# MAMMAL AND FLEA OCCURRENCE IN ASSOCIATION WITH BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) COLONIES: IMPLICATIONS FOR INTERSPECIFIC PLAGUE TRANSMISSION

By

ROBERT JORIS BRINKERHOFF

B.S. University of Illinois, Urbana/Champaign, 1998

M.S. North Carolina State University, Raleigh, 2002

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Written by Robert Joris Brinkerhoff

Has been approved for the department of Ecology and Evolutionary Biology

_____
Sharon Collinge (Committee Chair)

_____
Andrew Martin

_____
Jason Knouft

_____
David Armstrong

_____
Michelle Sauther

Date _____

The final copy of this thesis has been examined by the signatories, and I find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline

Brinkerhoff, Robert Joris (Ph.D. Ecology and Evolutionary Biology)

Mammal and Flea Occurrence in Association With Black-Tailed Prairie Dog (*Cynomys ludovicianus*) Colonies: Implications for Intraspecific Plague Transmission

Thesis directed by Associate Professor Sharon K. Collinge

Sylvatic plague is an enigmatic disease affecting over 200 mammalian species worldwide, yet its dynamics and ecology are poorly understood. Transmitted by fleas, plague was introduced into North America in the 19$^{th}$ century and threatens humans and wildlife species, most notably, the black-tailed prairie dog. This thesis focuses on elucidating potential mechanisms of plague transmission among mammals in Colorado by examining the community of alternate disease hosts and vectors in which plague exists in four ways. First, I examined spatial and temporal patterns of plague emergence in mammalian species most likely to acquire infection. Second, I described and evaluated occurrence patterns of fleas on a variety of mammalian species associated with black-tailed prairie dog colonies. Third, I tested for interactions within and between flea species on black-tailed prairie dogs. Fourth, I analyzed the population genetic structure of fleas collected from nine prairie dog colonies with different histories of plague occurrence. I detected universally low levels of plague exposure in mammals associated with prairie dog colonies, suggesting that plague is uncommon between epizootic events. Patterns of flea species occurrence among mammalian species suggest that the small rodents hypothesized to be involved in plague dynamics may play only a small role in plague

transmission, whereas predators of prairie dogs may be responsible for harboring and transporting plague-infected fleas among colonies. Flea species apparently do not compete for resources and the presence of one flea species on a host may facilitate colonization by other flea species. Finally, the population genetic structure of prairie dog fleas is reflective of plague history at a given site and lends insights into patterns of recolonization following plague events. Taken as a whole, these results provide a uniquely detailed assessment of patterns of host and vector occurrence in the context of plague and they challenge previously held beliefs about plague dynamics in wildlife communities.

## Dedication

To my parents, for their support, encouragement, and for never suggesting that I reconsider my career in ecology in favor of more traditional occupational pursuits.

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# Chapter 1

## INTRODUCTION

Since the time of Hippocrates, diseases of humans and other species have been viewed in a state of dynamic equilibrium, influenced by organismal and environmental factors (Mittman 2005). With the advent of germ theory in the late nineteenth century, the groundwork was laid for medical study of disease etiology (Mittman 2005), though general study of pathogens as biological entities did not occur until the early twentieth century (Anderson 2004). Early ecologists such as Charles Elton and Aldo Leopold recognized disease as an important factor in the regulation of populations and as a mediator of interspecific interactions, but it was Theobald Smith who first strongly advocated for approaching the study of disease in broadly biological terms (Anderson 2004). Smith was also among the first to draw an ecological link between parasitism and disease transmission, stating, "The forces controlling disease are a mixture of heredity, environment, and parasitism" (Smith 1921). Smith was both discouraged and excited by his observation that pathogen occurrence and maintenance are dependent on interactions among a multitude of biotic and abiotic factors (Smith 1921) and these relationships form the basis of many contemporary investigations of disease ecology.

The complexity of disease processes lies at the heart of modern disease ecology, where investigators aim to assimilate genetic, ecological, pathological and biochemical data to form clearer descriptions of pathogen dynamics. Wildlife disease ecology is a relatively recently emerged discipline (Hudson et al. 2002) though its

roots can be traced back at least a century. Much current investigation in disease ecology focuses on emerging pathogens, which may impact human and wildlife populations. Many recently emerged human pathogens are zoonotic in origin, and many threatened and endangered species are further imperiled by naturally occurring or introduced pathogens (McCallum and Dobson 1995, Daszak et al. 2000). Although much recent attention has been paid to the ecology of emerging pathogens, the factors that affect disease transmission and spread are incompletely understood in many cases. One such enigmatic pathogen is *Yersinia pestis*, the bacterium that causes sylvatic, pneumonic and bubonic plague. Given the importance of *Y. pestis* to human history, surprisingly little is understood regarding its ecology in natural systems, due in part to often contradictory results (Gage and Kosoy 2005), leaving much room for future investigations. Research into the ecology of plague also has practical significance: *Y. pestis* is a potential bioterrorism agent, affects human health with 10-20 human infections reported per year (NIAID fact sheet 2003), and threatens the persistence of several North American mammalian species including black-footed ferrets (*Mustela nigripes*) and prairie dogs (*Cynomys* spp.) (Reading et al. 2002).

Native to Eurasia, *Y. pestis* is relatively recently (1.5-20 kya) derived from its sister taxon *Y. pseudotuberculosis* (Erickson et al. 2007), a far less virulent disease agent native to Asia (Chain et al. 2005). It is likely that plague spread naturally through the Old World and was inadvertently introduced into North America by rats and their fleas transported on ships from Asia (Barnes 1982). Plague likely established sometime during the late 19$^{th}$ century in or around San Francisco, California, and has since spread eastward to about the 100$^{th}$ meridian (Adjemian et al.

2007). Although plague is not native to North America, its dynamics are very similar between the New and Old Worlds (Gage and Kosoy 2005). Plague is thought to be maintained in an enzootic, or maintenance, cycle, consisting of hosts and their fleas that are somewhat or completely resistant to infection (Pollitzer and Meyer 1961). Periodically, plague infection is then thought to shift to epizootic, or amplifying hosts that are much more highly susceptible to infection (Barnes 1982). Although resistance to plague clearly varies among mammalian species (Barnes 1982, Perry and Featherston 1997, Gage and Kosoy 2005), compelling evidence for the existence of separate enzootic and epizootic plague cycles is lacking (Gage and Kosoy 2005).

The high virulence of *Y. pestis* is likely to be due to a series of inserted plasmids that differentiate it from other *Yersinia* species, which are predominantly water-borne pathogens. These plasmids are composed of two virulence factors, which facilitate reproduction within a vertebrate host, and transmission factors, which facilitate parasite-mediated transmission (Hinnebusch 2005). Among the virulence factors in the *Y. pestis* genome are genes that encode the Fraction 1 (F1) capsule, a protein that forms a gel-like structure that increases resistance to monocyte phagocytosis (Perry and Featherston 1997). Other virulence factors result in immune suppression and fibrinolysis (Gage and Kosoy 2005). The presence of the transmission factors unique to *Y. pestis* differentiates this pathogen from other *Yersinia* species ecologically as well as genetically: other *Yersinia* are water-borne (Sharma et al. 2003) whereas *Y. pestis* is transmitted by fleas. The *Y. pestis* genome contains a series of genes that encode for outer membrane proteins that cause individual *Y. pests* bacteria to stick together in the form of a biofilm that blocks its

vector's digestive tract (Hinnebusch 2005). The presence of this biofilm allows *Y. pestis* to remain within a suitable vector, but also reduces the lifespan of the vector because the biofilm precludes feeding in most cases (Gage and Kosoy 2005). The blockage mechanism to account for flea-borne plague transmission was discovered by Bacot and Martin (1914) and has since been assumed to be the only reliable method by which fleas can transmit plague bacteria. Very few flea species regularly form blockages and have thus been generally regarded inefficient plague vectors (Eisen et al. 2006) when compared to the Oriental rat flea, *Xenopsylla cheopis*, which is the flea species most prone to blockage (Pollitzer and Meyer 1961, Gage and Kosoy 2005). However, recent theoretical explorations of plague dynamics suggest that blockage cannot account for some patterns of plague spread observed in nature (Webb et al. 2006) and that fleas may effectively transmit plague soon after exposure by other mechanisms (Eisen et al. 2006, Eisen et al. 2007).

Combined results from both early and recent investigations demonstrate that a great deal of variation exists among flea species in terms of plague transmission efficiency. Effects of *Y. pestis* infection in vertebrate hosts are similarly variable. Plague is rapidly fatal in many mammalian species but few effects of infection are seen in other species (Barnes 1982). Overall, however, *Y. pestis* is a highly virulent pathogen. The persistence of virulent pathogens generally requires a high rate of transmission; without an effective mode of transmission among individuals, it would be expected that such a pathogen would become extinct upon causing the local extinction of its host (Anderson and May 1978, Tompkins et al. 2002). Typically, highly virulent pathogens must balance reproduction and transmission: high rates of

reproduction increase the probability of transmission but decrease the host's life span, thus minimizing the window of opportunity that a suitable vector will become infected (Lorange et al. 2005). However, the interaction between generally resistant enzootic hosts and highly susceptible epizootic hosts could account for plague persistence in natural systems.

In grasslands of western North America, plague exists in a complex community context consisting of a wide variety of potential host and vector species, each theoretically differing in their respective susceptibility to plague and vector competence. Of all North American mammals affected by plague, mortality is highest in prairie dogs (genus *Cynomys*), often reaching 99% of exposed individuals (Ubico et al. 1988, Biggins and Kosoy 2001). The black tailed prairie dog (*Cynomys ludovicianus*) is a species of serious conservation concern, partly because its habitat has been reduced by 98% in the last 100 years (Cully and Williams 2001). Prairie dogs are also often considered keystone species or ecosystem engineers because they have a disproportionately large impact on the communities in which they occur; prairie dogs significantly affect plant (Weltzin et al. 1997, Winter et al. 2002, Johnson-Nistler et al. 2004) and animal community structure (e.g., Ceballos et al. 1999, Smith and Lomolino 2004, Collinge et al. *in press*, Cully et al. unpublished data) and have been shown to alter soil properties (Carlson and White 1987, Munn 1993) and influence nutrient cycling processes (Whicker and Detling 1988). Sylvatic plague has been listed as one of the key factors threatening the persistence of black-tailed prairie dogs, along with shooting, poisoning, and urbanization (Reading et al. 2002).

Plague epizootics are spatially and temporally unpredictable, although historical plague events have been associated with temperature, precipitation (Parmenter et al. 1999, Enscore et al. 2002, Collinge et al. 2005a) and landscape features (Collinge et al. 2005b) in mathematical models. A trophic cascade hypothesis (Parmenter et al. 1999, Collinge et al. 2005a) has been proposed as a mechanistic link between climatic factors and increased prevalence of potential enzootic hosts, though it is unclear which mammals serve as reservoirs for the plague bacterium when epizootic infections in prairie dogs are not evident (Engelthaler and Gage 2000, Cully and Williams 2001, Gage and Kosoy 2005). Carnivores, lagomorphs, and ungulates have been described as incidental hosts in the plague cycle (Gage and Kosoy 2005), but these species may be responsible for spreading plague or plague infected fleas among susceptible hosts (Barnes 1982, Anderson and Williams 1997, Antolin et al. 2002, Girard et al. 2004). Carnivores may become infected with plague either through bites from infected fleas or by ingesting infected prey (Thomas et al. 1989). Many species of carnivores are resistant or resilient to plague infection and thus may act as indicator or "sentinel" species for plague (Gese et al. 1997), though non-rodent mammals are rarely considered to be enzootic hosts (Salkeld and Stapp 1996). Similarly, identities of particular flea species involved in sylvatic plague transmission are unidentified (Pollitzer and Meyer 1961, Gage and Kosoy 2005). With the recognition of early-phase plague transmission in the absence of blockage (Eisen et al. 2006, Eisen et al. 2007), a number of flea species previously discounted as poor plague vectors must be reconsidered as potentially important to plague transmission.

To understand sylvatic plague dynamics, it is crucial to identify which hosts and vectors are involved, or likely to be involved, in the transmission cycle. In order to make inferences into plague transmission among black-tailed prairie dog colonies, I investigated the community of potential enzootic hosts and plague vectors that occur in association with prairie dog colonies. Specifically, I investigated, 1) whether spatial and temporal patterns of plague exposure in mammalian carnivores are associated with seasonality of plague emergence in prairie dogs and to see if such data can be used to identify local plague foci, 2) flea assemblages of mammalian species associated with prairie dog colonies to make inferences about interspecific flea and plague transmission, 3) the population genetic structure of the prairie dog flea, and potential plague vector, *Oropsylla hirsuta,* to infer patterns of plague movement, and 4) the patterns of occurrence of congeneric fleas on prairie dog hosts to infer interactions among different flea species. Chapters two through five of this dissertation focus each of these areas of research and the results are summarized in chapter six. The interpretation of the results from this dissertation make an important contribution to wildlife disease ecology and, specifically, to plague transmission dynamics among mammalian species.

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## Chapter 2

PATTERNS OF PLAGUE EXPOSURE IN MAMMALIAN CARNVIORES


### Abstract

Sylvstic plague, caused by the bacterium *Yersinia pestis*, is a flea-borne disease that primarily affects rodents but has been detected in over 200 mammalian species worldwide. Mammalian carnivores are routinely surveyed as sentinels of local plague activity, since most species of carnivores are apparently resilient to infection, showing few signs of morbidity. In Boulder County, Colorado, USA, periodic plague epizootic events occur in black-tailed prairie dogs; enzootic hosts are unidentified as are plague foci. For three years, I systematically sampled carnivores in two distinct habitat types to determine whether carnivores may play a role in maintenance or transmission of plague and to identify habitats associated with increased plague prevalence. I sampled 83 individuals representing six species of carnivores and found only two that had been exposed to *Y. pestis*. The overall low rate of plague exposure in carnivores suggests that plague may be very rare in this study system, or that carnivores are poor sentinels of plague. In either case, I cannot draw any conclusions regarding habitat-associated plague foci or temporal changes in plague activity. Plague epizootics involving prairie dogs were confirmed in this study system during two of the three years of this study, and I therefore suggest that the use of carnivores to survey for plague may be of limited value.

## Introduction

Plague is a vector-borne bacterial disease that affects humans as well as wildlife in the western United States. *Yersinia pestis*, the bacterium that causes plague, was introduced to North America around 1900 and has since spread eastward through most of the western United States (Gage et al. 1995, Adjemian et al. 2007). Plague is highly pathogenic to a variety of mammalian species, including the black-tailed prairie dog (*Cynomys ludovicianus*), which has been deemed a species of conservation concern (Reading et al. 2002) due to its role as a keystone species (Miller et al. 2000) and ecological engineer (Bangert and Slobodchikoff 2004). It is widely believed that plague is maintained by one or more enzootic, or maintenance, host species that are partially resistant to infection, although such reservoir species are unidentified in many systems (Gage and Kosoy 2005). Enzootic periods are punctuated by epizootic events whereby an amplifying host becomes infected and plague spreads rapidly (Webb et al. 2006), potentially over long distances (Girard et al. 2004). Although reservoir species for plague in North America have not been identified, likely candidates include rodents and carnivores that show variation in susceptibility to plague (Barnes et al. 1982) and are found in close association with epizootic hosts such as prairie dogs (Gage and Kosoy 2005).

Carnivores are attractive candidates as plague reservoirs because they can acquire plague by multiple routes of infection. Similar to other mammalian species, including rodents, carnivores may become infected by bites from infected fleas. At least a dozen flea species in western North America have been found to be naturally infected with plague (Hubbard 1968, Gage and Kosoy 2005) and carnivores routinely

harbor fleas that are generally specific to other mammalian species (Hubbard 1968, Lewis 2002, Brinkerhoff unpublished data). Carnivores often acquire the fleas of their prey, as fleas will abandon a newly-dead host and immediately begin questing for a new host (Gage et al. 1994, Salkeld and Stapp 2006). Carnivorous mammals may also become infected by ingesting infected prey (Barnes 1982, Thomas et al. 1989), and then serving as an infectious source for fleas. By this mechanism, carnivores may amplify plague prevalence in the environment; even if overall plague prevalence is very low, the act of sampling large numbers of prey may serve to increase the probability of encountering infected individuals (Salkeld and Stapp 2006). During plague epizootics, carnivores may be attracted to prairie dog colonies as infected prey may be more easily captured (Salkeld and Stapp 2006), thus increasing the likelihood of both flea-borne and ingestive routes of plague infection.

Carnivores may also be involved in plague transmission without becoming infected. Given that carnivores often acquire fleas from the mammals on which they prey (Hubbard 1968), it is reasonable to conclude that they are likely to become infested with plague-positive fleas during predation events on prairie dogs and other rodents when plague activity is high. Compared to rodents and other small-bodied mammals, carnivores have large home ranges and may travel long distances in relatively short periods of time (Rosatte 2002). During epizootic periods, plague can spread quite quickly over several kilometers; this pattern is more congruous with carnivore movement than with small rodent or prairie dog movement (Girard et al. 2004). Furthermore, carnivore movement patterns are known to change during

disease epizootics and long-distance transmission by carnivores of other diseases has been documented (Greenwood et al. 1997).

Given their relatively high likelihood of plague exposure, carnivores may also be useful as indicator species for plague (Gese et al. 1997). For example, high prevalence of plague antibodies in carnivores may signal a high degree of plague occurrence in the environment. As a result, species of carnivores are frequently surveyed to determine levels of local plague activity. However, most studies of carnivores as potential reservoirs or sentinels of plague are done opportunistically (Salkeld and Stapp 2006) so the use of such studies is limited as surveys are not carried out systematically with the goal of testing specific hypotheses. Another problem with using carnivores as sentinels of plague activity is that persistence of antibodies to plague in wild carnivores has not been established. Persistence of plague antibodies has been studied in feral and captive domestic dogs (Rust et al. 1971a, b, Taylor et al. 1981, Barnes 1982) as well as in captive wildlife species (Barnes 1982), but differences in stress levels among captive and free-ranging or wild and domestic species might cause differences in antibody response (Aviles and Monroy 2001). Given the uncertainty of persistence of plague antibodies in wildlife species, it cannot be concluded that a high proportion of plague-positive individuals necessarily indicates recent plague activity, because time of exposure cannot be accurately determined.

Epidemic plague outbreaks in prairie dogs are irregular, suggesting that plague occurs sporadically in the plains habitats in which prairie dogs are found (Cully and Williams 2001). However, data indicate that plague may be resident in the

Colorado Front Range; the timing of recent local plague outbreaks in prairie dogs in Boulder County, Colorado is suggestive of west-to-east transmission out of the Rocky Mountains with westernmost colonies becoming infected earlier in the season (S. Collinge et al., *unpublished data*). Mechanisms to account for this pattern have not been described and may depend on mammal movement and seasonality of plague occurrence in different habitat types. Because prairie dogs do not occur in mountainous sites, the movement of plague from the foothills to the plains must depend on other species if plague is indeed resident in the Colorado Front Range.

Plague surveillance typically is carried out by testing mammals for presence of antibodies to *Y. pestis* in serum samples by passive hemagglutination with the *Y. pestis*-specific F1 antigen (Chu 2000). *Y. pestis* is rarely isolated in bacterial cultures taken from wild mammals, though detection of antibody response is possible up to eight months after exposure to plague (Cavanaugh et al. 1965, Barnes 1982). Although duration of plague antibody persistence is unknown for wild carnivores, plague surveillance methods based on seroprevalence may provide reasonable evidence to indicate possible plague foci during a single time period. With the goal of identifying spatial patterns of plague prevalence and to determine seasonal patterns in plague emergence, I systematically tested wild-caught carnivores for plague exposure during spring and late summer months for three years. Specifically, my objective was to test the following hypotheses:

1) If the foothills of the Rocky Mountain Front Range serve as an enzootic focus of plague activity, carnivores sampled in the foothills should show

higher rates of plague exposure than carnivores sampled in grassland habitats.

2) Levels of plague exposure and plague prevalence in carnivores should be highest during spring and early months when plague is typically observed in prairie dog colonies.
3) Plague exposure in carnivores should vary by species, with those that prey routinely on prairie dogs showing highest rates of exposure.

**Methods**

I live trapped carnivores during spring (April – June) and late summer (August and September) trapping sessions at a total of six trapping locations in Boulder County, Colorado, from 2004 to 2006. All trapping sites were located within two kilometers of active prairie dog colonies. In 2004, two sites (A and B) were trapped for three-week sessions consisting of one week of pre-baiting and two weeks of active trapping. In 2005 and 2006, four sites (A, B, C and D) were trapped seasonally for two-week trapping sessions with no pre-baiting. Sampling sites were categorized as either foothills sites (A and C) or grassland sites (B and D) based on topography and elevation (Figure 2.1) with plains sites located at least 10 km from the foothills by straight-line distance. Foothills sites were characterized by relatively high elevation (roughly 1900 m), rocky outcrops, steep topography, and ponderosa pine forest. Grassland sites were characterized by relatively low elevation (roughly 1550 m), mixed agriculture consisting of cattle grazing and crops, and riparian woodland. Supplemental trapping in response to local prairie dog die-offs was also

conducted at one additional site in 2005 (site E) and 2006 (Site F); these sites were located less than 10 km from the foothills and were therefore not included in site-type comparisons of seroprevalence. These sites were not sampled seasonally and are also excluded from seasonal comparisons.

Animals were trapped with three sizes ( 17.8 x 17.8 x 50.8cm, 25.4 x 30.5 x 81.3cm, and 40.1 x 50.8 x 106.7cm) of wire box traps (Tomahawk Live Trap Inc, Tomahawk, WI) (2004 - 2006) and #1.5 and #3 Victor Coil Soft Catch$^{TM}$ leg-hold traps (Woodstream Corporation, Lititz, PA) (2005 and 2006). Box traps were placed in sets containing three traps, one of each size. Trapping sets were placed in locations predicted to experience carnivore visitation such as among rock outcrops, along creek beds, or along stream banks. Box trap sets were separated by at least 25 meters. Leg-hold traps were placed in sets of two traps and were located in areas predicted to be frequented by foxes and coyotes. Box traps were baited with raw chicken and commercial trapping lures were used for leg-hold traps. Traps were open and set for six consecutive days per week for two weeks at a time (12 total nights per trap per site). In all cases, traps were checked daily between 0600 and 0930. Equivalent numbers of box and leg-hold traps were used at all sites throughout the study.

To chemically immobilize captured animals, I used an intramuscular injection of Telazol (Fort Dodge Animal Health, Fort Dodge, IA), delivered by a 1 m pole syringe, at a rate of 10 mg/kg. Once animals were sedated, I applied a uniquely numbered aluminum ear tag (National Band and Tag Co., Newport, KY) and collected 2 - 5 ml of blood from either the lateral saphenous, cephalic, or jugular vein. A small volume of blood (~100µl) was first applied to a nobuto filter paper strip and

dried. Remaining blood was divided between 2 ml cryovials (Nalgene Co, Rochester, NY), stored at -20ºC, and 10 ml EDTA vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ), stored at 4 ºC. I also identified each animal to species and collected sex, measurement, and body weight data before the sedation wore off (30 - 90 minutes). All trapping was done in accordance with the guidelines set by and with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Colorado.

Blood samples on Nobuto strips were used to test for antibodies to *Y. pestis* by passive hemagglutination by the F1 antigen (Chu 2000). For samples that tested positive by hemagglutination, I used a Qiagen DNEasy blood and tissue kit (Qiagen, Inc., Valencia, CA) to extract DNA from the corresponding frozen blood sample and tested this for presence of *Y. pestis* DNA by multiplex polymerase chain reaction (PCR) (Stevenson et al. 2003). In addition to the carnivore trapping described above, I also received blood samples from domestic cats (*Felis catus*) collected by a local veterinarian. Samples were collected from pet cats which were admitted to the veterinarian with a variety of presenting conditions. All samples from domestic cats were analyzed in the manner described above.

**Results**

I collected and tested blood samples from a total of 83 individual carnivores. Of these samples, 76 came from five species of wild carnivores and the remaining seven came from domestic cats (Table 2.1). Because many of the domestic cat owners did not wish to provide complete addresses, I was not able to determine

precise sampling locations for these individuals. However, all domestic cat samples were collected within 3 months of and at a veterinary clinic located less than 3 km from a confirmed wildlife plague case. Two individual carnivores tested positive for exposure to plague: one striped skunk, *Mephitis mephitis* sampled at site B in 2004 and one red fox, *Vulpes vulpes* sampled at site C in 2005. Both individuals showed relatively high antibody titer counts: 1:128 and 1:512 for the *M. mephitis* for the *V. vulpes*, respectively. *Y. pestis* DNA was not detected in blood samples from either individual by PCR. A total of six individual carnivores (four *M. mephitis* and two *P. lotor*) were recaptured and resampled at least once in trapping sessions subsequent to the first trapping occasion. All such recaptures occurred at the study site of initial capture, though typically at a different trap set location. Individuals recaptured within a sampling session were released unprocessed.

