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bibkey: 52289

GENETIC VARIATION AMONG THREE PRAIRIE DOG
COLONIES (*CYNOMYS LUDOVICIANUS*) ON THE GREAT PLAINS:
A PRELIMINARY REPORT

by

Gillian Bowser

Department of Biology

University of Missouri-St. Louis

St. Louis, Missouri

and

National Park Service-Rocky Mountain Regional Office

Fort Collins, Colorado

September 1993

INTRODUCTION

"... at a time when ecology and genetics are each racing swiftly towards one new concept after another, yet with little contact of thought between the two subjects, there may be some advantage in surveying, if only synoptically and in a preliminary fashion, the largely uncharted territory between them."

Elton 1938

"Another big loser because of the current [prairie dog] management system is the wildlife ecosystem. Very little consideration is given to state and federally listed endangered species in my opinion. Indeed, one gets the impression they hope no endangered species will be found because it might upset the management system."

Sharps 1987

OVERVIEW

Prairie dogs (*Cynomys* spp) are found throughout North America's central mixed-and short-grass prairies (Forrest et al. 1988). As colonial, fossorial rodents, black-tailed prairie dogs (*C. ludovicianus*) create unique ecosystems preferentially used by a wide variety of organisms (Kruger 1986, Uresk and Bjustad 1980). Once common residents of prairie ecosystems, prairie dogs have been drastically reduced with the introduction of cattle and ranching operations during the early 1900s (Sharps 1987, Uresk and Paulson 1988). The decline of prairie dogs also contributed to the demise of black-footed ferrets (*Mustela nigripes*), an obligate predator on prairie dogs (Clark 1989). The listing of the ferret under the Federal Endangered Species Act (ESA) in 1984 has led to a recent interest in the conservation of prairie dogs (Clark 1989, Sharps and Uresk 1990).

Prairie dogs are key species within the prairie ecosystem. The colonization, extinction, or movement of prairie dog towns impacts a wide variety of threatened, endangered, and sensitive species including

black-footed ferrets (*Mustela nigripes*), ferruginous hawks (*Buteo regalis*), and burrowing owls (*Speotyto cunicularia*) (Kruger 1986, Uresk 1984). Because prairie dogs alter their environment and because their towns persist for fairly long periods, the spatial and temporal distribution of prairie dog towns have implications for the persistence of many other species. Species, like the black footed ferret, have fragmented distributions that mirror the distribution of large (or persistent) prairie dog towns. Spatial and temporal patterns of prairie dogs are thus also good predictors of the past distribution of many plants and animals.

I will describe the spatial and temporal prairie dog population-dynamics using genetic variability (heterozygosity, allele frequency and genetic distance) and environmental features. Such a description is important in understanding the spatial and temporal dynamics of prairie dog towns and the impact of such dynamics on other species. I also propose to tie historical and environmental factors together with a genetic database of present prairie dog population-dynamics. This tie represents a unique combination of population genetics and conservation biology linking genetic parameters through spatial and temporal patterns to landscape features (either natural or anthropogenic). These are the 'uncharted' territories imagined by Elton 50 years ago, which still await exploration. These territories link conservation biology, ecology, and genetics through a unique ecosystem (prairie dog colonies) that contain an endangered species (black-footed ferrets), and a breeding system that tends towards genetic differentiation.

This study is divided into three separate chapters; each one a separate element in a single model of dispersal. The three chapters relate to prairie dog-dispersal through the historic placement of populations in the landscape; a genetic confirmation of patterns of dispersal and exchange; and the influence of environmental factors. Each chapter is a separate entity; however, the results from the first two chapters (history and ecology) form the temporal and spatial database for the third chapter (model of dispersal).

CHAPTER ONE. THE HISTORY AND ECOLOGY OF PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*) ON THE GREAT PLAINS.

"...we put an amendment on the bounty bill for predators. I think South Dakota is the only state [in the nation] where prairie dogs are listed as predators."

Peterson 1987

OVERVIEW

Prairie dogs have a particularly extensive record of historical and ecological information due to their perceived role as an economic pest (Campbell and Clark 1981, Uresk et al. 1981, Sharp 1987). This chapter reviews the literature on the ecology and management history of black-tailed prairie dogs (*Cynomys ludovicianus*), and describes the spatial distribution of prairie dog populations. Specifically, I propose to use historic prairie dog distributions from documents and aerial photographs to examine population movement and growth as determined by directional or areal change in the boundaries of individual prairie dog towns. There are three questions addressing these towns: (1) do they move in a nonrandom manner; (2) what are some of the common environmental features among them; and (3) is there a statistical relationship between town growth and certain environmental features or management actions which would explain their spatial distribution.

BACKGROUND

Black-tailed prairie dogs (*Cynomys ludovicianus*) are colonial, fossorial, harem-polygynous rodents and one of the largest members of the squirrel family--weighing 700-1500 grams (Hoogland 1982, 1985). The colonial habit concentrates prairie dogs into aggregations (colonies) consisting of several clusters (towns) composed of family groups (coteries). This arrangement of coteries and towns enhances the

diversity of prairie ecosystems because the prairie dogs selectively remove vegetation from their territories. Prairie dogs are also important ecosystem regulators for mixed- and short-grass prairie ecosystems (Uresk 1985, Ceballos et al. 1993) as they change the species composition of both the flora and fauna within the boundaries of their extensive colonies (Bennett and Bock 1990, Sharps and Uresk 1990).

Breeding Structure and Dispersal

Coterie, as breeding and feeding territories, have several important characteristics which affect prairie dog population dynamics (King 1955, Chesser 1983, Hoogland 1985, Daly 1992). Each coterie is a defended area for a single family-unit with a dominant adult male, several adult females, yearling offspring of both sexes, and juveniles (Hoogland 1985, Tang-Halpin 1987, Garret and Franklin 1988). Since the females tend to have higher site philopatry than males, sisters and daughters are more likely to stay on the same territory which contributes to different dispersal rates for males and females (Hoogland 1987). Although the exchange of individuals between coterie within a colony or town occurs throughout the year, yearling males and adult non-breeding females (failure to breed may be an impetus for migration) disperse among towns within a colony predominantly in the early spring (Cincotta et al. 1987). Dispersal distance also changes with the season: longer intercolony dispersal occurs mostly in the late spring and early summer when yearling males are the dominant dispersers (Garret and Franklin 1988). The average dispersal distance for prairie dogs is 2.4 km (although Knowles [1985] measured distances of up to 10 km). However, successful dispersal is also influenced by intercolony separation, acceptance into a new coterie, and available territorial space (Garret and Franklin 1988, Devenport 1989, Daly 1992). Predation also limits the numbers of successful dispersers, being the primary cause of mortality to dispersing individuals (Garret and Franklin 1988). Despite all these barriers, dispersers, in some cases, account for up to 42% of the adult population, suggesting considerable gene flow both among- and within-colonies (Knowles 1985).

Ecosystem Regulators

Prairie dog towns are unique ecosystems maintained by the animal's fossorial habit and active removal of vegetation (Uresk and Bjugstad 1980). Prairie dogs preferentially graze buffalograss (*Buchloe dactyloides*), scarlet globemallow (*Sphaeralcea coccinea*), threadleaf sedge (*Carex filifolia*), blue grama (*Bouteloua gracilis*), and western wheatgrass (*Agropyron smithii*) (Collins et al. 1984, Apa et al. 1990). This preferential grazing alters the composition of forbs and grasses as a town ages--changing the plant community from one dominated by grasses to one more forb-dominated (Cincotta et al. 1989, Uresk 1989). However, towns are not static. Active coterries shift along the edge of the town, expanding outward in concentric rings and collapsing back with age (Cincotta et al. 1987). Thus, older towns have an inner circle (old town center) consisting of abandoned burrows and forb-dominated vegetation. As one moves away from the center, the vegetation shifts to a forb-grass mixture, then back to grass-dominated stands (new town) at the town's edge (Bennett and Bock 1990).

Many animals prefer prairie dog towns, attracted by the high forb-diversity, the abundance of tunnels, and the prairie dogs themselves (Apa et al. 1990). Mule deer (*Odocoileus hemionus*), Rocky Mountain bighorn sheep (*Ovis canadensis*), bison (*Bison bison*), black-tailed jackrabbits (*Lepus californicus*), white-tailed jackrabbits (*L. townsendii*), eastern cottontail (*Sylvilagus floridanus*), deer mice (*Peromyscus maniculatus*), and grasshopper mice (*Onychomys leucogaster*) are some of the herbivores who preferentially use prairie dog towns (Kruger 1986, Apa et al. 1990, Deisch et al. 1990). Resident predators include rattlesnakes (*Crotalus viridis*), burrowing owls (*Speotyto cunicularia*), ferruginous hawks (*Buteo regalis*), black footed ferrets (*Mustela nigripes*), badgers (*Taxidea taxus*), and coyotes (*Canis latrans*) (Uresk and Bjugstad 1980, Apa et al. 1990, Clark et al. 1982, Bennett and Bock 1990, MacCracken and Uresk 1990, McDonald et al. 1985, Cully 1991, Blake et al. 1992).

History

The stormy history of prairie dogs and western ranchers is colored by severe mis-interpretations of the prairie dog's role as an ecosystem

regulator (Sharps 1987). Prairie dogs were 'pests' to ranching early in the 19th century when cattle were first introduced to the western states (Uresk and Bjugstad 1980, Uresk and Paulson 1988, Daley 1992). From 1902 to 1971, prairie dog colonies were reduced from 283 million ha to less than 1.2 million ha in the United States (larger areas remain in northern Mexico) as prairie dogs were poisoned or shot on both public and private lands (Collins et al. 1984, Sharps 1987, Ceballos et al. 1993). In 1978, South Dakota legislated laws defining prairie dogs as a 'pest species' and a predator under predator bounty-bills (L. Peterson, former South Dakota legislator). The enactment of these laws contributed in a reduction of over 90% of the prairie dog-occupied acres in South Dakota alone (Sharps 1987).

