DISSERTATION

FACTORS AFFECTING FLEA DENSITIES IN PRAIRIE DOG COLONIES:

IMPLICATIONS FOR THE MAINTENANCE AND SPREAD OF PLAGUE

Submitted by

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ABSTRACT

FACTORS AFFECTING FLEA DENSITIES IN PRAIRIE DOG COLONIES:

IMPLICATIONS FOR THE MAINTENANCE AND SPREAD OF PLAGUE

Plague is a re-emerging, rodent-associated disease caused by the primarily flea-borne bacterium *Yersinia pestis*. The bacterium likely originated 1,500–20,000 years ago in Asia but has been transported by humans to multiple additional continents and islands where it degrades populations of a wide array of rodents. In the western United States, there is an urgent need to acquire a deeper understanding of plague because over half the rodent species of conservation concern occur within its introduced range.

This dissertation describes research on fleas in colonies of black-tailed prairie dogs (*Cynomys ludovicianus*), colonial rodents that amplify *Y. pestis* in the Great Plains. Adult fleas were combed from live-trapped prairie dogs during June–August 2010–2012 in the short-grass prairie of Vermejo Park Ranch, New Mexico, USA. We evaluated correlations between flea densities and the attributes of soils, prairie dog colonies, and weather.

Adult fleas were most abundant in portions of prairie dog colonies with coarse surface-soils and moderately textured subsurface-soils. Coarse surface-soils may allow precipitation to infiltrate to the depth of prairie dog nests, where the moisture could create humid microclimates that are preferred by fleas. Inside burrows, moderately textured soils may hold considerable amounts of water, some of which could evaporate into prairie dog nests, thereby creating humid microclimates. Although fleas tend to fare best under humid conditions, they were scarce in

areas with very wet subsurface-soils, presumably because sodden soils can facilitate the accumulation of fungi and mites, some of which are lethal to fleas.

We also studied the abundance of fleas in old colonies (initially 8-11 years-old) and young colonies (3–6 years). Fleas were 110% more abundant in old colonies and their abundance was positively correlated with the number of years since a colony was established. Fleas may accumulate to high densities in old colonies because prairie dogs have created deep burrows there, and deep burrows provide ectothermic fleas with humid microclimates and stable temperatures. Moreover, older burrows presumably contain a wealth of organic matter upon which flea larvae feed.

Fleas desiccate under dry conditions and, consequently, their densities are thought to decline during droughts. At Vermejo, February–June precipitation was relatively plentiful in 2010 and 2012 but scarce in 2011, the driest spring-summer on record for New Mexico. Unexpectedly, fleas were 250% more abundant in 2011 than in other years. During the dry 2011 field season, prairie dogs were in poor condition and devoted little time to grooming. In contrast, during 2010 and 2012, prairie dogs were in 27% better condition and, when controlling for month and observer variation, devoted 450% more time to grooming. Prairie dogs provided with supplemental food and water during March-May 2012 were in 18% better condition and carried 40% fewer fleas during June-August. Increased flea densities during droughts may provide context for the maintenance and spread of plague.

Three additional studies are presented herein. First, we developed a new method for combing fleas from hosts. The method and resulting data can be used with occupancy models to estimate prevalence rates for ectoparasites while accounting for imperfect detection.

Second, we used the combing new method to estimate prevalence rates for the generalist flea *Pulex simulans* during June–August 2012. Prevalence estimates were >30% higher than indices from studies with substantial sample sizes for prairie dogs. If *P. simulans* can attain high prevalence on prairie dogs, the species may commonly serve as a bridge vector between *Cynomys* and other mammalian hosts of *Y. pestis*, and even function as a reservoir of plague.

Third, a case study is presented to describe how *Y. pestis* can transform grassland ecosystems by devastating populations of prairie dogs and, thereby, causing (1) declines in native species abundance and diversity, including threatened and endangered forms, (2) alterations in food web connections, (3) alterations in the import/export of nutrients, (4) loss of ecosystem resilience to encroaching invasive plants, and (5) modifications of prairie dog burrows.

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CHAPTER ONE

LITERATURE REVIEW AND RESEARCH CONTEXT

Introduction

Animals are often highly susceptible to introduced pathogens with which they have no evolutionary history and invasive diseases can distort trophic relationships and negatively affect a plethora of species (Jones et al. 2008, Estes et al. 2011, Selakovic et al. 2014). There is an urgent need to acquire an increased understanding of introduced diseases so appropriate mitigation strategies can be identified and implemented in the field (Thomas et al. 2005).

This dissertation concentrates on plague, a reemerging zoonosis caused by the primarily flea-borne bacterium *Yersinia pestis*. While best known for causing devastating epidemics in humans (Orent 2004), plague degrades populations of a wide array of mammals, thereby transforming ecosystems (Biggins and Kosoy 2001a, b). Consider the effects of plague on prairie dogs (*Cynomys* spp.) and the grasslands of western North America:

> Prairie dogs are colonial, burrowing rodents that structure grassland communities
> as keystone species and ecosystem engineers (Fig. 1; Kotliar et al. 2006,
> Davidson et al. 2010). *Y. pestis* was introduced to North America around the year
> 1900 and spread rapidly in the western United States, expanding eastward >2,200
> km in 40 years (Barnes 1982, Adjemian et al. 2007). The pathogen was detected
> in prairie dogs during the 1930s (Eskey and Haas 1940) and is now endemic
> throughout the majority of their range (Cully et al. 2006, Antonation et al. 2014).

About 95–100% of prairie dogs succumb to plague during epizootics[1] and the disease appears to commonly reduce survival rates of prairie dogs by 30–40% or more during enzootic periods[2] (Biggins et al. 2010). Due to the accumulating deaths, prairie dogs occur at low densities in a small fraction of their historic areas of occupancy. Moreover, *Y. pestis* has affected, and continues to affect many species that associate with *Cynomys*, including the black-footed ferret (*Mustela nigripes*), an endangered, specialized predator of prairie dogs that is itself highly susceptible to plague (Fig. 2; Biggins and Kosoy 2001a, b, Antolin et al. 2002, Miller and Reading 2012). Even in the absence of epizootic outbreaks, enzootic plague can reduce annual ferret survival by at least 240% (Matchett et al. 2010).

Intensive effort is devoted to studying plague in hopes of acquiring information that can be used to manage the disease and mitigate its effects on prairie dogs and associated wildlife (Cully et al. 2006). As with other diseases (Dobson and Foufopoulos 2001), efforts to manage plague are facilitated by studies of factors that influence transmission rates (Eisen and Gage 2011). Recently, two lines of research have called for studies of fleas:

(1) Rates of plague transmission can be heightened if fleas are relatively abundant (Lorange et al. 2005) and fleas might help *Y. pestis* to persist in localized habitats during enzootic periods (Eisen and Gage 2009, Wimsatt and Biggins 2009). With an increased understanding of factors that affect the abundance and dispersion of fleas, plague might be managed by concentrating mitigation actions where flea densities are heightened, for instance using insecticides and

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[1] Epizootic: a die-off of hosts over relatively large expanses of land (e.g., complexes of prairie dog colonies) during a short timeframe (e.g., months).

[2] Enzootic: periods between plague epizootics. Plague-caused mortality can occur during enzootic periods, but at a slower rate, or at smaller spatial scales than observed during epizootics (Biggins et al. 2010).

vaccines (Seery et al. 2003, Rocke et al. 2008, 2010, Abbott et al. 2012).

Assuming a particular habitat is suitable for prairie dogs (or ferrets) but not

yet occupied, translocations might be concentrated in areas where fleas are

predicted to be scarce.

(2) Flea abundance is also important from the perspective of human health

because human plague cases usually result from infectious flea bites (Lowell

et al. 2005, Gage 2012).

In addition to these applied implications, fleas are intrinsically interesting because they are a

component of biodiversity and various factors constrain their densities at multiple scales

(Krasnov 2008). Regarding fleas that parasitize prairie dogs, habitat quality could vary

throughout the *Cynomys* range, between species of prairie dogs, between prairie dog colonies,

among portions of colonies, among prairie dog families, among individual prairie dogs, among

body regions of a host, among burrows, and among portions of burrow systems. Moreover,

habitat quality varies over time (Cooke 1990) and constraints vary at different stages of the flea's

holometabolous life cycle (Krasnov 2008).

This dissertation was motivated by concern for North American ecosystems and wildlife

affected by *Y. pestis* (including fleas), but concentrates specifically on fleas in prairie dog

colonies. Below, a literature review is presented to provide context for research chapters.

Review topics include (1) the plague bacterium and flea-borne transmission of *Y. pestis*, (2)

specific examples of the importance of flea abundance in plague ecology, and (3) hypotheses for

factors that may influence flea densities.

Literature Review

The Plague Bacterium and Flea-Borne Transmission

  Y. pestis originated about 1,500–20,000 years ago after genetic divergence from *Y. pseudotuberculosis*, a water- and food-borne enteropathgen (Achtman et al. 1999). These two *Yersinia* are about 97–98% DNA sequence similar (Chain et al. 2004). However, *Y. pestis* exhibits a pPla plasmid with a plasminogen activator gene that is often believed to allow the bacterium to escape to deeper tissues within mammalian hosts, and a pFra plasmid that impedes phagocytosis by host macrophages. These particular genes or the proteins they express also allow for detection of *Y. pestis* (Perry and Fetherson 1997, Sebbane et al. 2006, Antolin 2008).