In 2004 there were was no plague activity in Boulder County prairie dogs. In 2005, epizootic activity was observed in several prairie dog colonies and was also confirmed in samples collected from squirrels and fleas in the northern portion of the study area (Figure 2.2). In 2006, several more plague events were observed, most of which were located in relatively close spatial proximity to the plague events of 2005 (Figure 2.2). Twenty-one individual carnivores, representing five species, were sampled within 2 months and 2 kilometers of these observed plague events (Table 2.2). At site B, I trapped an adult female striped skunk (*M. mephitis*) on 9 September 2004 and recaptured the same individual on 15 May 2005 at the same site, though in a trapping set roughly 100 m from the location of the first trap occasion. The blood sample collected at the first capture tested positive for *Y. pestis* antibodies with a titer

of 1:128. The second blood sample, collected 248 days (~8 months) later, tested negative for antibodies to *Y. pestis*. Neither the blood samples nor the fleas collected from this animal tested positive for *Y. pestis* DNA by PCR.

**Discussion**

I detected very low rates of plague exposure among the six species of carnivores that I tested in this study. There was no apparent relationship between plague exposure and habitat type, suggesting that neither of the habitat types I sampled is a source for plague infection. I also failed to detect a relationship between plague exposure in carnivores and epizootic plague activity in black-tailed prairie dogs. Although one plague-positive carnivore was detected in close spatial and temporal proximity to a prairie dog die-off, the other was detected in a year and a location in which no plague activity was observed. On the contrary, many individual carnivores of several species were sampled in close spatial and temporal proximity to plague epizootics in prairie dogs and tested negative for exposure to *Y. pestis* (Table 2.2). Because I was able to re-test one individual striped skunk that previously had tested positive for exposure to *Y. pestis*, I am able to draw some preliminary conclusions about plague antibody persistence in carnivores that may be relevant to the observed low rates of seroprevalence.

Differences between the results from this study and published records of plague exposure in species of carnivores are intriguing and not easily explained. For example, plague exposure in coyotes (N = 17,403) sampled in the western United States, as summarized by Salkeld and Stapp (2006) indicated a mean seroprevalence

rate of 14%; coyote seroprevalence in this study was 0%. Similarly, I detected very low exposure to plague in species whose sample sizes were 2 - 3 times higher than coyotes: striped skunks showed seroprevalence of only 3.4% and I failed to detect any exposure to plague in raccoons. Previous reports of plague exposure for these species average 10% for striped skunks and 13% for raccoons (Salkeld and Stapp 2006). Data from 21,826 carnivores compiled from 28 studies indicated an overall seroprevalence rate of 15.9% (Salkeld and Stapp 2006) compared to only 2.4% in this study. It would be reasonable to conclude, given my results, that plague is generally absent from this study system. However, during two of the three years of our study (2005 and 2006), plague activity was confirmed in small rodents and prairie dogs.

The low rates of seroprevelance that I detected in carnivores could be explained by at least two causes. First, it is possible that, in this study system, *Y. pestis* is absent between epizootics or is present at nearly undetectable levels. Support for this hypothesis comes from the lack of detection of plague in other mammalian species in this study area during inter-epizootic periods; repeated sampling of the small rodent community assumed to contain enzootic host species has resulted very little evidence of plague exposure (S. Collinge, *unpublished data*). If a small rodent that fit the definition of a classic reservoir species (reference) were to exist in this system, at least some measure of plague exposure would be expected. In lieu of a persistent reservoir population, it is possible that plague is periodically re-introduced into this study system from other locations. Plague epizootics are difficult to predict, but may be associated with climatic and landscape factors (Stapp et al. 2004, Collinge et al. 2005a, b) and these factors could potentially influence long-

distance plague movement. For example, climate-related factors that result in increased rodent population sizes or higher flea loads could increase dispersal rates and therefore the probability of a plague-infected flea or mammal immigration. The observed patterns of plague activity in our system may be reflective of repeated introduction rather than low-level enzootic activity since virtually no evidence of plague exists between epizootic events.

Second, it is possible that antibody persistence in wild carnivores is such that exposed animals clear *Y. pestis* infection very quickly and the detection window of positive seroprevalence is very short, although this explanation seems unlikely. Laboratory studies of domestic and wild species of carnivores, as well as studies of free-ranging domestic dogs, indicate that positive titer responses are measurable for 4 - 8 months following an initial inoculation with *Y. pestis* (Rust et al. 1971b, Taylor et al. 1981, Barnes 1982). Given that physical stressors are known to affect the production and persistence of antibodies in animals (Aviles and Monroy 2001), it might be expected that wild carnivores would show variation in the persistence of an antibody response. My data suggest that antibody deterioration from a relatively high level titer count (1:128) to undetectable levels may be roughly equivalent between natural and laboratory conditions, though without intermittent samples between the initial positive sample and the negative sample collected several months later, it is impossible to assess exactly how long *Y. pestis* antibodies might persist in wild carnivores. It is, however, reasonable to conclude that antibody persistence in wild carnivores is not likely to be longer than in domestic or laboratory-housed wild species of carnivores. *Y. pestis* antibody persistence in wild species of carnivores is

rarely discussed as a factor that might influence the results of plague surveillance studies and precise estimates of antibody persistence are unavailable for wildlife species. However, the window of positive antibody detection is clearly long enough in many cases to result in non-zero levels of plague exposure (Hopkins and Gresbrink 1982, Thomas and Hughes 1992, McGee et al. 2006, Salkeld and Stapp 2006), so it is unlikely that a short window of antibody presence would explain the low levels of plague detection in this study. Furthermore, plague-exposed carnivores may be found when no plague activity is observed in other species (e.g., Hopkins and Gresbrink 1982, Salkeld and Stapp 2006) suggesting that antibody persistence may be enough to remain detectable well beyond epizootic events.

Given that some period of time is expected between the onset of a plague epizootic and antibody detection in incidental hosts such as carnivores, I expected that a multi-month lag time might be observed between plague die-offs in prairie dogs and high rates of exposure in carnivores. Laboratory studies indicate that circulating *Y. pestis* antibody levels tend to peak three to four weeks following exposure and slowly decline to undetectable levels over a period of 4-8 months (Rust et al. 1971b, Barnes 1982). However, I found no evidence of this phenomenon; of the two individuals that tested positive for plague exposure, neither was sampled in the months following an observed epizootic event. To explain this observation, I must assume that either very few individuals became exposed to *Y. pestis* during the epizootic events of 2005 and 2006 or that relatively short duration antibody persistence precluded any samples from testing positive. However, given that our

sampling occurred throughout the summer months when these epizootic events were observed, the latter explanation seems unlikely.

The unusually low levels of plague exposure found in carnivores in this study are perplexing. These results may be best explained by the hypothesis that an enzootic host is lacking in this study system and that carnivores rarely encounter plague-positive prey or fleas. Plague incidence is influenced by landscape features that may act as barriers to pathogen introduction (Collinge et al. 2005b). Boulder County is much more highly urbanized than other areas in which plague epizootics are common and anthropogenic barriers such as roads may effectively isolate the mammal populations in this study system from enzootic plague foci. Widespread plague epizootics may be dependent on synchronization of enzootic host populations (Kausrud et al. 2007) and the lack of connectivity between Boulder County and less anthropogenically influenced areas may preclude the introduction and maintenance of plague. Whether it is because a true enzootic host is absent and plague can therefore not persist, or if it is because a source of *Y. pestis* in our system does not exist, it seems apparent that carnivore surveillance is not a reliable measure of plague activity in this study system. Serosurveys of carnivores are a common method of assessing general prevalence levels for plague (Salkeld and Stapp 2006), as well as for other wildlife diseases (Gese et al. 1997). Such surveys are often convenient and can be relatively cost-effective when they are done opportunistically or when they are "piggybacked" onto other wildlife projects. However, such non-systematic sampling regimes often limit the usefulness of the final dataset as projects are not designed for *a priori* hypothesis testing. Carnivore sero-surveys may be used to generate reliable

measures of plague activity, though is some cases, plague prevalence in carnivores does not reconcile with disease rates in human or other mammalian species (Hopkins and Gresbrink 1982). The ultimate usefulness of carnivore surveillance for plague will be limited until reliable estimates of antibody persistence from wild-caught individuals are determined. Only then can reasonable spatial and temporal determinations of sylvatic plague activity be estimated.

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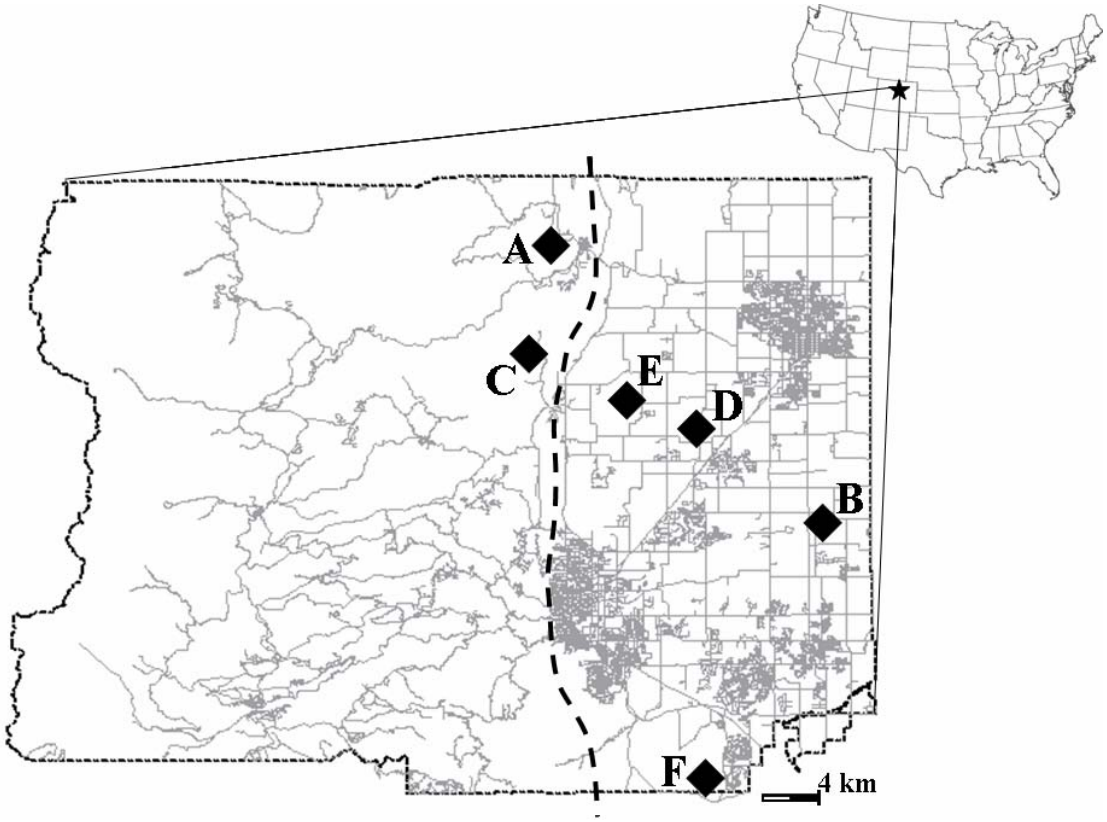
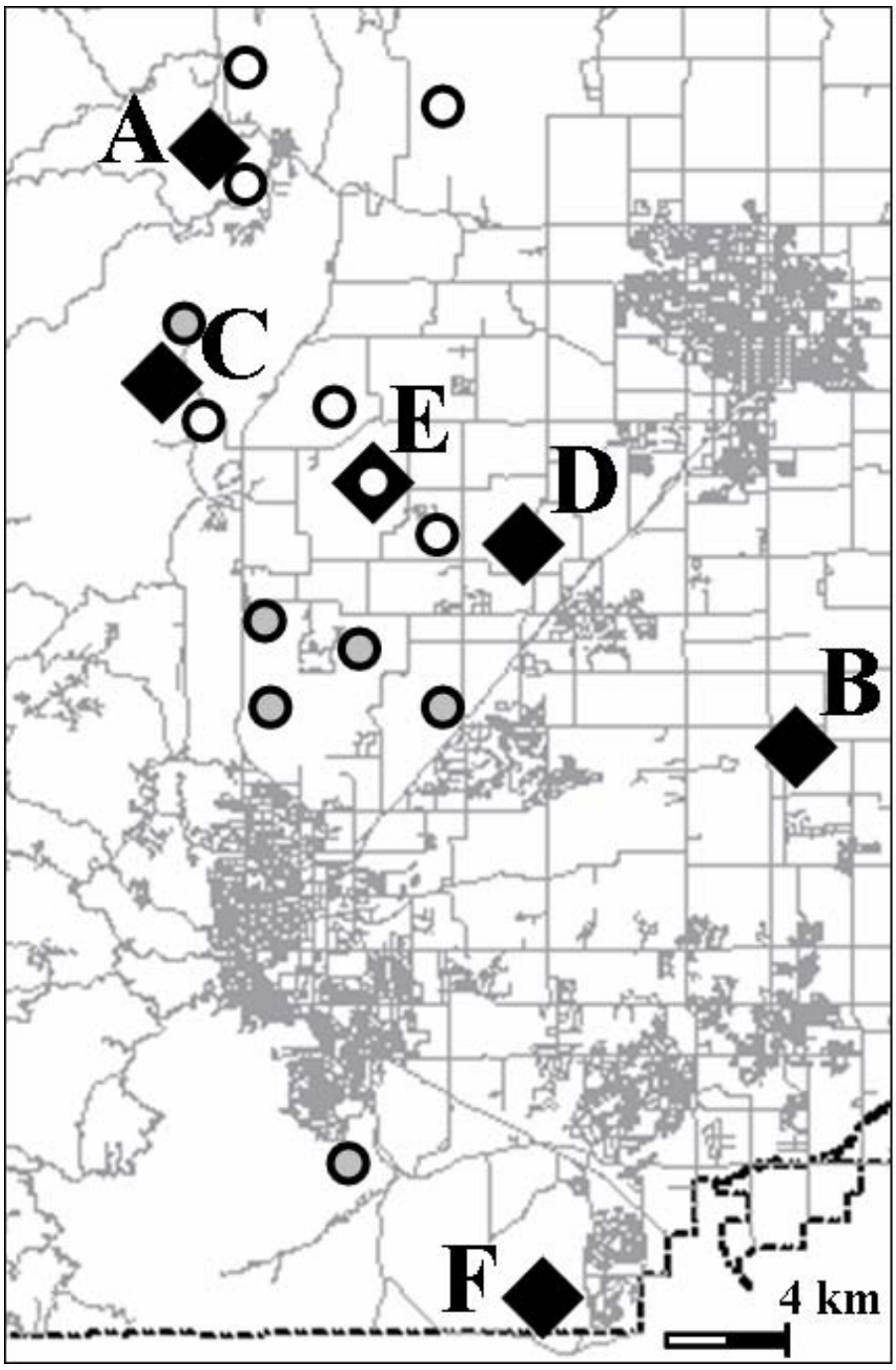
Figure 2.1




Map representing the location of Boulder County in Colorado as well as the locations of the six sites at which carnivores were sampled. The dashed vertical line approximates the eastward extent of the foothills of the Colorado Front Range.

Figure 2.2




Locations of sampling sites relative to sites at which plague was observed in 2005 (open circles) and 2006 (gray-filled circles).

Table 2.1: Proportions of each species of carnivores from each habitat type that tested positive for plague with sample sizes in parentheses.

| Species | Foothills habitat | Grassland habitat | Other habitat type | TOTAL |
|---|---|---|---|---|
| *Canis latrans* | 0 (0/2) | 0 (0/5) | 0 (0/4) | 0 (0/11) |
| *Vulpes vulpes* | 33.3 (1/3) | 0 (0/0) | 0 (0/0) | 33.3 (1/3) |
| *Mephitis mephitis* | 0 (0/19) | 11.1 (1/9) | 0 (0/1) | 3.4 (1/29) |
| *Spilogale gracilis* | 0 (0/5) | 0 (0/0) | 0 (0/0) | 0 (0/5) |
| *Procyon lotor* | 0 (0/2) | 0 (0/26) | 0 (0/0) | 0 (0/28) |
| *Felis catus* | 0 (0/0) | 0 (0/0) | 0 (0/7) | 0 (0/7) |
| TOTAL | | | | 2.4 (2/83) |

Table 2.2: Individuals sampled in close spatial and temporal proximity to prairie dog die-

offs or other confirmed plague occurrence

| Species of carnivores | Sampling site | Approximate distance to plague activity | Time between carnivore sampling and plague event |
|---|---|---|---|
| *Canis latrans* | E | 200m | 1 month |
| *Canis latrans* | D | 1.3 km | 1 month |
| *Canis latrans* | C | 1 km | 2 months |
| *Mephitis mephitis* | A | 500m | 2 weeks |
| *Mephitis mephitis* | A | 1 km | 2 months |
| *Mephitis mephitis* | C | 200 m | 2 months |
| *Mephitis mephitis* | C | 1 km | 2 months |
| *Mephitis mephitis* | C | 1.2 km | 1 month |
| *Procyon lotor* | C | 1 km | 1 month |
| *Procyon lotor* | C | 1 km | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |
| *Procyon lotor* | D | 1.3 | 2 months |

| | | | |
|---|---|---|---|
| *Procyon lotor* | D | 1.3 | 1 months |
| *Spilogale gracilis* | A | 1 km | 2 months |
| *Spilogale gracilis* | C | 1km | 2 months |
| *Vulpes vulpes** | C | 500m | 1 month |

## Chapter 3

# FLEA ASSEMBLAGES OF MAMMALS ASSOCIATED WITH BLACK-TAILED PRAIRIE DOG COLONIES: IMPLICATIONS FOR INTER-SPECIFIC PLAGUE TRANSMISSION


### Abstract

Inter-specific transmission of *Yersinia pestis*, the causative agent for sylvatic plague, is thought to be predominantly reliant on fleas. A number of species of mammals and fleas exhibit infection with *Y. pestis*, yet definitive vectors and reservoirs are unidentified in most plague systems. In western North America, black-tailed prairie dogs are particularly susceptible to plague infection and suffer high rates of mortality when plague infects a colony. I collected fleas from 13 species of mammals associated with black-tailed prairie dog colonies to determine which host species are most likely to spread plague-infected fleas to or from prairie dogs. I calculated community similarity indices and performed cluster and ordination analyses to determine which mammalian species had the most similar flea assemblages. Analyses indicated that the flea assemblages of prairie dogs diverged from those of small rodents but were very similar to certain species of carnivores as well as desert cottontail rabbits. These results are inconsistent with the paradigm that small rodents serve as reservoirs for *Y. pestis* and are sources for infection in prairie dogs. Rather I conclude that other mammals, including carnivores and lagomorphs are more likely to spread plague-infected fleas to and from prairie dogs.

## Introduction

Emerging vector-borne zoonotic diseases pose a threat to human health and, in some cases, imperil endangered or threatened species (Daszak et al. 2004). The emergence, persistence and dynamics of zoonotic diseases are highly dependent on the ecological communities in which they occur (Collinge and Ray 2006, Keesing et al. 2006). Interactions among host species, in particular, can determine whether a pathogen will establish or persist (Holt et al. 2003, Holt and Dobson 2006). In addition to abundance of and interactions among host species, vector prevalence and abundance are crucial to dynamics of pathogen transmission (Lorange et al. 2005, Webb et al. 2006). Although vector ecology may explain patterns of disease transmission and even pathogen evolution (Lorange et al. 2005, Krasnov et al. 2006), detailed information regarding vector occurrence is seldom incorporated into disease transmission models (Keesing et al. 2006). To fully describe and predict patterns of vector-borne pathogen spread it is crucial to understand and incorporate information regarding vector distribution and abundance within communities of susceptible and infectious hosts.

One such flea-borne zoonotic disease is sylvatic plague, caused by the bacterial pathogen *Yersinia pestis*. Plague is a disease that is naturally associated with at least 250 mammalian species worldwide in both its native range in Eurasia and Africa, and its introduced range in the New World (Pollitzer and Meyer 1961). Response to plague infection is highly variable both within and among species and

mortality is generally highest among colonial, burrowing rodents (Gage and Kosoy 2005). Plague is thought to persist in an enzootic, or maintenance, host that shows some degree of resistance to plague infection (Pollitzer and Meyer 1961). Mammalian species that are highly susceptible to infection are typically described as epizootic, or amplifying hosts, and usually experience rapid widespread die-offs upon plague emergence (Gage et al. 1995). However, true enzootic hosts have been identified in very few ecological communities in which plague occurs naturally (Pollitzer and Meyer 1961) and tangible distinction between enzootic and epizootic hosts may not exist (Gage and Kosoy 2005).

Flea species also vary in their ability to transmit plague effectively among mammals (Wheeler and Douglas 1945). The classical model of flea-mediated plague transmission is based on a study of the Oriental rat flea, *Xenopsylla cheopis*, and the observation that exposure to plague often results in the formation of a biofilm in the flea's proventriculus, located between the esophagus and midgut (Bacot and Martin 1914). The presence of this biofilm causes a blockage of the upper digestive tract, leading to regurgitation of subsequent blood meals and eventually resulting in death by starvation. Bacterial transmission as a result of blockage has traditionally been assumed to account for the rapid spread of *Y. pestis* among susceptible individuals (Pollitzer and Meyer 1961), but many flea species do not regularly form blockages (Gage and Kosoy 2005), including most fleas occurring in western North America (Esky and Haas 1940) where plague epizootics are regularly observed. Furthermore, blockage is not required for *X. cheopis* to transmit plague efficiently (Eisen et al. 2007a), thus calling into question the notion that proventricular blockage plays any

part in pathogen transmission. Early-phase plague transmission, whereby *Y. pestis* is transmitted among host individuals within 96 hours of initial infection in a flea and without resulting in a proventricular blockage, has been observed in several flea species and may explain patterns of disease spread that are observed in natural systems (Eisen et al. 2006, Eisen et al. 2007a, b, Wilder et al. in review). Therefore, prior assumptions about vectorial capacity based on whether a flea species is prone to blockage are likely irrelevant. As a result, many flea species previously dismissed as potential plague vectors must now have their potential roles in plague transmission reconsidered.

In western North America, prairie dogs (genus *Cynomys*) fit the description of epizootic hosts for plague and suffer high mortality (often upwards of 99%) upon infection (Cully and Williams 2001). Prairie dogs are colonial and highly social ground squirrels whose complex burrow systems alter ecosystem function (e.g., Whicker and Detling 1988) and affect plant (e.g., Winter et al. 2002) and animal community composition (e.g., Stapp 2007). Deer mice (*Peromyscus maniculatus*) are among the mammalian species whose occurrence is positively affected by prairie dog presence. Relative and absolute abundance deer mice are significantly higher at prairie dog colony sites than in adjacent grasslands where prairie dogs are absent (Agnew et al. 1986, Collinge et al. in press, Cully et al. unpublished data). *Peromyscus* and other small rodent genera are putative reservoir host species for *Y. pestis* as they show some degree of resistance or resilience to plague infection, though there is little hard evidence to support this assertion (Pollitzer and Meyer 1961, Gage and Kosoy 2005). Other mammals that have been implicated in plague transmission

include lagomorphs and squirrels (Pollitzer and Meyer 1961), species that share prairie dog burrows, as well as the species of carnivores that rely on prairie dogs as prey (Salkeld and Stapp 2006).

Plague epizootics in prairie dogs spread very rapidly within a colony and the high virulence of *Y. pestis* results in a very short period of time (6 - 8weeks; Webb et al. 2006) between exposure and complete extermination. Plague also spreads very quickly among prairie dog colonies (Rayor 1985) and is apparently capable of covering long distances in relatively short periods of time (Girard et al. 2004). In some locations, epizootic outbreaks may be triggered by increases in spring-time precipitation and relatively cool summers (Parmenter et al. 1999, Stapp et al. 2004, Collinge et al. 2005a), though specific mechanisms to account for these observations have not been directly identified. One possibility is that favorable conditions for plant growth result in increased densities of deer mice on prairie dog colonies, allowing for increased opportunities for flea or pathogen exchange between deer mice and prairie dogs (e.g., Collinge et al. 2005a). Another explanation is that flea populations increase under these circumstances as flea reproduction and survival are highly dependent on abiotic conditions (Krasnov et al. 2001). Landscape features may also be associated with plague emergence in prairie dogs; colonies that are isolated by natural or anthropogenic barriers may be less likely to be exposed to plague (Collinge et al. 2005b). Identification of such barriers as being important to plague emergence suggests that *Y. pestis* moves by terrestrial rather than air-borne means.

Because of the great uncertainty surrounding identities of the mammals and fleas that are responsible for plague transmission and movement, there is a clear need for systematic study of mammal and flea communities associated with prairie dog colonies. To evaluate possible reservoir hosts of *Y. pestis* and sources for plague transmission to prairie dogs, I systematically evaluated occurrence patterns of fleas on a wide variety of mammalian species associated with prairie dog colonies. Among my goals were 1) to identify mammalian species that are likely to be involved in plague transmission by measuring flea prevalence and abundance on each species, and 2) characterize relationships and associations between host and flea species.