Prairie dogs remain 'pest' species in most of the western ranching states. Zinc phosphide, a potent rodenticide, is still liberally used as a prairie dog control-agent despite studies showing economic unfeasibility (Uresk et al. 1981, Collins et al. 1984, Uresk et al. 1986). Additional studies showing an improvement of range and a preferential use by cattle and other ungulates of prairie dog colonies have not affected the prevailing rancher sentiment towards prairie dogs (Kruger 1986, Cincotta et al. 1987b). With a rapid prairie dog re-population rate of over 30% within a year after poisoning, ranchers are reluctant to relax control efforts or to explore more risky avenues like predator reintroductions (Clark 1990, Bessken pers. commun.). Furthermore, the hidden economic carrot is a hunting industry where sportsmen spend over 46,000 hunter-days per year shooting prairie dogs, thus generating 3.2 million dollars yearly in state revenues (Sharps 1987).

OBJECTIVES

In this study, I will examine temporal changes in the distribution of prairie dog towns in Badlands National Park, South Dakota over a 40 year period. Specifically, I will report on both areal changes over time as well as extinction and appearance of prairie dog towns. In addition, I will examine the association of prairie dog towns with specific landscape features such as land ownership, land use, roads, and cliffs. I

will use two distinct steps:

- (1) map changes in the town/colony perimeters over time using historic aerial-photography (1954-1992) from United States Geological Survey (USGS) National Aerial Photography Program (NAPP), and Soil Conservation Service (SCS) agencies; and
- (2) create a composite map of landscape features around/within each study colony/town from property and landuse maps (Department of Agriculture).

HYPOTHESES

- (1) Areal changes and extinctions of prairie dog towns occur non-randomly.
- (2) Prairie dog towns are significantly associated with certain landscape features.

STUDY AREA

There are four prairie dog populations (Wind Cave National Park, Devil's Tower National Monument, Theodore Roosevelt National Park and Badlands National Park) on the Great Plains used in the overall study; however, Badlands National Park, South Dakota, is the main focus for this chapter. Badlands (BADL) consists of four separate colonies in the northern unit of the park (Burns Basin, Conata Basin, Kocher Flats, and Tyree Basin), which are some of the larger colonies of prairie dogs remaining on the Great Plains. The largest BADL colony, Kocher Flats (700ha) is located in the Sage Creek wilderness area has four main towns with a total of over 70,000 individuals (Bessken and Vaughn 1991). Conata Basin, although almost as large in area as Kocher Flats, is still subject to periodic reductions by ranchers and land managers. This colony contains 20,000-30,000 individuals. Tyree Basin, the smallest of the three colonies, has fewer than 20,000 individuals in six towns and is controlled along its eastern boundary which borders private lands.

The other three sites are used in this study for contrast, because

there are more complete records of prairie dog density over time than for BADL. Wind Cave National Park (WICA) is located in the Black Hills region of South Dakota. This colony is much smaller than the Badlands colonies, and was used as a research colony for many years (Hoogland 1981-1990). Thus excellent records on dispersal rates, coterie size, breeding behavior, and genetics exist for this colony (King 1955, Hoogland 1981, 1985, Garret and Franklin 1988, Daley 1992). Since the history and behavior of these towns has been well documented, a detailed picture of the movement and exchange of individual animals among towns within the colony is available (J. Gyra and L. Hayes pers. commun.). In contrast, Devil's Tower (DETO) is a single town, isolated on the western edge of the Black Hills. Prairie dog numbers at DETO have been maintained at a low level (50 individuals) with biannual reductions (Gyra, pers. commun.). Similarly, Theodore Roosevelt (THRO), located in the badlands of North Dakota, is part of a larger population in the surrounding grassland. This colony is still reduced periodically by management. Both of these colonies have been restricted in both their growth and potential movement by boundaries between park and private lands (prairie dogs are still controlled by law along park boundaries to prevent the spread of these 'pests' onto private lands).

METHODS

Data will be compiled primarily for the BADL colonies. The following sections refer only to BADL.

Aerial Photography

Aerial photographs are available for the study region from three agencies--United States Geological Survey (USGS), National Aerial Photography Program (NAPP), and Soil Conservation Service (SCS). Photography is available for the BADL area for every year starting in 1945; however, as the resolution is variable (photographs taken at a higher altitude have lower ground resolution), I preferentially choose photographs from low-level flights for the best resolution (1:24,000) whenever possible (Uresk and Schenbeck 1987, Schenbeck and Myre 1986).

I picked photographs at approximately ten-year intervals for a set of five different years (Table 1).

Aerial photographs from USGS, NAPP, and SCS will be scanned into a digital database using a desk-top scanner. The scanner transfers spectral images (both black and white and color-infrared-CIR) into a digital database which can then be analyzed spatially and temporally using video-scanner software that measures the area of selected towns.

Statistical Tests

Badlands National Park has a number of towns in the northern unit. I will use 13 of these towns to test for nonrandom movements over time. I will generate the database for this test by scanning the set of aerial photography and recording the number of extant towns, the area and perimeter of each town, and the degree of change (positive or negative) in the perimeters of each town. I will then test the change in area (growth) by creating a random model where a town has an equal probability of growing in any given direction. For each set of aerial photographs I will then have two datasets: the first generated by measuring changes in town perimeters from one year to the next; and the second from taking the same amount of growth, but dividing towns into quadrants and assigning a random probability for growth to each quadrant. I would then test each years growth by overlaying these quadrants of random growth with actual growth and measuring the differences using a Chi Square statistic. I can repeat this test using a uniform growth model, where each quadrant has an equivalent probability of growth--total growth for a given year would be equally divided among the quadrants.

WORK TO BE COMPLETED

All of the aerial photographs have been purchased; however, I still need to scan the photographs using a PC based scanner. Each town will have to be scanned into the computer, overlaid by a grid to divide towns into quadrants--the number of quadrants will change with the size of each town. This will be done by re-scaling and then printing each image

so each photograph is the same scale. I can then overlay the grid using an identifiable center-point (like a road or building) that appears in each year of photography. I will then generate area of each town for each year of photography and the areal change among years.

Table 1. Summary of all black and white aerial photography available for three prairie dog colonies in the Badlands area of South Dakota (Jackson and Pennington counties; UTM zones 13 and 14). These data include UGSG, NAPP, SCS; high level NASA (1:1,000,000) is available for all years starting in 1973, but does not have the fine resolution needed to delineate prairie dog towns. Those photographs with poor resolution can be used to trace short-term fission and fusion events of towns that may not appear in the ten year-intervals.

Year	Population			Scale
	Badlands East	Badlands West	Conata Basin	
1939+	+	+	+	1:24,000
1941	+	+	+	1:24,000
1954**	+	+	+	1:24,000
1961	+	+		***1:100,000
1968**	+	+		1:24,000
1970	+	+		***1:100,000
1974	+	+		***1:100,000
1978**	+	+		1:80,000
1980*	+	+		***1:100,000
1981**			+	1:5,000
1989*&	+	+	+	1:24,000
1990**	+	+	+	1:40,000
1991	+	+	+	1:40,000

*= Color Infra-Red (CIR) photography available.

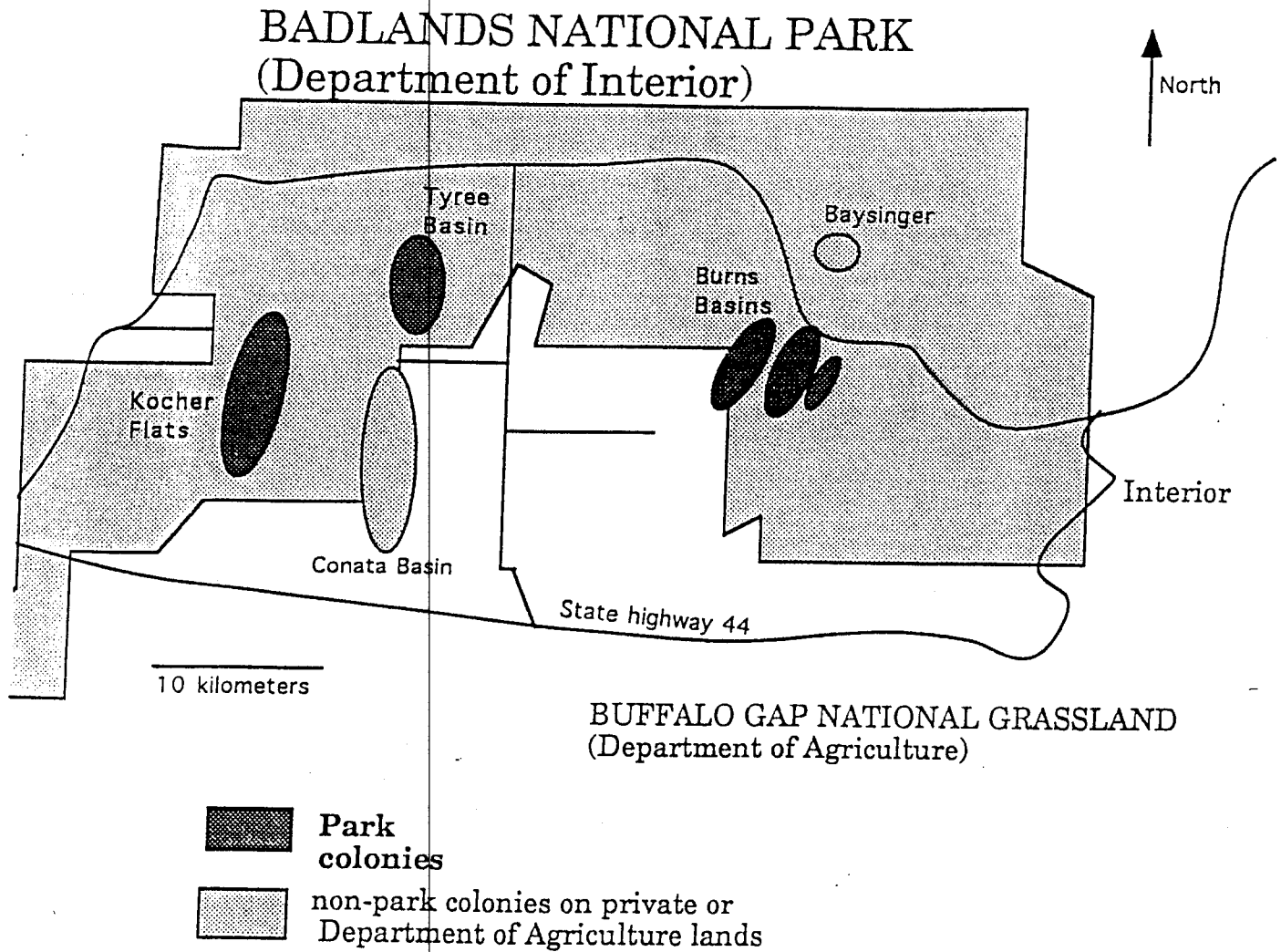
**=years used for historical comparison.