  Y. pestis also differs from *Y. pseudotuberculosis* in that plague is primarily flea-borne (Sun et al. 2014). Adult fleas become infected when feeding on blood from a highly bacteremic host (Lorange et al. 2005). *Y. pestis* resists the flea's immune response and up-regulates genes to facilitate infection (Vadyvaloo et al. 2010). For instance, *Yersinia* murine toxin (Ymt) facilitates survival in the flea's gut (Hinnebusch et al. 2002). With assistance from *Y. pestis* hemin storage (hms) genes, plague bacilli can generate a biofilm that accumulates on inward-directing, cuticle-coated spines of the flea's proventriculus (Hinnebusch et al. 1996, Hinnebusch 2005). Accumulating bacilli can eventually "block" passage of blood to the midgut, thereby inhibiting blood feeding. A blocked flea begins to starve and, consequently, increases its feeding efforts. The blockage impairs proventriculus functioning and *Y. pestis*-contaminated blood can reflux from the midgut and proventriculus to the foregut and mouthparts. To clear passage, the flea

regurgitates blood and bits of the biofilm blockage containing the plague bacilli. Such regurgitation can transmit *Y. pestis* to a host via the bite-site (Bacot and Martin 1914).

Blocked fleas were long presumed to be the principal contributors to plague transmission in rodent populations. However, many flea species found on hosts killed by plague do not easily form proventricular blockages (Eskey and Haas 1940, Burroughs 1947, Eisen et al. 2009). For fleas that do block, a bacteremia of greater than about 10^6 cfu/mL of blood is required for reliable infection (thus often requiring feeding on a highly bacteremic host) and sufficient blockage necessitates an extrinsic incubation period[3] of about five or more days (often weeks; Eskey and Haas 1940). Further, a single bite from a blocked flea is often insufficient for *Y. pestis* to infect a host (Eskey and Haas 1940, Burroughs 1947, Kartman and Prince 1956, Kartman et al. 1956, Engelthaler et al. 2000, Lorange et al. 2005). The extrinsic incubation period and inefficiency of transmission can hamper the rate at which *Y. pestis* spreads among hosts. Consequently, it seems blocked fleas are not solely responsible for the rapid spread of *Y. pestis* during epizootics (Webb et al. 2006).

Fleas are undoubtedly involved in epizootics, however, because widespread outbreaks are not observed when flea densities are reduced using insecticides[4] (Seery et al. 2003, Hoogland et al. 2004). With this information in mind, recent lab studies tested an earlier hypothesis that unblocked fleas transmit *Y. pestis* via contaminated mouthparts, or transmission of infectious blood before a complete blockage forms (if any blockage forms; Bacot and Martin 1914, Burroughs 1947, Kartman et al. 1958a, b). Fleas that consumed *Y. pestis*-infected rat blood were infectious at the next feeding opportunity for about 24–48 hours, <u>before</u> a proventricular

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[3] Extrinsic incubation period: the period of time elapsed from a flea becoming infected until it is capable of transmitting *Y. pesti*s (Eisen et al. 2009).
[4] Insecticides also reduce the rate of plague transmission during enzootic periods (Biggins et al. 2010, Matchett et al. 2010).

blockage would have formed (which requires a minimum of 5 days but much more often 10 or more days; Eisen et al. 2006, 2007a, b, 2009, Wilder et al. 2008). This phenomenon was termed "early-phase transmission" (Eisen et al. 2006) and its mechanisms are under research (Johnson et al. 2014).

Regardless of the underlying mechanisms, early-phase transmission may help to explain the rapid spread of *Y. pestis* during epizootics (Eisen et al. 2006). For instance, early-phase transmission can cease within 48-96 hours or so, but if a flea acquires a "booster-feeding" from an infectious host, the flea can become infectious once again and transmit *Y. pestis*, thus facilitating the spread of plague among hosts (Eisen et al. 2007a, Wilder et al. 2008).

While early-phase transmission may allow for a more rapid spread of plague among hosts, the efficiency[5] of early-phase transmission is often <25% (Eisen et al. 2009, Eisen and Gage 2011). Thus, an abundance of fleas is seemingly needed for continued spread of *Y. pestis* among susceptible hosts (Wilder et al. 2008).

If sufficient *Y. pestis* bacilli are transmitted to a mammalian host (in some cases as few as 1-10 bacteria), a plague infection could be induced. *Y. pestis* exhibits multiple changes during the process of infecting a mammal, including (1) synthesis of a system to uptake iron, (2) expression of the F1 capsular antigen that facilitates resistance to phagocytosis from host monocytes, (3) expression of pH 6 antigen that, along with the biofilm, facilitates adhesion to host cells, (4) use of hypodermic-like needles on *Yersinia* outer proteins to inject cytotoxic and immunosuppressive proteins into host macrophages, and (5) escape to host lymph nodes, liver, spleen, and blood stream (pla gene), which facilitates uptake of plague bacilli by additional fleas (Perry and Fetherson 1997, Antolin 2008).

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[5] Vector efficiency: the proportion of infected fleas that successfully transmit bacteria to susceptible hosts (Eisen et al. 2009).

Although some mammals exhibit heterogeneous responses to *Y. pestis*-infection, many rodent hosts die within 5–7 days (Kartman et al. 1962, Gage et al. 1995). After an infected host dies, fleas jump from its body and may seek, acquire, bite, and (if infectious) transmit *Y. pestis* to a new host. The new host may become infectious and, consequently, additional fleas on that host can become infected. As *Y. pestis* accumulates within the blood of infected hosts, fleas can acquire booster feedings that allow for repeated bouts of early-phase transmission. Over time, as more hosts die from plague, increasing numbers of potentially infectious fleas seek declining numbers of living hosts, thereby perpetuating the plague cycle (Poland and Barnes 1979, Tripp et al. 2009). Plague epizootics can ensue for months, often occurring as a "wave" of host mortality across the landscape (Girard et al. 2004, Adjemian et al. 2007, Gage 2012, St. Romain et al. 2013). Populations of some hosts begin to recover after epizootics, although plague-caused mortality can persist, sometimes to a large degree (Gage and Kosoy 2006), for instance as observed with endangered black-footed ferrets (Matchett et al. 2010).

The Importance of Flea Abundance in Plague Ecology

If infectious fleas are scarce or not present in an area, transmission of *Y. pestis* is reduced and perhaps eliminated (Seery et al. 2003, Hoogland et al. 2004, Biggins et al. 2010). Conversely, high flea densities can compensate for low vector efficiency and deaths of blocked fleas (Eskey and Haas 1940, Burroughs 1947, Engelthaler et al. 2000, Lorange et al. 2005). Moreover, high flea-burdens presumably increase the odds of infectious fleas finding susceptible hosts (Webb et al. 2006, Buhnerkempe et al. 2011, Reijniers et al. 2012). Thus, it seems plausible that if

conditions are conducive for transmission of *Y. pestis*, plague-caused mortality could be heightened in areas where fleas are abundant.

Data support this contention. For example, during periods when plague was most active, growth rates in populations of three species of *Cynomys* (*C. ludovicianus*, *C. leucurus*, and *C. parvidens*) were reduced in areas where fleas were most abundant (D.E. Biggins, unpublished data). Separate studies suggest coincidence between seasonal trends in the abundance of fleas and epizootics in black-tailed prairie dogs (Pauli et al. 2006, Tripp et al. 2009). The incidence of plague in rodents and humans in India correlated positively with seasonal indices for the abundance of *Xenopsylla cheopis* fleas (Hirst 1927) which are efficient vectors via blocked and early-phase transmission (Eisen et al. 2007b, 2009).

Fleas may also contribute to maintenance of *Y. pestis* between epizootics (Gage and Kosoy 2005, 2006, Eisen and Gage 2009, Wimsatt and Biggins 2009). In at least a few cases, infected adult fleas have been reported to survive long periods without blood meals and, in such cases, may have helped *Y. pestis* to persist in local habitats (e.g., 13 months in *Oropsylla* fleas; Kartman et al. 1962; 396 days in *Ctenopthalmus breviatus*, Golov and Ioff 1928; 558 days in *Oropsylla silantiewei*, Kozlov 1979). Infected *Ceratophyllus tesquorum altaicus* fleas survived the hibernation period of *Urocitellus undulates* and later transmitted *Y. pestis* to the susliks, thus reinitiating the host-flea cycle (Bazanova and Maevskii 1996).