## Methods

### *Host species and study sites*

With the assistance of many field assistants, I collected fleas from live mammals at a total of 30 study sites in Boulder County, Colorado, located between 105º18'33" and 105º5'43" west longitude and 39º 54'30" and 40º14'32" north latitude, beginning in April 2004 and ending in September 2006 (Figure 3.1). I independently targeted three types of mammals: small rodents, black-tailed prairie dogs, and carnivores. Small rodents and black-tailed prairie dogs were trapped exclusively at prairie dog colony sites and carnivores were trapped at locations that varied between 0 and 1000m from prairie dog colonies. Elevation at study sites ranged from roughly 1550 to 1920 m. The colonies sampled in this study represent a subset of those described in Collinge et al. (2005b) and ranged from a minimum of

roughly 8 to a maximum of over 25 hectares. Prairie dog colonies in this study system occurred in shortgrass prairies which are dominated by western wheatgrass (*Agropyron smithii*) and blue grama (*Bouteloua gracilis*) (Collinge 2000, Collinge et al. 2005a), although active prairie dog colonies have significantly higher cover of forbs and bare ground than grassland sites where prairie dogs are absent (Conlin 2005). Landscape context of study areas ranged from urban and suburban development to active agriculture to protected and managed wildlife habitat.

*Small rodent trapping*

Small rodents were trapped at 20 sites twice per year, in May/June and August/September, for periods of roughly four weeks at a time. I used non-collapsible Sherman live traps (H.B. Sherman Co., Tallahassee, FL) arranged in 7 x 7 grids with 20m spacing between traps. Each site was trapped for four consecutive nights during each trapping session. Traps were baited with rolled oats and were set in the evening between 1600 and 2000h. Traps were checked and closed between 0600 and 1100. Small rodents and their fleas were anesthetized with isoflurane in a transparent tube. Upon removal, each individual was brushed 10 times on the ventral and dorsal side with a toothbrush while being held over a white plastic tray. All observable fleas inside the anesthesia chamber, on the plastic tray and on the host's body were collected. I also collected weight, length, and sex data and shaved a small patch of fur from the hindquarters to identify recaptured individuals. Within-session recaptures were released unprocessed. I could not distinguish individuals recaptured from a previous session and so each non-marked animal (i.e. not captured previously

within the session) was processed as a new capture and the full set of samples and data was collected.

*Prairie dog trapping*

I trapped prairie dogs at 24 colonies (Figure 3.1) in June and July 2004-2006 using Tomahawk live-traps (Tomahawk Live Trap, Inc., Tomahawk, WI) baited with a mixture of corn, oats, barley and molasses. Trapping grids were either square or rectangular with 25m spacing between traps and consisted of 48, 49, or 50 traps, depending on the shape of each colony. Each trapping session lasted four days and was preceded by three days of pre-baiting. Traps were set either from 0630 – 0930 h or from 0900 – 1200 h with daily alternation between "early" and "late" trapping hours. Traps were baited and locked open during daytime hours to encourage prairie dog visitation.

I anesthetized prairie dogs and their fleas in the same way described above for small rodents. Fleas were collected from the host's body and from inside the anesthesia chamber using forceps. Individual prairie dogs were marked with uniquely coded passive integrated transponder (PIT) tags. I shaved a small patch of fur from behind the head so that recaptured animals could be identified on sight and I collected sex, weight, and length data from each captured prairie dog. Individuals that were recaptured during the same trapping session were released unprocessed. Individuals that were recaptured in subsequent years were processed as described above with the exception of PIT tag implantation.

*Carnivore trapping*

I live-trapped carnivores from 2004 to 2006 at a total of six study sites, each surveyed twice per year (Figure 3.1). In each year, carnivores were trapped from April to June (spring trapping sessions) and from August to September (late summer trapping sessions). In 2004, I used three-week trapping sessions at a total of two study sites which were trapped twice per year. In 2005 and 2006, I trapped at a total of four study sites for two-week periods during spring and late summer sessions. In each 2005 and 2006 I trapped at one additional study site for a single two-week trapping session (Figure 3.1).

All trapping was conducted with a combination of three sizes ( 17.8 x 17.8 x 50.8cm, 25.4 x 30.5 x 81.3cm, and 40.1 x 50.8 x 106.7cm) of wire box traps (Tomahawk Live Trap Inc, Tomahawk, WI) (2004-2006) and #1.5 and #3 Victor Coil Soft Catch™ leg-hold traps (Woodstream Corporation, Lititz, PA)(2005 and 2006 only). Captured animals were anesthetized with an intramuscular injection of Telazol (Fort Dodge Animal Health, Fort Dodge, IA) at a rate of 10 mg/kg and delivered by way of a 1 m pole syringe. I used fine-toothed combs and forceps to collect all observable fleas from each trapped individual. Flea collection continued until 5 – 10 minutes of continued combing resulted in no additional fleas being detected. All captured individuals were marked with an individually numbered eartag and sex, length measurements, and body mass data were noted. Each captured individual was observed until the effects of the sedative began to subside. All trapping was done in accordance with the guidelines set by and with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Colorado.

*Flea handling and identification*

All collected fleas were immediately deposited in vials containing a 2% saline solution with a small amount of Tween (polysorbate 80) and were stored at -80°C. Fleas were identified by light microscopy using dichotomous keys presented in Hubbard (1968) and Furman and Catts (1982). Species determinations for a subsample of fleas were confirmed at the Centers for Disease Control in Fort Collins, CO.

*Quantitative analyses*

I calculated flea prevalence (proportion of infested hosts), flea abundance (total number of fleas divided by total number of hosts), and flea intensity (total number of fleas divided by number of infested hosts) for each mammalian species that was sampled. For each pair of hosts, I calculated similarity indices using both incidence (presence/absenc, Sorensen's similarity index) and raw abundance (Morisita-Horn similarity index) data with EstimateS software (Colwell 2005). I tested for flea assemblage structure by comparing observed incidence data, measured as detection of flea species A on host species B, with a null incidence model. The null expectation was generated with EcoSim 7.72 (Gotelli and Entsminger 2001) through 5000 iterations of a reshuffling algorithm where the number of flea species per host species and number of hosts utilized by each flea species were held constant (i.e., row and column sums in the presence/absence data matrix were fixed).

I used nonmetric multidimensional scaling (NMS) ordination (Mather 1976) run with PC-ORD software to assess flea assemblages qualitatively on mammalian species. Because NMS is sensitive to the structure of the original dataset (McCune and Grace 2002), I natural log-transformed the data and used relative Euclidean distance to quantify dissimilarity of host species in terms of flea species assemblage. I also used incidence data to perform ordination analysis and measured dissimilarity among host species by Euclidean distance. For all ordination analyses, final stress and instability were determined after 50 runs with the real dataset, each started from a random initial seed. I used a randomized Monte Carlo simulation to determine dimensionality of the best ordination result; dimensions were added until results from real data ordination runs did not result in reduced stress when compared to the simulations (McCune and Grace 2002). In addition to ordination, I used cluster analysis to group host species hierarchically by flea assemblage similarity. Distance values among host species for cluster analysis were determined in the same manner as for NMS. Hierarchical clustering was achieved by Ward's method (Ward 1963) and cluster dendrograms were generated with PC-Ord.

**Results**

I trapped a total of 4,569 mammals, representing 13 species, and collected and identified a total of 18,341 fleas, representing 14 species (Tables 2.1, 2.2). A small number of fleas (fewer than 0.1%) were too damaged to identify and were excluded from all analyses. Discrimination between female *Pulex irritans* and *P. simulans* is

unreliable (Hopla 1980) so the species were grouped together as *Pulex* sp. Flea prevalence varied among mammalian species but was generally lowest among small rodents and highest among carnivores (Table 3.3). Flea prevalence, however, was not associated with host body mass ($P > 0.2$, adjusted $R^2 < 0.1$). Flea abundance and mean intensity ranged from 0.13 to 20 and from 1 to 20, respectively. Both were significantly positively related to natural-log-transformed host body mass (flea abundance: $F = 8.63$, d.f. = 12, adjusted $R^2 = 0.39$, $P < 0.05$; flea intensity: $F = 8.75$, d.f. = 12, adjusted $R^2 = 0.39$, $P < 0.05$).

Sample size varied by nearly three orders of magnitude between the most frequently sampled host species (*Peromyscus maniculatus*) and the least frequently sampled host species (*Vulpes vulpes*)(Table 3.3). The number of fleas collected from each host species was even more variable (range: 2 – 13,761; Table 3.3). This imbalance results in higher likelihood of rare flea species detection on host species from which more samples were collected. To correct for this variation in sampling effort, I conducted analyses of both the full dataset as well as a dataset where rarely occurring flea species were removed. A rarely occurring flea species was defined as one which constituted less than one percent of the total number of fleas collected from a host species.

*Flea assemblage similarity and flea species co-occurrence*

Sorensen's similarity index values for both the full and sample size-corrected datasets as well as the Morisita-Horn similarity index values are presented in Tables 3.4, 3.5, and 3.6 respectively. Sorensen's index values for the sample size-corrected

dataset were highest (0.8) between *C. ludovicianus* and *C. latrans*, and *C. latrans* and *V. vulpes*. Sorensen's index values were generally consistent between the full and sample-size-corrected datasets (Tables 3.4 and 3.5). The highest Morisita-Horn index value (0.961) was between *C. latrans* and *V. vulpes*. Null model analyses indicated that the flea species I collected from mammals associated with black-tailed prairie dog colonies co-occurred less frequently than would be expected by chance for both the full and reduced datasets (Table 3.7). Additionally, there was a higher number of checkerboard species pairs than would be expected by chance (Table 3.7), indicating that non-random flea species assemblage results in a significant number of flea species that never co-occur. The number of species combinations did not differ from the null model range for either the full or sample size-corrected dataset. Taken as a whole, the null model analyses indicate that flea-host associations are non-random and are suggestive of strong host-specificity in this system.

*NMS ordination*

Flea assemblages were strongly associated with specific and discrete groups of host species. Ordination of natural-log transformed flea abundances arranged by host species resulted in a 2-dimensional solution (Figure 3.2); addition of further dimensions to describe the dataset did not reduce the stress of the ordination more than would be expected by chance alone. Axes 1 and 2 of this ordination respectively accounted for 44.4 and 33.0 percent of the variation in the original dataset, with a cumulative $R^2$ value of 0.774. The ordination represented in Figure 3.2 has a stress value of 14.2, indicating that this is a fairly reasonable representation of the dataset

with modest risk of misinterpretation; stress values of 5-10 are considered to indicate good ordinations that should not lead to false inferences (Clarke 1993). Ordination stress was reached after 33 runs with a final instability less than $1x10^{-6}$.

Ordination of the sample size-corrected flea incidence dataset resulted in a 3-dimensional solution with a stress value of 6.09 and final instability of $1x10^{-5}$. The first three axes accounted for 40.9, 31.9, and 17.1 percent (cumulative $R^2 = 0.899$) of the variation in the dataset, respectively. Arrangement of hosts is presented on the first two axes, which account for 72.8 percent of the variation in the dataset and is presented in Figure 3. NMS ordination of the full (non sample size-corrected) incidence dataset resulted in a qualitatively similar arrangement of hosts yet produced a higher stress value (14.5) and lower cumulative $R^2$ (0.799).

*Cluster diagrams*

Similar to the ordination results, cluster analysis indicated strong associations between host species based on flea assemblage similarity. Hierarchical cluster diagrams for the natural log-transformed dataset and sample size--corrected incidence dataset are presented in Figures 3.4 and 3.5. Percent chaining, defined as the average path length between two units divided by the minimum possible average path length between sampling units, was 2.44 for the log-transformed dataset and 9.76 for the sample size-corrected incidence dataset; dendrograms resulting in extreme chaining (over 25%) have not reliably served to divide the dataset into small groups (McCune and Grace 2002). I also used the natural log-transformed dataset to group flea species based on which hosts they parasitized, using the same distance metric and clustering

method as for the analysis of hosts grouped by flea assemblage (Figure 3.6). Chaining for this analysis was also calculated to be 2.44.

**Discussion**

Flea-mediated plague transmission occurs within the context of multi-host communities, thus the richness, relative abundance of, and interactions among host and vector species are likely to determine pathogen transmission dynamics (Holt et al. 2003, Collinge and Ray 2006, Keesing et al. 2006). The results of this study demonstrate that relationships between the flea and mammalian species associated with prairie dog colonies are non-random with a greater-than-expected number of flea species that never co-occur on any host. Such patterns are suggestive of high host-specificity among flea species as each flea species is generally associated with one host species or group of ecologically similar species and many flea-host species combinations are not observed. Furthermore, both ordination and cluster analysis indicate that certain subsets of this mammal community can be defined by their flea species assemblages and that these groupings are generally concordant with taxonomic association and/or ecological characteristics; carnivores tended to have very similar flea species assemblages as did cricetid rodents (Figures 3.2, 3.3, 3.5, 3.6). The results of this study may be useful for identifying routes of transmission of *Yersinia pestis* among mammalian species and cast doubt upon the proposed presence of a small rodent plague reservoir that serves as a source of infection for epizootic hosts such as prairie dogs. Rather, these flea association data suggest that flea-mediated plague transmission to and from prairie dogs is more likely to involve a

non-rodent (lagomorph or carnivore) species. These data also suggest that flea species that are not specific to prairie dogs may play a role in interspecific plague transmission.

*Mammalian species likely to be involved in plague transmission*

As epizootic hosts, prairie dogs play a key role in plague ecology and dynamics (Collinge et al. *in press*). Given the rarity and apparent stochasticity of epizootic plague events, it is necessary to examine the host and vector communities in which prairie dogs exist in order to draw inferences and make predictions about mechanisms of *Y. pestis* transmission to and from prairie dogs. Similarity index values indicate that prairie dog flea assemblages overlap most with coyotes and desert cottontails, based on incidence data, and with pocket mice based on abundance data (Tables 3.4 – 3.6). These metrics provide a general assessment of which mammalian species share the most flea species. Morisita-Horn index values, which take into account the relative abundance of each flea species on a given host, are generally low for all pair-wise comparisons involving prairie dogs because prairie dog flea assemblages are dominated by one species (*O. hirsuta*; Figure 3.7) that is uncommonly found on any other host. The second most common prairie dog flea (*Pulex* sp.), on the other hand, is commonly collected from species of carnivores and is rarely found on other rodents (Figure 3.7). Based on examination of the two most common prairie dog fleas, substantial flea assemblage overlap exists between prairie dogs and their mammalian predators, whereas little overlap occurs between prairie dogs and small rodents (Figure 3.7).

Cluster analyses of host species, grouped by flea assemblage, indicate a deep bifurcation between a small rodent species group and a group containing carnivores, prairie dogs, and rabbits (Figures 3.4, 3.5). Ordination analyses also generate two more or less discrete groups with prairie dogs falling closer to the carnivore cluster than to the small rodent cluster (Figures 3.2, 3.3). These results indicate that the flea assemblages of small rodents are quite distinct from those of other mammalian species but are very similar among small rodent species. Likewise, the flea assemblages of carnivores, although slightly more variable than those of small rodents, are quite distinctive. The flea assemblages of ground squirrels (including prairie dogs) and desert cottontails are more similar to those of carnivores than to those of small rodent species (Figures 3.2 – 3.5). In the context of plague transmission, these results suggest that carnivores are more likely than small rodents to exchange plague-infected fleas with prairie dogs. Furthermore, flea prevalence on larger mammals is significantly higher than on smaller mammals. This means that larger mammals are more likely to have fleas, and that interactions between larger mammalian species are more likely to result in flea exchange than those between smaller mammalian species, given that there is a higher probability of two small-bodied species having no fleas to exchange than for similar interactions among larger-bodied mammals.

Carnivores in general, and coyotes in particular, are perhaps the most likely group to be involved in flea-mediated plague transmission to or from prairie dogs. I have shown here that roughly 2.4% (5/208) of fleas collected from coyotes are specific to prairie dogs and that over 97% (202/208) of coyote fleas belong to species

that also commonly occur on prairie dogs (Figure 3.7). The possibility that carnivores are involved in plague movement and transmission has been raised repeatedly (Barnes 1982, Cully and Williams 2001, Harrison et al. 2003, Salkeld and Stapp 2006, Salkeld et al. 2007), partly due to relatively high rates of plague exposure in many carnivore taxa (see Salkeld and Stapp 2006 for review) and partly because of the relatively large home ranges and potential for long-distance dispersal among mammalian carnivores species (Rosatte 2002). Carnivores often acquire the fleas of their prey, as fleas will abandon a recently-dead host (Salkeld and Stapp 2006) or a host whose body temperature and/or blood oxygen level have begun to fall (pers. obs.). Carnivores may also be indirectly involved in plague transmission to prairie dogs by preying upon rabbits. Both foxes and coyotes were observed to carry rabbit fleas suggesting that rabbits constitute a portion of the prey base for each predator. Rabbits and prairie dogs have moderately similar flea species assemblages, so plague-infected fleas could move from carnivores to rabbits to prairie dogs, or vice-versa.

Although a variety of species of carnivores have been found to carry fleas typically associated with prairie dogs (Harrison et al. 2003, McGee et al. 2006), their role as dispersers of plague-infected fleas is often underplayed. McGee et al. (2006) suggested that, although they found foxes carrying prairie dog fleas, foxes are likely to play only a minor role in plague transmission to or from prairie dogs because none of the prairie dog fleas collected from foxes tested positive for presence of *Y. pestis*. The data presented in this paper, however, indicate that carnivores are much more likely to acquire prairie dog fleas than are other mammals typically assumed to be reservoirs for *Y. pestis* (i.e. *P. maniculatus*) and are therefore the most likely group to

spread plague-positive fleas to or from prairie dogs, especially in light of their daily movement patterns.

*Flea species likely to be involved in plague transmission*

Fleas are generally highly host-specific and many flea species have morphological adaptations suited to a particular host's behavior and biology (Traub 1985). Burrowing and fast-moving mammalian species tend to harbor fleas that possess a large number of coarse hairs and spines that are presumed to keep the flea lodged in its host's pelage (Traub 1985). Fleas may respond to olfactory (Krasnov et al. 2002a), visual (Cox et al. 1999), or thermal (Benton and Lee 1965, Rust and Dryden 1997) cues in order to locate a host and in many cases, these responses are only elicited by one or a few host species (Krasnov et al 2002a). The vast majority of the 18,341 fleas I collected were associated with the host to which they are specific, though host specificity is apparently looser in some flea species than in others. Relationships between small rodents and their fleas, for example, were not as clear-cut as relationships between squirrels and their fleas. Each flea species in the suite of small rodent fleas I sampled (*E. wenmani*, *A. wagneri*, *C. pseudagertes*, and *O. leucopus*) was associated with as many as five small rodent species, but was rarely collected from squirrels and was never collected from rabbits or carnivores. These results suggest that the cues small rodent fleas use to find their hosts may be common to all of the small rodent species I sampled. The relative taxonomic similarity between murids, sciurids and lagomorphs compared to those between sciurids and carnviores, may be offset by the behavioral and anatomical similarity between

sciurids, lagomorphs, and carnivores in explaining flea species-host species mismatches. For example, the fast-moving and burrowing nature of prairie dogs may preclude infestation by some small rodent fleas that are not able to remain lodged in a prairie dog's pelage.

Revisiting the flea-host association data from the perspective of host groups utilized by each flea species provides insights into which flea species might be involved in transmitting plague among host species. Cluster analysis of flea species, grouped by host association (Figure 3.6) is indicative of the host specificity of flea species analyzed. For example, the small rodent fleas *E. wenmani*, *A. wagneri*, *C. pseudagertes*, and *O. leucopus* fall into one cluster, as do the rabbit fleas *E. glacialis* and *C. inaequalis*. This analysis also indicates that the host utilization of the prairie dog flea *O. hirsuta* is most similar to that of *Pulex* sp. and is quite distinct from the host utilization of any of the small rodent fleas. Taken as a whole, these results suggest that prairie dogs are more likely to share fleas with non-rodent mammals and that small rodents, such as deer mice, are less likely to be involved in plague transmission to or from prairie dogs than are some other mammalian species.

Fleas of the genus *Pulex* were collected from prairie dogs, carnivores, and rabbits (Table 3.1, Figure 3.7) and the presence of *Pulex* on these hosts was largely responsible for the grouping of these mammals in ordination and cluster analysis (Figures 3.2 – 3.5). *Pulex* is one of only a few flea genera that are truly host generalists (Traub 1985) and the results of this study are consistent with previous studies finding that carnivores are commonly infested with *Pulex irritans* and *simulans* (Hubbard 1968). Although *Pulex* are not specific to any host taxon, they are

often recovered from prairie dogs (Hubbard 1968, Trevino-Villareal et al. 1998, Stevenson et al. 2003), and, in at least one study, were found to be the most common prairie dog flea, accounting for up to 74% of fleas collected (Nascarella et al. 2005). Very few carnivore-specific fleas exist (Traub 1985) and carnivores often acquire fleas of their prey, either during predation events or through foraging activity in prey burrows, nests, or other microhabitats (Harrison et al. 2003, Salkeld and Stapp 2006). In this study, I recovered wood rat-specific fleas (*Anomiopsyllus nudatus* and *Orchopeas neotomae*) and ground squirrel-specific fleas (*Oropsylla montana*) from striped and western spotted skunks, rabbit-specific fleas (*Cediopsylla inaequalis* and *Euhoplopsyllus glacialus*) from spotted skunks, raccoons, foxes and coyotes, and prairie dog-specific fleas (*Oropsylla hirsuta*) from coyotes (Table 3.1).

*Pulex* sp. have typically been discounted in hypotheses regarding plague transmission because they rarely form blockages and are thought to be inefficient vectors (Gage and Kosoy 2005, Drancourt et al. 2006). However, recent recognition of early-phase plague transmission in other flea species that were also considered poor vectors suggests that proventricular blockage may not be required for plague transmission in natural systems (Eisen et al. 2006, 2007, Wilder et al. in review). Furthermore, *Pulex irritans*, which was routinely collected from wild canids in this study, has recently been implicated in human plague cases in Tanzania; areas with recurrent plague activity and high plague frequency were associated with high abundance of *Pulex irritans* (Laudisoit et al. 2007). Although direct evidence linking *Pulex* sp. to plague transmission is lacking, circumstantial evidence suggests that *Pulex* sp. may play a role in spreading plague within and among mammalian species.

*Conclusion*

Plague is predominantly a flea-borne disease (Gage and Kosoy 2005) and recent research suggests that proventricular blockage may not be required for effective flea-mediated transmission among host species (Eisen et al. 2006, 2007a, Webb et al. 2006). New data indicate that early-phase transmission, in lieu of blockage, may be common and could account for rapidly-spreading plague epizootics (Eisen et al. 2007, Wilder et al. in review). The recognition of early-phase plague transmission suggests the possibility that any flea species might be capable of transmitting plague in natural systems. Thus, flea species previously rejected as potential plague vectors may, in fact, be largely responsible for plague transmission. Even though *Pulex* spp. are considered poor plague vectors, their high prevalence and abundance on prairie dogs, in addition to their tendency to occur on a wide variety of mammal host species, suggests that they play a role in interspecific plague transmission.

It should be noted, however, that high rates of flea exchange among mammalian species may not be necessary for plague transmission among mammalian species. It is possible that even rare occurrences of flea exchange among mammalian species are sufficient to account for patterns of plague emergence that are observed in natural systems. For example, as few as one to three infected fleas may be required to initiate an epizootic event, according to simulation models (C. Webb, pers. comm.). Thus, even exceedingly rare cases of flea exchange from small rodents to prairie dogs or vice-versa could potentially account of observed patterns of plague occurrence.

However, given the rapidity with which plague epizootics spread across the landscape, it is unlikely that small rodents are solely responsible for initiating and propagating plague events.

The data presented here were collected prior to a plague epizootic in Boulder County, Colorado, and it has been argued that during or after plague events, host specificity in fleas breaks down, leading to higher rates of flea exchange among mammalian species (McGee et al. 2006). Although this phenomenon is likely to occur, at least to some extent, Salkeld and Stapp (unpublished data) observed no significant increase in occurrence of *O. hirsuta* on deer mice in the weeks and months following prairie dog die-offs due to plague. Carnivores, however, may be attracted to prairie dog colonies that are suffering from plague as plague morbidity in prairie dogs results in lethargy, making infected animals more susceptible to predation (McGee et al. 2006). This behavior would likely result in prairie dog predators acquiring infected fleas, thus increasing the probability of long-distance plague dispersal. The lack of flea assemblage overlap between prairie dogs and associated small rodents is somewhat surprising. Deer mice are often found in higher abundance on prairie dog colonies than on nearby grassland sites where prairie dogs are absent (Agnew et al. 1986, Cully et al. unpublished data) and often share borrows with prairie dogs (Agnew et al. 1986).