*** This scale is too high to distinguish prairie dog colonies (Schenbeck and Myre 1990).

&= most recent management maps of prairie dog towns are based on this year's photography.

Blanks indicate incomplete photographic coverage for that area.

Figure 1. The distribution of three prairie dog (*Cynomys ludovicianus*) populations in Badlands National Park, South Dakota.



CHAPTER TWO. THE POPULATION GENETICS OF PRAIRIE DOGS (*CYNOMYS LUDOVICIANUS*) ON THE GREAT PLAINS.

"...the fundamental reality is the information that is carried by the chromosomes coiled up in the reproductive cells, not the characters which the individuals manifest."

Jacquard 1974

DRAFT

OVERVIEW

The genetic structure of prairie dog populations on the Great Plains, as measured by gene flow, allele frequencies, and genetic distance, can be used to describe the effects of linear distance on genetic differentiation. There are two questions addressed in this chapter: what is the genetic structure of prairie dog populations within the Great Plains, and what is the effect of geographic distance on their genetic relatedness. To address these questions, I used starch-gel electrophoresis on prairie dog blood-samples to obtain mean allele frequencies of prairie dog towns located in the Great Plains. These allele frequencies were used to estimate genetic distance among towns and to test the hypothesis that more distant towns are less similar in allele frequencies than towns located closer together.

BACKGROUND

The genetic structure of colonial animals, such as prairie dogs, is directly linked to dispersal, acceptance, and territory vacancies (Foltz and Hoogland 1983, Cincotta et al. 1987b). Dispersal here is defined the number of animals leaving a colony as some function of colony density or territoriality (coterie occupancy); and acceptance is defined as the proportion animals arriving at a new colony which become integrated into the colony's genome. If the colonial habit restricts breeding

opportunities, then the chance that two colonies will genetically diverge increases. If the numbers of dispersers are severely restricted (local family groups aggressively kill or chase off migrant individuals [Apa et al. 1990]), then the probability that two adjacent populations (colonies) will differ genetically is high (Schaal 1980, Smith and Patton 1980, Slatkin 1981, 1987, Garcia and Gardenal 1991).

Genetic Differentiation

Genetic differentiation has two evolutionary elements that act in opposition: gene flow and genetic drift (Slatkin 1985). Gene flow retards speciation by 'gluing' populations together through the exchange of genetic material (Schaal 1980, Smith and Patton 1980, Hartl and Clark 1989). Genetic drift, in turn, pulls isolated populations apart by the gradual accumulation of genetic differences or random loss of alleles (Wayne et al. 1991). The influence of genetic drift and gene flow on the genetic structure of a population depends on dispersal or lack thereof; hence, an estimate of successful dispersal is an important aspect of explaining genetic patterns among populations (Wayne et al. 1991, Britten and Brussard 1992, Stewart et al. 1993).

The measurement of genetic drift and gene flow was first developed by Wright (1965) with a group of statistics based on neutral evolutionary drift. If two populations randomly inter-breed, allele frequencies within the population will have a normal distribution. This distribution, the Hardy-Weinberg (HW) assumes mendelian inheritance patterns and represents an expected value where, all else being equal, populations should have an allele distribution and fixation index representative of random mating and exchange (Slatkin 1981, Gale 1990).

F-statistics

When allele frequencies deviate from HW, either more alleles are fixed than expected, or there are more heterozygotes than expected. These deviations from random mating can be measured using Wright's F-statistics (Slatkin 1993). F-statistics provide a way of describing the genetic variation of a population based on the average heterozygosity of polymorphic loci.

There are two types of F-statistics that relate to gene flow and dispersal, F_{IS} and F_{ST} , that represent differences in allele distributions among/within populations (Wayne et al. 1991). F_{IS} is the inbreeding coefficient of an individual relative to its subpopulation and measures the deviation of observed heterozygotes in a population from expected frequencies under HW. F_{IS} should decline (approaches zero) with migration because new alleles would be brought into a population (higher gene flow), and increase as populations become more inbred.

In contrast, F_{ST} measures the effect of population subdivision, and describes the reduction in heterozygosity due to genetic drift within populations (Patton and Feder 1981). F_{ST} increases (approaches one) as populations differentiate--especially if the populations are small and/or isolated (Liengiri et al. 1990). As F_{ST} declines, populations become more similar (have a higher gene flow).

F-statistics are calculated directly from measures of heterozygosity. Individuals are scored based on allele frequencies such that H_i = heterozygosity of individual in a subpopulation (or the average heterozygosity across loci for an individual) and H_s is the average heterozygosity of a random mating population in the i^{th} subpopulation, where $H_s = 2p_iq_i$ (where p and q are allele frequencies). H_t is the total heterozygosity of a randomly mating population $= 2P_0Q_0$. Thus the F-statistics can be calculated from heterozygosity:

$$F_{IS} = (H_s - H_i) / H_s$$

$$F_{ST} = (H_t - H_s) / H_s$$

From F-statistics, dispersal can be estimated (in the form of gene flow) by the mathematical relationship between the probability of alleles going to fixation among subpopulations (F_{ST}) and the number of migrants or dispersers (N_m) per generation:

$$F_{ST} = 1 / (1 + 4N_m)$$

In this study, I will concentrate on dispersal as one of several mechanisms influencing genetic differentiation. Other elements

(selection, founder effect) also play important roles in shaping genetic differentiation. For example, if there is a significant founder effect, there may be a deficiency in heterozygosity among populations. If there is a decline in heterozygosity, founder effects or selection for homozygosity cannot be ruled out. Furthermore, there is a potential for population bottlenecks to affect populations in a similar pattern as population sub-division; however, the recent history of the population could help to distinguish possible bottleneck events that occurred due to man's fragmentation of prairie dogs range on the Great Plains.

OBJECTIVES

I plan to describe genetic structure and estimate dispersal among prairie dog colonies on the Great Plains and determine if geographic separation of the towns accounts for variation in genetic distance (as calculated from allele frequencies) between local towns and colonies. There are three objectives:

- (1) To describe genetic variation for each town by an allele frequency, and average heterozygosity.
- (2) To determine genetic distance among towns from allele frequencies and examining any groupings of towns by cluster analysis and principal component analysis.
- (3) To test the isolation by distance hypothesis on a social colonial species.

HYPOTHESES

I will test two hypotheses.

- (1) Patterns of genetic variation differ among towns, colonies, and regions resulting in genetic structuring of prairie dog populations.
- (2) The rate of gene flow (dispersal) should match the geographic separation among colonies. Corollary: dispersal is controlled primarily by linear distance.

STUDY SITES

This study was conducted in four regions on the Great Plains: Theodore Roosevelt National Park (THRO), North Dakota; Wind Cave National Park (WICA), South Dakota; Devil's Tower National Monument (DETO), Wyoming; and Badlands National Park (BADL), South Dakota (Figure 1). Three of the four regions (THRO, DETO, WICA) had a single colony with fewer than ten towns or less, while the fourth region, BADL, had several colonies with an average of ten or more towns per colony (Table 1). Each of these regions is isolated by at least 100 kilometers of uninhabitable (for prairie dogs) lands or by areas where prairie dogs are severely reduced by humans.

METHODS

Twenty individuals, on average, were sampled from each of 17 towns from four regions (smaller towns may have sample sizes less than 20). At BADL, collections were stratified such that each colony was represented by four or five towns. Animals were sampled along variable-length transects that bisected each town. Individuals were collected along these transects at flagged coterie centers (for the smaller towns) or at 200-meter intervals (to ensure samples were from separate coterie) for the larger towns. The approximate center of each coterie was determined by behavioral observations for smaller towns (animals were observed by telescope or binoculars for territorial displays [Tang-Halpin 1987]). The territorial displays were more difficult to observe in larger towns, so 200-meter intervals were flagged as a distance greater than published accounts of coterie size (Hoogland 1981, Knowles 1985, Garret and Franklin 1988, Tang-Halpin pers. commun.) and the cluster of mounds nearest to the flagged-interval was marked. Animals were destroyed or trapped within a 20m radius of these marked clusters. The locations of each sample collected were recorded by the distance and bearing from cluster center and transect interval. These locations were also mapped so I could later determine the possibility of two samples coming from the same coterie.

Sample Collections

Blood and tissue samples were collected by either trapping with National Double Door live-traps (WICA, DETO, Tyree 91 [one of the towns within BADL]), or by destroying the animals by shotgun. Blood samples were collected from trapped animals by clipping a rear toe-nail or foot puncture (Hoogland pers. commun.). Blood from destroyed animals was collected with a syringe inserted in the central body cavity or neck region. All blood samples were stored in 250ul pipettes, treated with EDTA for blood stabilization, and immediately placed on dry ice to prevent protein degradation. Liver samples were collected from any animals destroyed during the sampling or from areas where mangement reductions were taking place (BADL, THRO). Liver samples were stored in small sample bags and immediately frozen.

