periphery ($M = 35.4$) outnumber migrants from the periphery to the core ($M = 11.8$). Although this result isn't surprising given the larger colonies and populations in the core compared to the periphery, differences in grassland productivity may contribute to this asymmetrical migration pattern among the regions we sampled. If so, fewer migrants move across the higher productivity, mixed grass prairie than the short grass prairie. It should be noted, however, that productivity and proximity to the periphery of this species distribution were confounded variables.

Objective 2a. Determine habitat suitable for dispersal among colonies but within regions.

We first investigated whether isolation by distance could be detected at a fine spatial scale: within locations and among complexes. We conducted Mantel tests of pairwise genetic and geographic distances separating colonies within our hierarchically sampled regional locations: Cimarron, Comanche, and Kiowa-Rita Blanca National Grasslands and Logan County, KS (Figure 6). All Mantel test were conducted in Genalex 6.5. We found that, in general, isolation by distance was not overwhelmingly predictive ($R^2=0.05-0.17$) of genetic distance at this fine spatial scale. This result was not surprising, given that the greatest distance separating colonies within the same region was approximately 40 km, which was within the neighborhood distance detected by our spatial autocorrelations.

Given the poor overall performance of geographic distance as a predictor of genetic distance among colonies and complexes within the same region, we next investigated whether including habitat information could improve our model. Using program Circuitscape (McRae 2006), we created isolation-by-resistance models to predict gene flow among colonies within the same region. A recent study by Sackett et al. (2012) found that the best fit isolation-by-resistance models included water bodies and wetlands as complete barriers to movements, while there was marginal evidence supporting the inclusion roads in these models as semipermeable barriers. We created this habitat model for each region to determine whether this model also performed well in our rural locations. Our results indicate that only one region, Kiowa-Rita Blanca National Grassland, supported the best model of Sackett et al. (2012). For all other regions, there was little to no improvement over isolation-by-distance models. This suggests that, while the best model from Sackett et al (2012) may perform well in urbanized locations, in locations that experience less traffic volume, roads may not act as barriers to dispersal.

Objective 2b. Determine the ecological processes responsible for maintaining connectivity or isolation among ecoregions.

We investigated whether isolation by distance alone could explain the observed genetic structure at our broadest spatial scale: among regions. We conducted a Mantel test, comparing pairwise F_{st} values (Genalex 6.5) and pairwise geographic distance (km, ArcMap 10) among all colonies in which >2 individuals were collected (Figure 7A). We found a significant, strong positive correlation ($R^2=0.41$) between geographic and genetic distance at this broad scale. Consequently, we concluded that isolation by distance was likely the most significant ecological process affecting connectivity among ecoregions.

Next, we examined the point distribution of the pairwise F_{st} values among colonies within the western portion of black-tailed prairie dog distribution (core), in which grassland productivity is relatively low, and among colonies within the eastern portion (periphery), in which grassland productivity is relatively high (Figure 7B). We observed a trend that colonies in the eastern periphery of black-tailed prairie dog range showed greater spread along the F_{st} axis and higher F_{st} values in general than did core colonies in the same distance class as expected for colonies that receive fewer immigrants per generation.

Conclusions

At broad spatial scales, genetic differentiation among black-tailed prairie dog colonies conforms to the expectations of isolation by distance. Isolation by distance occurs when migration and the associated gene flow are at equilibrium with isolation and the associated genetic drift. This equilibrium condition suggests that there are no better landscape level phenomena that describe the connectivity or isolation among regions than the distance that separates them. From a conservation perspective, this suggests that the best possible solution for maintaining isolated, western populations, is to create and maintain a stepping stone network of colonies across the central Great Plains. Our analysis also suggests that even isolated, small colonies contribute substantially to the overall metapopulation dynamic of this species and should be conserved.

At finer spatial scales, our results suggest that prairie dogs are highly mobile, capable of maintaining connectivity up to 40 km from their natal colony. This connectivity lessens the effect of isolation by distance among colonies separated by less than 40 km due to high levels of admixture. Our results also support the expectation that colonies within the eastern periphery of prairie dog distribution are more isolated than colonies within the core distribution; however, whether these differences are due to differences in levels of habitat fragmentation, habitat distribution, or grassland productivity is unclear. Colonies within Boulder County, CO, which lies in the extreme western portion of black-tailed prairie dog range and in a highly fragmented, urbanized area, have shown similar levels of isolation as colonies in the eastern periphery of this study (Magle et al 2010, Sackett et al 2012). Therefore, it is not necessarily grassland productivity alone driving the patterns we describe.

In conclusion, we were highly successful at achieving our aim to provide managers with information to predict the likelihood of connectivity between populations at multiple spatial scales, as well as provide a quantitative assessment of how variation in population proximity, range productivity, and landscape fragmentation affects the connectivity and isolation of populations (**in accordance with Performance Measure 11**). We demonstrated that colonies within mixed grass prairie along the periphery of black-tailed prairie dog range are more isolated than colonies within the short grass prairie core region; however, colonies within mixed grass prairie are not inbred, but rather frequently exchange migrants with other colonies in the periphery and with colonies from the core. In support of this statement, we provide concrete estimates of migration rates among all regions in our study, as well as F_{IS} for each colony sampled. These baseline data can be used in future studies to forecast how ecological and climatic change will affect black-tailed prairie dogs (**Performance Measure 14**). Finally, our hierarchical design addressed the landscape scale conservation strategies that need to be developed for this species (**Performance Measure 5**). At the broadest spatial scale to conserve black-tailed prairie dogs throughout the Great Plains Landscape Conservation Cooperative, we recommend maintaining metapopulations of prairie dogs throughout their distribution and creating additional stepping stone populations when possible. We found that the influence of isolated colonies in maintaining gene flow throughout the metapopulation should not be discounted, and thus even very small colonies that are isolated by large distance or landscape barriers contribute in a meaningful way to the overall functioning of the metapopulation including the recolonization of extirpated colonies.

On a smaller spatial scale, we provide an estimate of 40-60 km as the diameter of the genetic neighborhood of black-tailed prairie dogs within the GPLCC. This estimate provides managers with an approximation of the appropriate size for a prairie dog management unit. Additionally, specific results from our habitat and distance models of Cimarron, Comanche, and Kiowa-Rita Blanca National Grassland and Logan County, KS provide managers of those regions with information on factors that affect or, conversely, do not affect connectivity within their specific management unit.