Sylvatic plague is an enigmatic disease that is likely to involve a suite of reservoir hosts and vectors. Roles of particular species of fleas and mammals in the ecology of plague are undefined, but flea-mediated plague transmission to and from prairie dogs is critical to understanding initiation and continuation of epizootic events

in western North America. I have shown that flea exchange to or from prairie dogs does not commonly involve small rodents. Rather, coyotes and desert cottontails have flea species assemblages that are most similar to those of prairie dogs. Furthermore, the movement and behavior of coyotes in particular may reconcile with patterns of plague emergence in prairie dog colony complexes (e.g., Girard et al. 2004). In light of recent laboratory experiments and field observations, I suggest that fleas of the genus *Pulex* might play a larger role in plague transmission than was previously considered.

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Table 3.1: List of mammalian species sampled and flea species collected from each.

| Host species | Host species common name | Flea species |
|---|---|---|
| *Chaetodipus hispidus* (CHHI) | Hispid pocket mouse | AWAG, MTEL, ONEO, OHIR |
| *Cynomys ludovicianus* (CYLU) | Black-tailed prairie dog | AWAG, CINA, EWEN, MTEL, EGLA, OHIR, PULE |
| *Microtus ochrogaster* (MIOC) | Prairie vole | CPSE, MTEL, OLEU, EWEN |
| *Microtus pennsylvanicus* (MIPE) | Meadow vole | AWAG, MTEL, OLEU, OHIR |
| *Peromyscus maniculatus* (PEMA) | Deermouse | AWAG, MTEL, OLEU, OHIR, TFOT, ONEO, EWEN |
| *Reithrodontomys megalotis* (REME) | Western harvest mouse | AWAG, OLEU |
| *Spermophilus tridecemlineatus* (SPTR) | Thirteen-lined ground squirrel | OHIR, TFOT |
| *Sylvilagus audubonii* (SYAU) | Desert cottontail | CINA, EGLA, OHIR, PULE |
| *Canis latrans* (CALA) | Coyote | CINA, EGLA, OHIR, PULE |
| *Mephitis mephitis* (MEME) | Striped skunk | ONEO, OMON, PULE, ANUD |
| *Procyon lotor* (PRLO) | Raccoon | EGLA, PULE |
| *Spilogale gracilis* (SPGR) | Western spotted skunk | EGLA, ONEO, OMON, ANUD |
| *Vulpes vulpes* (VUVU) | Red fox | CINA, PULE |

Table 3.2: List of flea species encountered in this study and host species from which each was collected.

| Flea species | Host species |
|---|---|
| *Aetheca wagneri* (AWAG) | CHHI, CYLU, MIPE, PEMA, REME |
| *Cediopsylla inaequalis* (CINA) | CYLU, SYAU, CALA, VUVU |
| *Ctenopthalmus pseudagertes* (CPSE) | MIOC, MIPE, PEMA |
| *Epitidea wennmani* (EWEN) | CYLU, MIOC, MIPE, PEMA |
| *Euhoplopsyllus glacialus* (EGLA) | CYLU, SYAU, CALA, PRLO, SPGR |
| *Malareus telchinum* (MTEL) | CHHI, CYLU, MIOC, PEMA |
| *Orchopeas leucopus* (OLEU) | MIOC, MIPE, PEMA, REME |
| *Oropsylla hirsuta* (OHIR) | CHHI, CYLU, MIPE, PEMA, SPTR, SYAU, CALA |
| *Thrasis fotus* (TFOT) | PEMA, SPTR |
| *Orchopeas neotomae* (ONEO) | CHHI, PEMA, MEME, SPGR |
| *Oropsylla montana* (OMON) | MEME, SPGR |
| *Pulex* sp. (PULE) | CYLU, SPTR, CALA, MEME, PRLO, VUVU |
| *Anomiopsyllus nudatus* (ANUD) | MEME, SPGR |

Table 3.3. Samples size for each host species with number of fleas collected, total flea prevalence, mean abundance, and mean intensity.

| Mammalian species | Total number sampled | Number of fleas collected | Flea prevalence | Mean flea abundance | Mean flea intensity |
|---|---|---|---|---|---|
| *Chaetodipus hispidus* | 44 | 7 | 0.14 | 0.16 | 1.17 |
| *Cynomys ludovicianus* | 1702 | 13761 | 0.84 | 8.09 | 9.66 |
| *Microtus ochrogaster* | 20 | 22 | 0.65 | 1.10 | 1.69 |
| *Microtus pennsylvanicus* | 13 | 23 | 0.54 | 1.77 | 3.29 |
| *Peromyscus maniculatus* | 2667 | 3907 | 0.53 | 1.46 | 2.77 |
| *Reithrodontomys megalotis* | 7 | 2 | 0.29 | 0.29 | 1.00 |
| *Spermophilus tridecemlineatus* | 16 | 9 | 0.31 | 0.56 | 1.80 |
| *Sylvilagus audubonii* | 19 | 176 | 0.79 | 9.26 | 11.73 |
| *Canis latrans* | 11 | 208 | 1.00 | 18.91 | 18.91 |
| *Mephitis mephitis* | 32 | 109 | 0.53 | 3.41 | 6.41 |
| *Procyon lotor* | 30 | 4 | 0.13 | 0.13 | 1.00 |
| *Spilogale gracilis* | 5 | 54 | 1.00 | 10.80 | 10.80 |
| *Vulpes vulpes* | 3 | 60 | 1.00 | 20.00 | 20.00 |

Table 3.4: Matrix indicating Sorensen's similarity index values for each pair of host species calculated from the raw incidence dataset.

| | CHHI | CYLU | MIOC | MIPE | PEMA | REME | SPTR | SYAU | CALA | MEME | PRLO | SPGR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CYLU | 0.545 | | | | | | | | | | | |
| MIOC | 0.25 | 0.364 | | | | | | | | | | |
| MIPE | 0.571 | 0.4 | 0.286 | | | | | | | | | |
| PEMA | 0.727 | 0.571 | 0.545 | 0.6 | | | | | | | | |
| REME | 0.333 | 0.222 | 0.333 | 0.8 | 0.444 | | | | | | | |
| SPTR | 0.333 | 0.222 | 0 | 0.4 | 0.444 | 0 | | | | | | |
| SYAU | 0.25 | 0.727 | 0 | 0.286 | 0.182 | 0 | 0.333 | | | | | |
| CALA | 0.25 | 0.727 | 0 | 0.286 | 0.182 | 0 | 0.333 | 1 | | | | |
| MEME | 0.25 | 0.182 | 0 | 0 | 0.182 | 0 | 0 | 0.25 | 0.25 | | | |
| PRLO | 0 | 0.444 | 0 | 0 | 0 | 0 | 0 | 0.667 | 0.667 | 0.333 | | |
| SPGR | 0.25 | 0.182 | 0 | 0 | 0.182 | 0 | 0 | 0.25 | 0.25 | 0.75 | 0.333 | |
| VUVU | 0 | 0.444 | 0 | 0 | 0 | 0 | 0 | 0.667 | 0.667 | 0.333 | 0.5 | 0 |

Table 3.5: Matrix of Sorensen's similarity index values for each pair of host species calculated from the raw incidence dataset.

| | CHHI | CYLU | MIOC | MIPE | PEMA | REME | SPTR | SYAU | CALA | MEME | PRLO | SPGR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CYLU | 0.333 | | | | | | | | | | | |
| MIOC | 0.25 | 0 | | | | | | | | | | |
| MIPE | 0.667 | 0.286 | 0.667 | | | | | | | | | |
| PEMA | 0.571 | 0 | 0.571 | 0.75 | | | | | | | | |
| REME | 0.4 | 0 | 0 | 0.333 | 0.5 | | | | | | | |
| SPTR | 0.333 | 0.5 | 0 | 0.286 | 0 | 0 | | | | | | |
| SYAU | 0.286 | 0.4 | 0 | 0.25 | 0 | 0 | 0.4 | | | | | |
| CALA | 0.286 | 0.8 | 0 | 0.25 | 0 | 0 | 0.4 | 0.667 | | | | |
| MEME | 0.25 | 0.333 | 0 | 0 | 0 | 0 | 0 | 0 | 0.286 | | | |
| PRLO | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.4 | 0.4 | 0.333 | | |
| SPGR | 0.222 | 0 | 0.222 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.667 | 0.286 | |
| VUVU | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.4 | 0.8 | 0.333 | 0.5 | 0 |

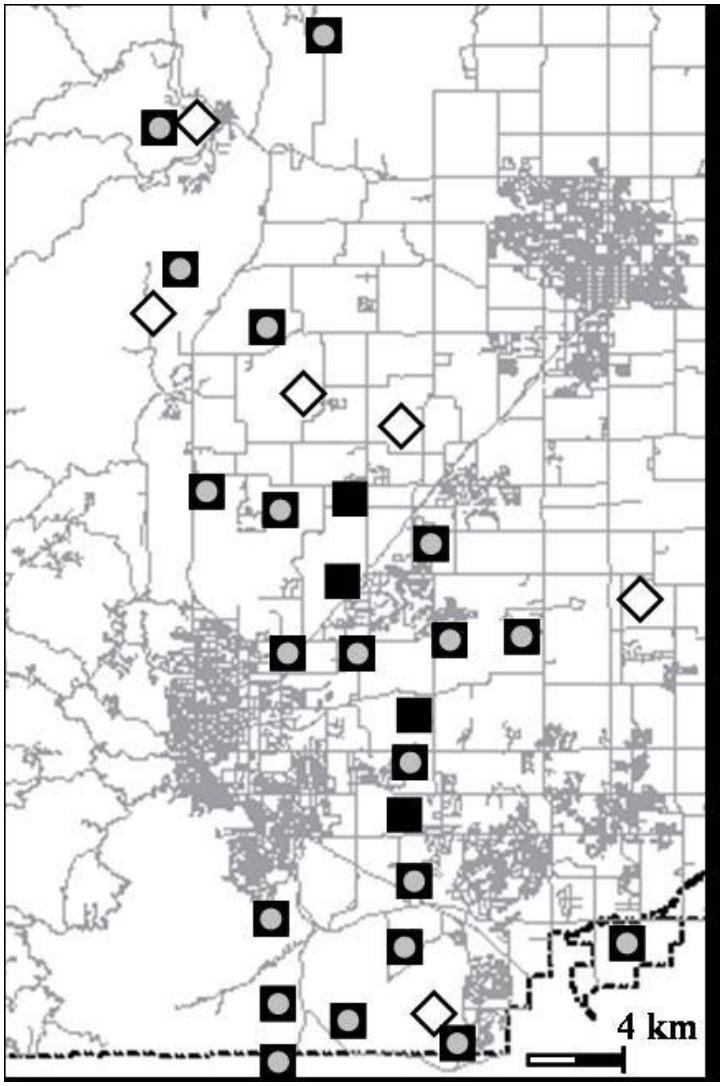
Table 3.6: Matrix indicating Morisita-Horn similarity index values for each pair of host species calculated from natural-log transformed data.

| | CHHI | CYLU | MIOC | MIPE | PEMA | REME | SPTR | SYAU | CALA | MEME | PRLO | SPGR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CYLU | 0.208 | | | | | | | | | | | |
| MIOC | 0.012 | 0 | | | | | | | | | | |
| MIPE | 0.884 | 0.085 | 0.259 | | | | | | | | | |
| PEMA | 0.858 | 0.001 | 0.023 | 0.946 | | | | | | | | |
| REME | 0.644 | 0 | 0.692 | 0.862 | 0.694 | | | | | | | |
| SPTR | 0.027 | 0.124 | 0 | 0.011 | 0 | 0 | | | | | | |
| SYAU | 0.016 | 0.07 | 0 | 0.006 | 0 | 0 | 0.009 | | | | | |
| CALA | 0.006 | 0.048 | 0 | 0.002 | 0 | 0 | 0.004 | 0.027 | | | | |
| MEME | 0.007 | 0.018 | 0 | 0 | 0 | 0 | 0 | 0.008 | 0.952 | | | |
| PRLO | 0 | 0.017 | 0 | 0 | 0 | 0 | 0 | 0.433 | 0.875 | 0.865 | | |
| SPGR | 0.138 | 0 | 0 | 0 | 0 | 0 | 0 | 0.105 | 0.001 | 0.094 | 0.049 | |
| VUVU | 0 | 0.019 | 0 | 0 | 0 | 0 | 0 | 0.084 | 0.961 | 0.934 | 0.86 | 0 |

Table 3.7: Results from null model analyses indicating statistical significance of C-score,
number of checkerboard species pairs and species combinations for both raw and sample size-corrected datasets.

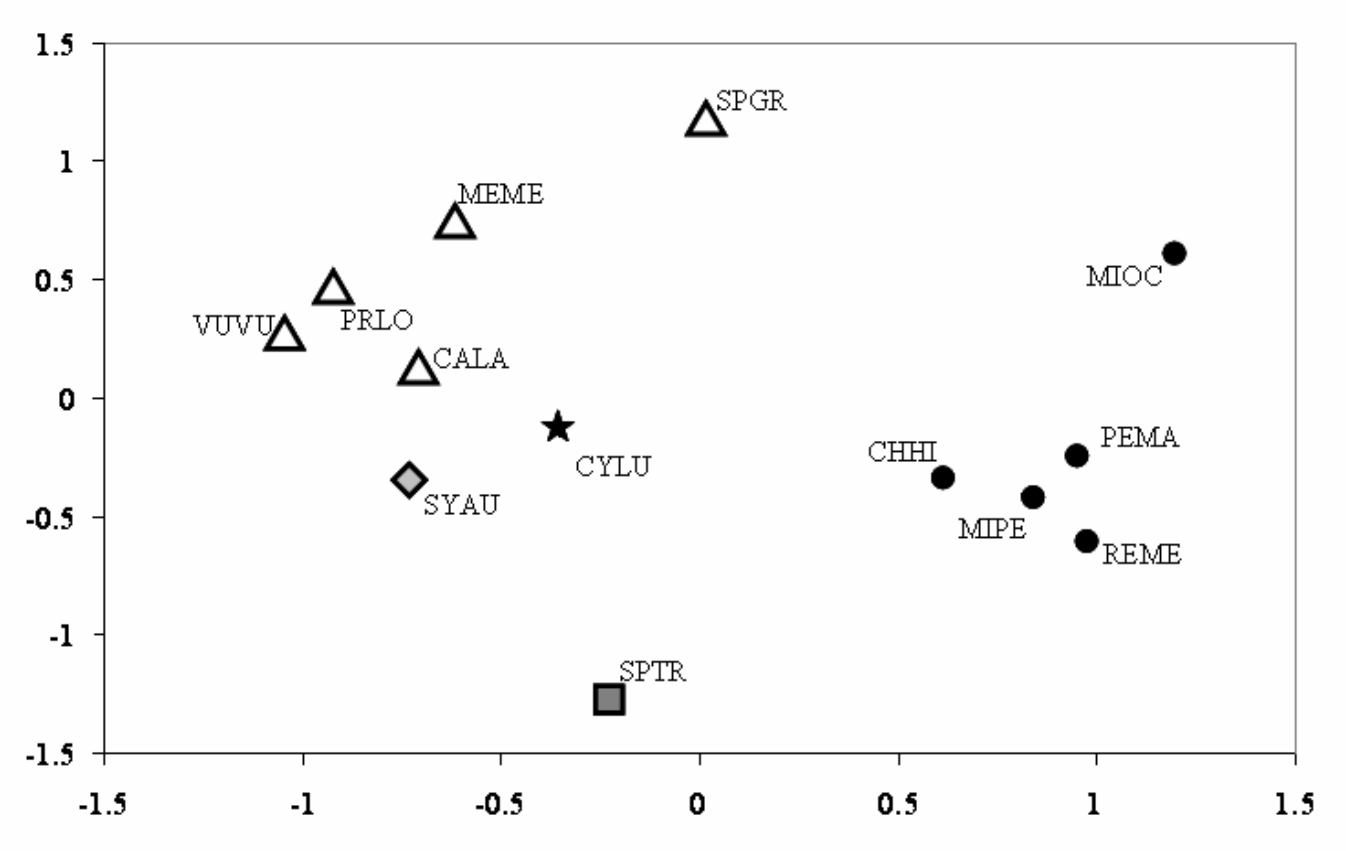
| | C-Score | Checkerboard Pairs | Species Combinations |
|---|---|---|---|
| Incidence data, full dataset | < 0.01 | < 0.025 | P > 0.5 |
| Sample size-corrected incidence data | < 0.001 | < 0.05 | P > 0.5 |

Figure 3.1



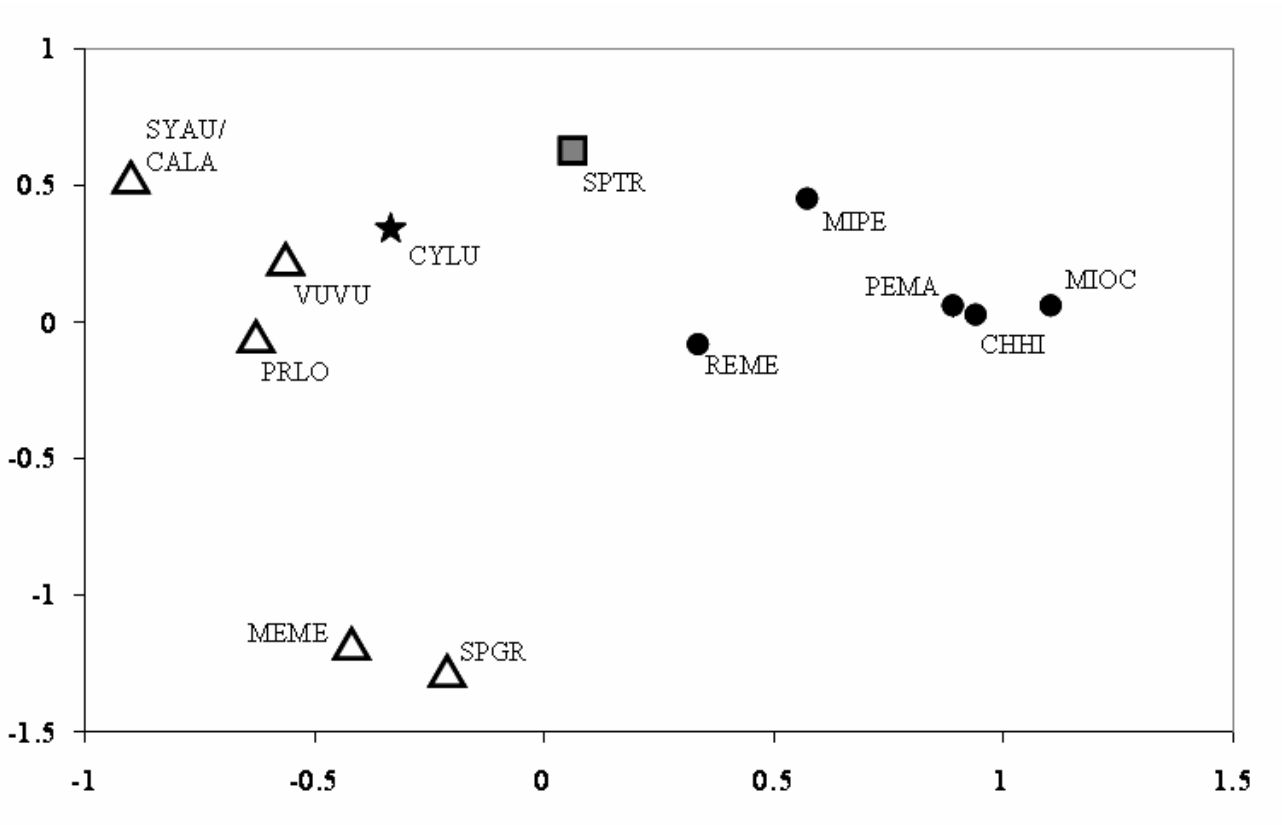

Map indicating locations of study sites in Boulder County, Colorado. Filled squares represent sites where only prairie dogs were sampled. Squares filled with white circles represent sites where prairie dogs and small rodents were sampled. Open diamonds represent sites where carnivores were sampled

Figure 3.2



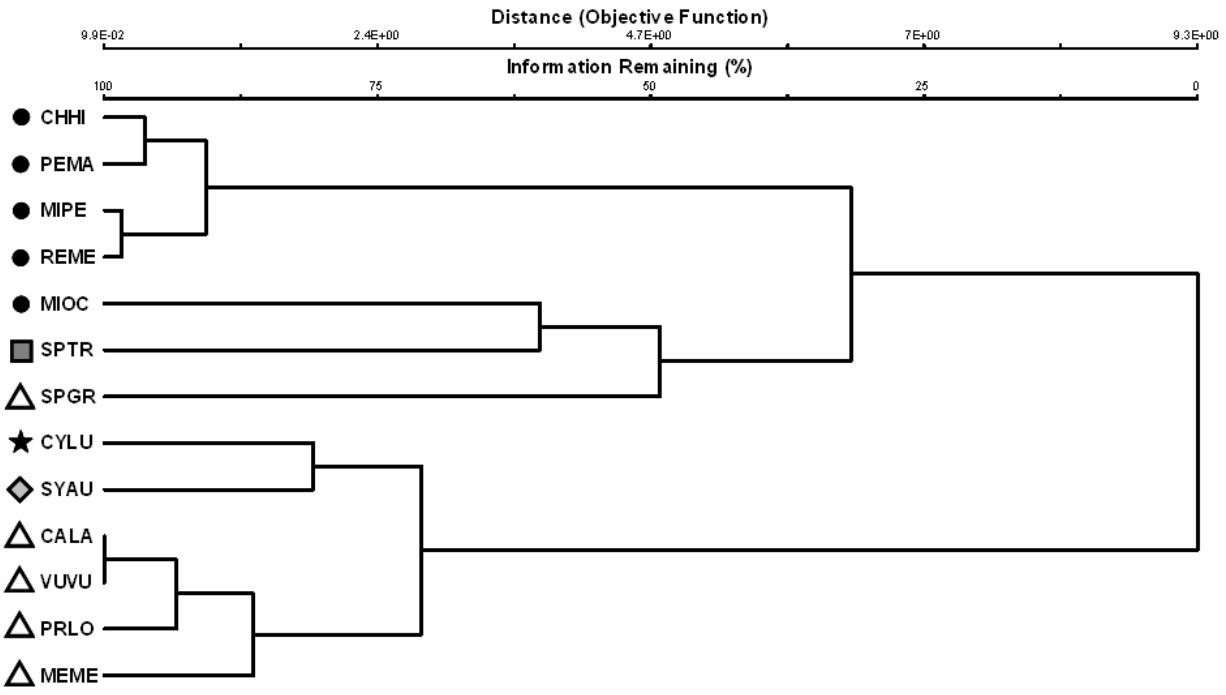

Non-metric multidimensional (NMS) ordination representing host species grouped by flea assemblage based on incidence (presence/absence) data. Small rodent species are indicated by black dots and species of carnivores are indicated by open triangles. The black-tailed prairie dog (CYLU) is indicated by a black star, the desert cottontail (SYAU) is represented by a gray diamond, and the thirteen-lined ground squirrel (SPTR) is represented by a gray square

Figure 3.3



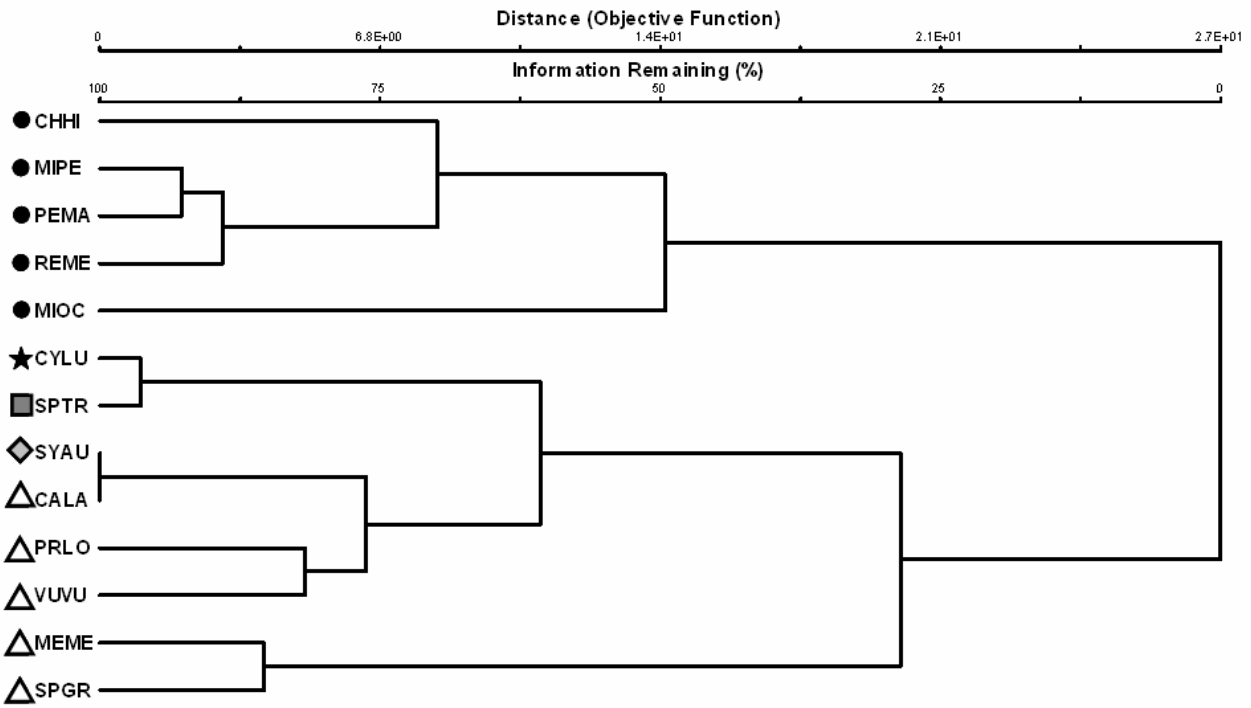

NMS ordination representing host species grouped by flea assemblage based on the sample size-corrected incidence dataset. Small rodent species are indicated by black dots and species of carnivores are indicated by open triangles. The black-tailed prairie dog (SPTR) is indicated by a black star, and the thirteen-lined ground squirrel is represented by a gray square. In this ordination, desert cottontail (SYAU) shares the same coordinates as the coyote (CALA)