All samples were stored on dry ice and shipped to University of Missouri-St Louis (UMSL) for long-term storage in a -80° C ultracold freezer.

Electrophoresis

Electrophoresis procedures followed those of the laboratories of V. Sork, A. Templeton, and published accounts (Richardson et al. 1986, Hamrick and Godt 1990, Hillis and Moritz 1990, Liengsiri et al. 1990, Acquaah 1992). Blood samples were thawed and buffered with distilled water, detergent, and NADP; and then refrozen at -80° C. Samples were loaded onto filter paper-wicks and run on 12% or 10% starch gels (enhanced with $MgCl_2$, NADP, and 10% sucrose) for 8-14 hours at 75mA using Poulik (ph 8.0), Tris Citrate discontinuous (ph 6.3/6.7), and continuous (ph 8.0) buffer systems (Richardson et al. 1986, Pasteur et al. 1988).

Blood samples were intially screened for polymorphic loci while buffering systems, run times, and staining techniques were still being developed. Therefore, only samples with larger amounts of blood were used during these initial trials; hence, the full compliment of 20 samples per town was not completed for this proposal.

All blood samples were stored for future runs with a stabilizing detergent to prevent the dehydrogenases from degrading. Some samples

(DETO) were also centrifuged at 100gs for three minutes in an attempt to reduce smearing on the gels.

DATA ANALYSIS

Data analysis focused on two questions: (1) what is the genetic variability among prairie dog populations; and (2) is there a relationship between genetic distance (as determined from allelic frequencies) and geographic distance for individual prairie dog towns.

Genetic Structure

The first question is to determine what types of patterns are indicated from the allele frequencies and what is the probability of genetic fixation for the individual colonies or towns. There are five components to this question: (1) the genetic variation of individuals within towns and colonies; (2) the hierarchical F-statistics to describe the inbreeding, genetic fixation, and migration among towns (as sub-populations); (3) the deviation of allele frequencies from HW expectations; (4) Modified Rogers distance calculation to cluster towns based on their genetic identities; and (5) the calculation of the first two principal component axes (based on the frequency of the most common allele).

Hierarchical F-statistics are based on the genetic variation of individuals (allele frequencies) and they characterize populations based on inbreeding and exchange. F_{is} , as a measure of the inbreeding (genetic fixation) within a population, approaches zero as the correlation of genes within an individual within a population declines (Weir and Cockerham 1984). F_{st} can be used to determine sub-population differentiation and to estimate the number of migrants among those sub-populations per generation. F_{st} also relates to the correlation of genes of different individuals within the same population (Weir and Cockerham 1984). Hierarchical F-statistics and heterozygosity (the relative proportion of heterozygotic individuals within a population) can determine fixation rates and genetic variability for each 'unit'--town, colony, or population.

F-statistics are only one aspect of a population's genetic structure.

The separation among populations (genetic distance), and deviations of these populations from expected allele frequencies based on random mating, are two other aspects of the genetic structure of a population. The genetic separation among populations can be used to generate clusters of similar (neighboring) populations, while deviations from random mating can be indicative of genetic isolation, fixation, founder effect or selection (Leberg 1993).

I can examine the effects of individual enzymes on the cluster patterns by using principal component analysis and ordination techniques. The principal component analysis (PCA) ordines prairie dog towns based on multivariate analysis of allelic frequency. Genetically similar towns are closer together on a PCA axis, those located further apart on the same axis are more genetically distinct. Factor scores can be used with later analyses to examine environmental and population processes affecting genetic structure (Stewart and Baker 1992, White and Svenson 1992, Stewart et al. 1993).

Distance Effects

The second question tests a genetic distance measure against geographic distance. Genetic distance describes the probability of choosing an identical pair of alleles from two populations (Huang 1992) and characterizes the frequencies of various genotypes among population members such that if the "...distribution of numbers of individuals among the various geneotypes in two populations is the same in both, the distance between the two is zero or one" (Jacquard 1974). Modified Rogers and Nei's distance are two such measures; calculated from a pairwise comparison among sub-population allele frequencies. These distances can be used to discern patterns (based on the probability of a pair of alleles being identical) among and within each unit based on a clustering algorithm (Swofford and Selander 1981). Both Modified Rogers and Nei's distance use allele frequencies to determine the genetic similarity between two populations. However, I used each measure for a different reason: Modified Rogers (the number of heterozygotic alleles that are fixed at different loci) is more sensitive to variation among related towns (such as two towns within the same colony), while Nei's

(the number of alleles that are fixed in both populations) is more accurate when estimating the relationship among widely separated populations (i.e. towns among regions). Furthermore, Modified Rogers distance, as a metric data set can be used in ordination techniques to look at the distribution of alleles along different distance classes (Stewart and Baker 1992, Stewart et al. 1993).

Genetic distance data can be presented visually by drawing a dendrogram--a pictorial representation of the genetic distance that clusters populations together based on which ones have the shortest genetic distance, or the shortest distance among clusters. The clustering algorithm used for this study was UPGMA based on a euclidean distance coefficient. The dendrogram was generated by BIOSYS using Modified Rogers distances to draw the clusters (Swofford and Selander 1981).

The geographic distance between each pair of towns was calculated from USGS topographical maps (1:50,000). A center point was marked for each town, and linear distance between these midpoints was measured and converted from centimeters to kilometers. Total distance between each region was estimated from road atlases and all towns within a region received the same distance score when compared to a separate region. The relationship between geographic and genetic distances will be examined using simple linear regression. Data were tested for normality, log-transformed, and analyzed with UNIX/STAT on a SUN workstation.

RESULTS

Work Completed

Twenty-two presumptive loci were scored on 199 gels (10-20 samples per gels) from December 1992 to April 1993 (gels that were not scorable or were repeat runs are not included in this total, and plant enzymes [three] tested during the initial runs were also dropped). Loci were called 'consistent' when sequential buffers confirmed the existence of variable individuals (and thus were not artifacts of a particular gel run or stain). Five loci were polymorphic and consistent: 6-phosphate glucose dehydrogenase (6PGD), phospho glucomutase (PGM-2), isocitrate dehydrogenase (IDH), fluorescent esterase (Fest-1,-2), and phosphoglucose isomerase (PGI-2) (Table 2). There were two loci that were consistently monomorphic: lactate dehydrogenase (LDH), lactate acid phosphorylase (LAP); both showed consistent clear bands and were scoreable for all samples run (note exceptions for LAP in Table 2). These loci, with the exception of LDH, which was not run for all populations, were used in the analyses of allele frequencies. Malate dehydrogenase (MDH) was included in the analysis because the allele was initially consistent, but apparently was degraded during an accidental warming to -6°C for 48 hours. The remainder of the loci tested fell into two categories: (1) inconsistent--polymorphic individuals could not be resolved on sequential buffers, or were not visualized during repeat runs; or (2) degraded samples--loci that were visualized earlier in the study, but did not appear in later runs (after samples were warmed for 48 hours). The remaining loci were either not screened for all populations or could not be resolved on any of the buffer systems tested (Table 3).

Sample Distributions

Numbers of males and females collected varied among study sites (Figure 2). One population, Devil's Tower (DETO), was particularly skewed: of the 20 captures, 65% were juveniles (13 individuals), 20% were adult males (4 individuals) and 15% adult females (3 individuals). These animals were trapped from approximately nine coterries in one isolated town, and showed a potential for high levels of inbreeding--only

one loci (PGM-1) was variable. In general DETO had more fixed alleles (alleles with the same pattern--monomorphic or polymorphic--in all individuals) than any other tested population.

Wind Cave (WICA), another small isolated population, had three heterozygotic loci (PGM-2, PGI-1, F_{est} -2) from a sample size of 12 individuals (8 adult males, 1 adult female, 3 unidentified) from approximately three coteries. Theodore Roosevelt (THRO) with 15 juveniles, 4 adult males and 1 adult female (unknown number of coteries) was much more variable than either WICA or DETO (six loci were heterozygotic for THRO). The sex and age ratio for Badlands (BADL) was close to 50%; however these animals were shot, so we were able to choose animals to some degree (to avoid oversampling juveniles or adults).

Sex and age distribution is a potential confounding factor for allele frequencies. Because prairie dog females are less likely to move than males, there is a higher chance of inbreeding among the females. However, since most of the samples were skewed with higher numbers of males, this should not exert a strong influence on the results.

Genetic measures

The following sections summarize the genetic measures for prairie dog populations from horizontal starch gel electrophoresis. Allele frequencies for the loci used in the analysis are in Table 4.

F-Statistics

F_{is} , the level of inbreeding among individuals within a subpopulations, was close to zero for all four regions ($F_{is} = 0.017$), yet the BADL region alone deviated from random with higher levels of inbreeding among the towns ($F_{is} = 0.176$). Differences between regions, and among towns within a region were greater than that found between isolated island populations in other mammals (Wayne et al. 1991).

F_{st} , a measure of the correlation (fixation index) among gametes for the total of all regions, was high (0.381), with 38% of the total allele variation found explained by genetic differentiation among populations (Hartl and Clark 1989). When BADL data are separated from the other

regions, 40 % ($F_{st}=0.406$) of the total allele variation can be explained by differences among towns (Table 4). F_{st} for the three regional populations was higher than expected: 28% ($F_{st}=0.281$) of the allelic variance observed could be explained by differences among populations.