Figure 1. Red dots represent the 14 locations in which samples were collected, while black dots with red crosses represent locations in which more than one colony was sampled.

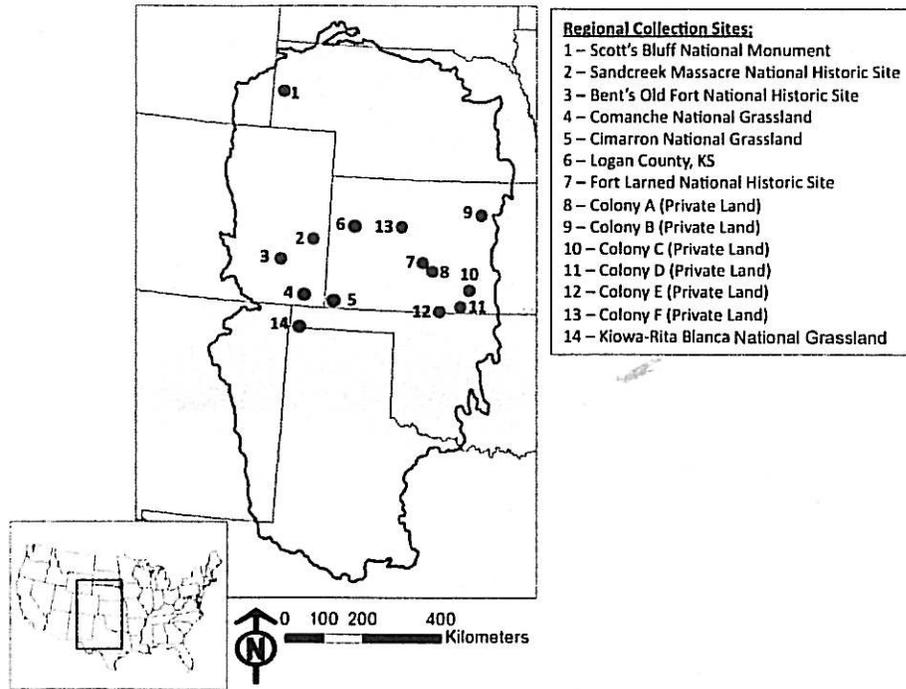


Table 1. Shown are numbers of individuals collected from each of the 14 locations shown in Figure 1.

State	Location	Number Collected
Nebraska	Scott's Bluff National Monument	20
Colorado	Sandcreek Massacre National Historic Site	2
	Bent's Old Fort National Historic Site	20
Kansas	Comanche National Grassland	210
	Cimarron National Grassland	218
	Logan County	322
	Fort Larned National Historic Site	20
	Colony A (Private Land)	30
	Colony B (Private Land)	16
	Colony C (Private Land)	18
	Colony D (Private Land)	9
	Colony E (Private Land)	30
	Colony F (Private Land)	30
New Mexico/Oklahoma/Texas	Kiowa/Rita Blanca National Grasslands	187

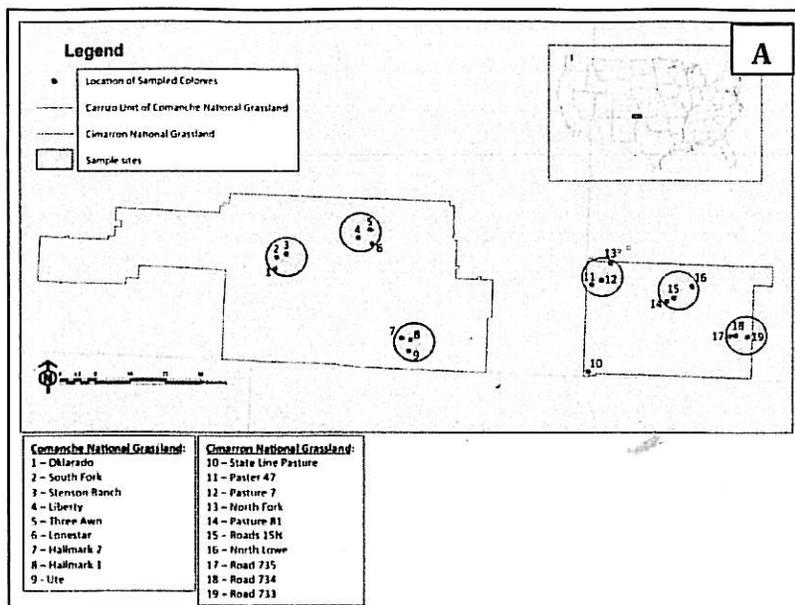


Figure 2. Shown are the specific locations of colonies sampled within (A) Comanche and Cimarron National Grasslands, (B) Logan County, Kansas, and (C) Kiowa-Rita Blanca National Grassland. Green dots represent the colonies. Cream circles represent the 10-km diameter sample sites. Within Cimarron National Grassland, Colony 10 (State Line Pasture) was sampled due to initial uncertainty as to whether or not Colonies 17-19 were active

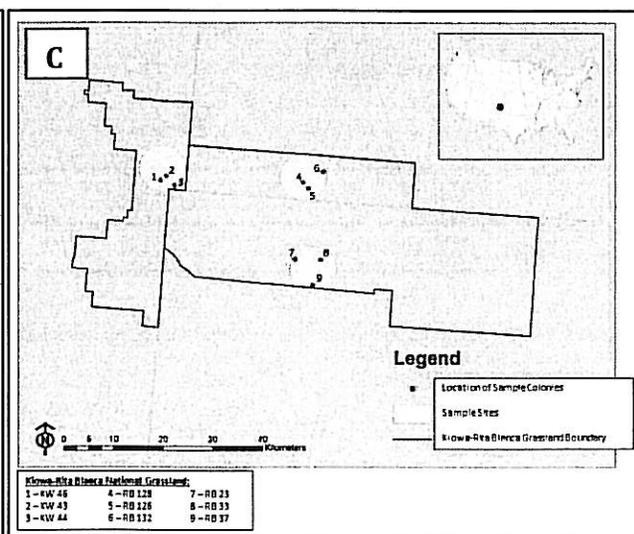
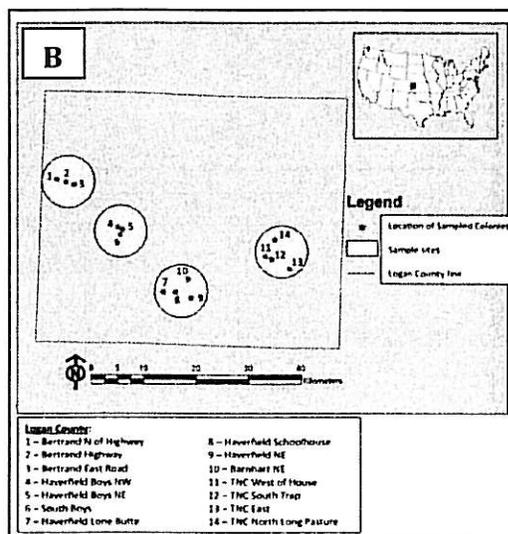