Young fleas have been hypothesized to contribute to plague maintenance (Wimsatt and Biggins 2009). For instance, adult fleas might provision (Smit 1973, Silverman and Appel 1994) and infect larvae with partially digested blood, and the infected larvae may facilitate persistence of *Y. pestis* in an area. Larval fleas might also acquire *Y. pestis* from the burrow substrate where the bacterium can survive for >400 days (Burroughs 1953, Baltazard 1964, Molyneux 1969,

Bazanova et al. 1997). Larval *Oropsylla montana* fleas have been observed to cling to

laboratory mice with their mouthparts and might pierce the skin and feed on host blood (perhaps

allowing for plague transmission),[6] thereby allowing *Y. pestis* to re-enter the transmission cycle

involving adult fleas and hosts.

 If fleas can maintain *Y. pestis*, persistence of the bacteria and subsequent transmission may

be heightened where fleas are abundant. What factors influence the abundance of fleas across a

landscape and, in particular, on prairie dog colonies? As discussed below, studies suggest the

attributes of soils, prairie dog colonies, and weather might be important.

Flea Abundance and Soil Attributes

 Fleas transition through a life cycle that includes egg, larval, pupal, and adult stages. Eggs

and larvae develop in host nests that, for rodents, are typically constructed in a soil substrate and

have a variety of vegetative debris (Hoogland 1995, Sheets et al. 1971, Gedeon et al. 2010). In

these chambers, larvae feed on organic debris and, perhaps, flea eggs, larvae, pupae, and feces

deposited by adults (Sharif 1937, Silverman and Appel 1994, Krasnov 2008). At the last instar

stage, larvae encase themselves in a silken cocoon derived from, and reinforced by silk and

substrate, thus transitioning to the pupae stage. Pupae develop within the substrate-bounded

cocoons, later emerging as adults that spend some time on hosts to acquire blood meals or breed,

but other time off hosts, inside host nests and elsewhere.

 The texture of soils can influence rates of development and survival for larvae, thereby

affecting the distribution of fleas across a landscape. For example, during lab trials with

Xenopsylla fleas from Israel, survival of larval *X. conformis* was greater in sand compared to

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[6] J.A. Montenieri, Centers for Disease Control and Prevention, personal communication.

loess, and this species was absent from loess habitats in the wild, despite the availability of suitable hosts (Krasnov et al. 1997, 2002). In contrast, larval *X. ramesis* fleas survived longer in loess and this species was absent from sandy habitats in the wild (Krasnov et al. 1997, 2002). Thus, given spatial variation in the texture of soils that line rodent nests (Shenbrot et al. 2002), fleas might not establish a population, or might occur at relatively low numbers in some areas but be abundant in others.

Another important factor for fleas is the ability of soils to retain water. Fleas are highly susceptible to desiccation (Krasnov 2008). If the water-holding capacity of soils is low, then burrow environments might be excessively dry and extract moisture from fleas, thereby killing them (Ebeling and Wagner 1959). In contrast, if soils tend to retain water and soil moisture is very high, molds can accumulate and kill flea pupae (Hirst 1927, Bruce 1948). Also, if soils are very moist, parasitic mites (Acarina) can increase in abundance and possibly kill fleas (Wimsatt and Biggins 2009). Thus, fleas might be most abundant in areas with soils of intermediate water-holding capacities, or actual levels of moisture (Stark 2002).

By influencing the abundance and dispersion of fleas, edaphic factors could also influence plague dynamics (Savage et al. 2011). A component of this dissertation investigates relationships between the attributes of soils and the abundance of adult fleas in colonies of black-tailed prairie dogs (Chapter 2). This dissertation also evaluates the importance of colony ages.

Flea Abundance in Old and Young Prairie Dog Colonies

The ages of prairie dog colonies and temporal changes in burrow habitats could affect fleas in many ways. For instance, old colonies contain burrows that have been used by prairie dogs

for many years, suggesting the burrows may contain an abundance of organic debris on which flea larvae feed. An increased abundance of food would benefit larvae, and larvae of greater condition could better survive to the adult, breeding stage, thereby facilitating growth in flea populations (Krasnov 2008).

The depth of burrows may also differ between old and young colonies, and those differences could be important for fleas. The depth of sciurid burrows tend to increase over time (Longhurst 1944, Fitch 1948), suggesting burrows in old prairie dog colonies would be deeper than burrows in young colonies. Deep burrows are buffered from above ground conditions (Schmidt-Nielsen 1964, Clark 1971, Smith 1982, Shenbrot et al. 2002), suggesting burrow temperatures in old prairie dog colonies would tend to be relatively stable. In addition, deep burrows are often more humid than shallow burrows (Reichman and Smith 1990). Stable microclimates and humid conditions could be beneficial to fleas that are ectothermic, sensitive to changes in temperature, and highly susceptible to desiccation (Krasnov 2008).

With the above information in mind, Chapter 3 of this dissertation compares flea densities in old and young prairie dog colonies. Chapter 3 also investigates the seasonality of flea abundance, and compares flea-loads on different age-sex classes of hosts.

In addition to soils and colony ages, weather patterns can affect flea densities and, in doing so, influence the spread and maintenance of plague (Gage et al. 2008, Ben Ari et al. 2011, Eisen and Gage 2012). Chapter 4 of this dissertation describes an investigation of how weather may influence prairie dogs, fleas, and plague ecology. An abbreviated literature review is presented here to provide context for that chapter.

Accumulating evidence suggests plague epizootics are most common during years with warmer, wetter springs and cooler summers, and least common during droughts (Ben Ari et al. 2011, Gage 2012, Eisen and Gage 2012). This trend has been explained using information from lab studies in which fleas exhibited increased rates of survival under mild temperatures and humid conditions, but tended to lose excessive amounts of water and die if temperatures were too hot and humidity too low (Krasnov 2008). Flea densities in the wild are assumed to increase during warm-wet springs and cool summers, thereby providing context for, and sometimes sparking and sustaining plague epizootics. In contrast, flea densities are assumed to decline during droughts.

A long-term dataset from northeastern Colorado appears to lend support to these assumptions. Plague epizootics in black-tailed prairie dogs were most common during warm-wet springs and cool summers, suggesting mild weather stimulated growth in flea populations, thereby causing an increase in rates of plague transmission (Savage et al. 2011). Additional fieldwork is needed for confirmation, however.

Chapter 4 of this dissertation uses field data on flea abundance and weather patterns to develop a conceptual model of flea and plague ecology under a unified framework for prairie dogs. In general, the data suggest prairie dogs can suffer declines in body condition, and increases in flea-loads during droughts. The model posits that increased flea-loads during droughts might provide context for the maintenance and spread of plague.

Brief Synopsis of Dissertation

We hypothesized that surface- and subsurface-soils would influence flea abundance (Chapter 2). We hypothesized that flea abundance would differ between old and young prairie dog colonies (Chapter 3). We also hypothesized that flea densities would vary in accordance with weather variables, including annual amounts of precipitation (Chapter 4).

Although not discussed above, we developed a new method that allows parasitologists to account for imperfect detection when studying the prevalence of ectoparasites on hosts (Chapter 5). We used that method to estimate rates of prevalence for *P. simulans*, a generalist flea that may function as a bridge vector between prairie dogs and other mammals (Chapter 6). Chapter 7 extends the "transformer species" concept of Richardson et al. (2000) to *Y. pestis*, with special reference to prairie dogs and the grasslands of western North America.

Figure 1 Black-tailed prairie dogs (*Cynomys ludovicianus*) in the Conata Basin, South Dakota. These colonial, burrowing rodents were once abundant, and functioned as keystone species and ecosystem engineers in the grasslands of western North America. Due to the effects of plague and other factors (e.g., poisoning campaigns), prairie dogs are no longer capable of serving their historic, ecologic functions at most sites. Proposals have been submitted for their listing under the Endangered Species Act. Photo: Copyright M. Forsberg Photography (http://shop.michaelforsberg.com/).

Figure 2 An adult female black-footed ferret (*Mustela nigripes*) carrying her prey, a juvenile black-tailed prairie dog (*Cynomys ludovicianus*), in the Conata Basin, South Dakota. Ferrets are specialized predators of prairie dogs. Plague can negatively affect ferrets by reducing the abundance of prairie dogs (prey), but also by directly killing ferrets via infection from flea bite or consumption of infectious meat. Photo: D.A. Eads.

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CHAPTER TWO

FLEA DENSITIES CORRELATE WITH THE ATTRIBTUES OF SOILS IN

BLACK-TAILED PRAIRIE DOG COLONIES: EVIDENCE FROM NEW MEXICO

Plague is a re-emerging, rodent-associated disease caused by the primarily flea-borne bacterium *Yersinia pestis*. Soils can affect the abundance and dispersion of fleas in rodent populations, suggesting edaphic factors may affect the spread and maintenance of plague. We investigated relationships between soil attributes and the abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August 2010–2012, in Vermejo Park Ranch, New Mexico, USA. Fleas were most abundant in areas with coarse surface-soils and moderately textured subsurface-soils. Coarse surface-soils may allow precipitation to infiltrate to the depth of prairie dog nests, where the moisture could create humid microclimates that are preferred by fleas. Inside burrows, moderately textured soils may help flea pupae construct firm, long-lasting cocoons, resulting in increased numbers of emerging adult fleas. Moreover, moderately textured soils can hold considerable amounts of water, some of which could evaporate into prairie dog nests and, in doing so, create humid microclimates. While fleas tend to fare well under humid conditions, they were scarce in areas of prairie dog colonies with very wet subsurface-soils, presumably because sodden soils can facilitate the accumulation of fungi and mites, some of which are lethal to fleas. Continued research on soils and fleas is warranted because an increased understanding of these relationships may facilitate efforts to manage plague.