The number of migrants among colonies was lower than expected based on previous studies (Chesser 1983). For the regional populations, the migration (gene flow) was high ($N_m=0.640$), despite the large distances between the regions. Such a level of migration among the regional colonies is comparable with the migration within a colony (BADL: $N_m=0.366$), or among all samples (among and within regions) in the study ($F_{st}= 0.381$, $N_m= 0.406$). These rates are much higher than those reported for isolated populations with 0.2 migrants per generation ($N_m=0.02$, Wayne et al. 1991), yet lower than rates for other prairie dog populations with five migrants per generation ($N_m=5.31$, Chesser 1983).

Private and Regional Alleles

Several populations appeared to have regional alleles that could influence the F-statistics and contribute to genetic differentiation (Britten and Brussard 1992). Private alleles, alleles segregating in one sample that are not present in others (Britten and Brussard 1992), were found in several prairie dog regions on the Great Plains. LAP segregated in two towns in two different colonies in BADL (Conata West and Baysinger), although this locus was monomorphic in most other populations. Regional alleles, those unique to a given region (Britten and Brussard 1992), appeared in the BADL-east colony. MDH segregated with four alleles in this colony, yet was fixed for all other locations with two alleles. Figure 2 illustrates the segregation of private, regional, and different alleles for the entire study population.

Genetic Distance

There were no clear relationship among towns as demonstrated by two different measures (Table 5). Towns within colonies were, in some cases, more dissimilar than different colonies. However, if the three regional populations are removed from the analysis, then some neighboring towns (Tyree and Tyree 91) did 'appear' closer in their

genetic distance than towns farther apart (Kocher and Baysinger for example).

Towns were clustered by genetic distance and the resulting cluster dendrogram (Figure 3) did not show any patterns indicating geographic separation. While Tyree and Tyree91--two towns separated by less than 5 km--cluster together, Haybutte, a town 7 km north of Tyree91, was quite distinct. Similarly, Baysinger (a controlled town on private land) and Bigfoot (a town on park land) are geographically quite close (5km), but they are distinct genetically. In fact, DETO, a separate colony from Wyoming (over 500 km apart), clusters within a group of BADL towns before Baysinger does, indicating that Baysinger may be quite distinct from the rest of the BADL complex.

Distance Hypothesis

I used linear regression analysis to test geographic distance as a predictor of genetic distance. If this hypothesis is supported then geographic distance among towns or colonies should be positively related to genetic distance--towns closer together should be more similar genetically than those farther apart. There was no significant relation between log-transformed genetic and linear distance ($R^2=0.01$, $p>0.74$, $df=25$) for towns within BADL. Moreover, there did not appear to be any pattern suggesting a non-linear relationships between genetic and geographic distance data. Given the distribution of towns in the cluster analysis, this regression suggests that factors other than geographic distance control the genetic differentiation among prairie dog towns.

Comparative Studies

Isozyme variability in prairie dog populations has been well-documented (Chesser 1983, Foltz and Hoogland 1983, Daley 1992, Hoogland 1992). Table 6 summarizes the results of these past studies, along with preliminary results from this study. The number of polymorphic loci, the total number of loci screened, and the number of samples were similar in all of the studies (Table 7). Four polymorphic loci were found in all the studies on *Cynomys* ; however, each study found a different set of polymorphic loci from screening the same number of

loci, suggesting local differentiation (founder effect) or selection.

PRELIMINARY CONCLUSIONS

This work demonstrates that genetic variation does exist among populations and towns sampled on the Great Plains. To perform the dispersal analyses in Chapter Three, genetic variation had to be demonstrated at the town and colony level. Preliminary results show variation in and among all towns with the exception of DETO--a restricted colony/town isolated by more than 10 km of uninhabitable (for prairie dogs) rangeland (*Gyrodactylus communis*). Although my results parallel those of other studies on isolated populations (Wayne et al. 1991), my F -statistics are noticeably higher ($0.381 F_{st}$ and $0.406 F_{is}$) than those found for prairie dogs in the southwest (Chesser 1983), or the smaller WICA population (Daley 1992--see Table 8). These differences may dissipate as sample size increases with re-analysis.

Overall I am confident that sufficient variation exists to generate the information needed for Chapter Three. Although F_{st} and F_{is} values currently reflect only a segment of the samples, the distribution of heterozygotes indicates that there are patterns within the genetic variation that could be teased out with additional spatial analysis. Furthermore, the distribution of towns on the cluster dendrogram indicate, by preliminary analysis, that there is a potential for other environmental factors to influence the genetic distribution. Geographic distance does not predict genetic distance among towns within a colony, hence other factors in the environment such as topography, soil, land use, etc., may be important controls on prairie dog dispersal.

Table 1. Prairie dog (*Cynomys ludovicianus*) populations on the Great Plains used for genetic analyses by starch gel electrophoresis. Animals were collected by trapping or shooting during the summers of 1991 and 1992.

Location ¹	# colonies ²	#towns ³	approx # individuals ⁴	#samples collected	#samples analyzed ⁵
DETO	1	1	75	20	12
WICA	1	1	200	12	12
THRO	1	1	75	20	20
BADL	4	14	+70,000	200	109

1. DETO=Devil's Tower National Monument, Wyoming, WICA=Wind Cave National Park, South Dakota, THRO=Theodore Roosevelt National Park, North Dakota, BADL=Badlands National Park, South Dakota.

2. Total number of colonies within a region.

3. Number of towns within a location.

4. Approximate number of individuals in each location based on the number of active burrows (Bessken and Vaughn 1991).

5. Number of blood samples analyzed as of April 1993.

Table 2. Summary of loci classification from electrophoresis studies of four prairie dog (*Cynomys ludovicianus*) populations on the Great Plains. Loci were classified based on the ability to obtain repeatable results using sequential buffers--buffers that varied in their pH and pore size --the inner space within a gel that the proteins move in based on their electrical charge (Liengiri et al. 1991). Consistent loci were those loci that had repeatable results on sequential buffer runs (Richardson et al. 1986).

Classification	Loci	#populations monomorphic	# populations heterozygotic	Grade/comment
Consistent and polymorphic	6PGD, PGM-2, IDH, Fes-1,-2 PGI-	2	12	(A to A+) Bands clear and easy to read. Repeatable on different buffer systems.
Consistent and monomorphic	LDH, LAP**	8	3	(A- to B+). Bands generally clear, some blurred.
Inconsistent ¹	MDH*, ALD, PGM-1, PGI-1,	12	15	(D) Bands blurred, smeared, but readable to some degree.
Inadequately screened ²	Per, ACPH, SDH, ADH, GOT, MPI, SOD			(D-) bands missing or absent on all buffers tried, or only appeared once or twice.
Degraded or unreadable ³	ME, TPI, GDH, NP	4	11	(F) Bands were resolvable earlier, but could not be repeated on same buffer. systems at a later date.

1= not repeated on sequential buffers, or unable to get results on current buffers.

2= enzymes were not tested on all populations or gel/staining system was problematic

3= samples were accidentally warmed to -6C for 48 hours.

*MDH had different numbers of alleles in some populations (from 2 to 4 bands). However, since MDH could not be resolved after accidental warming for 48 hours, earlier results could not be repeated.

** LAP was polymorphic in two towns and is included in the following analysis; however, LAP has not been found to vary in the literature so this result will have to be validated.

Figure 1. The historic distribution of black-tailed prairie dogs (*Cynomys ludovicianus*) in North America. Dots indicate regional study sites (1=DETO, 2=WICA, 3=THRO, 4=BADL).

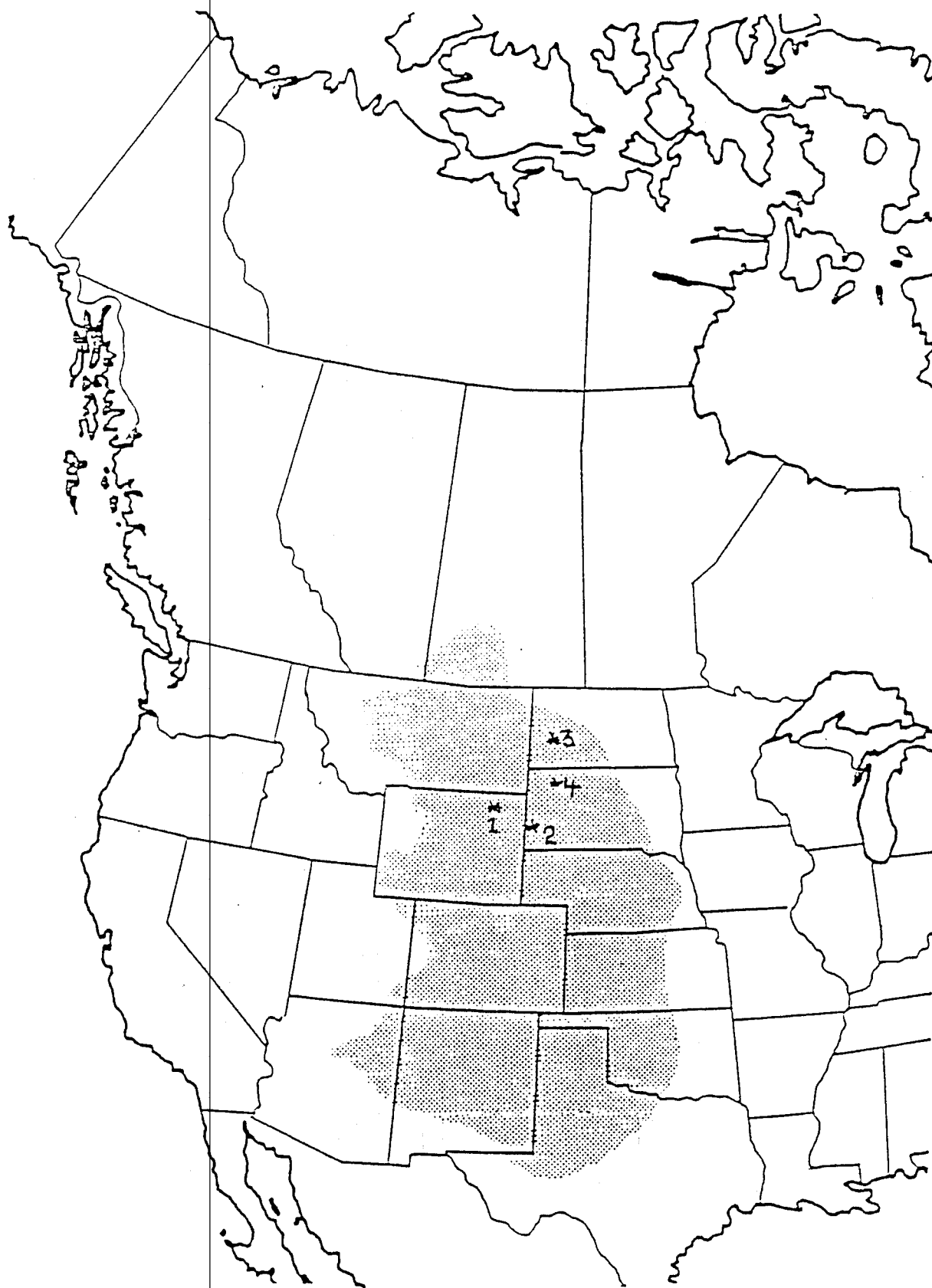


Figure 2. Sex and age distributions of prairie dogs (*Cynomys ludovicianus*) sampled from 17 towns (in four regions) on the Great Plains of North America.