Figure 3.4



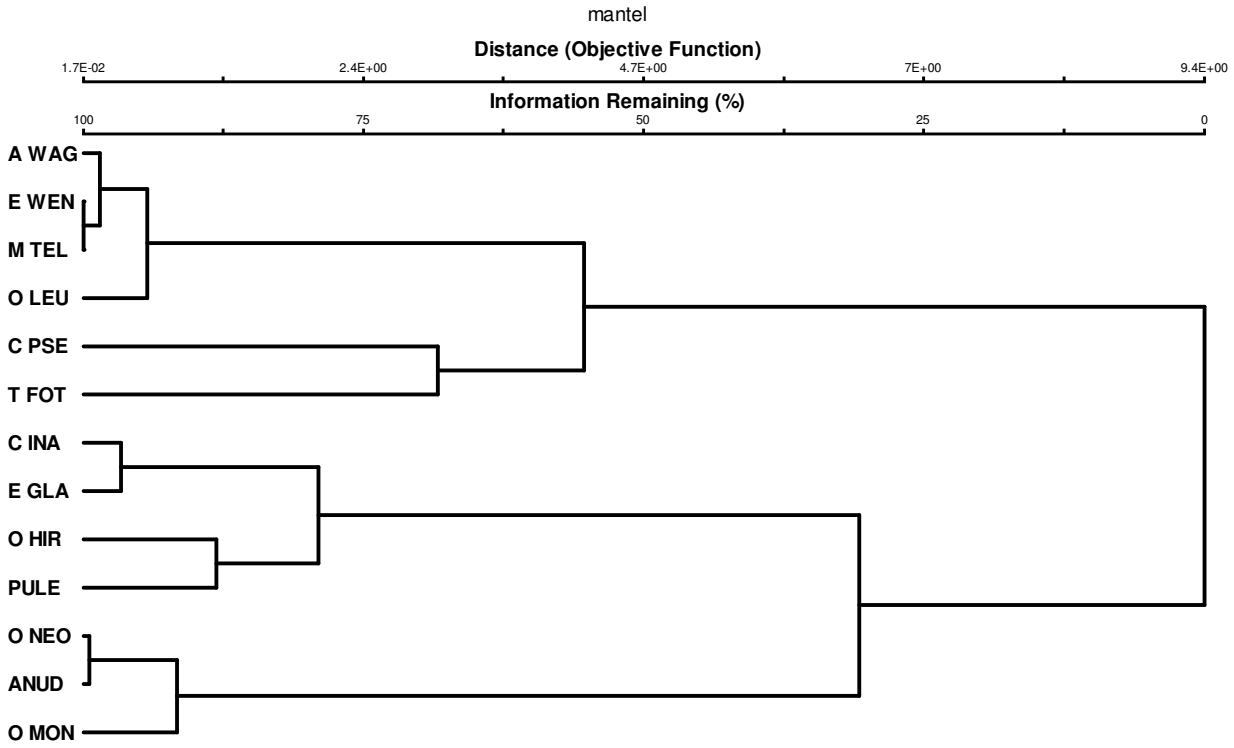

Host species clustered based on similarity of flea assemblage: natural log-transformed abundance dataset. Small rodent species are indicated by black dots and species of carnivores are indicated by open triangles. The black-tailed prairie dog is indicated by a black star, the desert cottontail is represented by a gray diamond, and the thirteen-lined ground squirrel is represented by a gray square.

Figure 3.5



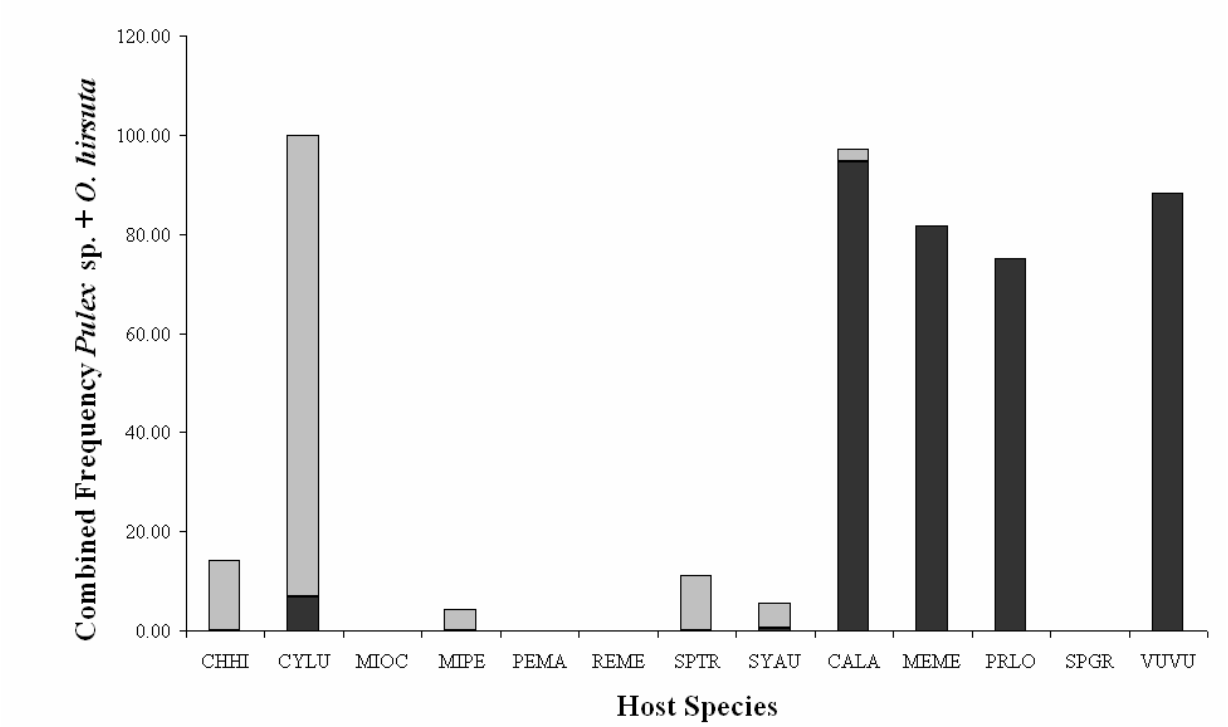

Host species clustered based on similarity of flea assemblage: sample-size corrected incidence dataset. Small rodent species are indicated by black dots and species of carnivores are indicated by open triangles. The black-tailed prairie dog (CYLU) is indicated by a black star, the desert cottontail (SYAU) is represented by a gray diamond, and the thirteen-lined ground squirrel (SPTR) is represented by a gray square.

Figure 3.6.




Flea species clustered based on similarity of host species utilized: natural log-transformed dataset.

Figure 3.7.




Proportion of total flea species assemblage on each host species represented by *Pulex* sp. (black portion of bar) and *Oropsylla hirsuta* (gray portion of each bar).

## Chapter 4

# POPULATION GENETIC STRUCTURE OF THE PRAIRIE DOG FLEA, *OROPSYLLA HIRSUTA*, SHOWS SIGNALS OF PLAGUE HISTORY


### Abstract

Plague is a flea-borne, bacterial disease that threatens black-tailed prairie dogs in western North America. The identities of flea species responsible for spreading plague are unknown, but the prairie dog flea, *Oropsylla hirsuta*, is assumed to play a role in intraspecific plague transmission. I examined the population genetic structure of *O. hirsuta* fleas collected from nine prairie dog colonies, five of which had experienced a plague-associated die-off in Boulder County, Colorado. I expected that fleas collected from colonies with a history of recent plague activity would have less genetic diversity and would be less distinctive genetically than fleas collected from colonies with no recent history of plague. I also expected that genetic differentiation would be dependent on distance, with fleas from recently plague-affected colonies most similar to their nearest neighbors. Three plague-negative colonies showed significant differentiation from all other colonies, as did two of the five recently plague-positive colonies. I found that all five recently plagued colonies showed signs of recent population expansion whereas two of the four plague-negative colonies showed patterns consistent with stable population sizes. I did not detect a signal of isolation by distance, suggesting that *O. hirsuta* may be able to disperse relatively quickly over long distances. These results suggest that *O. hirsuta* populations are affected by plague-induced prairie dog die-offs and that flea dispersal among prairie

dog colonies may be accomplished by multiple mechanisms.

## Introduction

Parasitic organisms are tremendously diverse in taxonomy, life history, and biology and can adversely affect their hosts in a variety of ways, such as by draining resources (e.g., Price 1980), reducing fecundity (e.g., Hall et al. 2007), and by serving as vectors for protozoan, bacterial, and viral pathogens (e.g., Stevenson et al. 2003). Parasitological research can be highly valuable in gaining insights into the ecological and evolutionary processes of host organisms, and parasite conservation measures have been advocated for this purpose (Whiteman and Parker 2005). Molecular tools have tremendous potential for providing insights into host specificity, population genetic structure, phylogeography, and gene flow, although such methods are infrequently applied to parasites (Criscione et al. 2005). However, a handful of researchers have made strong inferences of parasite phylogeography (e.g., Perkins 2001), parasite dispersal (e.g., McCoy et al. 2005), and parasite acquisition (e.g., Walker et al. 2007) using molecular methods. Parasite occurrence patterns and genetic structure may also be valuable for inferring host population history as parasites generally have shorter generation times and therefore evolve more rapidly than their hosts (Whiteman and Parker 2005). Given that many parasitic organisms are pathogenic, or serve as vectors for pathogenic microorganisms, the use of molecular tools might be especially valuable in tracking pathogen movement and making inferences and predictions about pathogen dynamics.

*Yersinia pestis* is a zoonotic pathogen whose transmission is primarily dependent on fleas (Gage and Kosoy 2005). The etiological agent for plague, *Y. pestis* is a globally-occurring pathogen that naturally infects over 200 mammalian

species, yet its ecology and transmission dynamics are poorly understood (Gage and Kosoy 2005, Salkeld and Stapp 2006, Webb et al. 2006)). Effects of *Y. pestis* infection are highly variable among host species, ranging from high degrees of resistance in some species to nearly complete mortality in others (Barnes et al. 1982). Plague is thought to be maintained in an enzootic cycle, consisting of small rodents and their fleas, that periodically shifts to epizootic, or amplifying, hosts causing massive and rapidly moving die-offs before returning to the enzootic cycle (Gage and Kosoy 2005). Mammalian host species responsible for maintaining *Y. pestis* are unidentified in most systems, as are the flea species responsible for inter- and intraspecific transmission (Krasnov et al. 2006, Eisen et al. 2006). Prairie dogs (genus *Cynomys*) are among the mammalian species most severely impacted by *Y. pestis* often suffering mortality in excess of 99% (Cully and Williams 2001). Although plague is thought to be primarily a flea-transmitted disease, specific mechanisms to account for plague transmission during epizootic events are unclear (Eisen et al. 2006, Webb et al. 2006).

In Boulder County, Colorado, epizootic plague events affecting the majority of black-tailed prairie dog (*Cynomys ludovicianus*) colonies occur roughly every five to ten years, although the likelihood of plague reaching a particular colony may be influenced by the landscape context in which the colony exists (Collinge et al. 2005b). Plague is thought to be dependent on abundance and prevalence of fleas that are competent vectors for effective transmission (Lorange et al 2005). Vector and host abundances may, in turn, be dependent on climatological processes and, in some systems, relatively cool summers and relatively high precipitation are effective

predictors of plague epizootics (Parmenter et al. 1999, Stapp et al. 2004), though such variables are not useful predictors of plague in Boulder County, Colorado (Collinge et al 2005a).

Black-tailed prairie dogs in Boulder County are primarily parasitized by two kinds of fleas: *Oropsylla hirsuta* and *O. tuberculata cynomuris* (Brinkerhoff et al. 2006). Flea parasitism on prairie dogs is seasonal with *O. hirsuta* reaching peak abundance in summer months and *O. t. cynomuris* reaching peak abundance in February and March (Wilder et al. in review). In general, fleas are highly host specific with anatomical adaptations suited to their primary host (Marshall 1981, Traub 1985) and *O. hirsuta* and *O. tuberculata* are rarely collected from mammalian species other than black-tailed prairie dogs (Brinkerhoff, unpublished data). Flea survival is highly dependent on temperature and relative humidity (Rust and Dryden 1997, Krasnov et al. 2001) but flea lifespan does not typically exceed 21 days, even under ideal conditions (Krasnov et al. 2002) In rare cases, flea survival in the absence of host induced mortality may exceed 300 days (Amin et al. 1993, Rust and Dryden 1997) but typical longevity is substantially shorter, particularly for individuals that have taken their first bloodmeal (Krasnov et al. 2002).

Dramatic reductions in prairie dog population size resulting from plague would be expected to negatively affect populations of highly host specific parasites, such as fleas, which are not prone to utilizing other host species (Brinkerhoff unpublished data). Therefore, the population genetic structure of prairie dog fleas may be reflective of that of their hosts, assuming flea populations are tightly coupled with prairie dog populations. Furthermore, flea migration and movement events inferred from molecular data could be used as a proxy for prairie dog migration and

movement. A study of seabirds and their ticks revealed insights into host population dynamics and migratory patterns (McCoy et al. 2005) and range expansion of guinea pigs was inferred from a study of the population genetic structure of guinea pig fleas in Peru (Dittmar-de la Cruz and Whiting 2003). The population genetic structure of prairie dog fleas may thus lend insight into plague transmission dynamics and mechanisms given the high host-specificity shown by prairie dog fleas. In order to elucidate patterns and processes of inter- and intraspecific plague transmission, I explored whether population genetic structure of *O. hirsuta*, 1) shows signals of recent plague events, 2) can be used to infer flea migration and dispersal patterns, and 3) can be used to make inferences regarding plague transmission and movement. Specifically, I predicted that colonies with no recent history of plague would show significant genetic differentiation from neighboring colonies whereas colonies that had recently experienced plague would not be differentiated from nearest-neighbor colonies. I also predicted that fleas sampled from recently-plagued colonies would show signals of recent population expansion, whereas colonies without recent plague history would support relatively stable flea populations.

## Methods

*Study sites and species*

I collected fleas from live-trapped black-tailed prairie dogs (*Cynomys ludovicianus*) at nine study colonies in Boulder County, Colorado (Figure 4.1). *C. ludovicianus* is a social and colonial burrowing ground squirrel native to west-central North America, east of the Rocky Mountains. Flea assemblages of *C. ludovicianus*

are dominated by *Oropsylla hirsuta* and *O. tuberculata cynomuris* (Brinkerhoff et al. 2006), which make up roughly 98% of all prairie dog fleas in this study system (Brinkerhoff, unpublished data). *O. hirsuta* is a warm-season flea which can be collected all year long but whose peak abundance comes in summer months. *O.t. cynomuris* is a cool season flea which is primarily collected during late winter and spring months with peak abundance in March (Wilder et al. in review). All study colonies (Figure 4.1) occurred between 1,550 and 1,900 m elevation and were generally similar in area and vegetation (Conlin 2005).

Study sites were selected such that equivalent numbers of historically plague-free and recently plagued colonies were sampled. Plague history was determined in consultation with local land and wildlife managers who have records of plague activity in Boulder County dating from the mid-1980s (Collinge et al. 2005a, Markeson 2005). Colonies included in the plague positive group are known to have been affected by a major plague epizootic in 1994 whereas plague negative colonies persisted through this epizootic. Another widespread epizootic affecting most colonies in the study system occurred in 1986 and, though plague history is unknown for some colonies, colonies 18A and 60 A (Figure 4.1) are known to have survived.

*Sample collection*

Prairie dog sampling using Tomahawk live-traps (Tomahawk Live Trap, Inc., Tomahawk, WI), arranged in square or rectangular grids, was conducted in June and July of 2005 and 2006. Each colony was sampled for four-day trapping periods preceded by three days of pre-baiting. Traps were separated by 25 m and trapping

grids consisted of 48, 49, or 50 traps arranged in a 6 x 8, 7 x 7, or 5 x 10 pattern depending on which arrangement best fit the shape of the target colony. Two study sites were trapped at a time and traps were set for three hours per day (0630 – 0930 h or 0900 – 1200 h) with daily alternation between early and late morning trapping. Between active trapping times, all traps were left open, but not set, to encourage visitation.

All trapped individuals were sedated with isoflurane to facilitate flea collection. All visible fleas were collected from each prairie dog using forceps and a fine-toothed comb. Fleas were placed in vials containing a 2% saline solution and a small amount of the surfactant Tween (polysorbate 80). The vials were stored at -80°C and the fleas were later identified to species by light microscopy using dichotomous keys presented in Hubbard (1968) and Furman and Catts (1982).

*PCR amplification and sequence analysis*

One *Oropsylla hisrsuta* from each captured prairie dog was haphazardly selected for genetic analysis. DNA from selected fleas was extracted using a DNEasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) following the included standard protocols. Extracted DNA Polymerase chain reaction (PCR) was used to amplify a portion of the cytochrome oxidase II gene using primers Fleu and Blys (Whiting 2002). I used Amplitaq Gold LD (Applied Biosystems, Inc., Foster City, CA) DNA polymerase and the following PCR conditions: 95ºC for 12 minutes and 37 cycles of 94ºC for 45 seconds, 42ºC for 45 seconds and 68ºC for 2 minutes, followed by a final elongation step of 7 minutes at 68ºC. PCR product purification was done with

ExoSap (USB Corp., Cleveland, OH) solution and sequencing was performed on an ABI 3730xl with BigDye chemistry 3.1. Sequence data were also collected from two individuals of the congeneric flea species *O. tuberculata cynomuris* for use as an outgroup in phylogenetic analysis.

Raw sequence data were edited with Sequencher software and were aligned with Clustal X and unique haplotypes and redundant sequences were identified using MacClade 4.08. I constructed a neighbor-joining phylogram, rooted with *O. t. cynomuris* sequence data and following the Kimura two-parameter model of measuring DNA sequence divergence using PAUP 4.0. I used Arlequin 2.0 (Schneider, Roessli, and Excoffier 2000) to calculate pairwise $F_{st}$ values between all pairs of sampling sites and to perform an exact test of population differentiation, done by comparing the observed distribution of haplotypes among populations to those generated from 10,000 Markov steps. I used a sign test to test the hypothesis that plague-negative colonies would show significant differentiation from neighboring colonies whereas plague positive colonies would not. Arlequin 2.0 was also used to test observed mismatch distributions for signals of recent population expansion and a sign test was used to test the hypothesis that plague negative colonies would show signals of recent population expansion and plague negative colonies would not. I tested the relationship between linearized pairwise $F_{st}$ distance using a Mantel test with statistical significance determined by 5,000 randomized Monte Carlo runs. A haplotype network diagram was constructed with TCS 3.1.

**Results**

I collected COII sequence data from a total of 201 *O. hirsuta* (Table 4.1) sampled from nine prairie dog colony sites in Boulder County, Colorado (Figure 4.1). Sequences collected from all but three individuals provided clear and unambiguous data consisting of a 480 base-pair section of the COII gene. I identified a total of 21 unique haplotypes, twelve of which were detected at least twice (Table 4.2). Haplotype H1 was the most common and was detected in 75 individuals dispersed among all nine sampling sites (Table 4.2). The second most common haplotype (H2) was detected 35 times and was localized entirely in the southern region of the study area except for one detection at site 30A (Figure 4.1, Table 4.2). Several other haplotypes (H3, H5, H6, H7, and H11) were only found in the southern half of the study area whereas one haplotype (H10) was only detected in the northern region. Two haplotypes only occurred at one study area each: H5 was only detected at site 20A and H11 was only detected at site 3A (Figure 4.1, Table 4.2). A neighbor-joining tree representing phylogenetic relationships among these haplotypes with respect to *O. t. cynomuris* is presented in Figure 2.

Pairwise linearized $F_{st}$ values for all pairs of sampling sites are presented in Table 4.3 and are plotted against distance in Figure 4.3. Non-differentiation tests show a strong signal of overall population genetic structure ($P < 0.0001$), though a non-differentiation hypothesis could not be rejected ($P > 0.05$) at four pairs of sampling sites: 2A and 19A, 2A and 30A, 18A and 19A, and 19A and 30A (Figure 4.4). However, a sign test indicated that plague history was not a significant predictor of genetic differentiation between any given population of fleas and neighboring populations ($P > 0.1$). I detected no statistical evidence of isolation-by-distance in

these data (Mantel test $P > 0.1$, $r = .204$; Figure 4.3). I found that six of the nine sites I sampled showed mismatch distributions consistent with recent population expansion (Table 4.1). Two sites failed goodness-of-fit (GOF) tests with the population expansion model at $P < 0.05$ and one site failed at $P<0.1$ (Table 4.1). Sign tests indicated that the probability of finding flea population histories consistent with predictions at seven of nine study colonies is marginally significant ($P = 0.09$). A haplotype network is presented in Figure 4.5 where lines joining haplotypes represent a change of one nucleotide. The relative size of each node is proportional to the number of individuals carrying each haplotype and the relative size of each shaded area is proportional to the number of individuals represented from each sampling site.

**Discussion**

The results of this study indicate clear population genetic structure in *O. hirsuta* collected from active prairie dog colonies. The observed pattern of non-differentiation indicates that colonies in the northern part of the study system are not distinguishable in terms of haplotype distribution whereas the colonies in the southern part of the study system are genetically distinct from each other. The two most commonly observed haplotypes in this study, both of which are widely distributed throughout the study area, are several nucleotide changes from the common ancestor to these lineages (Figure 4.5), suggesting a recent overall population expansion. I detected evidence for recent population expansion in six of nine study colonies with two colonies showing strong, and one colony showing marginally significant evidence of stable populations. The population genetic structure determined from

prairie dog fleas in this study system is consistent with the history of recently observed epizootic plague events, suggesting that prairie dog flea populations may be affected by prairie dog die-offs. These data also lend insights into patterns of flea repopulation following plague events and suggest that multiple mechanisms might account for flea dispersal.

The global test of non-differentiation in prairie dog fleas sampled among the nine colonies tested in this study indicates significant overall population differentiation. Given that fleas are incapable of dispersing in the absence of a mammalian host, it would be expected that local micoevolutionary processes would eventually result in genetic differentiation among discrete sampling sites. However, pairwise non-differentiation tests indicate that the colonies in the northern part of this study system are not distinguishable in terms of relative haplotype frequency (Figure 4.4). This result likely stems from the fact that haplotype H1 occurs at high frequency at each of the northern sampling sites (2A, 19A, 30A and 18A; Table 4.1, Figure 4.6); although haplotype H1 was detected at every sampling site, its average relative frequency at northern sites was 0.66 compared to 0.18 at southern sites (60A, 106A, 3A, 20A, and 10A). Tests of non-differentiation for all pairs of southern colonies failed, indicating that each colony in this set supports fleas with distinct haplotype frequencies. Patterns of genetic differentiation could arise for a variety of reasons including, but not limited to, founder effects, population bottlenecks, reduced gene flow stemming from physical isolation, differential selective pressure, or genetic drift. The limited dispersal abilities of fleas are likely to affect gene flow and founder effects or bottlenecks resulting from prairie dog die-offs are ecologically relevant

factors that could have led to the general patterns of genetic differentiation observed in fleas in this system.

Mismatch distributions and relative haplotype frequencies at each site are noteworthy and suggestive of different population histories at each site. The mismatch distributions observed at six of the nine colonies (2A, 3A, 10A, 19A, 20A and 30A) sampled in this study are consistent with recent population expansion (Table 4.1), though the probability of observing mismatch distributions consistent with plague history at seven of nine sites is not significant at alpha = 0.05. Two colonies (18A and 106A) showed signs of relatively stable flea populations whereas one colony (60A) showed a marginally significant departure from the recent expansion model (Table 4.1). These observed patterns are generally consistent with plague history at each study colony (Figure 4.1) and suggest that colonies that escaped prairie dog die-offs maintained viable flea populations as well. On the other hand, colonies that were affected by plague appear to have also experienced reduced flea populations and are currently undergoing population expansion stemming from a relatively small initial population.