Table 2A. Shown are the collections, heterozygosity, and fixation index (F_{IS}) from each of 44 colonies within the core range of the black-tailed prairie dog: Kiowa/Rita Blanca National Grasslands, Comanche National Grassland, Cimarron National Grassland, Logan County, Kansas, and Bent's Old Fort and Sandcreek Massacre National Historic Sites, Colorado.

Location	Colony Name	Number Collected	Number Genotyped	H_O	H_E	UH_E	F_{IS}
Comanche	Oklarado	20	20	0.675	0.674	0.691	0.003
	South Fork	21	21	0.639	0.601	0.615	-0.069
	Stenson Ranch	26	26	0.706	0.689	0.702	-0.031
	Liberty	31	29	0.691	0.683	0.695	-0.005
	Three Awn	21	21	0.689	0.710	0.727	0.029
	Lonestar	22	22	0.669	0.667	0.682	-0.006
	Hallmark 2	27	27	0.682	0.660	0.672	-0.028
	Hallmark 1	20	20	0.734	0.680	0.698	-0.070
	Ute	22	22	0.667	0.679	0.695	0.030
Cimarron	State Line Pasture	20	20	0.721	0.702	0.720	-0.026
	Pasture 47	35	35	0.715	0.698	0.708	-0.028
	Pasture 7	22	20	0.737	0.692	0.710	-0.062
	North Fork Pasture	22	22	0.742	0.721	0.738	-0.032
	Pasture 81	20	20	0.700	0.690	0.708	-0.022
	Roads N15	23	25	0.736	0.710	0.725	-0.037
	North Lowe Pasture	20	20	0.737	0.710	0.728	-0.050
	Road 735	18	18	0.737	0.692	0.712	-0.066
	Road 734	20	20	0.757	0.724	0.743	-0.049
	Road 733	18	18	0.713	0.680	0.700	-0.056
Logan County	Bertrand Highway	9	9	0.752	0.699	0.740	-0.080
	Bertrand East Road	15	15	0.698	0.703	0.727	-0.002
	Bertrand North of Highway	15	15	0.747	0.705	0.729	-0.064
	Haverfield Boys Northwest	13	13	0.713	0.698	0.726	-0.022
	Haverfield Boys Northeast	23	23	0.739	0.712	0.728	-0.037
	South Boys	29	29	0.761	0.720	0.732	-0.067
	Haverfield Northeast	32	32	0.745	0.721	0.732	-0.037
	Barnhart Northeast	25	25	0.771	0.728	0.743	-0.062
	Haverfield Schoolhouse	29	29	0.720	0.706	0.718	-0.023
	Haverfield Lone Butte	30	30	0.743	0.715	0.727	-0.039
	TNC West	24	24	0.767	0.716	0.731	-0.082
	TNC South Trap	30	0	NA	NA	NA	NA
	TNC North Long Pasture	16	16	0.760	0.690	0.712	-0.103
	TNC East	32	31	0.749	0.714	0.726	-0.054
Kiowa/Rita Blanca	KW 46	25	25	0.749	0.737	0.752	-0.019
	KW 43	22	22	0.739	0.712	0.729	-0.037
	KW 44	25	25	0.789	0.703	0.718	-0.124
	RB 128	23	23	0.785	0.731	0.747	-0.080
	RB 126	24	24	0.787	0.719	0.735	-0.097
	RB 132	15	15	0.783	0.716	0.741	-0.106
	RB 23	1	1	0.579	0.289	0.579	-1.000
	RB 33	25	25	0.762	0.727	0.740	-0.049
	RB 37	27	27	0.735	0.708	0.722	-0.036
Bent's Old Fort National Historic Site, CO	20	20	0.726	0.669	0.686	-0.087	
Sandcreek Massacre National Historic Site, CO	2	2	0.658	0.441	0.588	-0.467	

Table 2B. Shown are the collections, heterozygosity, and fixation index (F_{IS}) from each of 8 colonies in the eastern peripheral range of the black-tailed prairie dog and Scott's Bluff National Monument, Nebraska.

Location	Number Collected	Number Genotyped	H_o	H_E	UH_E	F_{IS}
Colony A, Kansas	30	30	0.728	0.669	0.680	-0.093
Colony B, Kansas	16	16	0.503	0.445	0.459	-0.116
Colony C, Kansas	18	18	0.406	0.409	0.420	0.001
Colony D, Kansas	9	9	0.544	0.496	0.526	-0.068
Colony E, Kansas	30	30	0.609	0.631	0.641	0.028
Colony F, Kansas	30	30	0.661	0.582	0.591	-0.145
Fort Larned National Historic Site, Kansas	20	20	0.637	0.680	0.697	0.072
Scott's Bluff National Monument, Nebraska	20	20	0.566	0.591	0.606	0.048

Table 3. Of the 1127 samples collected, 1099 prairie dogs were successfully genotyped at the 19 microsatellite loci shown below. Heterozygosity was calculated using Genalex 6.5 (Mean H_E = average estimated heterozygosity across populations; Mean H_o = average observed heterozygosity across populations)