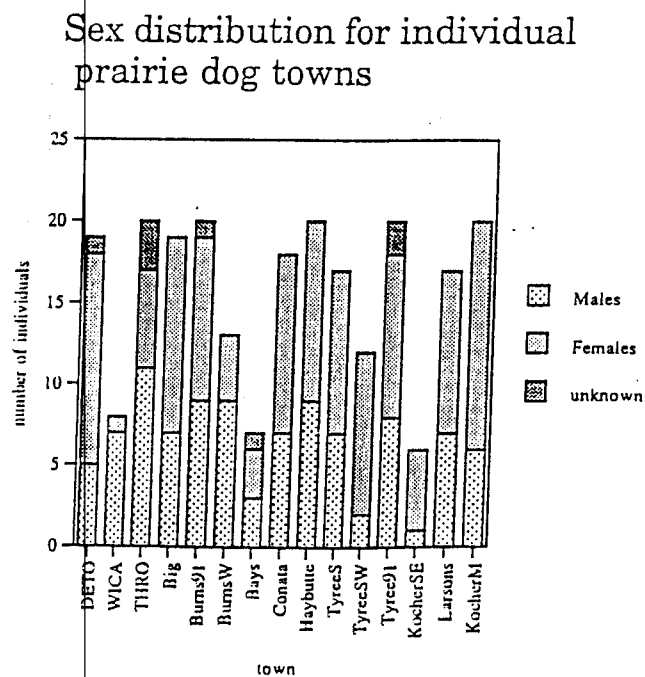
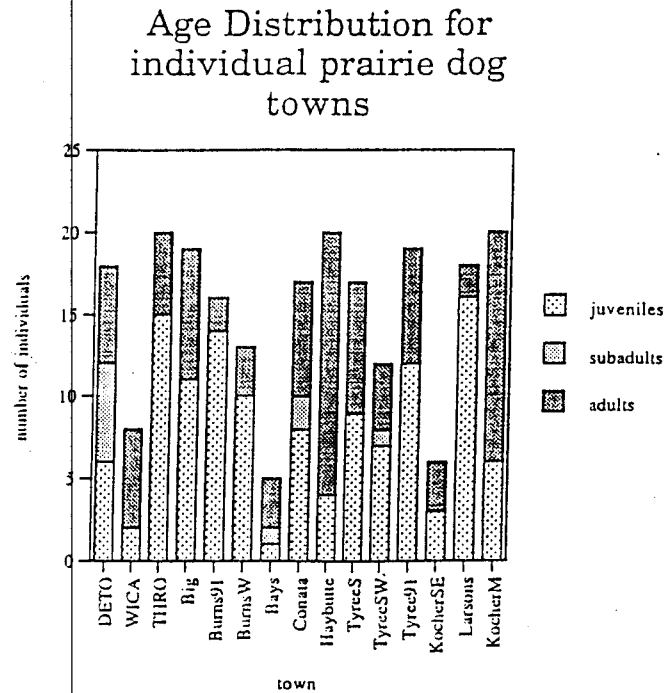


Table 3. Summary of electrophoresis results for all loci tested from four populations of prairie dogs on the Great Plains. Enzymes were tested based on available receipts as well as published studies (Richardson et al. 1986, Pasteur et al. 1990). All enzymes listed below were tested on blood samples (liver samples will be analyzed at a later date).

Enzyme	Koch	BurnsW	Big	Bay	Burns	Cona	Tyr91	Tyree	Hay	Tyrees	Cona2	THRO	DETO	WICA
6PGD	H	H	H	H	H	H	H	M	H	H	H	H	M	H
PGM-1	H	M	M	M	H	-	H	-	H	H	H	H	H	M
PGM-2	-	-	H	-	-	-	-	-	-	-	-	-	M	H
ME	M	-	NR	H	-	H	-	H	H	NR	NR	NR	NR	-
MDH	M	M	-	M	-	M	H	M	-	H	M	H	M	-
LAP	M	M	-	H	-	H	-	-	M	M	-	M	M	M
PGI-1	M	-	-	-	-	H	H	H	H	M	M	NR	H	M
PGI-2	-	-	-	-	-	-	-	-	-	H	-	-	-	H
IDH	H	-	-	M	-	-	M	M	H	M	H	H	-	M
NP	-	-	-	-	-	H	-	H	H	-	-	H	-	NR
GDH	NR	NR	-	-	H	M	H	H	M	NR	M	H	-	-
TPI									NR					
Fest-1										H	H	-		H
Fest-2										M	M			M
LDH										M	M			M
SDH	-	NR	-	-	-	NR	-	-	NR	NR	NR	-	NR	-
MPI														M
ALD					H									
ACPH		NR												
GOT		NR												
Per		NR												
ADH														

NR= no reaction in any buffer system tested.

- = sample tested, results not repeated or inconsistent.

H=heterozygotic.

M=monomorphic.

codes: Koch=Kocher Flats (BADL), Big= Bigfoot, BurnsW= Burns Basin West, Bay= Baysinger, Burns= Burns Basin East and center, Cona= Conata Basin (two smaller towns are all included), Tyr91= Tyree Basin 1991, Tyree= Tyree Basin 1992, Hay= Haybutte, Tyrees= Tyree South 1992 (two towns), Cona2= Conata Basin second town, THRO= Theodore Roosevelt National Park, DETO= Devil's Tower National Park, WICA= Wind Cave National Park.

Blanks indicate that the enzyme was not tested for that population. These blanks will be filled once all of the samples are re-run on the same buffer series (Tris discontinuous and continuous).

Table 4. Allele frequencies for prairie dog (*Cynomys ludovicianus*) towns on the Great Plains. Data are presented by the number of samples analyzed (N) and the two predominant alleles (those alleles used for scoring). Sample size will vary with the number of bands that could be scored on a given gel. Some samples degraded or smeared during the gel-run and were not scored. Conata towns and Tyree south and southwest were combined in this table since both had small sample sizes due to degraded samples (Conata) or insufficient (low volume) samples (Tyree).

Locus	Hay	Burns West	Bay	Conata	Big	Kocher	Tyree	Tyree South	THRO	WICA	DETO
6PGD											
N	20	12	5	5	18	13	20	20	18	13	12
a ¹	.225	.042	.400	.100	.250	.077	.000	.125	.417	.154	.000
b ²	.775	.958	.600	.900	.750	.923	1.00	.875	.583	.846	1.00
IDH											
N	20	12	5	5	18	13	20	20	14	13	18
a	.950	.000	.000	.000	.000	.038	.118	.050	.643	.000	.000
b	.050	1.00	1.00	1.00	1.00	.962	.882	.950	.357	1.00	1.00
PGM											
N	20	12	5	5	18	13	17	20	20	13	18
a	.075	.000	.000	.000	.139	.692	.118	.050	.500	.192	.056
b	.925	1.00	1.00	1.00	.861	.308	.882	.950	.500	.808	.944
LAP											
N	20	12	5	5	18	13	20	20	20	13	18
a	.000	.000	.100	.600	.000	.000	.000	.000	.000	.000	.000
b	1.00	1.00	.900	.400	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH*											
N	20	12	5	5	18	13	20	20	20	13	18
a	.025	.000	.000	.400	.000	.000	.000	.000	.150	.000	.000
b	.975	1.00	1.00	.600	1.00	1.00	1.00	1.00	.850	1.00	1.00
PGI											
N	20	12	5	5	18	13	20	20	20	13	16
a	.025	.000	.000	.400	.000	.000	.100	.150	.000	.000	.313
b	.975	1.00	1.00	.600	1.00	1.00	.900	.850	1.00	1.00	.688

a=fast allele on gel.

b=slow allele on gel.

*= MDH is a tetramer. Only two alleles were used in this analyses (the others were faint and/or inconsistent).

Table 5. Genetic distance for prairie dog (*Cynomys ludovicianus*) populations on the Great Plains. Modified Rogers distance below the diagonal, Nei's genetic distance above the diagonal.