Introduction

Plague is a reemerging zoonosis caused by the primarily flea-borne bacterium *Yersinia pestis*. While best known for causing epidemics in humans (Orent 2004), *Y. pestis* poses a direct risk to many mammals, including endangered and threatened species (Gage and Kosoy 2005, 2006). Moreover, by reducing the abundance of mammals (rodents in particular), the plague bacterium can distort trophic relationships and indirectly affect a multitude of species (Biggins and Kosoy 2001b). Thus, in addition to its implications for human health, *Y. pestis* is important in conservation biology (Biggins and Kosoy 2001a, Antolin et al. 2010).

In North America, plague poses a significant risk to prairie dogs (*Cynomys* spp.), colonial, burrowing rodents that once occupied vast expanses of the Great Plains (Proctor et al. 2006). Humans inadvertently introduced *Y. pestis* to California (most likely San Francisco) in 1900 and the bacterium quickly expanded eastward (Adjemian et al. 2007), reaching prairie dogs by the 1930s or earlier (Eskey and Haas 1940). Plague devastated prairie dog populations, causing 95–100% mortality during epizootics (Cully et al. 2006) and fragmenting populations into degraded metapopulations (Antolin et al. 2006).

Current evidence suggests *Y. pestis* persists within prairie dog colonies between outbreaks (during enzootic periods; Matchett et al. 2010), therein causing chronic rates of mortality for 3-15 years or so (Barnes 1982) until conditions are suitable for another epizootic (Biggins et al. 2010). The accumulating mortalities force prairie dogs into smaller areas and their densities are chronically reduced, sometimes resulting in permanent colony extinctions, an accumulation of which may threaten metapopulations with extinction in the long-term (Antolin et al. 2006, Cully et al. 2010, George et al. 2013).

The conservation implications of plague are exemplified by how the disease affects species that associate with prairie dogs. For example, plague reduces the availability of habitat for, and directly kills black-footed ferrets (*Mustela nigripes*), endangered predators of prairie dogs (Godbey et al. 2006, Matchett et al. 2010). Plague also reduces the availability of habitat for birds that prefer to nest on prairie dog colonies, such as burrowing owls (*Athene cunicularia*) and mountain plovers (*Charadrius montanus*), additional species of conservation concern (Desmond et al. 2000, Dinsmore and Smith 2010). As a result, conservation biologists increasingly study plague in hopes of acquiring information that can be used to manage the disease (Cully et al. 2006).

Numerous factors could affect the spread and maintenance of plague, but current evidence suggests flea densities are important (Eisen and Gage 2009, Wimsatt and Biggins 2009). For instance, vector efficiency is often <25% and, therefore, an abundance of fleas is usually needed to infect a host (Lorange et al. 2005, Eisen et al. 2009). Moreover, high flea-burdens increase the odds of infectious fleas finding susceptible hosts (Webb et al. 2006, Buhnerkempe et al. 2011, Reijniers et al. 2012). Thus, it seems rates of plague transmission may be heightened in areas of prairie dog colonies where fleas are most abundant.

Given the importance of fleas in plague ecology, the disease might be best managed by first targeting mitigation actions in those areas where fleas are predicted to be most abundant, and then extending protective actions into areas where fleas occur at lower densities. Such an approach might dampen the spread of plague, while also allowing conservation groups to strategize funds devoted to plague management (the costs of which may exceed $13 million or more annually).

Prairie dogs occupy areas with varying soils (Munn 1993) and spatial variation in soil attributes can affect the abundance and dispersion of fleas (Fig. 3; Osacar-Jimenez et al. 2001, Krasnov 2008). We studied adult fleas in colonies of black-tailed prairie dogs (*C. ludovicianus*) in New Mexico, USA, and posited the following:

(1) Adult fleas will be most abundant in areas with coarse surface-soils. Coarse surface-soils allow considerable amounts of precipitation to infiltrate belowground (Rawls et al. 1992), perhaps to the depth of prairie dog nests where the moisture might create humid microclimates. Humid microclimates can benefit fleas, because these insects are prone to desiccation if humidity levels are low (Krasnov 2008).

(2) Fleas will be most abundant in areas with finely-textured subsurface-soils (with few sand particles). Fleas are thought to spend most of their time in prairie dog nests (e.g., Krasnov et al. 2004). Sandy soils have a low water-holding capacity (Rawls et al. 1992) and, therefore, might create dry microclimates in burrows, which could be detrimental to fleas (Krasnov 2008). Moreover, "sandy nesting substrates have the potential to abrade the waxy epicuticle of flea larvae, increasing water loss and larval mortality" (Wimsatt and Biggins 2009:92). Conversely, finely-textured soils, with high proportions of clay and silt, may create humid, smooth burrow environments.

(3) Fleas will be most abundant in areas with subsurface-soils of intermediate moisture. Dry soils can extract water from insects (Ebeling and Wagner 1959) and excessively moist soils facilitate the accumulation of fungi and mites, some of which are lethal to fleas (Silverman and Rust 1983, Wimsatt and Biggins

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2009), suggesting fleas might attain relatively high densities in areas with

subsurface-soils of intermediate moisture (Stark 2002).

Materials and Methods

Host Species, Study Site, and Sampling Plots

Excavations of black-tailed prairie dog burrows suggest flea larvae and pupae develop in

prairie dogs nests, which are usually situated about 2 m underground (Sheets et al. 1971). Black-

tailed prairie dogs (hereafter "prairie dogs") spend over half of their daytime hours in burrows

and rarely emerge aboveground at night, except when escaping nocturnal predators (Hoogland

1995, Eads et al. 2010). Thus, regardless of the amount of time adult fleas spend on prairie dogs,

they would presumably spend most of their time in burrows.

We completed our study during June–August, 2010–2012, at the Vermejo Park Ranch,

Colfax County, New Mexico. The Vermejo short-grass prairie is characterized by gently rolling

holes and interceding arroyos. We studied prairie dogs in a complex situated in the southeast

portion of Vermejo, on 24,300 ha of short-grass steppe dominated by blue grama (*Bouteloua*

gracilis) and dispersed patches of cholla cacti (*Cylindropuntia imbricata*).

Prairie dogs were live-trapped and sampled in 1.54 or 2.25 ha plots distributed among 13

colonies (Fig. 4; *n* = 19 plots in 2010; 20 in 2011; 19 in 2012). Each plot contained 25 or 37

live-traps ($16 \times ha^{-1}$) distributed among burrows with fresh prairie dog scat (Tomahawk single-

door live traps, Hazelhurst, Wisconsin, USA). Each year, the plots were categorized into five

groups of 2–5 plots. We sequentially sampled the groups in randomized order during 10-day

work periods.

We did not observe rapid die-offs of prairie dogs, which are indicative of plague epizootics

(Cully et al. 2006). Therefore, we suggest plague epizootics did not occur in prairie dogs

occupying our study areas. Fieldwork was completed under Colorado State University (CSU)

Institutional Animal Care and Use Committee Protocol #10-1785A.

Trapping Prairie Dogs and Sampling Fleas

Live-trapping and sampling procedures are described in Eads et al. (2013).[1] Briefly, we

aged, sexed, and weighed a prairie dog; anesthetized it and fleas on its body with isoflurane;

combed it as thoroughly as possible for 45 seconds to collect fleas; marked each of its ears with

tags for permanent identification; and measured the length of its right hind foot (Hoogland 1995,

Gage 1999, Biggins et al. 2010, Martin et al. 2013). We released each prairie dog at its trapping

location and used hand-held global positioning units to record Universal Transverse Mercator

(UTM) coordinates as spatial reference points for all capture events.

Fleas were identified to species using a Leica Microsystems (Buffalo Grove, Illinois) WILD

M3Z stereo zoom microscope and keys in Stark (1958) and Hubbard (1968). Two species were

most prevalent[2] and their abundances are analyzed collectively and separately herein: *Oropsylla*

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[1] Chapter 5 in this dissertation.
[2] Four flea species were detected: *Oropsylla hirsuta* (a prairie dog flea), *Oropsylla tuberculata cynomuris* (another prairie dog flea), *Pulex simulans* (a generalist), and *Echidnophaga gallinacean* (a stick-tight flea). *O. hirsuta* and *P. simulans* were highly prevalent, whereas *O. t. cynomuris* and *E. gallinacean* were scarce. *O. t. cynomuris* tends to be most abundant on prairie dogs in winter, as reported by Maupin (1970) and affirmed by Tripp et al. (2009) and Wilder et al. (2008). In most cases, *E. gallinacean* is collected from a very small proportion of captured prairie dogs.

hirsuta (a prairie dog specialist[3]) and *Pulex simulans* (a generalist). All male *Pulex* were

identified as *P. simulans*, so we assumed all *Pulex* were *P. simulans* (Tripp et al. 2009).