Publication	Species	Locus	Number of Alleles	Size Range	Mean H_E	Mean H_o
Jones et al. 2005	Black-tailed prairie dog	A2	14	220-248	0.716	0.755
		A8	13	265-291	0.649	0.706
		A104	10	189-207	0.716	0.749
		A111	10	181-199	0.630	0.670
		A115	9	189-205	0.681	0.700
		A119	10	111-133	0.687	0.844
		C116	14	190-242	0.725	0.615
		D1	8	192-218	0.654	0.738
		D2	8	300-328	0.677	0.717
		D6	7	186-206	0.556	0.640
		D12	7	204-228	0.670	0.773
D115	16	193-225	0.674	0.776		
Stevens et al. 1997	Columbian ground squirrel	GS14	18	237-275	0.736	0.625
May et al. 1997	Northern Idaho ground squirrel	IGS-1	9	103-119	0.668	0.728
Sackett et al. 2009	Prairie dog spp.	A105	6	204-216	0.600	0.618
		A109	9	324-346	0.533	0.559
		C101	17	300-356	0.757	0.786
		D109	17	401-489	0.672	0.688
		TAGA27	9	220-252	0.645	0.674

Table 4A. Shown is the analysis of molecular variance (AMOVA, Arlequin 3.5) when only regional locations in which multiple colonies were samples included in the analysis (i.e., Cimarron National Grassland, Comanche National Grassland, Kiowa/Rita Blanca National Grassland, and Logan County, Kansas). Individual colonies in which <3 prairie dogs were genotyped were not included in this analysis.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Components	Percentage of Variation Explained
Among locations	3	370.161	0.23156	3.16
Among complexes within locations	36	690.481	0.27761	3.79
Among individuals within complexes	863	5798.846	-0.08963	-1.22
Within individuals	903	6229.500	6.89867	94.27
Total	1805	13088.987	7.31821	

Table 4B. Shown is the analysis of molecular variance (AMOVA, Arlequin 3.5) when 13 regional locations were included in the analysis (i.e., Cimarron National Grassland, Comanche National Grassland, Kiowa/Rita Blanca National Grassland, and Logan County, Kansas). Individual colonies and/or regional locations in which <3 prairie dogs were genotyped were not included in this analysis.

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Components	Percentage of Variation Explained
Among locations	12	1068.985	0.46510	6.31
Among complexes within locations	36	703.759	0.29126	3.95
Among individuals within complexes	1047	6780.133	-0.13392	-1.82
Within individuals	1096	7391.000	6.74361	91.55
Total	2191	15943.876	7.36605	

Figure 3. Of the 1099 genotyped prairie dogs, the sex of 796 individuals was known from field data (females = 439, males = 357). Spatial autocorrelations produced by Genalex 6.5 demonstrate that the limits of dispersal for female (A) and male (B) prairie dogs are similar. Both sexes are capable of moving approximately 40 km between colonies. (r = relatedness).

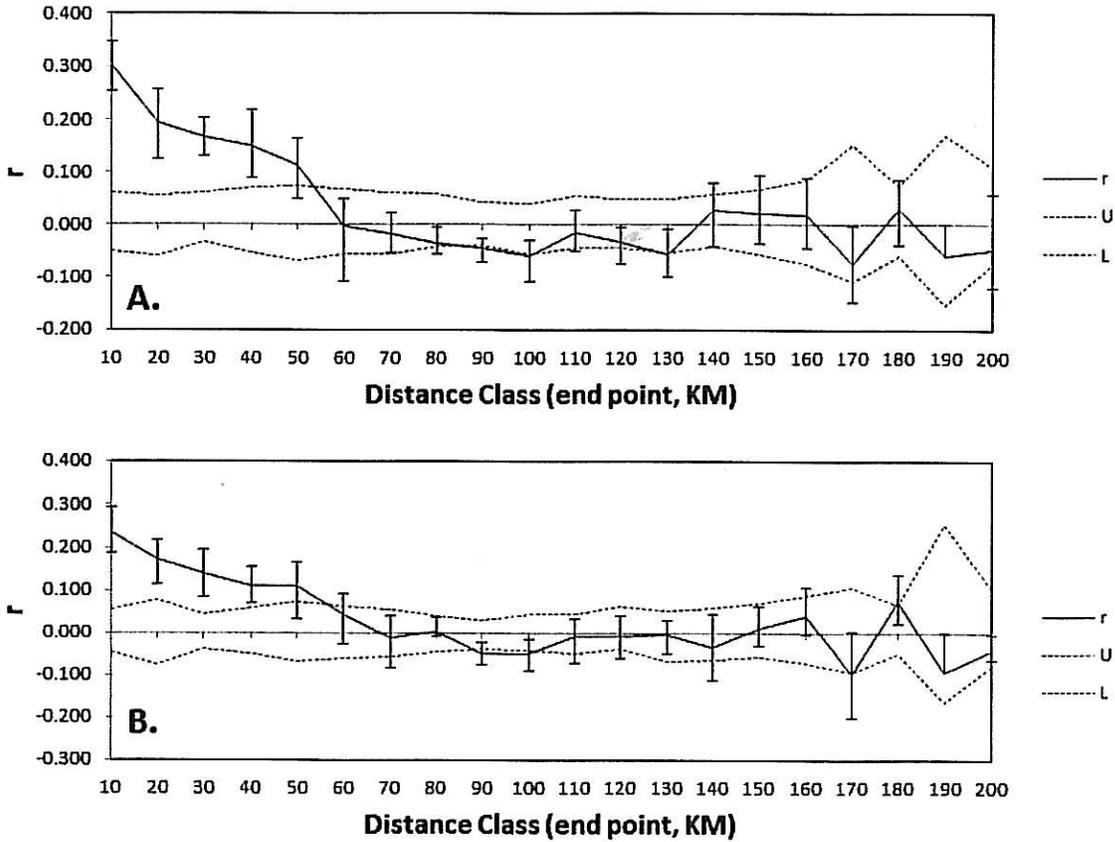


Figure 4. Of the 1099 genotyped prairie dogs, the sex of 796 individuals was known from field data (females = 439, males = 357). To determine whether sex-biased dispersal occurs among black-tailed prairie dog colonies, we ran single sex assignment test in Genalex 6.5. Our results suggest that sex-biased dispersal does not occur among colonies. A boxplot of the mean assignment bias of males and females (A) and the frequency distribution of the assignment bias (B) are shown below.