Population	Hayb	WICA	BurnsW	Bay	Conata	Big	Kocher	THRO	DETO	Tyrees	Tyree
Hayb	--	.188	.182	.196	.385	.186	.263	.065	.213	.190	.193
WICA	.392	---	.008	.020	.153	.002	.049	.125	.026	.008	.007
BurnsW	.396	.091	---	.024	.131	.010	.088	.164	.017	.005	.004
Bay	.398	.134	.152	---	.141	.010	.118	.150	.051	.021	.036
Conata	.511	.346	.337	.332	---	.155	.267	.371	.110	.121	.128
Big	.389	.045	.102	.093	.347	---	.065	.125	.031	.009	.013
Kocher	.454	.207	.283	.315	.440	.237	---	.113	.099	.085	.065
THRO	.234	.316	.371	.341	.474	.315	.300	---	.198	.160	.159
DETO	.416	.153	.131	.212	.300	.167	.292	.388	---	.008	.009
Tyrees	.394	.085	.073	.136	.312	.088	.270	.353	.084	---	.004
Tyree	.400	.081	.065	.180	.325	.110	.241	.357	.090	.062	---

Modified Rogers demonstrates increasing fixation at different alleles for two populations (as approaching 1), while Nei's demonstrates an decrease in similar allele frequencies (as approaching 0).

Figure 3. Rare and regional alleles in six polymorphic loci in prairie dogs (*Cynomys ludovicianus*) on the Great Plains. Squares represent each region and the numbers within the squares represent private alleles (when in area of the square that does not overlap with another square). Those areas overlapped by several squares (bold numbers) are shared alleles (present to those populations at the same frequencies). Regional alleles for a particular population are indicated in outlined numbers.

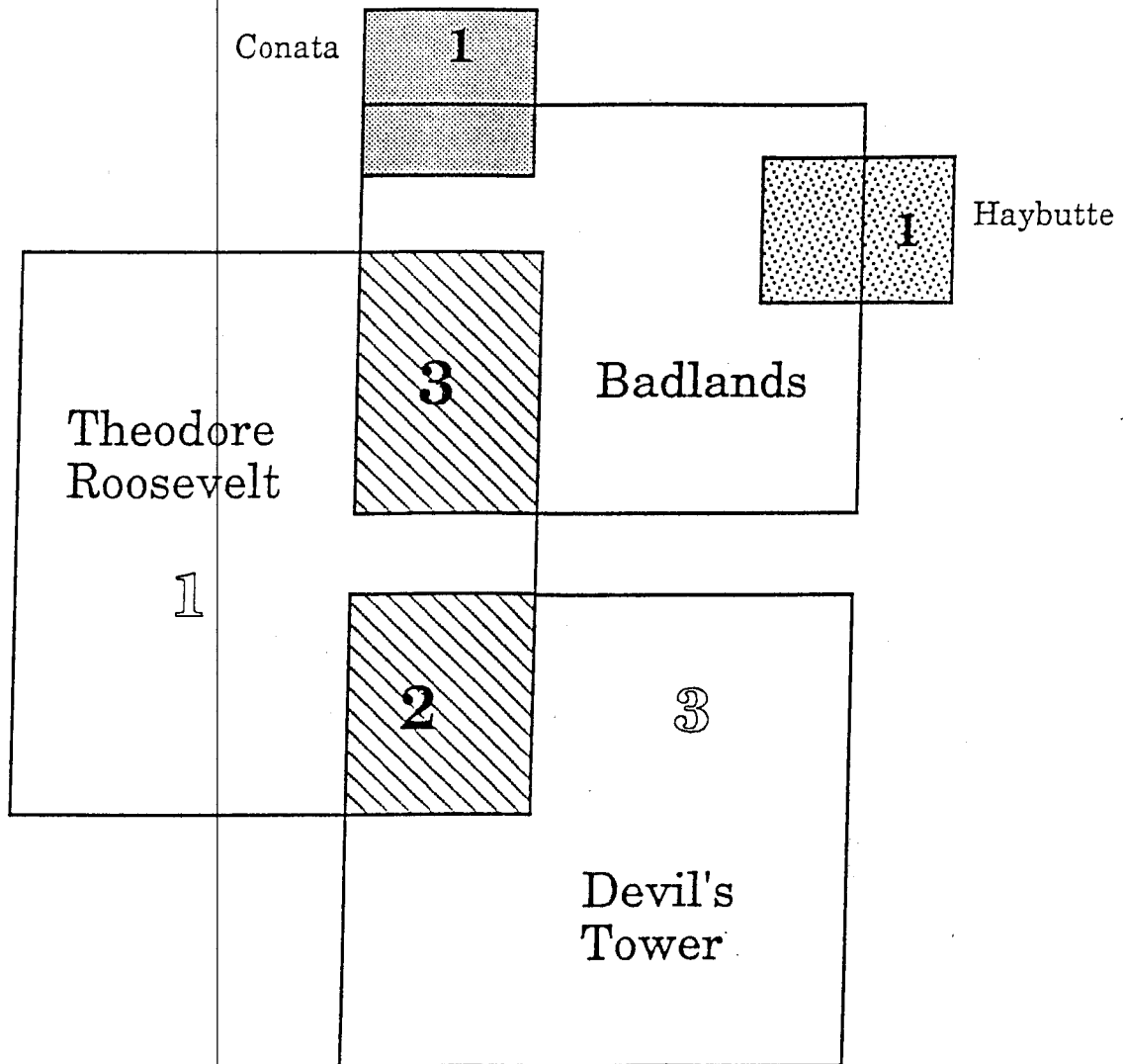


Table 6. Summary of published work on prairie dog (*Cynomys ludovicianus*) genetic differentiation based on starch gel electrophoresis.

Study	publi- cation year	N	sample	# loci screened	# poly- morphic	F _{st}	F _{is}	H
Chesser	1983	509	liver	16	7 *	0.1031	0.3297	0.1143
Foltz & Hoogland Daly	1983	167	blood	28	4	- -	1.867	- -
	1992	377	blood	57	4	0.115	0.0359	0.027- 0.040
Bowser* *	1993	284	blood	23	4	0.381	0.490	- -

* This number reflects polymorphic loci from both liver and blood samples.

**Pilot results from blood samples--not all samples were analyzed in the pilot study.

Table 7. Polymorphic loci found in four separate studies of prairie dogs (*Cynomys ludovicianus*) from starch gel electrophoresis.

Study	Polymorphic Loci				
	6-PGD	NP	Fest-1	Trf	PGM-2
Foltz & Hoogland	✓	✓	✓	✓	
Chesser*	✓	✓			✓
Daly	✓	✓		✓	✓
Bowser**	✓		✓		✓

*These three loci were from blood samples alone. Additional polymorphic loci were found in liver samples.

**IDH, LAP, MDH, and Fest-2 were also polymorphic for this study; however, these loci were monomorphic in the other studies.

Table 8 . Comparative F-Statistics for prairie dogs (*Cynomys ludovicianus*) and other genera. See text for sample numbers for individual towns and each region. Blanks indicate data not available.

Population	N	6PGD	IDH	PGM	LAP	MDH	PGI	Mean
BADL ¹								
F _{is}	100	-.165	.545	-.155	.697	.149	-.014	.176
F _{it}		-.029	.956	.312	.843	.441	.423	.491
F _{st}		.117	.902	.405	.483	.383	.415	.406
ALL ²								
F _{is}	153	-.012	-.032	-.174	.697	.298	-.112	.017
F _{it}		.132	.767	.219	.847	.495	.138	.391
F _{st}		.142	.774	.335	.497	.281	.225	.381
Regional ³	53							
F _{is}		.365	-.244	-.197	-	.608	-.164	.023
F _{it}		.487	.434	.024	-	.049	.107	.297
F _{st}		.192	.545	.185	-	.108	.233	.281
Chesser 1983								
F _{is}	400	.0986		.433				.33
F _{it}		-.267		.480				
F _{st}		.1873		.084				.045
Daly 1990								
F _{is}		.182		.238				-.023
Patton and Feder*								
F _{is}		.044	-.017					
F _{st}		.077	.093					
Wayne et al. 1990**								
F _{st}	201	.004	.026			.436		.564
Stewart and Baker 1992*								
F _{st}	246					-.036		.238

1=Badlands colonies only.

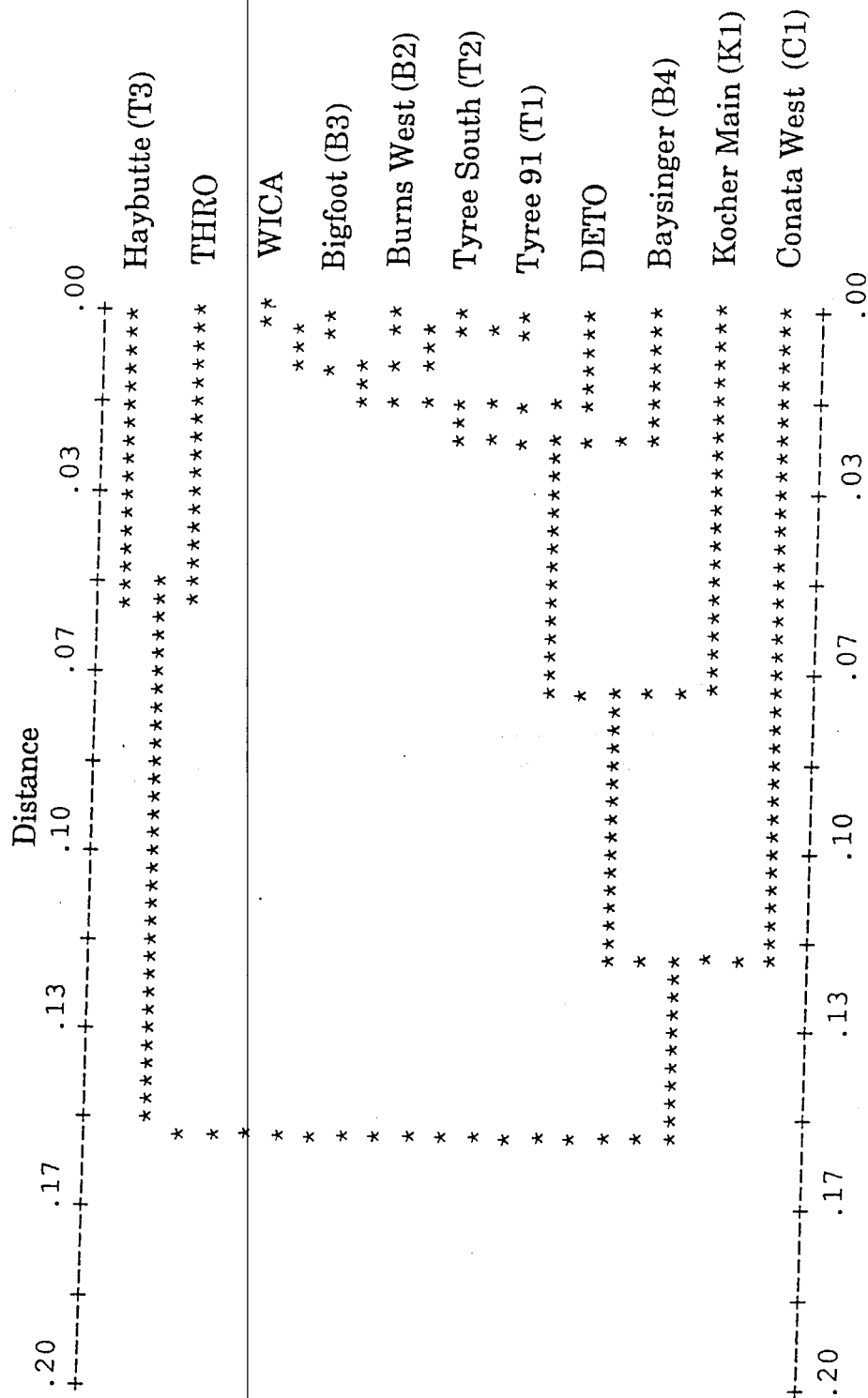
2=All four regional colonies (Badlands data entered as individual towns).