Attributes of Soils

The attributes of surface-soils (0.25 m depth) were derived from a Soil Survey Geographic

Database map (SSURGO, U.S. Department of Agriculture, Natural Resource Conservation

Service; Anderson et al. 1982). We overlaid UTM coordinates for the combing events on the

SSURGO map and categorized each event with the texture of surface-soils (U.S. Department of

Agriculture) at the corresponding locations in ArcGIS 9.2 (Environmental Systems Research

Institute, Redlands, California).

The attributes of subsurface-soils were derived from samples collected adjacent to trapping

locations using a hydraulic machine (Giddings Machine Company, Windsor, Colorado). Each of

the samples originated from 2 m depth, the average depth of prairie dog nests excavated by

Sheets et al. (1971). Initial samples were collected during June–August 2010. We randomly

selected 212 UTM coordinates at which we trapped prairie dogs and collected subsurface-soils

adjacent to those locations ($\bar{x}$ = 11 samples/plot, range = 4–25). Before collecting a sample, we

noted the descending angle of a focal burrow opening and positioned a 3-in auger in that

direction, 1 m from the center of the opening. We drilled to 1.8-m depth, used a 0.15 m carbide

saw-tooth core sampler to remove excess soils, and collected a cored sample at 2-m depth.

Each cored sample was immediately transferred to a plastic bottle and the "wet weight" was

measured with a balance scale. We dried groups of 20–25 soil samples in an oven (110 °C) until

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[3] Results from this study should prove applicable not only to prairie dogs, but also to black-footed ferrets, because *O. hirsuta* commonly parasitizes ferrets (Harris et al. 2014).

a stable dry weight was obtained for 5 replicates (drying time = about 48 h), after which each

sample was weighed on a digital scale. We assessed soil moisture as the percentage of water on

a dry-weight basis ((wet weight–dry weight)/dry weight) × 100; Gardner 1986). We used the

hydrometer method (Gee and Bauder 1986) to estimate percentages of sand, silt, and clay

particles in each sample (clay= < 2 μm, silt = 2–50 μm, and sand = >50 μm; U.S. Department of

Agriculture).

We collected subsurface-soils in June 2011 ($n = 19$) and May 2012 ($n = 15$) to investigate the

possibility that the moisture of subsurface soils changed during the 3-year study. Locations for

these samples were selected randomly from the pool of 2010 samples, with the restriction that

each new sample within a given year must be collected from a different plot (for spatial

replication) and from a location that had not been re-sampled (to eliminate pseudoreplication).

Each new sample was collected 1 m away from a focal burrow opening at an angle perpendicular

to the descending angle of the opening. We used paired tests (analogous to t-tests) to compare

moisture levels in the original 2010 samples and the 2011 or 2012 samples. For each t-test, we

calculated AICc for the null hypothesis (H_0, effect size = 0.00) and the alternative hypothesis

(H_a, effect size = $\bar{d}$), calculated differences between AICc values for H_0 and H_a, and calculated

the probability of each model given the data (Anderson 2008). The moisture of subsurface-soils

remained similar during 2010–2011 (Prob(H_0|data) = 0.99) and 2010–2012 (Prob(H_0|data) =

1.00). Thus, we related the 2010–2012 data to moisture-levels in the 2010 soil samples.

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Data Analyses

Data analyses were completed in two steps. The first used the entire dataset to evaluate relationships between flea abundance and surface-soils. The second used a subset of data to evaluate relationships between flea abundance and subsurface-soils.

Detection of fleas is likely to be imperfect (Chapters 5 and 6), especially when considering detection of individual fleas. Consequently, our data on flea abundance should be considered indices. Because we standardized the combing method, we assume our data can be used for relative comparisons of flea abundance in areas with differing soils.

Generalized linear mixed models (GLMMs) were used in SAS$^®$ 9.3 ('PROC GLIMMIX', SAS Institute Inc.) to relate flea abundance (*sensu* Bush et al. 1992)[4] to various predictor variables. Maximum likelihood estimates of the negative binomial parameter k for flea abundance were always <1.00 ('theta.ml' package, Program R version 2.13.2, R Development Core Team 2011), so we used the negative binomial family for aggregated count data (Wilson et al. 2002). Goodness-of-fit X^2 statistics of the most general models were ~1.00, suggesting the negative binomial distribution accounted for overdispersion in the data (Burnham and Anderson 2002). Coefficient 95% confidence intervals for interpreted variables never encompassed 0.00.

The GLMMs included random-effects that accounted for the nested-nature of our data. According to likelihood ratio tests (McCullagh and Nelder 1989), inclusion of a random effect for "Prairie Dog ID" never increased model fit, suggesting little repeatability of flea abundance within individual prairie dogs (see also Krasnov et al. 2006).[5] Therefore, we did not include

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[4] Results remained the same if flea loads were expressed as the number of fleas per unit of a host's body surface to control for host size (mass$^{-0.67}$; Heusner 1985, Hawlena et al. 2005).

[5] Similar results have been found with black-tailed prairie dogs in Colorado (M.F. Antolin, personal communication).

"Prairie Dog ID" as a random effect. We included "Plot ID" as a random effect, however, to account for repeated samples from plots. Moran's I analyses (Moran 1950) with GLMM residuals (Cliff and Ord 1981) suggested the random effect accounted for spatial dependencies in the data.

In GLMMs, we included variables that explained variation in flea abundance during other analyses (Chapters 3 and 4, Eads et al. 2013) including year-of-sampling, month-of-sampling, age-of-prairie dog, sex-of-prairie dog, host condition (mass:foot ratio; Krebs and Singleton 1993), and prairie dog densities (minimum number alive, divided by plot area; Krebs 1966). These variables served as controls in all models and are not discussed herein.

As additional controls, we included binomial variables for colony age (old = initially ≥ 8 years-old; young = ≤ 6 years) and the presence or absence of insecticide-treatments (Eads et al. 2013). For those colonies previously treated with insecticides, at least four years had elapsed between insecticide treatment and the start of our study.

Surface-soils and fleas

In the exercise that related surface-soils to flea abundance, we evaluated a main-effect for the texture of surface-soils (SURFACE-TEXTURE) and a SURFACE-TEXTURE × YEAR interaction to determine if the SURFACE-TEXTURE effect varied among field seasons. We fit models with or without SURFACE-TEXTURE or the SURFACE-TEXTURE × YEAR interaction and ranked the three models by an adjusted Akaike's Information Criterion (AICc). Akaike weights (w_i) were also calculated (Burnham and Anderson 2002). Likelihood ratio tests

($\alpha = 0.100$; McCullagh and Nelder 1989) were used to compare nested submodels with more general models (differences would suggest the more complex model was useful).

Subsurface-soils and fleas

In the exercise that related subsurface-soils to flea abundance, we restricted the data to trapping events that occurred within 5 m of subsurface-soil samples collected in 2010. We fit GLMMs using main-effects for subsurface-soil texture (SUBSURFACE-TEXTURE) and moisture (SUBSURFACE-MOISTURE). SUBSURFACE-TEXTURE was expressed as the percentage of sand particles (i.e., coarse particles). Interactions between SUBSURFACE-TEXTURE and YEAR, or SUBSURFACE-MOISTURE and YEAR were also considered. Control variables included those mentioned above and SURFACE-TEXTURE from the SSURGO map. We fit all possible models with SUBSURFACE-TEXTURE, SUBSURFACE-MOISTURE, and their individual interactions with YEAR ($n = 9$ models), used AICc for model ranking, and used likelihood ratio tests to compare nested submodels with more general models.

Results

Surface-Soils and Fleas

The GLMM investigation with surface-soils included 1,800 combing events. We collected 9,623 fleas ($\bar{x} = 5.35$ fleas/host, $SD = 8.72$) which were aggregated among prairie dogs ($k =$

0.63). Trapping locations fell within five categories of the SSURGO map, including (from fine

to coarse texture) silt, silt-clay-loam, silt-loam, loam, and sandy-loam.

With the two flea species combined, the SURFACE-TEXTURE variable increased fit ($X^2_4 =$

26.13, $P < 0.001$) whereas the log-likelihood declined (and AICc increased by 13.15) when the

SURFACE-TEXTURE × YEAR interaction was included (Table 1). Thus, we interpreted the

SURFACE-TEXTURE variable with all data combined. Fleas were most abundant in areas with

loam, silt-loam, and sandy-loam surface-soils. Fleas were relatively scarce in areas with silt-

clay-loam and silt surface-soils (Fig. 5).

For *O. hirsuta* ($n = 5,082$, $\bar{x} = 2.82$, $SD = 4.89$, $k = 0.45$), the SURFACE-TEXTURE variable

increased fit ($X^2_4 = 44.09$, $P < 0.001$) whereas the SURFACE-TEXTURE × YEAR interaction

did not (the interaction increased AICc by 7.26; Table 2). *O. hirsuta* was most abundant in areas

with sandy-loam surface-soils (Fig. 5).