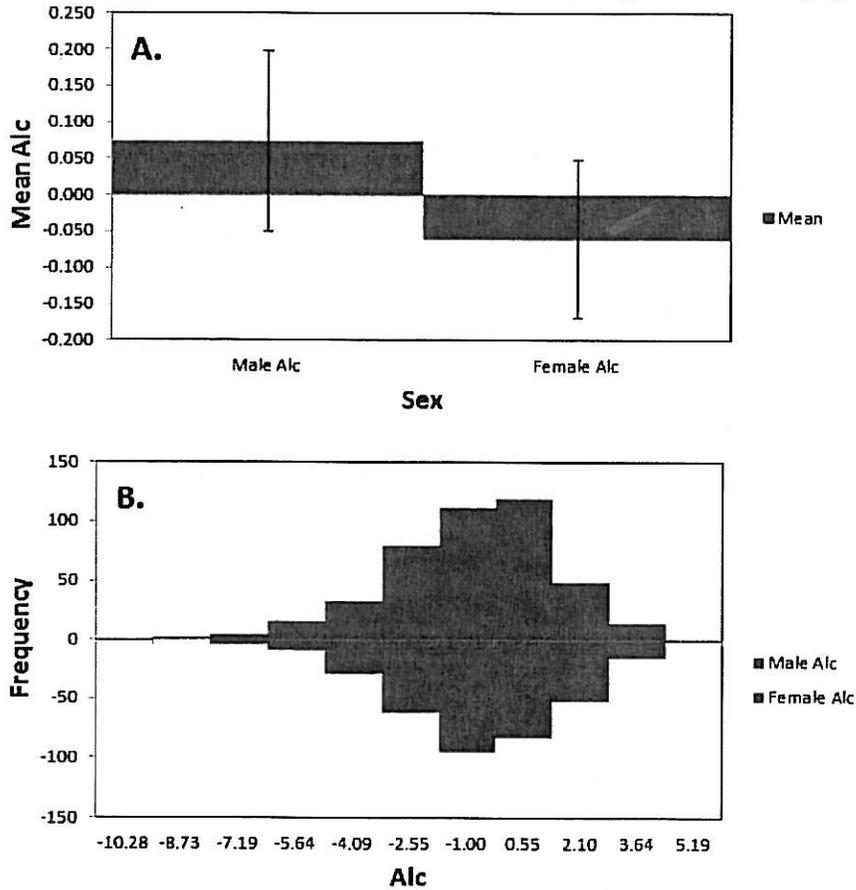


Figure 5. To visualize connectivity among and within our regional locations in graphical space, we ran a principal coordinates analysis (PCoA) in Genalex 6.5 based on observed allele frequencies. Results of this PCoA (Figure 6) suggest the presence of a latitudinal dispersal barrier.

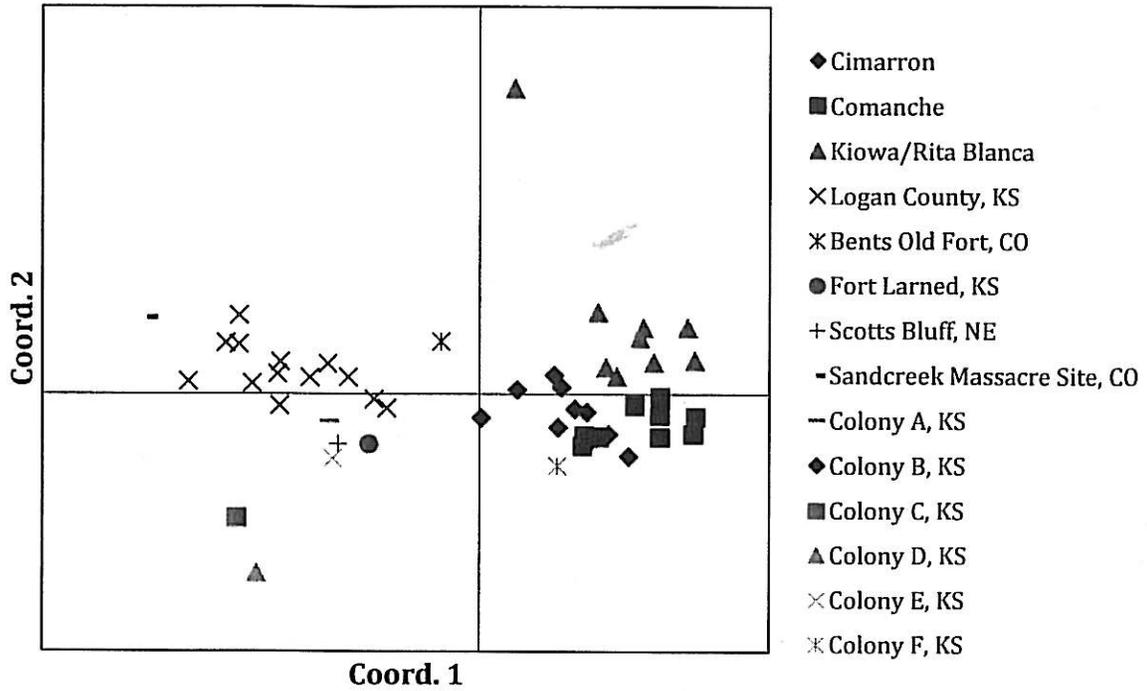


Table 5. Shown are pairwise migration rates (M) among 13 of our regional locations. Row headings correspond to the origin of the migrant, while column headings correspond to the destination. Theta (Θ) for each region are given along the diagonal. All estimates were calculated in program Migrate using the infinite alleles model. (CIM = Cimarron National Grassland; COM = Comanche National Grassland; KRB = Kiowa/Rita Blanca National Grassland; LOG = Logan County, KS; A through F = Colony A - F, KS; FL = Fort Larned National Historic Site, KS; BOF = Bent's Old Fort National Historic Site, CO; SB = Scott's Bluff National Monument, NE)