In this study system, periodic plague epizootics result in local prairie dog extinctions. All plague-positive colonies in this study (2A, 3A, 10A, 19A, and 20A) are known to have experienced prairie dog die offs during the same year (1994) and were possibly affected by another widespread epizootic event in 1986. Elimination of 99% or more of available primary hosts would be expected to substantially reduce flea population sizes, though this reduction could vary among sites depending on local impacts of plague. However, the patterns of population expansion are generally

consistent with plague history: all plague positive colonies show genetic signals of recent population expansion whereas three of the four plague negative colonies show haplotype mismatch distributions consistent with stable population size. Among the plague negative colonies, 30A and 106A are only known to have survived the 1994 plague epizootic whereas colonies 18A and 60A are known to have remained active though the major epizootic events of 1994 and 1986 as well as through smaller-scale epizootics in 1991 and 1999. Thus the detection of strong departures from the recent expansion model might be expected to be strongest in colonies 18A and 106A. Although colonies 30A and 60A are known to have survived the 1994 plague epizootic, it is possible that prairie dog and flea populations at these sites were reduced due to plague and therefore flea population genetic structure shows evidence of recent expansion.

The population genetic structure of each sample colony is generally consistent with plague history suggesting that flea populations are affected by plague epizootics in prairie dogs. However, inferences into repopulation events and gene flow are also possible with this dataset. Prairie dog and flea populations that persist during plague events are likely to serve as sources for repopulation of extirpated sites. Some such source colonies are located in the central part of this study system (colonies 18A, 60A and 106A; Figure 4.1). For example, the high relative abundance of haplotype H1 at site 18A is repeated at nearby sites showing signs of recent population expansion (19A, 30A and 2A). This pattern could have been caused by founder individuals carrying haplotype H1 that dispersed north to newly vacant sites. A similar mechanism could account for the high relative abundance of haplotype H2 at sites

60A, 106A, 10A and 3A (Figure 4.6); this haplotype is absent or present in very low relative abundance at northern sites and its distribution could be indicative of southward gene flow from colony 18A (Figure 4.6).

Haplotypes H5 and H11 were each detected at only one colony; 20A and 3A, respectively (Table 4.2). Haplotypes H3, H6, and H7 were also only detected at southern colonies (3A, 10A, 20A and 60A). Three of these haplotypes (H5, H7 and H11) were not detected at the plague-negative site 60A. Sample size is certain to influence which haplotypes are detected at a particular site and it is likely that increased sampling effort would lead to detection of addition haplotypes at each site. Therefore, it cannot be concluded that fleas carrying haplotypes H5, H7 and H11 are not present at site 60A. However, it is also possible that these haplotypes were introduced by individual fleas originating from outside the sampling area. Although the data presented here suggest a radiation of flea emigration from the plague negative sites in the central part of the study area, certainly fleas from other sources could repopulate a prairie dog colony following a plague epizootic. The presence of such unidentified sources could account for the genetic differentiation detected in the southern portion of the study system. The detection of uniquely-occurring haplotypes also points to the probable existence of unsampled pools of fleas in this landscape and suggests that haplotype richness in this system is higher than was observed (Chao 1984).

The data presented in this study indicate that plague history among prairie dog colonies can be inferred from the haplotype distributions of prairie dog flea populations, as can patterns of repopulation by prairie dog fleas following a plague

event. However, the mechanism(s) by which fleas disperse are unclear. Given the high host-specificity shown by prairie dog fleas (Brinkerhoff unpublished data), it would be expected that *O. hirsuta* is reliant upon prairie dogs for dispersal to new colonies. However, prairie dog dispersal is spatially and temporally limited, typically occurring only in late spring and covering a straight-line distance average of 2.4 kilometers (Garrett and Franklin 1981). I detected no isolation-by-distance effect in the genetic structure of prairie dog fleas (Figure 4.3), suggesting that flea dispersal is not highly distance-limited. Thus, long-distance dispersal mechanisms are likely present in this study system. Mammalian prairie dog predators are known to acquire prairie dog fleas (McGee et al. 2006, Salkeld et al. 2007), often in the course of predation or foraging activities. Movement distances of predators, including coyotes (*Canis latrans*) and foxes (genus *Vulpes*), are substantially greater than those of prairie dogs (Rosatte 2002) and may increase during epizootic events (i.e. Greenwood et al. 1997). In this study system, *O. hirsuta* makes up roughly 2.5% of flea species collected from coyotes sampled near prairie dog colonies (Brinkerhoff, unpublished data), which suggests that coyotes or other species of carnivores could be involved in long-distance flea dispersal.

Plague events are likely to impact flea populations, though some *O. hirsuta* may be able to persist in the absence of prairie dog hosts. *O. hirsuta* have been collected from prairie dog burrows up to one year after a plague event (Kartman et al. 1962) though mechanisms by which they survive such in the absence of a primary host are unknown. *O. hirsuta* is highly host-specific and is rarely collected from hosts other than prairie dogs (Brinkerhoff, unpublished data). Although there is

evidence for host-switching by *O. hirsuta* following prairie dog die-offs (McGee et al. 2006, Salkeld and Stapp unpublished data), these events are quite rare and suggest that *O. hirsuta* does not commonly parasitize alternate hosts when prairie dogs are unavailable. After first feeding, fleas die quickly from starvation, typically in a matter of days or weeks, depending on temperature and relative humidity (Dryden 1989, Krasnov et al. 2001). However, records exist of fleas persisting for over 365 days without feeding under certain conditions (Dryden 1989) and plague-positive fleas have been recovered over a year after plague-induced local extinction of prairie dogs (Kartman et al. 1962), though such longevity is not common (Krasnov et al. 2001). Prairie dogs may begin to repopulate colonies as soon as one year following a plague epizootic (pers. obs.) making it possible for flea populations to remain continuous through a plague epizootic that locally extirpates prairie dogs, however the biology of fleas makes it unlikely that substantial numbers of fleas would persist in the prolonged absence of primary host individuals.

The population genetic structure of the primary prairie dog flea, *O. hirsuta*, may be influenced by local plague history and lends insights into flea dispersal and migration patterns. However, it should be noted that other mechanisms could lead to the patterns of haplotype distribution observed in this study. As few as three infected fleas may be sufficient to initiate a plague epizootic in prairie dogs (C. Webb, unpublished data) and it is possible that the population genetic patterns observed in *O. hirsuta* could result from *O. hirsuta*-mediated plague dispersal and transmission. Specifically, the persistence of infected founder individuals through an epizootic that kills off all resident fleas and prairie dogs would be indistinguishable from

repopulation following a plague-induced die-off in many ways. It seems unlikely, however, that individual plague-infected fleas would survive an epizootic to found an expanding flea population while all other flea lineages present at a colony would become locally extinct. More plausible is the scenario whereby a portion of the pre-epizootic genetic diversity would be preserved through a population bottleneck while colonizing individuals would import genotypes representative of colonies that were not affected by the plague epizootic.

Although the relationship between prairie dog die-offs and flea population genetic patterns is fairly straightforward, mechanisms to account for flea dispersal and migration are less clear. Landscape features have been shown to influence plague occurrence in prairie dogs in this study system; high cover of roads, reservoirs, and streams surrounding prairie dog colonies is associated with lower probability of plague occurrence (Collinge et al. 2005b). Such features might serve as movement barriers for terrestrial mammals carrying *Y. pestis* infection or *Y. pestis*-infected fleas, though this hypothesis remains untested. The data presented in this study do not seem to indicate that prairie dog fleas are influenced by landscape boundaries. For example, prairie dog colonies 18A and 19A are separated by one of two major highways in this study system, yet do not show signs of population differentiation (Figure 4.4). Furthermore, I did not detect a signal of isolation by distance in prairie dog fleas, suggesting that dispersal in fleas is not strongly distance-limited. The data presented here instead suggest that *O. hirsuta* population genetic structure is more strongly influenced by recent plague history than by landscape features.

In terms of plague transmission, these data suggest that, because prairie dog flea dispersal is not apparently affected by the factors that influence plague occurrence, a mechanism other than *O. hirsuta*-mediated plague transmission is likely to exist. The results of this study suggest that population genetic structure of the primary prairie dog flea and purported plague vector, *O. hirsuta,* can provide information regarding past epizootic events but contribute less information in terms of identifying mechanisms of plague transmission. More specifically, these data suggest that *O. hirsuta* populations are affected by epizootic plague events, but *O. hirsuta* may not be responsible for plague dispersal. Further studies of prairie dog flea genetic structure, as well as explorations of alternate mechanisms for long-distance plague dispersal, should do much to elucidate patterns and processes of sylvatic plague transmission.

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Table 4.1: Numbers of *O. hirsuta* tested and number of unique COII haplotypes detected

at each of nine study sites in Boulder County, Colorado. The rightmost column indicates results from goodness-of-fit testing of observed mismatch distributions compared to a recent population expansion model. Underlined site numbers indicate prairie dog colonies that are known to have escaped a widespread plague epizootic in 1994.

| Sampling site | Number *O. hirsuta* analyzed | Number of haplotypes observed | Number of singletons detected | Mismatch distribution SEM GOF test p-value |
|---|---|---|---|---|
| 2A | 14 | 4 | 1 | 0.3 |
| 3A | 25 | 11 | 1 | 0.69 |
| 10A | 25 | 9 | 1 | 0.29 |
| 18A | 21 | 5 | 1 | 0.01 |
| 19A | 30 | 8 | 4 | 0.15 |
| 20A | 22 | 5 | 0 | 0.26 |
| 30A | 23 | 5 | 0 | 0.50 |
| 60A | 15 | 5 | 0 | 0.06 |
| 106A | 23 | 4 | 1 | 0.03 |

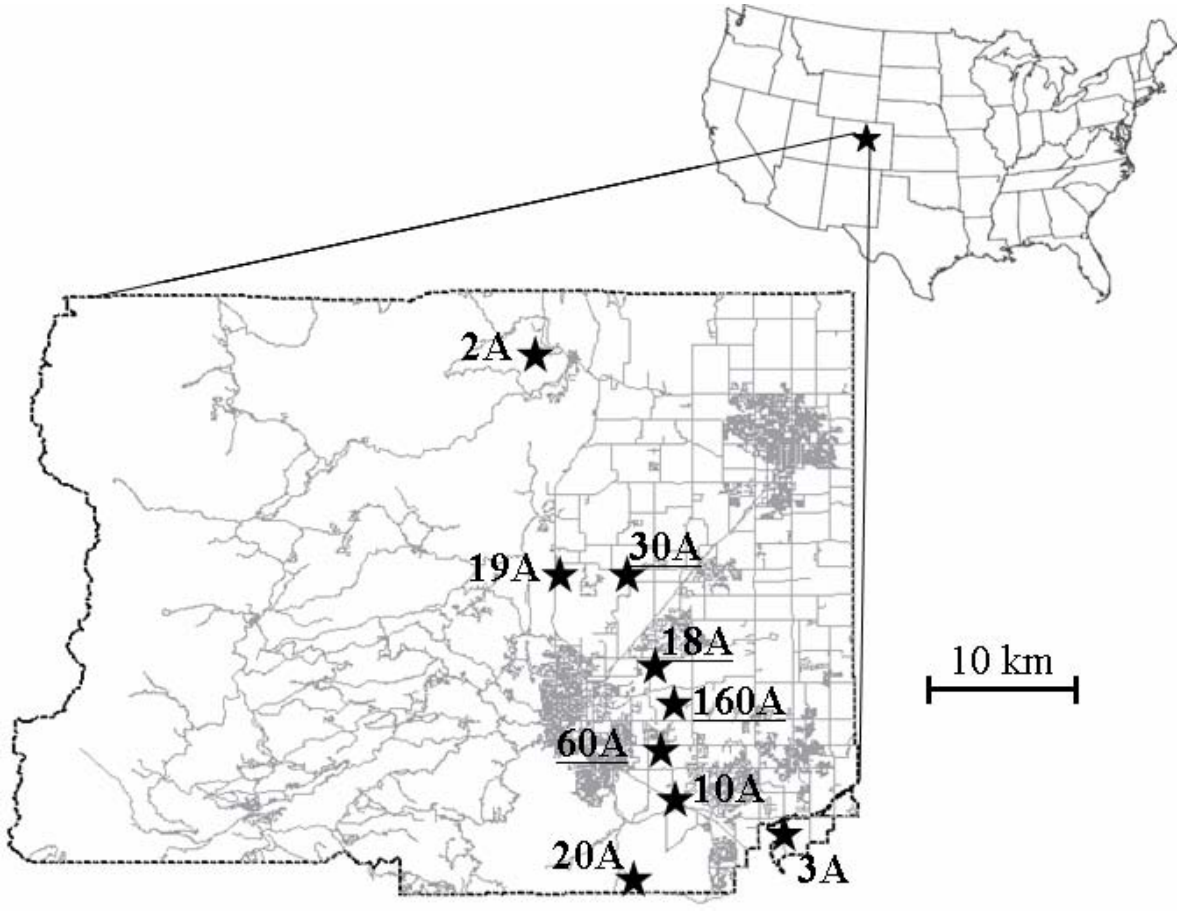
Table 4.2: *O. hirsuta* haplotype frequencies detected at nine prairie dog colonies in Boulder County, Colorado. Singleton haplotypes are not included in this table. Underlined site numbers indicate prairie dog colonies that are known to have escaped a widespread plague epizootic in 1994

| | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2A | 10 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| 3A | 8 | 5 | 0 | 3 | 0 | 2 | 3 | 0 | 0 | 0 | 4 | 1 |
| 10A | 3 | 6 | 2 | 1 | 0 | 8 | 0 | 0 | 1 | 0 | 0 | 1 |
| 18A | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 5 |
| 19A | 18 | 0 | 0 | 1 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 |
| 20A | 4 | 1 | 0 | 2 | 4 | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| 30A | 17 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 2 |
| 60A | 1 | 6 | 5 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 106A | 5 | 16 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Table 4.3: Linearized pairwise $F_{st}$ values of *O. hirsuta* collected at nine prairie dog colonies in Boulder County, Colorado. Underlined site numbers indicate prairie dog colonies that are known to have escaped a widespread plague epizootic in 1994. Values in bold indicate colony pairs which were not statistically differentiated by exact test at alpha 0.05.

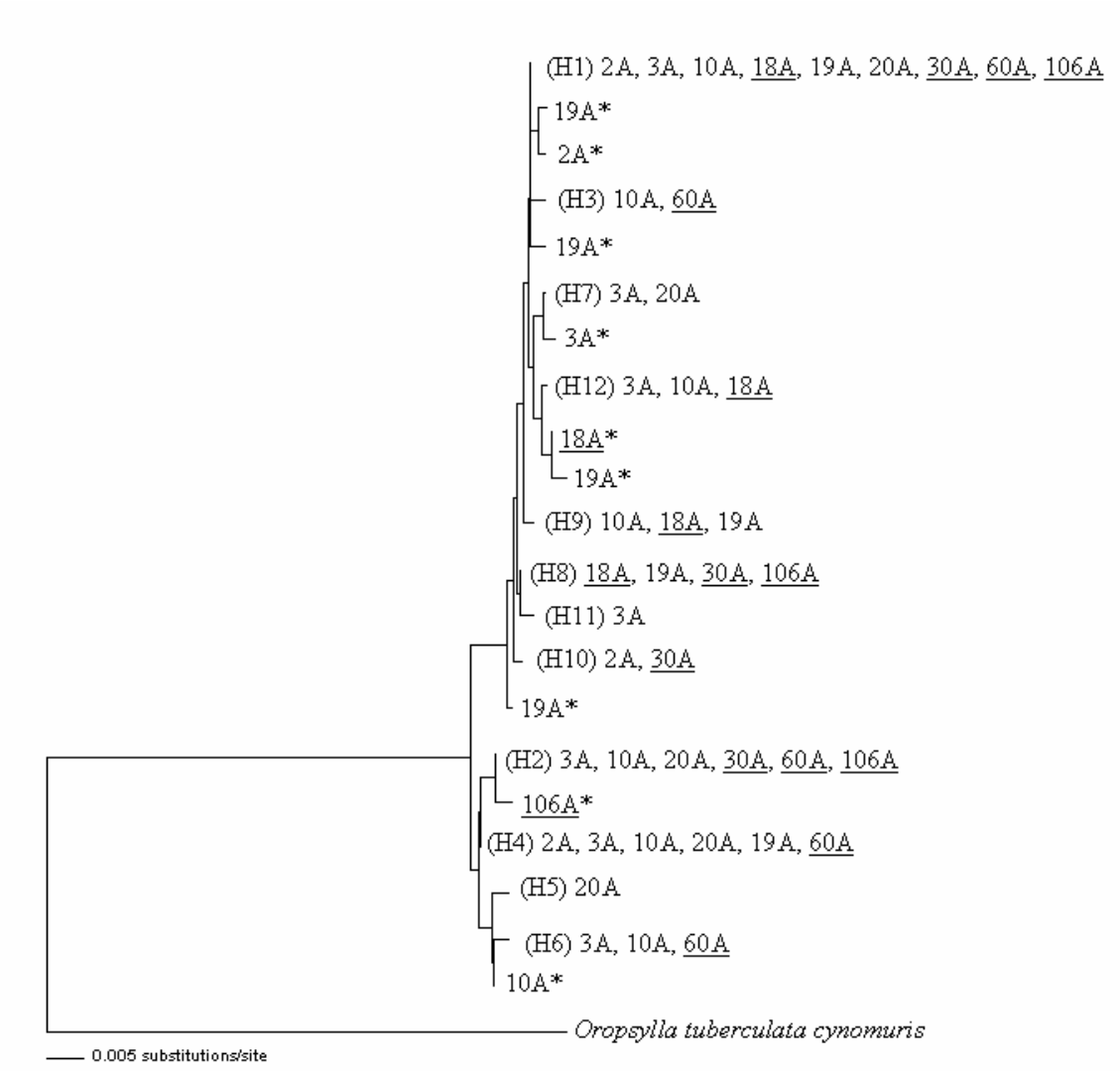
| | 2A | 10A | 18A | 19A | 20A | 30A | 60A | 106A | 3A |
|---|---|---|---|---|---|---|---|---|---|
| 2A | - | | | | | | | | |
| 10A | 0.332 | - | | | | | | | |
| 18A | 0.103 | 0.171 | - | | | | | | |
| 19A | 0.**022** | 0.278 | 0.**041** | - | | | | | |
| 20A | 0.417 | 0.170 | 0.265 | 0.351 | - | | | | |
| 30A | **-0.017** | 0.380 | 0.**079** | 0.008 | 0.492 | - | | | |
| 60A | 0.507 | 0.076 | 0.291 | 0.417 | 0.316 | 0.585 | - | | |
| 106A | 0.725 | 0.214 | 0.460 | 0.555 | 0.562 | 0.712 | 0.155 | - | |
| 3A | 0.133 | 0.076 | 0.071 | 0.112 | 0.123 | 0.158 | 0.112 | 0.190 | - |

Figure 4.1



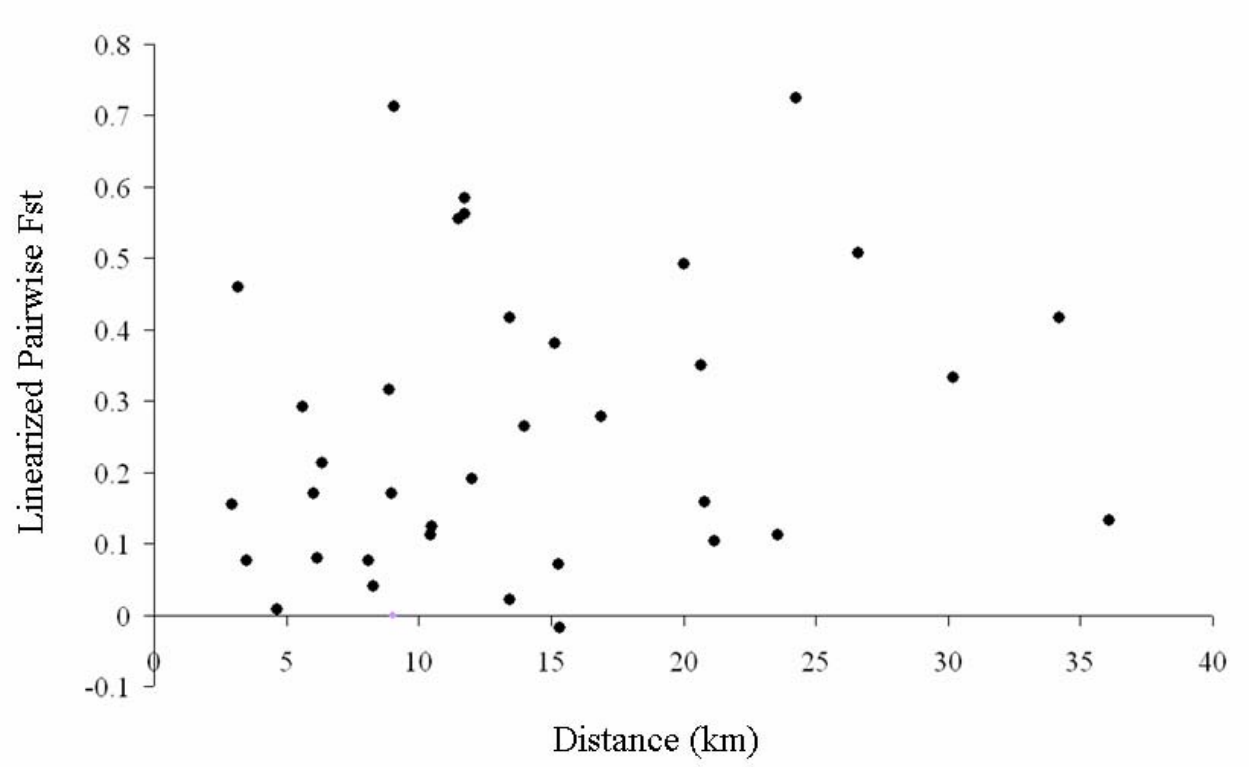

Locations of sampling sites from which *Oropsylla hirsuta* samples were collected. Sites for which the number is underlined escaped a widespread plague epizootic in 1994 and are referred to in the text as 'plague negative'. All other sites are referred to as 'plague positive.'

Figure 4.2



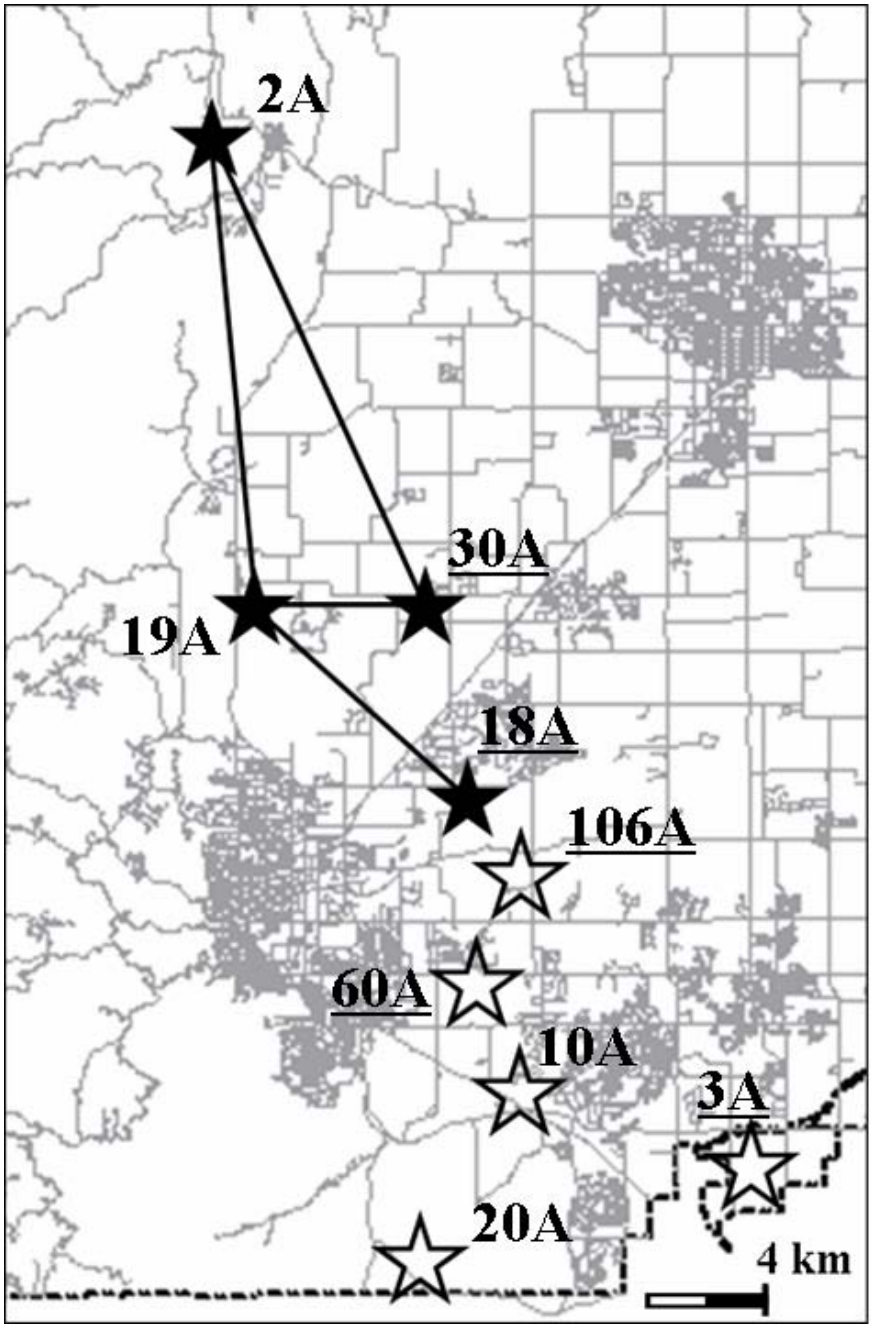

Neighbor-joining tree representing relationships among the 23 haplotypes detected in this study. Haplotype names are indicated in parentheses followed by the sites at which each haplotype was detected. Asterisks indicate haplotypes that were detected only once.