For *P. simulans* ($n = 4,541$, $\bar{x} = 2.52$, $SD = 6.16$, $k = 0.22$), the SURFACE-TEXTURE

variable increased fit ($X^2_4 = 40.75$, $P < 0.001$); the SURFACE-TEXTURE × YEAR interaction

increased AICc by 9.33 units and did not increase fit (Table 3). *P. simulans* was most abundant

and primarily concentrated in areas with loam surface-soils[6] (Fig. 5).

Subsurface-Soils and Fleas

On average, the subsurface-soil samples from 2010 were 44.6% sand, 20.6% silt, and 34.8%

clay, with 24% moisture (9% by dry-weight). SUBSURFACE-TEXTURE was not significantly

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[6] *P. simulans* was consistently most abundant in particular colonies (D.A. Eads, unpublished data).

correlated with SUBSURFACE-MOISTURE (linear regression $r^2 = 0.013$)[7] suggesting we could investigate these two variables separately.

The investigation with flea species combined included 389 combing events, during which 1,312 fleas were collected ($\bar{x} = 3.38$, $SD = 6.04$, $k = 0.57$). Preliminary analyses demonstrated that a quadratic form of SUBSURFACE-TEXTURE and a linear form of SUBSURFACE-MOISTURE increased fit. Three of the GLMMs competed (Table 4). The SUBSURFACE-TEXTURE × YEAR interaction increased fit ($X^2_4 = 29.38$, $P < 0.001$) as did inclusion of SUBSURFACE-MOISTURE ($X^2_1 = 4.16$, $P = 0.041$). Inclusion of the SUBSURFACE-MOISTURE × YEAR interaction did not substantially increase fit ($X^2_2 = 1.56$, $P = 0.458$). Therefore, we interpreted the most supported model (Table 4). Fleas were most abundant in areas with about 15% SUBSURFACE-MOISTURE, and were scarce in areas with relatively dry or wet subsurface-soils (Fig. 6).

We separated the exercise by YEAR to investigate the SUBSURFACE-TEXTURE × YEAR interaction. A quadratic form of SUBSURFACE-TEXTURE was most supported for 2010 ($k = 0.65$) and 2012 ($k = 0.71$) and a linear form was supported for 2011 ($k = 0.66$). SUBSURFACE-TEXTURE increased fit for each year (2010: $X^2_2 = 13.97$, $P = 0.001$; 2011: $X^2_1 = 4.22$, $P = 0.040$; 2012: $X^2_2 = 5.65$, $P = 0.060$). Fleas tended to be most abundant in areas with low to medium proportions of sand particles at 2-m depth throughout the study. Fleas were least abundant in areas with sandy subsurface-soils (Fig. 7).

In the investigation for *O. hirsuta* ($n = 802$, $\bar{x} = 2.07$, $SD = 4.31$, $k = 0.35$), a preliminary assessment suggested use of quadratic forms of SUBSURFACE-TEXTURE and SUBSURFACE-MOISTURE. The most general model gained 100% of Akaike weight. The

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[7] This simple analysis did not account for spatial and temporal variation in precipitation that could produce correlations between the textures of surface- and subsurface-soils.

SUBSURFACE-TEXTURE × YEAR interaction ($X^2_4 = 33.16$, $P < 0.001$) and the SUBSURFACE-MOISTURE × YEAR interaction ($X^2_4 = 29.70$, $P < 0.001$) both increased fit (thus, a table is not presented), so we separated the analysis by YEAR.

With *O. hirsuta*, a quadratic form of SUBSURFACE-TEXTURE was most supported for 2010 ($k = 0.30$), whereas linear forms were supported for 2011 ($k = 0.65$) and 2012 ($k = 0.55$). SUBSURFACE-TEXTURE increased fit for 2010 ($X^2_2 = 15.39$, $P = 0.001$) and 2012 ($X^2_1 = 5.69$, $P = 0.017$) but not 2011 ($X^2_1 = 0.14$, $P = 0.708$). In 2010 and 2012, *O. hirsuta* was most abundant in areas with medium proportions of sand particles at 2-m depth (Fig. 8). Linear forms of SUBSURFACE-MOISTURE were supported for all three years. SUBSURFACE-MOISTURE increased fit for 2010 ($X^2_1 = 5.07$, $P = 0.024$) and 2012 ($X^2_1 = 10.84$, $P = 0.001$) but not 2011 ($X^2_1 = 2.48$, $P = 0.115$). In 2010 and 2012, *O. hirsuta* was most abundant in areas with about 5-10% SOIL-MOISTURE and scarce in areas with relatively dry or wet subsurface-soils (Fig. 9).

In the investigation for *P. simulans* ($n = 510$, $\bar{x} = 1.31$, $SD = 3.35$, $k = 0.21$), a preliminary assessment suggested use of quadratic forms for SUBSURFACE-TEXTURE and SUBSURFACE-MOISTURE. One model carried 96% of the Akaike weight (Table 5). SUBSURFACE-MOISTURE did not substantially increase fit ($X^2_2 = 3.38$, $P = 0.185$); however, when considering biological significance (Johnson 1999), *P. simulans* exhibited a trend for reduced abundance in areas with very moist subsurface-soils, especially during 2010 and 2011 (Fig. 9). In the most supported model (Table 5), the SUBSURFACE-TEXTURE × YEAR interaction justified separation of the data by year ($X^2_4 = 17.38$, $P = 0.002$). A quadratic form of SUBSURFACE-TEXTURE was most supported for 2010 ($k = 0.23$), 2011 ($k = 0.23$) and 2012 ($k = 0.31$). SUBSURFACE-TEXTURE increased fit with each yearly dataset (2010: $X^2_2 =$

10.91, $P = 0.004$; 2011: $X^2_2 = 7.67$, $P = 0.022$; 2012: $X^2_2 = 11.13$, $P = 0.004$). *P. simulans* was most abundant in areas with medium proportions of sand particles at 2-m depth (Fig. 8).

Discussion

Soil Attributes and Flea Abundance

Our results complement previous studies of fleas on rodents (Krasnov et al. 1997, 2002) and lagomorphs (Osacar-Jimenez et al. 2001) and suggest edaphic factors may affect the abundance and dispersion of fleas in prairie dog colonies. Adult fleas were most abundant in areas of prairie dog colonies with coarse surface-soils. This correlation may relate to the rate at which precipitation infiltrates belowground to the depth of prairie dog nests. Coarse particles create large pores within soils, thereby increasing capillary action and water infiltration (Kay and Whitford 1978, Reichman and Smith 1990, Rawls et al. 1992), suggesting that, all else being equal, rates of water infiltration would be heightened in areas of prairie dog colonies with coarse surface-soils. The infiltrating water could moisten soils around prairie dog nests, thus creating humid microclimates for fleas.

While coarse soils may hasten the rate at which water infiltrates to the depth of prairie dog nests, the majority of water from precipitation events within the short-grass prairie of North America is lost to evaporation or plant absorption (Sala 1982). The remaining water tends to accumulate <1 m belowground and it usually takes years (sometimes >10) for the moisture to infiltrate to the depth of prairie dog nests (Sala et al. 1992, Dodd and Lauenroth 1997, Singh et al. 1998). Despite annual variation in precipitation during our study (Chapter 4), the moisture of

subsurface-soils did not change substantially during 1- and 2-year intervals on prairie dog

colonies. The results of Wilcomb (1954) suggest recent precipitation events do not affect the

moisture-content of soils that line prairie dog nests. Thus, the positive correlation between

coarse surface-soils and flea abundance in our study might reflect an effect of long-term

precipitation on nesting environments[8] (see also Hall and Myers 1978, Osacar-Jimenez et al.

2001, Ben-Ari et al. 2011).[9]

Considering soils at the depth of prairie dog nests, we initially hypothesized that fleas would

be most abundant in areas with subsurface-soils comprised of few sand particles. Prairie dog

nests are lined at the bottom with loose subsurface-soils (Wilcomb 1954) in which flea larvae are

thought to develop (Krasnov 2008). Sandy soils may damage a larval flea's epicuticle, thereby

causing water loss and, thus, increasing rates of mortality (Wimsatt and Biggins 2009).[10] During

our study, fleas were scarce in areas with very sandy soils, as hypothesized, but attained their

highest densities in areas with subsurface-soils comprised by ~50% sand particles, suggesting a

moderate proportion of sand particles may somehow benefit the species we studied.

For instance, larval fleas push against surrounding objects to propel themselves through soils

(K.L. Gage, personal communication), and large soil particles may facilitate such movement. If

subsurface-soils are moderately textured, clay and silt particles can adhere to sand particles,

especially if organic matter is available to facilitate soil clumping, thereby by creating non-

abrasive aggregates (Gee and Bauder 1986). In doing so, the clay and silt may provide larval

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[8] Our results suggest recent precipitation events are unlikely to affect soil moisture at the depth of prairie dog nests, but could indirectly affect fleas by affecting food availability to prairie dogs, the condition of prairie dogs, and their defenses against fleas (Chapter 4).
[9] Hall and Myers (1978), Osacar-Jimenez et al. (2001), and Ben-Ari et al. (2011) suggest burrow humidity is primarily influenced by past precipitation (and soil texture), not recent precipitation.
[10] Some studies suggest sandy soils are abrasive and can damage the cuticle of larval insects (e.g., Brust and House 1990). Nonetheless, larvae from some flea species exhibit increased rates of survival in sandy substrate (Krasnov et al. 2002).

fleas with large, smooth soil aggregates that can be used for propulsion through soils, thus increasing their mobility and, perhaps, access to food resources within prairie dog nests.