	CIM	COM	KRB	LOG	A	B	C	D	E	F	FL	BOF	SB
CIM	0.09792	65.874	112.038	87.109	41.872	34.329	27.294	21.184	23.243	36.135	61.742	35.140	65.390
COM	132.705	0.09767	125.421	88.303	28.930	33.276	52.750	30.804	30.734	38.574	63.707	25.726	25.450
KRB	1.555	73.602	0.09797	75.873	72.576	5.948	28.486	46.515	34.465	5.783	50.496	29.299	22.100
LOG	158.644	137.069	163.677	0.09743	39.349	32.937	20.117	90.985	79.355	73.517	69.689	35.577	32.145
A	14.617	17.650	16.370	13.691	0.00882	6.826	14.874	7.124	11.592	17.538	35.189	11.978	10.899
B	7.498	11.816	13.913	11.045	7.897	0.00074	11.646	3.658	7.514	10.774	9.902	9.952	23.312
C	12.148	9.648	14.026	11.513	6.801	8.370	0.00119	18.264	2.605	6.105	8.106	9.246	9.184
D	5.498	6.035	11.822	3.734	11.232	4.535	12.042	0.00203	5.508	7.161	13.264	8.080	19.802
E	14.987	14.940	15.000	16.159	14.116	6.033	15.616	3.120	0.01237	4.577	6.315	6.555	6.979
F	15.999	16.519	17.151	10.643	14.131	14.189	11.783	27.561	2.556	0.00003	16.007	9.527	13.650
FL	5.360	19.245	14.591	11.041	19.403	18.671	11.558	1.636	8.057	21.385	0.07794	9.751	9.011
BOF	16.559	8.729	14.754	9.192	12.979	5.700	7.362	31.136	6.801	10.869	20.630	0.00003	6.077
SB	10.162	15.875	12.729	12.129	9.622	6.926	18.489	10.084	4.251	15.331	12.686	13.078	0.02178

Figure 6. We conducted Mantel tests of pairwise genetic and geographic distances to investigate the effect of isolation by distance among colonies within core prairie dog range. The four regional locations shown include: Cimarron (A), Comanche (B), Logan County, KS (C), and Kiowa-Rita Blanca (D). Results suggests that genetic distances among colonies at intermediate and broad distances in Comanche have a wider spread than is seen in other regional locations, while Cimarron is the only region in which there exists a strong, significant isolation by distance effect.

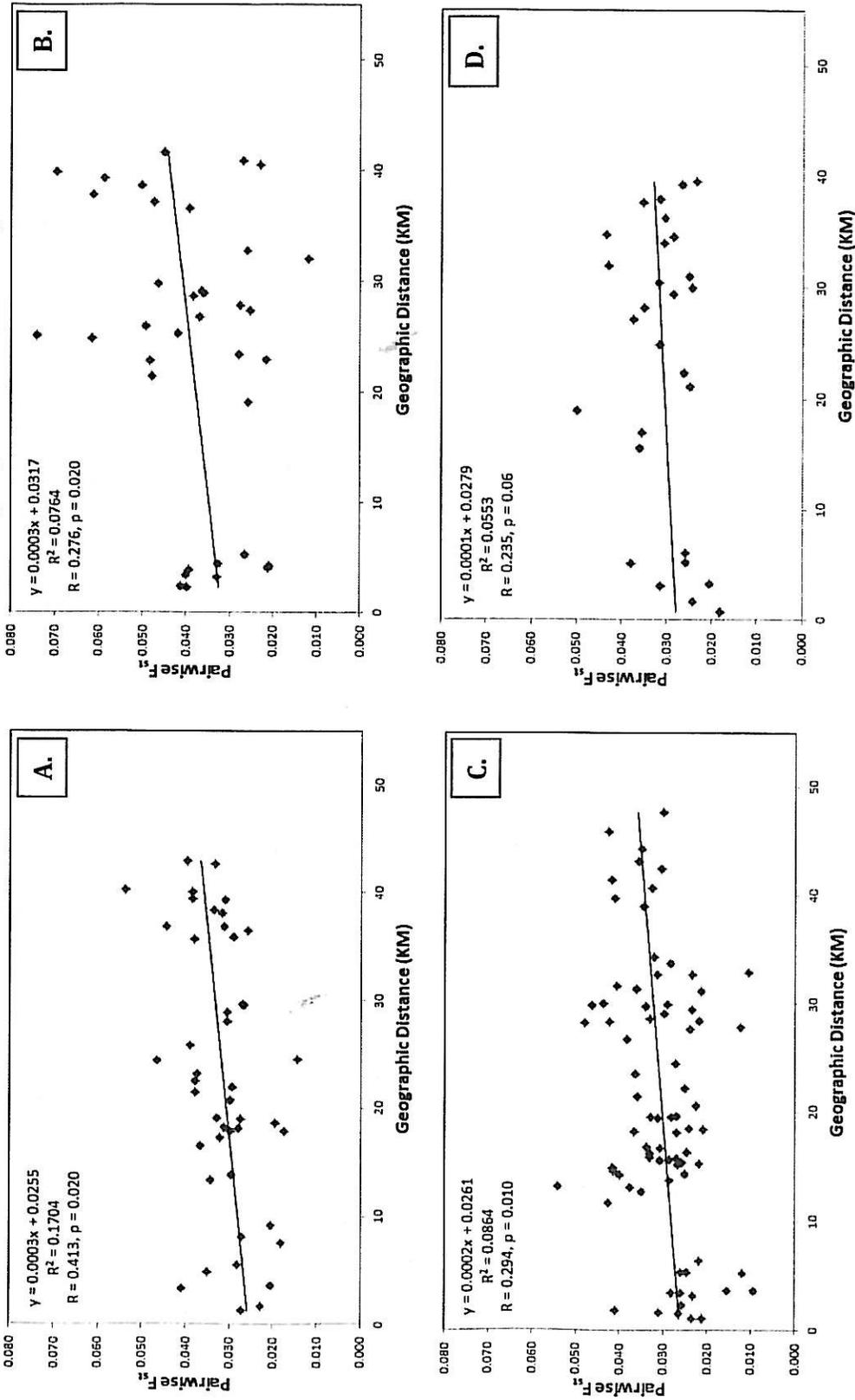


Table 6. Shown are Mantel test results for the three models predicting genetic distances among colonies within each of four regional locations: Cimarron, Comanche, Kiowa-Rita Blanca National Grasslands and Logan County, KS. Our habitat model was created using program Circuitscape (McRae 2006). This model was based on the best models from a study of prairie dog colonies in Boulder County, CO by Sackett et al. (2012). The baseline conductance value was set at 100; the higher the conductance value assigned to a landscape feature, the lower resistance of that feature to prairie dog movement. Conductance values of 0 are impermeable barriers to movement.