Figure 4.3



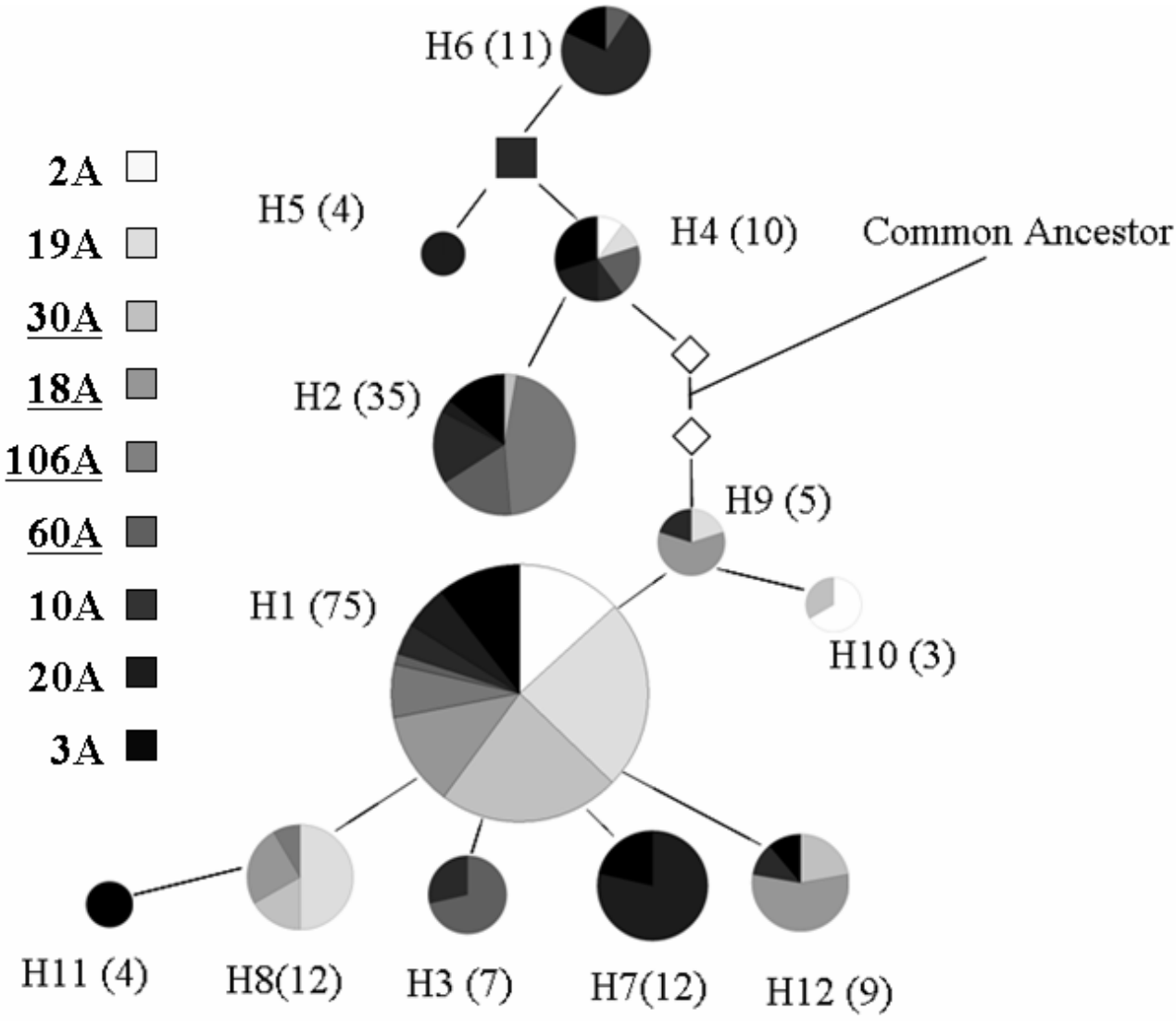

Mantel test of the relationship between linearized pairwise $F_{st}$ and distance: r = 0.2, P > 0.1

Figure 4.4



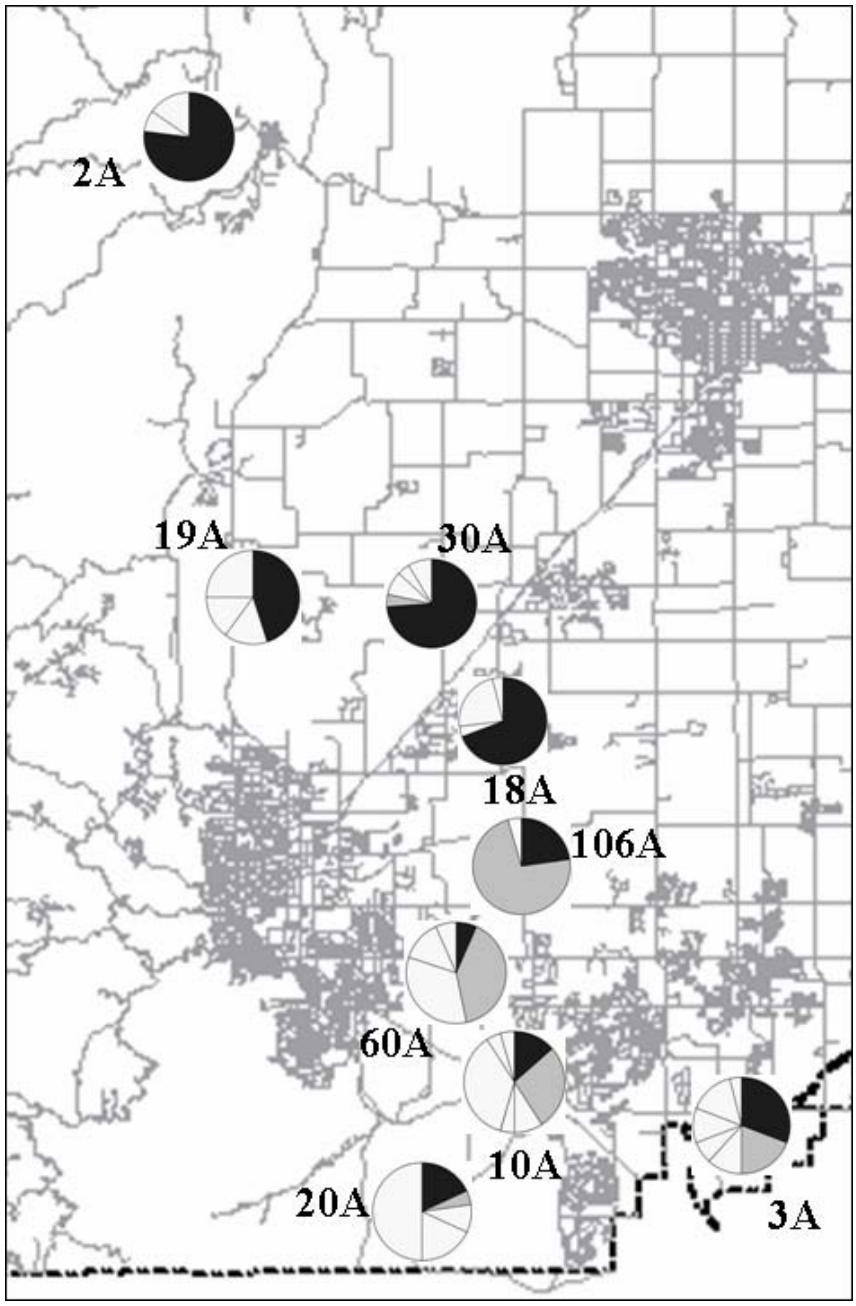

Filled stars connected by lines were not statistically different by pairwise $F_{st}$ at alpha 0.05. Open stars indicate sites that were statistically different from all other sites by pairwise $F_{st}$

Figure 4.5




Haplotype network diagram where the relative size of each node is proportional to the number of individuals in which each haplotype was detected. The square node indicates a haplotype that was only detected once and the diamonds indicate haplotypes that were not sampled but were inferred to exist. The location of the common ancestor was determined from the phylogenetic tree in Figure 4.2

Figure 4.6




Relative abundance of the two most common haplotypes detected (H1 and H2) in this study at each of the nine study sites. Haplotype H1 is indicated by the black sections of each pie chart and haplotype H2 is indicated by the gray sections

## Chapter 5

# OCCURRENCE PATTERNS OF TWO *OROPSYLLA* (CERATOPHYLLIDAE: SIPHONAPTERA) SPECIES ON BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) HOSTS


### Abstract

Behavioral, genetic, and immune variation within a host population may lead to aggregation of parasites whereby a small proportion of hosts harbor a majority of parasites. In situations where two or more parasite species infect the same host population there is the potential for interaction among parasites that could potentially influence patterns of aggregation through either competition or facilitation. I studied the occurrence and abundance patterns of two congeneric flea species on black tailed prairie dog (*Cynomys ludovicianus*) hosts to test for interactions among parasite species. I live- trapped prairie dogs on ten sites in Boulder County, Colorado and collected their fleas. I found a non-random, positive association between the two flea species, *Oropsylla hirsuta* and *O. tuberculata cynomuris*; hosts with high loads of one flea species had high loads of the second species. This result suggests that there is no interspecific competition among fleas on prairie dog hosts. Host weight had a weak negative relationship to flea load and host sex did not influence flea load, though there were slight differences in flea prevalence and abundance between male and female *C. ludovicianus*. Although genetic and behavioral variation among hosts may predispose certain individuals to infection, these results indicate apparent facilitation

among flea species that may result from immune suppression or other flea-mediated factors.

## Introduction

Parasites may negatively affect their hosts by acting as resource drains (Price 1980), interfering with host behavior (Cox et al. 1999), or directly inducing host mortality (Fischer and Kelso 1988, Martin et al. 2001). Ectoparasites also serve as vectors for bacterial and viral pathogens. In general, the number of parasites per host is directly related to the severity of the consequences of parasite infection (Marshall 1981) either through the cumulative negative effects of individual parasites or by increasing the probability of pathogen transmission. Hosts, in turn, have developed a variety of behavioral (Grutter 2001), physiological (Raouf et al. 2006) and immunological (Wilson et al. 2002a) responses in order to deal with parasite infection.

Fleas are common ectoparasites of mammals in a variety of habitats throughout the world. Most flea species are highly specific to a host species or host group and have anatomical adaptations that correspond to the ecology and behavior of their host(s) (Traub 1985). Adult fleas feed on the blood of the host individual and thus potentially reduce the host's fitness (Price 1980). Closely related species of fleas may share anatomical adaptations that predispose them to a particular host or host group (Traub 1985) but the presence of congeners may also have a negative affect on overall abundance of a flea species (Marshall 1981). Apparent competitive exclusion has been documented in fleas (Barnes 1965) and bat flies (Wenzel and Tipton 1966, Marshall 1981). The presence of one group of ectoparasites may preclude the presence of another group (Hopkins 1949) and crowding may reduce survival of

immature fleas (Marshall 1981). Furthermore, flea larvae may compete for resources and lead to the eventual exclusion of some species (Krasnov et al. 2005a).

Within species, fleas and other ectoparasites commonly exhibit an aggregated distribution among hosts; most host individuals in a population harbor a small number of parasites while a few hosts are heavily infected (Hudson and Dobson 1995). The degree to which parasites are aggregated depends highly on the transmission rate of parasites between hosts and the parasite's life cycle. Variation in parasite aggregation can result from differences in host behavior or body condition, parasite behavior, or environmental factors that affect parasite development. Host sex (Bundy 1988), age (Hudson and Dobson 1995), and body condition (Hamilton and Zuk 1982) have all been shown to be associated with differences in intensity of parasite infection. In some cases, heterogeneity in parasite infestation can be explained by behavioral differences between sexes or ages of host individuals (Hudson and Dobson 1995). In other cases, host physiology or immune response to parasites leads to variation in parasite prevalence and abundance (Khokhlova et al. 2004a, b).

Fleas use olfactory (Krasnov et al. 2002a), visual (Cox et al. 1999), and thermal (Benton and Lee 1965, Rust and Dryden 1997) cues to locate hosts and individual variation among hosts in pelage color or body chemistry could influence probability of infestation. Fleas tend to respond very specifically to the cues of a particular host or host group (Krasnov et al 2002a) though some species of fleas are much more catholic in their host associations (Traub 1985). The composition of flea or other parasite species present on a particular host is dependent on a variety of factors including, but not limited to, host size (Marshall 1981), host habitat (Krasnov

et al. 1997, Oscar-Jimenez et al. 2001), and host distribution (Perez-Ponce de Leon and Choudhury 2005, Krasnov et al. 2005b, c). In some cases, however, host specificity of fleas may also vary geographically (Krasnov et al. 2004, 2005b).

Prairie dogs (genus *Cynomys*) are social, burrowing ground squirrels occurring in western North America that are primarily parasitized by fleas of the genus *Oropsylla* (Jellison 1939, Lewis 2002). Some flea species are known to be seasonal, occurring in either hot and dry or cool and wet times of year. During summer months, the primary prairie dog flea in north-central Colorado is *Oropsylla hirsuta*, while in spring months, prairie dogs are parasitized by both *Oropsylla hirsuta* and *O. tuberculata cynomuris* (Collinge et al., unpublished data; Markeson et al., unpublished data). I tested observed occurrence patterns of these two closely related flea species on black-tailed prairie dog (*Cynomys ludovicianus*) hosts to determine patterns in host-flea associations. First, I examined measures of aggregation for all fleas and for each flea species independently. Second, I looked for differences in patterns of infestation among different host sizes and between sexes of *C. ludovicianus*. Finally, I tested the patterns of co-occurrence of the two flea species to determine apparent competitive or other interspecific interactions.

## Methods and Study Sites

### *Host species and study sites*

With the help of several field assistants, I collected fleas from live *Cynomys ludovicianus* on ten sites in Boulder County, Colorado, in February and March 2004 (Figure 5.1). *C. ludovicianus* is a highly social, burrowing squirrel historically

ranging across much of the western North American Great Plains (Hoogland 1995). *C. ludovicianus* is diurnal and active year-round, unlike other *Cynomys* species (Harlow and Braun 1995). Mating occurs in late winter or early spring with exact timing dependent on primarily on latitude; young are born about 34 days later (Hoogland 1995). *C. ludovicianus* life span may be up to 5-8 years (Hoogland 1995). *C. ludovicianus* prefers short-grass prairie and occurs in dry, open habitats to roughly 6400 feet elevation (Fitzgerald et al. 1994). All study colonies occur at roughly 5300 feet elevation and are similar in overall vegetative structure (Conlin et al. *unpublished data*). Study colony size varies from roughly 4 to over 20 hectares and colony landscape context ranges from mixed native/non-native grassland to suburban development.

Female fleas tend to begin laying eggs following their first feeding and can continue to oviposit for the duration of their lives (Rust and Dryden 1997). Flea eggs are often laid upon the host and then proceed to fall off, typically where the host rests or sleeps (Rust and Dryden 1997). Egg hatching and larvae and adult survival are highly dependent on temperature and vary by flea species (Krasnov et al. 2001). Adult survival also depends highly on host behavior; grooming by hosts can cause as much as 50% adult flea mortality in one week (Wade and Georgi 1988).

*Prairie dog sampling and flea collection*

I used Tomahawk live-traps (Tomahawk Live Trap, Inc., Tomahawk, WI) baited with a mixture of corn, oats, barley and molasses to capture prairie dogs. Traps were set in grids with 25m spacing between trap locations. Most sites were

trapped with 49 traps in square grids containing seven rows. However, some colonies were to narrow for this configuration and were trapped using rectangular grids containing 48 (8 x 6) or 50 traps (5 x 10). All traps were pre-baited for two days prior to each four-day trapping session.

During trapping sessions, two sites were trapped at a time and traps were set for three hours per day. Traps on each site were set at either 0630 or 0900 and were wired open when they were not set to encourage visitation. I alternated between early (0630) and late (0900) trap setting times for the four days of trapping per site. Any species captured other than *C. ludovicianus* were released unprocessed.

In order to collect fleas from the hosts, I sedated all captured *C. ludovicianus* using isoflurane, a volatile anesthetic. Using forceps, I removed all fleas from each host and stored them in a 2% saline solution. In addition to collecting fleas, I recorded the sex, length (cm), and weight (g) of each host and shaved a small patch of fur from the back of the neck as a visual indication that the animal had been captured. Fleas were stored at -80º C until they could be identified.

*Statistical analyses*

I calculated flea load as the mean number of parasites per infested host; I calculated mean flea abundance as the mean number of parasites across all hosts. I normalized flea abundance data by natural log transformation (Shapiro-Wilk W-test, $P > 0.1$). Total normalized flea abundance across sites was compared by one-way ANOVA. I used analysis of covariance (ANCOVA) to test for effects of host sex and weight (weight may be used as a proxy for age e.g., Cattadori et al. 2005) on

normalized mean flea abundance. I used correlation to test the relationship between host length and weight. I evaluated our dataset prior to analysis and determined that the *equality of slopes* assumption of ANCOVA was not violated. Normalized mean flea loads and mean flea abundance were compared between host sex using t-tests.

In collaboration with another researcher, I tested patterns of flea species co-occurrence on prairie dog hosts using a null model. I tested the observed mean ratio of co-occurring flea species pairs against a null model of co-occurrence generated from flea abundance data of uniquely occurring flea species. The null expectation was based on the frequency distribution of each flea species that occurred by itself. The expected abundance ratio was calculated by drawing one abundance value from each null distribution for each pair of species that were observed to co-occur. The number of random pairings in the null model was the same as the number of times each species pair was observed in the real dataset. To generate a null distribution of mean abundance values, this process was repeated 1000 times. The observed species ratio was then compared to the null distribution to determine statistical significance. Competitive interactions between species pairs are likely to explain cases where observed species ratios are larger than expected based on the null distribution; smaller-than-expected species abundance rations could indicate facilitation.

**Results**

In partnership with other researchers, I sampled 153 prairie dogs and collected 1,722 fleas from our ten study sites. I trapped a total of 94 female and 59 male prairie

dogs. Prairie dog abundance varied from 3 to 30 individuals trapped per site (mean = 16.7, SD = 9.81). Flea abundance varied from 1.67 to 38.25 fleas per host across our 10 sites (mean = 12.5, SD = 13.41) and normalized total flea abundances differed significantly among sites (F = 2.27, P = 0.02). Prairie dog abundance, quantified as minimum number of individuals known alive, and normalized flea abundance were not significantly correlated (r = -0.24, P = 0.51) across sites.

There was no significant difference (Fisher's Exact Test, P = 0.37) between female (90.4%) and male (94.9%) flea prevalence rates. The majority of the flea species collected (1716 of 1722) belonged to two species, *Oropsylla hirsuta* and *Oropsylla tuberculata cynomuris*. The remaining fleas collected belonged to the genus *Pulex*. The fleas in our study system were highly aggregated among hosts (Figure 5.2). I calculated the sample size-corrected aggregation coefficient, *k*, for all fleas following Elliot (1977). The overall calculated value of 0.24 indicates a high degree of aggregation; values of *k* ~20 indicate Poisson (random) distribution and values of $k < 1$ indicate aggregated distribution (Wilson et al. 2002b). Calculated values of *k* were 0.21 and 0.09 for *O. hirsuta* and *O. t. cynomuris*, respectively.

Normalized average flea abundance on males (11.5 fleas per host) was marginally higher than on females (7.11 fleas per host) (t = 1.91, P = 0.057) but normalized flea load (average number of fleas on infested hosts only) did not differ by host sex (t = 1.97, P = 0.11). Host weight was found to be a significant predictor of flea load (F = 7.38, P = 0.007) with heavier animals having fewer parasites for both males and for females (Figure 5.3). Neither host sex nor the host weight*sex interaction term was found to influence flea load (F = 2.64, P = 0.106; F = 1.87, P =

0.174). Host length was not a predictor of flea load ($r^2 = 0.002$, P = 0.29) although host weight and length were significantly positively correlated (r = 0.56, P = 0.03).

*Oropsylla hirsuta* and *Oropsylla tuberculata cynomuris* were the only species that commonly occurred together. This species pair was observed 63 times in the real dataset with a mean abundance ratio of 3.379 ($\sigma = 3.679$). The expected abundance ratio based on the null distribution was 5.212 ($\sigma = 0.910$), indicating that *O. hirsuta* and *O. t. cynomuris* occurred together significantly more often than would be expected by chance alone (P = 0.020).

**Discussion**

The results presented here show that fleas on *C. ludovicianus* in this study area, like most macroparasites (Shaw and Dobson 1995), are aggregated among hosts, and that there is a significant positive relationship between the relative abundance of two congeneric flea species on prairie dogs. This result suggests that there is no competition between *Oropsylla hirsuta* and *O. tuberculata cynomuris* for space or resources on prairie dog hosts given that high numbers of one species are associated with high numbers of the other species on the same host animal. Variation in parasite infestation among hosts may be due to host-specific factors, parasite-specific factors, and environmental factors and I will discuss the results of this study in the context of each of these factors.

*Host factors*

I found no significant difference between male and female *C. ludovicianus* in flea load. Flea prevalence was roughly 5% higher on males than females which likely accounts for the slightly higher flea abundance on males. Male hosts are often found to have more parasites than females (Poulin 1996, Shalk and Forbes 1997) for a variety of reasons including differences in diet (Poole et al. 1983), behavior, or body size (Zuk and McKean 1996). My result is, however, similar to other studies of prairie dogs and their fleas; Holmes (2003) found no difference in flea loads between male and female *C. ludovicianus*.

My results show a significant, albeit weak, negative relationship between *C. ludovicianus* body weight and flea load. Other studies (Holmes 2003, Collinge et al. unpublished data) have found adult *C. ludovicianus* to have more fleas than juveniles. This apparent discrepancy among studies is probably best explained by the fact that my trapping was conducted before offspring were born in the spring and thus the youngest individuals in the population were near maturity. Interestingly, I found no significant relationship between host body length and flea abundance or flea load even though host weight and length are tightly correlated.

I chose to use weight as a proxy for age because weights were not bimodally distributed and I could not distinguish among age classes. Host age is also often related to macroparasite load (Anderson and May 1991, Hudson an Dobson 1995) though patterns are not consistent across systems or even among populations of the same host and parasite. In many cases, parasite load increases with host age until it reaches an asymptote suggesting a balance between parasite acquisition and parasite mortality (Hudson and Dobson 1995, Wilson et al. 2002b). Adults often have higher

parasite loads simply because they have been alive longer and have had more time to acquire parasites. Another explanation for this phenomenon is that adults have larger bodies and thus provide more suitable habitat for parasites. My data, however, show the opposite pattern where smaller (younger) individuals have more parasites than larger individuals. Flea species vary in their preference for host age with some preferring younger hosts and other preferring older hosts (Marshal 1981). Furthermore, physiological factors can influence flea prevalence and abundance on different age classes of hosts. Cattadori et al. (2005) suggested that such a pattern where younger animals are most heavily infested may be caused by acquired immunity to parasites. Immune response to different flea species has been documented in gerbils (Khokhlova et al. 2004a) but I did not measure the immune response of prairie dogs and thus cannot support or refute the hypothesis that older prairie dogs have acquired resistance to fleas.