In addition to providing non-abrasive environments for flea larvae, silt-clay particles in moderately textured soils might easily adhere to silk from larvae, and allow for construction of smooth, firm, and long-lasting cocoons at the pupae stage, resulting in heightened numbers of emerging adult fleas. In contrast, if subsurface-soils are excessively sandy, developing fleas may frequently abandon their cocoons, because large soil particles create pores through which the pupae could lose water (Krasnov et al. 1997, 2002), perhaps resulting in increased rates of mortality due to water loss or the energetic costs of repeated bouts of cocoon-construction.

Moderately textured subsurface-soils could have the additional benefit of creating moist, humid microclimates in burrows. Clay soils tend to absorb and retain water (Rawls et al. 1992) and may reduce humidity levels in burrows, and even absorb water from insects and cause desiccation and death (Ebeling and Wagner 1959). However, if silt and, more importantly, small proportions of sand are also present, as in moderately textured soils, the larger particles could create pores and capillaries that may allow water to evaporate into prairie dog nests, thereby creating humid microclimates.

The correlation between the texture of subsurface-soils and flea abundance relates well to a study in Colorado. Using a 23-year dataset on the occurrence of plague epizootics in colonies of prairie dogs in Pawnee National Grassland, Savage et al. (2011) related the incidence of local, within-colony epizootics to the attributes of soils at 1.5 m depth. Plague epizootics were most common in colonies with fine to moderately textured subsurface-soils characterized by high water-holding capacities. Our results suggest fleas may have been abundant in those particular colonies.

Moisture is not always beneficial for fleas. For example, molds can accumulate on moist soils with high organic content, and some molds are lethal to fleas (Hirst 1927, Bruce 1948, Silverman and Rust 1983, see also Olson 1969). In addition, mites that parasitize fleas are sometimes abundant in moist soils (Wimsatt and Biggins 2009), including moist soils in prairie dog burrows (Wilcomb 1954). These factors, separately or in unison, may help to explain why fleas were scarce in areas with very moist subsurface-soils during our study.

Conclusion

Our results suggest fleas may attain their highest densities in areas of prairie dog colonies with relatively coarse surface-soils, moderately textured subsurface-soils, and moist but not excessively wet subsurface-soils. Additional study is needed to evaluate the validity of our findings. In particular, replication is needed at other sites, including areas in mixed- and tall-grass prairies, and sites occupied by different *Cynomys* species. Also, data are needed from winter periods when another flea, *O. tuberculata cynomuris*, is commonly found on prairie dogs (Maupin 1970, Barnes 1982, Tripp et al. 2009, Wilder et al. 2008).

Additional soil attributes could play important roles in plague ecology and are deserving of attention. For instance, Rotshild (2001) found spatial correlations between plague epizootics in Russia and concentrations of trace metals in soils. Edaphic factors, including but not limited to trace metals, may affect the plague bacterium's ability to persist in soils (Eisen et al. 2008). Further, exposure to biota within soils (including bacteria other than *Y. pestis*) may affect plague transmission by adult fleas (Jones et al. 2013).

We have emphasized prairie dogs in this paper but encourage continued study of fleas and edaphic factors throughout the global range of plague, because many rodents and fleas exhibit an intimate association with soils. As we acquire a deeper understanding of relationships between edaphic factors and flea densities, we could begin to target plague management in areas where fleas are predicted to be abundant, thereby potentially reducing the ability of *Y. pestis* to persist and spread among mammalian hosts. We hope our study provides a springboard for such progress.

Table 1 Generalized linear mixed models investigating correlations between the abundance of adult fleas (species combined) on black-tailed prairie dogs (*Cynomys ludovicianus*), various control variables (see text), the texture of surface-soils (SURFACE-TEXTURE), and year of sampling (YEAR). A random effect was included in all models to account for repeated measurements from the same sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Data were collected during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Control variables + SURFACE-TEXTURE	13069.00	0	1.00
Control variables + SURFACE-TEXTURE + (SURFACE-TEXTURE × YEAR)	13082.15	13.15	0
Control variables	13095.00	26.00	0

Table 2 Generalized linear mixed models investigating correlations between the abundance of adult *Oropsylla hirsuta* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*), various control variables (see text), the texture of surface-soils (SURFACE-TEXTURE), and year of sampling (YEAR). A random effect was included in all models to account for repeated measurements from the same sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Data were collected during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Control variables + SURFACE-TEXTURE	10271.87	0	0.97
Control variables + SURFACE-TEXTURE + (SURFACE-TEXTURE × YEAR)	10279.13	7.26	0.03
Control variables	10312.30	40.43	0

Table 3 Generalized linear mixed models investigating correlations between the abundance of adult *Pulex simulans* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*), various control variables (see text), the texture of surface-soils (SURFACE-TEXTURE), and year of sampling (YEAR). A random effect was included in all models to account for repeated measurements from the same sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Data were collected during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Control variables + SURFACE-TEXTURE	7319.07	0	0.99
Control variables + SURFACE-TEXTURE + (SURFACE-TEXTURE × YEAR)	7328.40	9.33	0.01
Control variables	7342.95	23.88	0

Table 4 Generalized linear mixed models investigating correlations between the abundance of adult fleas (species combined) on black-tailed prairie dogs (*Cynomys ludovicianus*), various control variables (see text), the texture of subsurface-soils (SUBSURFACE-TEXTURE), the moisture of subsurface-soils (SUBSURFACE-MOISTURE), and year of sampling (YEAR). A quadratic form of SUBSURFACE-TEXTURE was most supported. A random effect was included in all models to account for repeated measurements from the same sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc), and model weights (w_i). Nine models were fit; only those models with $w_i > 0.00$ are listed here. Data were collected during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Control variables + SUBSURFACE-TEXTURE + (SUBSURFACE-TEXTURE × YEAR) + SUBSURFACE-MOISTURE	2361.22	0	0.62
Control variables + SUBSURFACE-TEXTURE + (SUBSURFACE-TEXTURE × YEAR)	2363.12	1.90	0.24
Control variables + SUBSURFACE-TEXTURE + (SUBSURFACE-TEXTURE × YEAR) + SUBSURFACE-MOISTURE + (SUBSURFACE-MOISTURE × YEAR)	2364.23	3.01	0.14