Location	Model	Model description	Mantel's R	p-value
Cimarron	Isolation by Distance	Only distance affects genetic distance	0.413	0.020
	Water and Roads as Barriers	Water impermeable barrier (conductance = 0), roads as semipermeable barrier (conductance = 50)	0.353	0.019
Comanche	Isolation by Distance	Only distance affects genetic distance	0.276	0.020
	Water and Roads as Barriers	Water impermeable barrier (conductance = 0), roads as semipermeable barrier (conductance = 50)	0.271	0.001
Kiowa-Rita Blanca	Isolation by Distance	Only distance affects genetic distance	0.235	0.060
	Water and Roads as Barriers	Water impermeable barrier (conductance = 0), roads as semipermeable barrier (conductance = 50)	0.420	0.001
Logan County	Isolation by Distance	Only distance affects genetic distance	0.294	0.010
	Water and Roads as Barriers	Water impermeable barrier (conductance = 0), roads as semipermeable barrier (conductance = 50)	0.338	0.010

Figure 7A. To determine whether isolation by distance alone could explain patterns of genetic differentiation among the 13 regional locations, we conducted a Mantel test comparing pairwise F_{ST} values to pairwise geographic distance (km). Results indicate a significant positive relationship between distance and genetic differentiation. ($R = 0.636$, $p = 0.010$, Genalex 6.5).

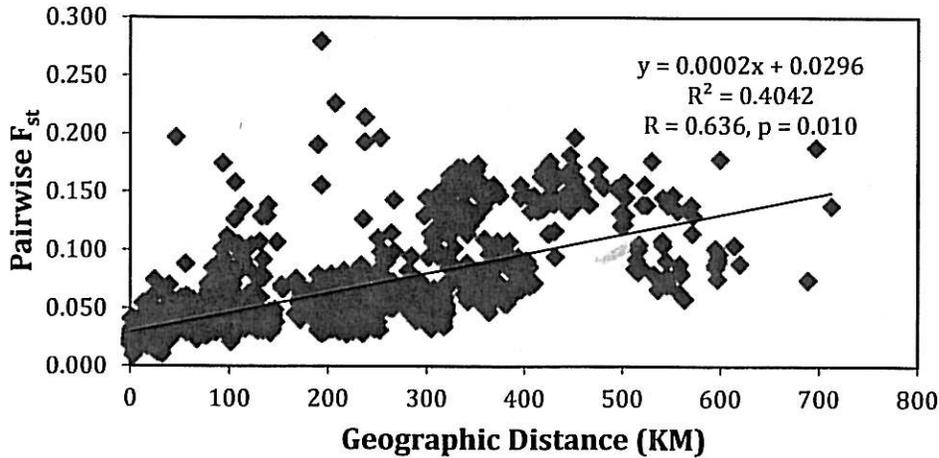
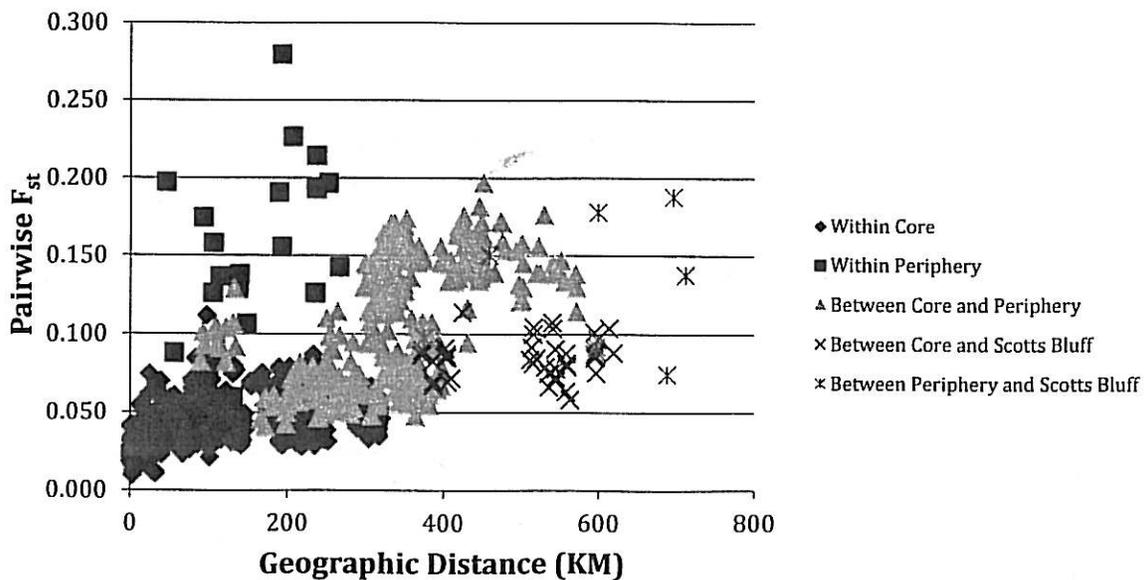


Figure 7B. Shown is the point distribution from Figure 6A, in which specific points are labeled as follows: (1) Within Core, if both colonies compared are found in regions from Table 2A, (2) Within Periphery, if both colonies compared are in regions from Table 2B, and (3) From Core to Periphery, if the colonies compared are not within the same portion of black-tailed prairie dog range. Pairwise comparisons between all colonies and Scott's Bluff National Monument are shown separately, as the distance separating this site from others represents a latitudinal rather than a longitudinal gradient. Results suggest that the effect of distance on genetic differentiation among colonies is amplified in the peripheral range of the black-tailed prairie dog.