3=All four regions, Badlands data lumped as three separate colonies (Badlands-east, Badlands-west, and Kocher Flats).

* other studies on rodent species (*Thomomys* and *Sorex*).

** study on mammals (*Vulpes*).

Figure 3. Cluster diagram using Modified Roger's Distance for allele frequencies of four regional prairie dog populations the Great Plains. Initials and numbers indicate colony relationships for the towns: T=Tyree Basin, B=Burns Basin, C=Conata Basin, K=Kocher Flats. Abbreviations for regions follow park designations in the text.



CHAPTER THREE. THE EFFECT OF ENVIRONMENTAL PARAMETERS ON GENE FLOW AMONG PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) POPULATIONS.

"We need a mapping, not only of the critical taxonomies of species [populations], but of the various agricultural, industrial processes that threaten them"

Dahlberg 1989

"Landscape ecology investigates the interplay between spatial patterns and ecological processes"

O'Neill, Gardner, and Turner 1992

OVERVIEW

Populations can be distributed randomly in space, or there may be some element that structures their spatial distribution. Elements such as environmental factors can control and direct the movement of populations by influencing their establishment and persistence and the dispersal of individuals. Here I examine the effects of different environmental factors on dispersal (an estimate of gene flow) among prairie dog towns in Badlands National Park, South Dakota. In particular, I will address two questions: (1) which environmental factors influence gene flow among populations; and (2) can current patterns of gene flow be explained by the presence or distribution of these environmental factors? I plan to test these effects on prairie dog towns by using spatial models that calculate the shortest dispersal path given environmental barriers and habitat permeability. Dispersal path length will be generated by different models (i.e. suitability measures) and will be compared with genetic distance among towns to find the best fit/explanation of genetic structure.

BACKGROUND

Landscape ecology is the study of "...spatially explicit patterns of landscape mosaics [patches] and the interactions among their environmental elements" (Wiens et al. 1993). The distances between

patches, and probability that an animal can cross intervening habitats, structure a population's genetic composition by affecting gene flow. Gene flow also can be controlled by environmental factors that are both extrinsic (soil, vegetation, slope, aspect, etc.) and intrinsic (behavior, etc.). Therefore, the pattern of genetic components for a population in a landscape can be predicted by delimiting environmental factors.

Land use and land ownership also impact dispersal and thus affect genetic similarity among different prairie dog towns. Roads, fences, and land use, can act as barriers to dispersal causing genetic differentiation among neighboring towns. Similarly, synergistic effects among different environmental factors, like topography and distance, may contribute to an apparently complex pattern of genetic differences among towns (Wayne et al. 1991, White and Svenson 1992, Kienast 1993, Stewart et al. 1993).

OBJECTIVES

The objective of this study is to explain the genetic differentiation among prairie dog towns as a function of environmental factors. There are three steps for this objective:

- (1) to determine if gene flow among prairie dog populations is a function of the intervening habitat types;
- (2) to explain current prairie dog town distributions by the spatial distribution of environmental factors; and
- (3) to manipulate environmental features (changing the probability that an animal can successfully cross that environmental feature) and model the effects on genetic differentiation and gene flow.

HYPOTHESES

I plan to test two hypotheses on the spatial distribution of prairie dog population genetic differentiation by integrating the genetic information from allele frequencies (Chapter two) and historic prairie dogs distributions (Chapter one) into a spatial analysis (Figure 1). Here I will use a realized dispersal path as the route an individual would take between two populations that has the least cost associated with crossing different environmental features (ESRI ARC/GRID manual 1992). In

particular, I will test the following hypothesis:

Environmental variables determine the ability of animals to exchange among towns. The shortest dispersal path between two towns, as realized by the environmental variables between the towns and the suitability of those variables (the probability that an animal can successfully cross a particular habitat type), best explains the genetic distance between the same towns. Alternative: geographic distance is the best predictor of genetic distance.

STUDY SITE

The northern unit of Badlands National Park, South Dakota, contains several large colonies of prairie dogs. Each of these colonies consists of towns scattered within a defined geographic area. Fourteen towns from three of the colonies were sampled to estimate gene flow based on F-statistics (see Chapter Two). All of the towns have recent density estimates (Bessken and Vaughen unpublished data), and all have been mapped onto USGS topographic maps (Bessken unpublished data).

Land use and land ownership varies among towns. There are four types of land use: (1) recreational, (2) sport hunting (prairie dogs and antelope), (3) private ranching (cattle and sheep), (4) croplands (Schenbeck and Myre 1985, Bessken pers. commun.). Of the four, recreational and ranching are the two most prevalent types of land use. Land ownership can be divided into four categories (for the purpose of this study categories are based solely on prairie dog control methods): (1) private ranching--prairie dogs controlled every two-three years by poisoning; (2) leased land (from the Department of Agricultural-DOA)--prairie dog controlled by shooting; (3) park lands--limited control (poisoning), if any; and (4) recently acquired park-lands (Department of Interior-DOI)--prairie dogs heavily controlled in the past.

Badlands National Park (DOI) is surrounded to the south, east, and west by the Buffalo Gap National Grassland (DOA) which has cattle allotments all along the park's boundary. These allotments, along with geographic features such as the badlands wall along the northern edge of

the park, create potential boundaries to the dispersal of prairie dogs both within the park and between the park and grasslands. For example, the eastern colony, Burns Basin, is isolated from the western colony by both the badlands wall to the north and grasslands to the south and west.

METHODS

There are two parts to the analysis: (1) a summary of the environmental factors around and within individual towns into a computer generated environmental profile; (2) creation of environmental models that vary in suitability measured such that dependent hypothetical distances can be generated and tested against genetic distances using linear regression.

To determine if towns are associated with any environmental feature, I will examine the location of prairie dog towns with respect to random (expected) distributions using a chi square test (Appendix 1 contains lists of possible predictor and response variables).

Environmental Models

Environmental features of BADL are described and integrated into a composite map using GIS software (Figure 2). This map is in raster format such that each cell within the map receives a score or value for a particular environmental feature (Baker and Cai 1992). I can generate a minimum dispersal path (based on the weights of individual environmental factors) between each pair of colonies using Geographic Information Software (GIS) and PATH analysis in ARC/GRID (ESRI Software). Path analysis generates a dispersal distances using all possible pairs of towns and colonies. The resultant data matrix would have towns (as rows), genetic components (PCA scores), a dispersal path, and geographic distance (as columns).

I can fit the model of environmental suitabilities against town genotypes by two different methods: regressing genetic distance (observed) against path length, and a multiple linear regression using PCA scores (dependent scores) in conjunction with several independent variables. First, I will use a simple regression to regress genetic

distance against path length. I will create several models by changing the ability of an animal to cross a given habitat type (suitability) until I get the best fit.

Given a path length from that model of best fit, I will test the final dispersal path lengths against the PCA scores generated in Chapter two. I will use the first two PCA scores (explaining 75% of the total variance) as the dependent variable, and test those scores against the dispersal path length

Land Use/ Land Ownership

Prairie dog management can be quantified by assigning category labels to each type of land use or land ownership. First, maps of land use (cattle allotments, grazing permits, cropland) and land ownership (federal, private, leased, preserves) will be over-laid on maps of prairie dog town distributions. Each town will be assigned to a category for land use and land ownership to generate a $N \times 4$ matrix (N =number of towns; 4=number of columns [town, ownership, use, genetic component]). This matrix will be tested using parametric tests such as ANOVA (genetic components are the dependent variable) to see if towns differ in genetic components by land use/ownership (each type of use/ownership as a separate treatment).

WORK TO BE COMPLETED

I have compiled a composite map of the environmental features for the BADL area. This composite consists of soil, slope, aspect, transportation, hydrology, and elevation. This composite map is stored in digital format on 9mm magnetic tape. I have the composite maps of land ownership and allotment rights (for cattle) from Buffalo Gap National Grassland, along with written and verbal information on the control efforts around the towns. Town perimeters have been mapped onto USGS 7.5 minute topographic maps, but this information still needs to be transferred to a digital format compatible with ARC/GRID.

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