All ectoparasites are, to some extent, habitat limited although this limitation is not necessarily availability of feeding sites (Marshall 1981). The high degree of flea aggregation in our data suggests that *C. ludovicianus* may have behavioral or physiological mechanisms to cope with flea infestation. Host grooming behavior is a major source of flea mortality (Marshall 1981) and allogrooming behavior in social or colonial animals such as *C. ludovicianus* helps alleviate parasite burden on individuals. Coteries are typified by one or two reproductive adult males, three or four reproductive females, and several juveniles and yearlings (Nowak 1999). The social structure of *C. ludovicianus* may explain the reduction of parasite load with increasing body size in that the larger and older animals tend to be dominant within

the coterie. This explanation conflicts with Johnson et al.'s (2004) conclusion that non-reciprocal allogrooming leads to higher group parasite burden. Since I did not quantify host behavior or physiological response to flea infestation I am unable to explain this result definitively. Similarly, I am unable to completely address the result that male *C. ludovicianus* have marginally higher abundances of fleas than females. Hormonal composition or behavior or size may influence flea abundance by sex (Marshall 1981) but I do not have the data to address these hypotheses.

*Parasite factors*

The non-random, positive association between *Oropsylla hirsuta* and *O. tuberculata cynomuris* suggests that certain host individuals are more suitable to fleas or that hosts vary in their susceptibility to flea infestation. This result is also contrary to the hypothesis that there is competition between these two flea species. Furthermore, if competition existed between or within these fleas, it would be expected that larger hosts would support larger numbers of fleas. Stone and Roberts (1991) described how species interactions that are assumed to be directly competitive may actually lead to indirect facilitation in a community context and under heterogeneous environmental conditions. Given the overall limited understanding of the actual interactions between *O. hirsuta* and *O. tuberculata cynomuris*, I am unable to definitively determine whether the positive pattern of interspecific association is due to direct facilitation.

In many host populations immune response is highly variable; the typical response to blood-feeding ectoparasites is hypersensitivity where arteries close to the

skin contract to reduce blood flow and thus further feeding (Marshall 1981). Such variation would explain the heterogeneity in flea loads among *C. ludovicianus*. Krasnov et al. (2005d, 2006) stated that patterns of apparent facilitation among flea species on a host individual may be due to suppression of the host's immune response. Hypersensitivity and other forms of acquired resistance or immunity may also be cross-reactive whereby the reaction to one species of parasite serves to reduce the burden of other parasite species (Marshall 1981, Khokhlova et al 2004b). Cross-reactivity may occur in this study system and might account for reduced loads of both *O. hirsuta* and *O. tuberculata cynomuris* on certain hosts, whereas immunochallenged individuals might have increased flea loads of both species. However, the immune challenges represented by *O. hirsuta* and *O. tuberculata cynomuris* may differ enough that a separate defense is required for each parasite species. Under this scenario, maintaining several lines of immune defense may be too energetically costly for the host, especially if the defenses are only marginally effective (Jokela et al. 2000)

*Environmental factors*

Flea survival (Krasnov et al. 2001) and development (Rust and Dryden 1997) are dependent on temperature and humidity and prairie dog burrows may vary in their suitability for maintenance of flea populations. Prairie dogs tend to share nests and burrows only within groups of related individuals called coteries (Hoogland 1995) and thus burrow systems and nests harboring different numbers of fleas may lead to heterogeneities in exposure among hosts. Male territoriality and female territoriality during rearing of young may also lead to differences in space-use and exposure to

parasites. Differences in temperature or relative humidity within a study site, as well as among sites, may influence the number of fleas available to a host but I did not measure these variables. The result that flea abundance per host varied significantly among the ten study colonies in this system may be reflective of site-level environmental differences.

Seasonality in fleas is a common phenomenon (Oscar-Jimenez et al. 2001, Krasnov et al. 2002b) and may partly account for the lack of competitive interactions between *O. hirsuta* and *O. tuberculata cynomuris*. *O. tuberculata cynomuris* is absent from *C. ludovicianus* during summer months when flea assemblages are dominated by *O. hirsuta* (Collinge et al., unpublished data) and these species may only co-occur as adults during part of the year.

Plague, the disease caused by the bacterium *Yersinia pestis*, periodically emerges in black-tailed prairie dogs and causes upwards of 99% mortality of exposed individuals (Ubico et al. 1988). *Y. pestis* is vectored by fleas and the emergence of plague may profoundly affect both prairie dog and flea abundances. The most recent widespread plague epizootic in Boulder County occurred in 1994 and affected roughly half of the prairie dog colonies in this study system. However, overall flea abundances were not significantly different between historically plague positive and plague negative sites (Mann-Whitney U-test, $P = 0.38$) suggesting that sufficient time may have passed to overcome any lingering effects of plague on flea abundance. I did not estimate prairie dog population sizes among the different sites because I did not trap enough animals to make these estimates reliable. Furthermore, other

methods of prairie dog abundance such as visual counts may provide more accurate estimates (Menkens et al. 1990).

*Conclusion*

Parasites play an important role in animal community ecology influencing the behavior (Tripet et al. 2002), fitness (Bize et al. 2005) and potential gene flow (Latta, 2003) of their hosts. In many cases of arthropod ectoparasitism, the severity of infestation is proportional to the effect on the host (Marshall 1981). Therefore, understanding heterogeneity in parasite load among host individuals may provide insight into host population biology. I have documented intriguing patterns of occurrence of two sympatric flea species on *C. ludovicianus* hosts but at this point am unable to offer conclusive explanations of these results. I showed a significant co-occurrence of two closely related flea species on *C. ludovicianus* hosts and interpreted this to mean that there is no evidence for competition between fleas. This assumption, however, may be too simplistic. Vandermeer et al. (2002) have shown through simulation modeling that patterns of species coexistence may be explained by competitive interactions.

The conservation status of *C. ludovicianus* is contentiously debated (Reading et al. 2002) partly due to the role this species may play in the maintenance of zoonotic diseases. *C. ludovicianus* and its fleas are periodically infected with plague (Gage and Kosoy 2005) and therefore understanding patterns of host-flea association may be important to public health. Furthermore, there is a growing movement of researchers

focusing on parasite conservation for the sake of inferring host genealogies and population genetics through parasite studies (e.g., Windsor 1990, Whiteman and Parker 2005). Arthropod parasites such as *O. hirsuta* and *O. t. cynomuris* are potentially very important to disease ecology, wildlife conservation, and human health, though host-host interactions and host parasite relationships are poorly understood in most cases. Here, I present intriguing patterns of flea abundance on a mammalian host species as a first step towards a broader understanding of interactions between parasites and their host.

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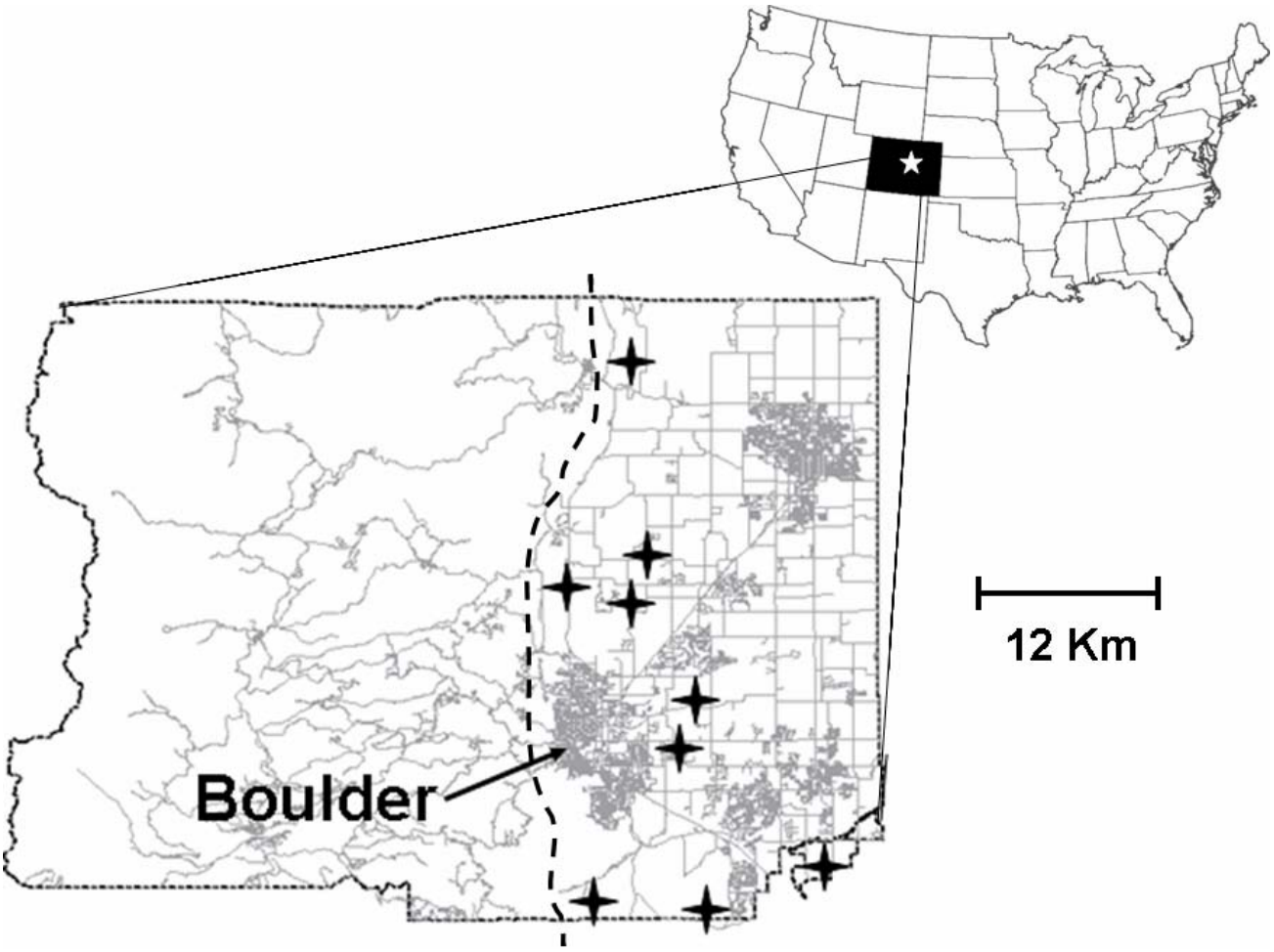
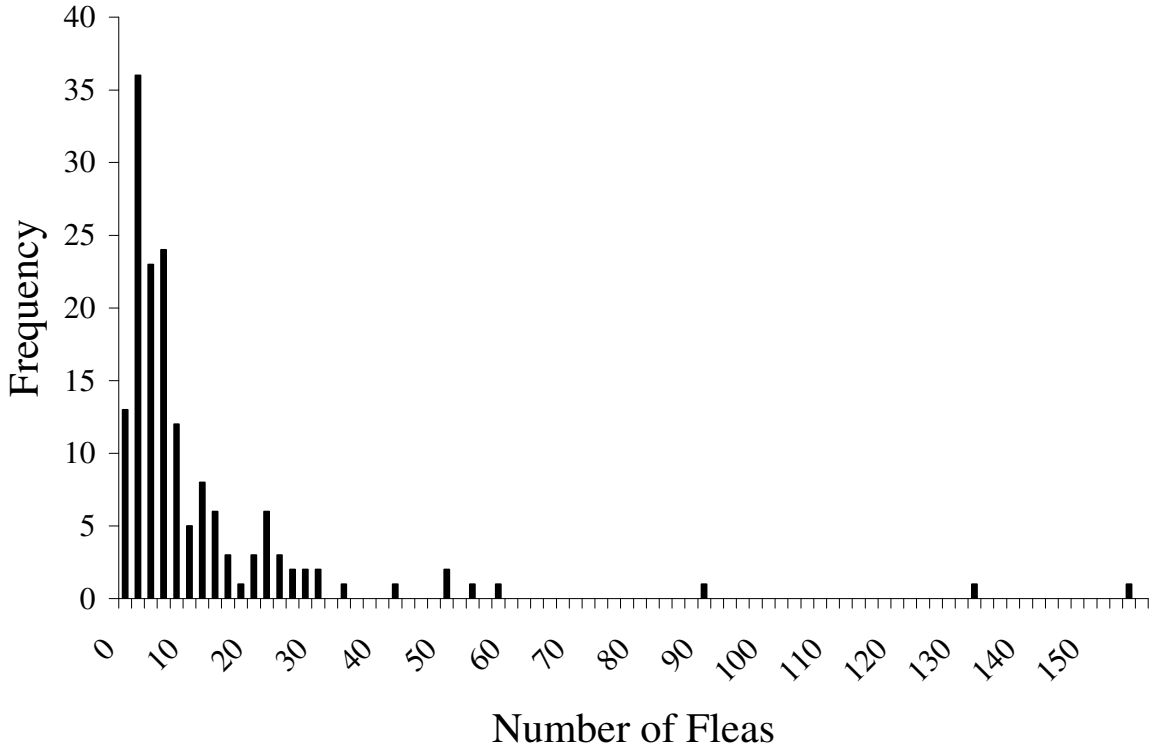
Figure 5.1



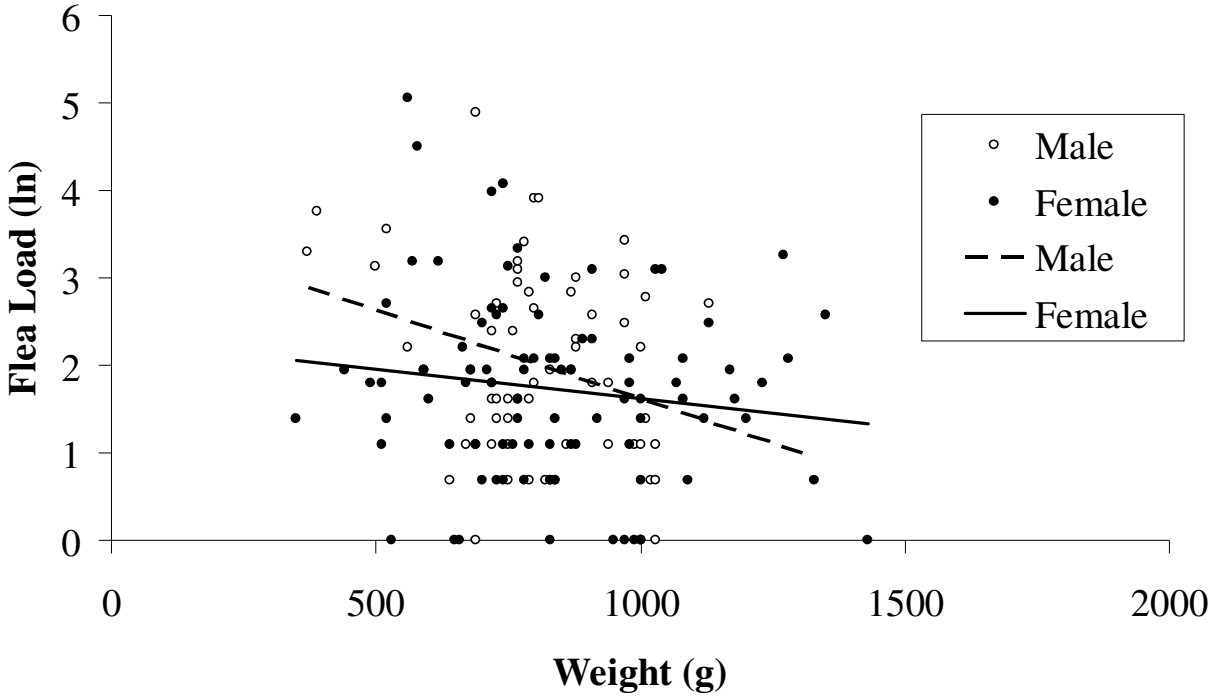

Location of Boulder County, Colorado and study sites. Lines represent roadways and watercourses. Densely lined areas represent the city of Boulder and surrounding urbanized areas. The dashed vertical line through Boulder County approximates the eastward extent of the foothills of the southern Rocky Mountains.

Figure 5.2




Histogram of *Oropsylla* abundance on black-tailed prairie dog hosts showing a highly aggregated distribution (k = 0.24).

Figure 5.3




Relationship between prairie dog weight and ln flea load. Host weight was a significant ($p < 0.01$) negative predictor of flea load while host sex and host weight*sex interaction terms were not significant predictors ($p = 0.11$ and 0.18, respectively).

# Chapter 6

## CONCLUSIONS

Sylvatic plague is a disease that exists within complex ecological communities. As such, its behavior and dynamics may be highly variable among ecological systems and difficult to predict. Given the importance of *Yersinia pestis* to human history, surprisingly little is known about its ecology, though this is surely due partly to its range of interactions with potential hosts and vectors. Traditionally, plague has been thought to exist in an enzootic, or maintenance, cycle consisting of rodents that are at least partly resistant to infection, and their fleas. Periodically, plague then shifts to an epizootic, or amplifying, cycle whereby an epizootic host species becomes infected. Epizootic hosts are characterized by high susceptibility to plague infection and they serve to increase overall plague prevalence, as infected epizootic hosts serve as pathogen sources from which vectors may become infected. Plague may be transmitted through the air or by direct physical contact, but flea-borne transmission is predominant.

The Oriental rat flea, *Xenopsylla cheopis*, has long been considered to be the most competent and efficient plague vector due to the fact that plague exposure in *X. cheopis* often leads to the formation of a bacterial biofilm in the flea's proventriculus, effectively blocking the digestive tract at the base of the esophagus. The formation of this blockage serves to increase rates of plague transmission as the starving flea increases its bite frequency, regurgitating *Y. pestis* bacilli into the host's bloodstream

with each unsuccessful feeding attempt. However, most flea species do not form blockages upon exposure to *Y. pestis* and, as a result, other mechanisms must account for plague transmission in systems where *X. cheopis* does not exist.

Recent studies have demonstrated that a several flea species can efficiently transmit plague among host individuals within four days of exposure without forming blockages. Therefore, flea species previously rejected as being involved in plague transmission must have their roles reexamined. Similarly, recent data suggest that the small rodent species purported to serve as plague reservoirs show very low levels of plague exposure before, during, and after plague epizootics. The existence of such data that contradict the existing plague paradigm necessitates closer examination of the community ecology of systems affected by plague.

In western North America, black-tailed prairie dogs (*Cynomys ludovicianus*) serve as epizootic hosts for plague. Prairie dogs are semi-fossorial ground squirrels that live in complex networks of burrows and suffer uniformly high mortality upon exposure to plague, which typically results in colony-wide die-offs. Sources of plague infection in prairie dogs are unknown, as are the flea species responsible for its transmission. Given the uncertainty surrounding plague ecology in western North America, I explored key aspects of the mammal and flea assemblages associated with black-tailed prairie dog colonies to gain insights into interspecific plague transmission.

First, I investigated the possibility that non-rodent mammals might play a role in plague transmission to prairie dogs or movement of plague among prairie dog colonies. Specifically, I surveyed mammalian prairie dog predators to test for spatial

and seasonal patterns of plague exposure. I expected that carnivores located near potential plague foci might show higher rates of plague exposure than carnivores sampled in areas that do not regularly experience plague activity. I also predicted that plague exposure in carnivores might be highest in spring months immediately prior to the onset of plague epizootic events. I detected uniformly low rates of plague exposure in species of carnivores and therefore could not draw conclusions regarding spatial or temporal patterns of plague emergence. However, low levels of plague exposure in carnivores suggest that carnivores are not reservoirs for plague infection and that plague prevalence may be very low in all mammalian species in this study system.

Second, I evaluated patterns of flea occurrence on thirteen mammalian species associated with prairie dog colonies to make inferences about interspecific plague transmission. I found that the flea species assemblages of prairie dogs were very similar to those of coyotes, foxes, and rabbits, and were very different from the small rodent species that share prairie dog burrows. Although there are a limited number of possible explanations for these results, they imply that plague transmission to or from prairie dogs is more likely to involve carnivore or lagomorph species than small rodents.

Third, I explored the population genetic structure of the prairie dog flea species most likely to be involved in spreading plague among individual prairie dogs. I sampled fleas from nine prairie dog colonies, four of which had escaped recent plague epizootics and five that suffered extensive die-offs in the past 15 years. I predicted that colonies that were closer together would have more similar genetic

structure, but I found that distance was not a significant predictor of flea genetic similarity. I was not able to directly infer movement rates or direction of fleas among these colonies but I did find patterns consistent with plague history at each colony: flea populations on colonies recently affected by plague showed signals of recent population expansion whereas flea populations from plague-negative colonies showed signals of population stability. Furthermore, spatial patterns of genotype occurrence were consistent with the hypothesis that plague-negative prairie dog colonies serve as sources for repopulation events following plague epizootics.

Finally, I explored relationships between two closely-related flea species on prairie dog hosts. I predicted that competitive interactions among flea species might result in patterns whereby only one flea species would be found on any given host individual. However, I found that the two primary prairie dog flea species occurred together more often than would be expected by chance, showing no signs of competitive interactions. This pattern is consistent with facilitative interaction, but other factors such as variation in immune response among host individuals or differential probability of encountering fleas could also produce the same result.

This body of work makes novel contributions to the study of the community ecology of sylvatic plague. The data I collected suggests that plague either exists at extremely low levels or is absent from Boulder County, Colorado during periods between epizootic events. The absence of high levels of plague exposure in carnivores is contrary to a large body of research indicating that carnivores may be useful sentinels of local plague activity. Because carnivores typically show high levels of plague exposure before, during, and after plague events, the discrepancy

between this and previous studies likely owes to very low levels of plague activity in Boulder County, Colorado.

My data also suggest that carnivores may play a bigger role in plague transmission and movement than was previously assumed based on associations between flea and mammalian species. The flea-host association data presented here also challenge the notion that small rodents serve as reservoirs for plague as flea exchange between small rodents and prairie dogs is very rare relative to flea assemblage overlap between prairie dogs and species of carnivores. Patterns of flea population genetic structure suggest that flea populations are likely to decline following plague events and that recolonization may originate from nearby or far-away sources. These results are also suggestive of carnivore-mediated flea dispersal; prairie dog movement is likely to be too infrequent and occur over relatively short distances, whereas carnivores are prone to regularly moving long distances. Furthermore, it seems unlikely that competitive interactions among flea species would restrict plague transmission among hosts; rather, apparent facilitation might result in certain individuals being more likely to acquire a variety of flea species, thus increasing the likelihood of plague transmission from one host species to another.

Plague is an enigmatic disease and although my results have provided some insights into its transmission among mammalian species, they also raise additional questions. Enzootic hosts and flea species responsible for plague transmission to and from prairie dogs are still unidentified, as are mechanisms of plague movement. On one hand, my data are suggestive of relatively high probability of flea exchange between prairie dogs and carnivores, yet I also failed to detect plague exposure in the

vast majority of carnivores sampled during years of plague epizootic activity. Similarly, my data suggest that flea populations are impacted by plague events in prairie dogs, yet patterns of recolonization by fleas following plague events do not seem to be sensitive to the landscape features that are associated with plague occurrence in prairie dogs. In other words, plague is assumed to move across the landscape by way of fleas, yet flea populations separated by putative barriers to plague cannot be differentiated in terms of population genetic structure.

Although I was not able to produce any definitive data to identify key species involved in plague transmission or provide indisputable evidence of specific transmission mechanisms, this study elucidates the necessity of approaching disease ecology from a community perspective. The plague pathogen, *Yersinia pestis*, interacts with a variety of potential host, reservoir, and vector species, which in turn interact with each other. For example, the event of a coyote or fox preying upon a prairie dog could result in the movement of a plague–infected flea from one host species to another, thus propagating plague infection and expanding the geographic scope of an epizootic. A study restrictive to one host or flea taxon or group might not take such interactions into account and could miss key factors crucial to disease dynamics.

To illustrate this point, a group of investigators recently concluded that swift foxes must play only a small role in plague transmission among prairie dog colonies because foxes at prairie dog colony sites sampled following a plague event carried only uninfected prairie dog fleas. These researchers conceded that acquisition of prairie dog fleas by foxes suggests that foxes could carry plague-infected fleas among

colonies, but that this was unlikely given that prairie dog fleas made up less than ten percent of swift fox flea species assemblages. The results presented in this dissertation, however, indicate that coyotes and foxes are much more likely than small rodent species to acquire prairie dog fleas, thus making them among the most likely mammalian species to be involved in interspecific plague transmission. This is but one example of how, in absence of a community-level investigation, the likelihood of missing key components to disease transmission dynamics is elevated as is the possibility of reaching potentially erroneous conclusions.

In more general terms, the results of my study may be applicable to other systems in which a pathogen may be transmitted by multiple competent vector species and occurs in a variety of hosts. In addition to relationships between hosts and vectors, interactions among vector species may shed light on disease transmission dynamics. I did not detect evidence for competition among vector species, but it is conceivable that vectors compete for resources on hosts in other systems. Furthermore, patterns of host and vector population genetic structure may lend insights into mechanisms of pathogen movement. The community ecology of disease is a burgeoning field and only recently have researchers investigated how interactions among host species influence disease dynamics. Effects of vector interactions on disease processes are less-studied still, and community-level investigations of host-vector-pathogen dynamics are all but non-existent.