References

- Antolin, M. F., L. T. Savage, and R. J. Eisen. 2006. Landscape features influence genetic structure of black-tailed prairie dogs (*Cynomys ludovicianus*). *Landscape Ecology* 21:867-875.
- Antolin, M.F., D.E. Biggins, C.J. Brand, J. F. Cully, L.E. Ellison, K.L. Gage, and T.E. Rocke. 2010. *Eds. Special Issue: Symposium on the Ecology of Plague and its Effects On Wildlife. Vector-borne and Zoonotic Diseases* 10(1):1-103.
- Beerli, P. 2002. Migrate, documentation and program: Version 1.5. <http://evolution.genetics.Washington.edu/lamarck/migrate.download.html>.
- Collinge, S. K., C Ray, and J. F. Cully, Jr. 2008. Effects of disease on keystone species, dominant species, and their communities. Pps 129-144. In: Ostfeld, Evener, and Keesing (eds.) *Effects of Ecosystems on Disease and of Diseases on Ecosystems*. Princeton University Press.
- Cully, J. F. and E. S. Williams. 2001. Interspecific comparisons of sylvatic plague in prairie dogs. *Journal of Mammalogy* 82: 894-905.
- Cully, J. F., Jr., S. K. Collinge, R. E. VanNimwegen, C. Ray, W. C. Johnson, B. Thiagarajan, D. B. Conlin, and B. E. Holmes. Spatial variation in keystone effects: Small mammal diversity associated with black-tailed prairie dog colonies. *Ecography* 33:667-677.
- Cully, J. F., Jr., T. L. Johnson, S. K. Collinge, and C. Ray. 2010. Disease limits populations: Plague in black-tailed prairie dogs. *Vector Borne and Zoonotic Diseases* 10:7-15.
- Excoffier, L., G. Laval, and S. Schneider. 2005. Arelequin version 3.0: an integrated software package for population genetics data analysis. *Evolutionay Bioinformatics Online* 1: 47-50.
- Garrett, M. G. and W. L. Franklin. 1988. Behavioral ecology of dispersal in the black-tailed prairie dog. *Journal of Mammalogy* 69:236-250.
- Hanski, I. 1998. Metapopulation dynamics. *Nature* 396: 41-49.
- Harrell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158-2162.
- Holland, M. M. and W. Parson. 2011. GeneMarker HID: a reliable software tool for the analysis of forensic STR data. *Journal of Forensic Science* 56: 29-35.
- Hoogland, J. L. 1995. *The black-tailed prairie dog: the social life of a burrowing mammal*. University of Chicago Press, Chicago.
- Johnson, T. L., J. F. Cully, Jr, S. K. Collinge, C. Ray, C. M. Frey, and B. K. Sandercock. 2012. Spread of plague among black-tailed prairie dogs is associated with colony spatial characteristics. *Journal of Wildlife Management* 75:357-368.
- Jones, R. T., A. P. Martin, A. J. Mitchell, S. K. Collinge, and C. Ray. 2005. Characterization of 14 polymorphic microsatellite markers for the black-tailed prairie dog (*Cynomys ludovicianus*) *Molecular Ecology Notes* 5:71-73
- Knowles, C. J., J. D. Proctor, and S. C. Forrest. 2002. Black-tailed prairie dog abundance and distribution in the Great Plains based on historic and contemporary information. *Great Plains Research* 12: 219-254.
- Kotliar, N. B., B. W. Baker, A. D. Whicker and G. Plumb. 1999. A critical review of assumptions about the prairie dog as a keystone species. *Environmental Management* 24: 177-192.

- Lomolino, M. V. and G. A. Smith. 2001. Dynamic biogeography of prairie dogs (*Cynomys ludovicianus*) towns near the edge of their range. *Journal of Mammalogy* 82: 937-945.
- Lomolino, M. V., G. A. Smith, and V. Vidal. 2003. Long-term persistence of prairie dog towns: insights for designing networks of prairie reserves. *Biological Conservation* 115: 111-120.
- Magle, S. B., E. W. Ruell, M. F. Antolin, and K. R. Crooks. 2010. Population genetic structure of black-tailed prairie dogs, a highly interactive species, in fragmented urban habitat. *Journal of Mammalogy* 91:326-335.
- May, B., T. A. Gavin, P. W. Sherman, and T. M. Korves. 1997. Characterization of microsatellite loci in the Northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). *Molecular Ecology* 1997:399-400.
- Miller, B. J., R. P. Reading, D. E. Biggins, J. K. Detling, S. C. Forrest, J. L. Hoogland, J. Jarersak, S. D. Miller, J. Proctor, J. Truett, and D. W. Uresk. 2007. Prairie dogs: an ecological review and current biopolitics. *Journal of Wildlife Management* 71: 280-2810.
- Mossman, C. A. and P. M. Waser. 1999. Genetics detection of sex-biased dispersal. *Molecular Ecology* 8: 1063-1067.
- Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel, population genetic software for teaching and research. *Molecular Ecology Notes* 6:288-295.
- Pearse, D. E., and K. A. Crandall. 2004. Beyond F_{ST} : Analysis of population genetic data for conservation. *Conservation Genetics* 5:585-602.
- Roach, J. L., P. Stapp, B. Van Horne, and M. F. Antolin. 2001. Genetic structure of a metapopulation of black-tailed prairie dogs. *Journal of Mammalogy* 82:946-959.
- Sackett, L. C., L. K. Etchberger, M. N. Mazzella, D. D. Lim, and A. P. Martin. 2009. Characterization of 18 microsatellite loci for three species of prairie dogs. *Molecular Ecology Resources* 10: 232-236.
- Sackett, L. C. T. B. Cross, R. T. Jones, W. C. Johnson, K. Ballare, C. Ray, S. K. Collinge, A. P. Martin. 2012. Connectivity of prairie dog colonies in an altered landscape: inferences from analysis of microsatellite DNA variation. *Conservation Genetics* 13:407-418.
- Sala, O. E., W. J. Parton, L. A. Joyce, and W. K. Lauenroth. 1988. Primary production of the central grassland region of the United States. *Ecology* 69: 40-45.
- Sala, O. E., F. S Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global Biodiversity Scenarios for the Year 2100. *Science* 287: 1770-1774.
- Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9:615-629.
- Spong, G & Creel, S 2001. Deriving dispersal distances from genetic data. *Proceedings of the Royal Society of London: Biological Sciences* 268:2571-2574.
- Stevens, S., J. Coffin, and C. Strobeck. 1997. Microsatellite loci in Columbian ground squirrels (*Spermophilus columbianus*). *Molecular Ecology* 6:493-395.
- Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. *Nature* 427: 145-148.