Table 5 Generalized linear mixed models investigating correlations between the abundance of adult *Pulex simulans* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*), various control variables (see text), the texture of subsurface-soils (SUBSURFACE-TEXTURE), the moisture of subsurface-soils (SUBSURFACE-MOISTURE), and year of sampling (YEAR). Quadratic forms of SUBSURFACE-TEXTURE and SUBSURFACE-MOISTURE were most supported. A random effect was included in all models to account for repeated measurements from the same sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc), and model weights (w_i). Nine models were fit; only those models with $w_i >$ 0.00 are listed here. Data were collected during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Control variables + SUBSURFACE-TEXTURE + (SUBSURFACE-TEXTURE × YEAR)	1120.30	0	0.96
Control variables + SUBSURFACE-TEXTURE + (SUBSURFACE-TEXTURE × YEAR) + SUBSURFACE-MOISTURE	1128.22	7.92	0.02
Control variables + SUBSURFACE-TEXTURE + SUBSURFACE-MOISTURE	1128.77	8.47	0.01
Control variables + SUBSURFACE-TEXTURE	1129.43	9.13	0.01
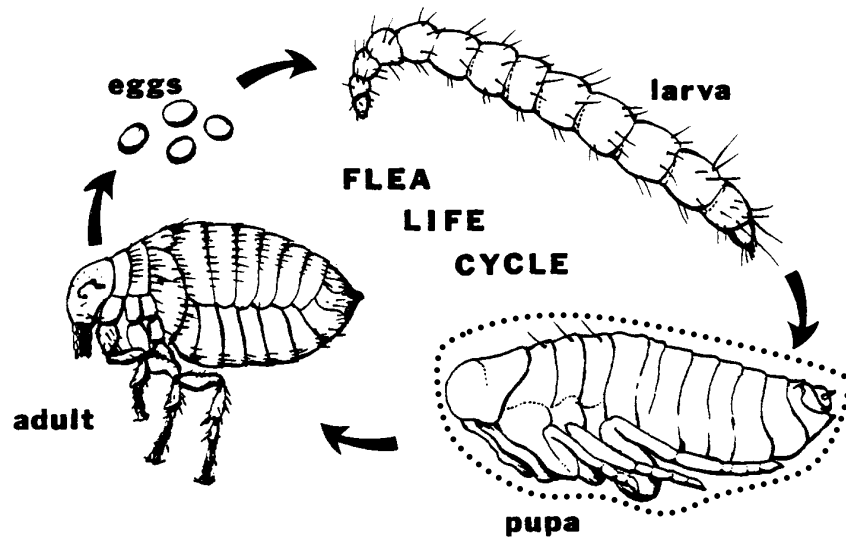
Figure 3 Flea eggs and larvae develop in prairie dog nests, which are lined by vegetative debris and soils (Wilcomb 1954, Sheets et al. 1971). Flea larvae crawl through soils, therein feeding on organic debris. At the last instar stage, larvae encase themselves in a silken cocoon reinforced by surrounding soils, thus transitioning to the pupae stage (Krasnov 2008). Flea pupae develop within these substrate-bounded cocoons and later emerge as adults that spend some of their time on hosts to acquire blood meals (and/or mate) but other time off hosts, in association with soils in host nests (Krasnov 2008). Image modified from: http://www.fleabusters-rxforfleas.com/.
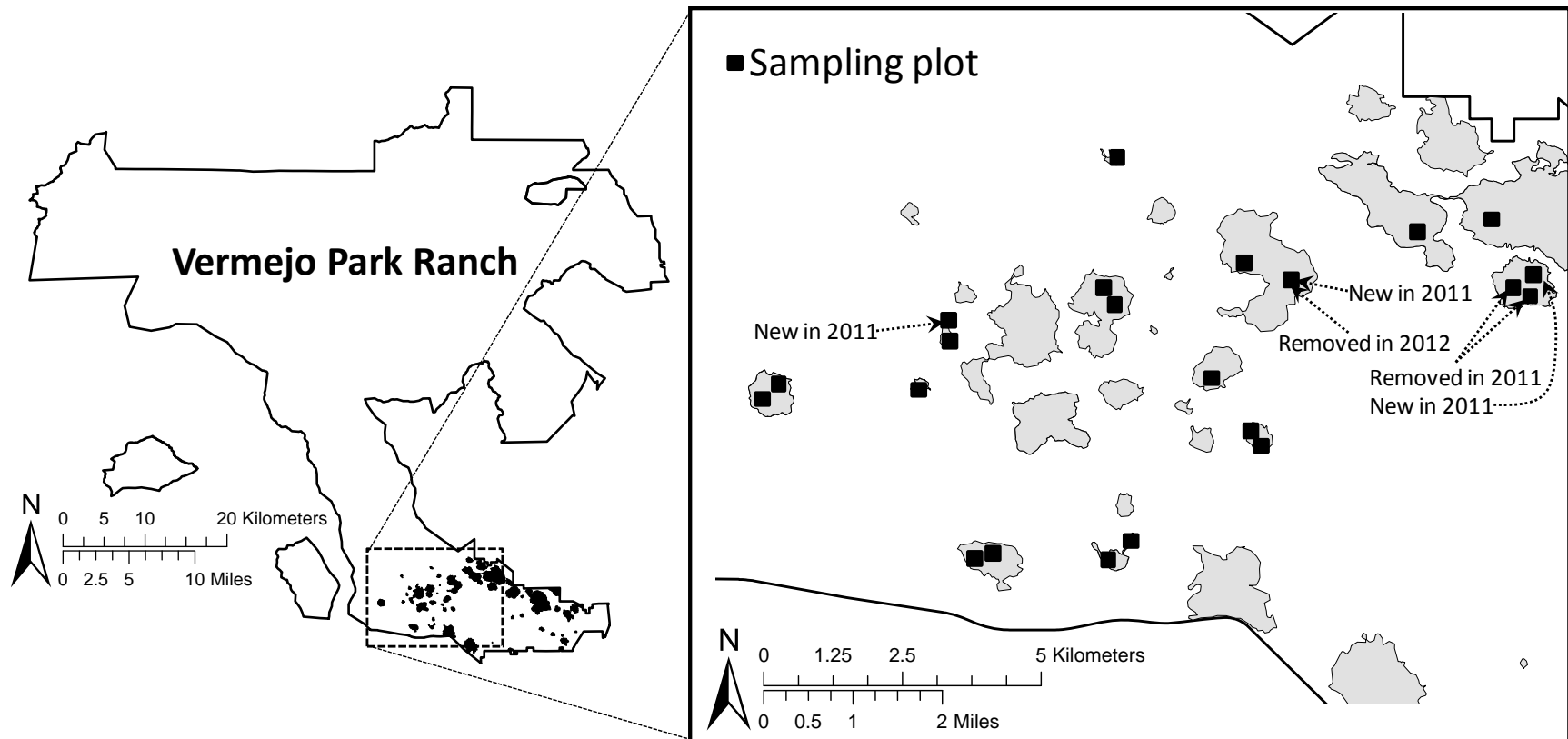
Figure 4 We studied black-tailed prairie dogs (*Cynomys ludovicianus*) and their fleas in the Vermejo Park Ranch, Colfax County, New Mexico, USA, during June–August, 2010–2012. Vermejo is a 240,000 ha ranch managed by Turner Enterprises Incorporated. On the right, prairie dog colonies are shaded gray.
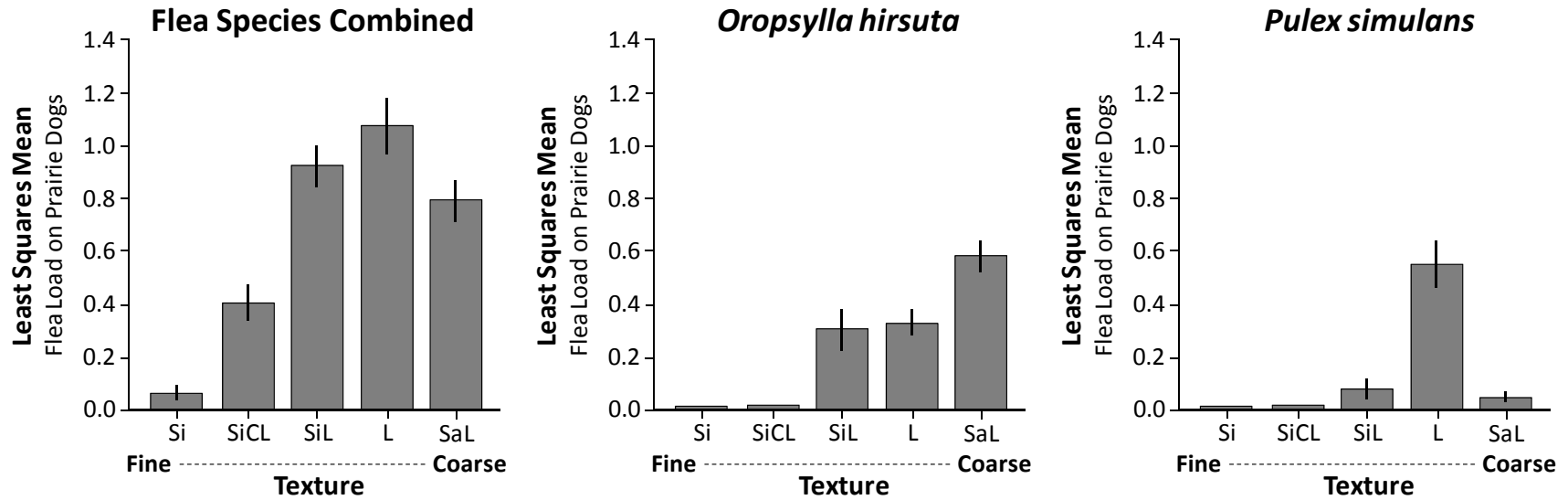
Figure 5 Least squares mean values from multivariate analyses of flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*) in areas with surface-soils of differing texture during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. Least squares mean values are presented to control for other variables in generalized linear mixed models. Si = silt, SiCL = silt-clay-loam, SiL = silt-loam, L = loam, SaL = sandy-loam.

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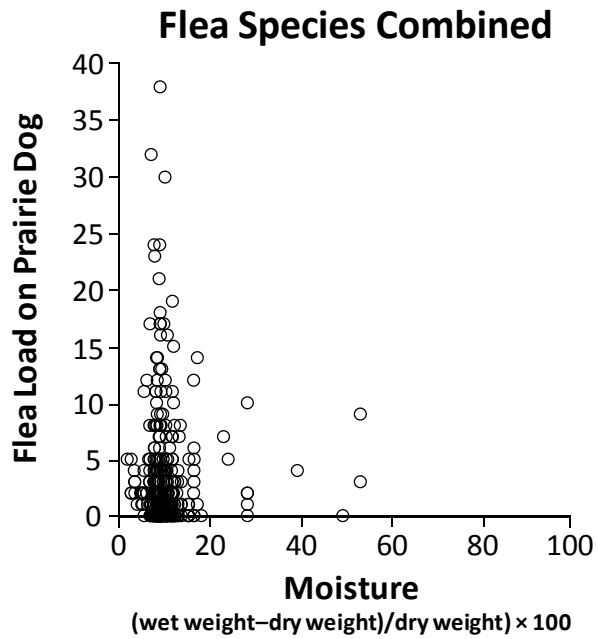
Figure 6 Abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) in areas with subsurface-soils that differed in moisture-content during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA.

Flea Species Combined
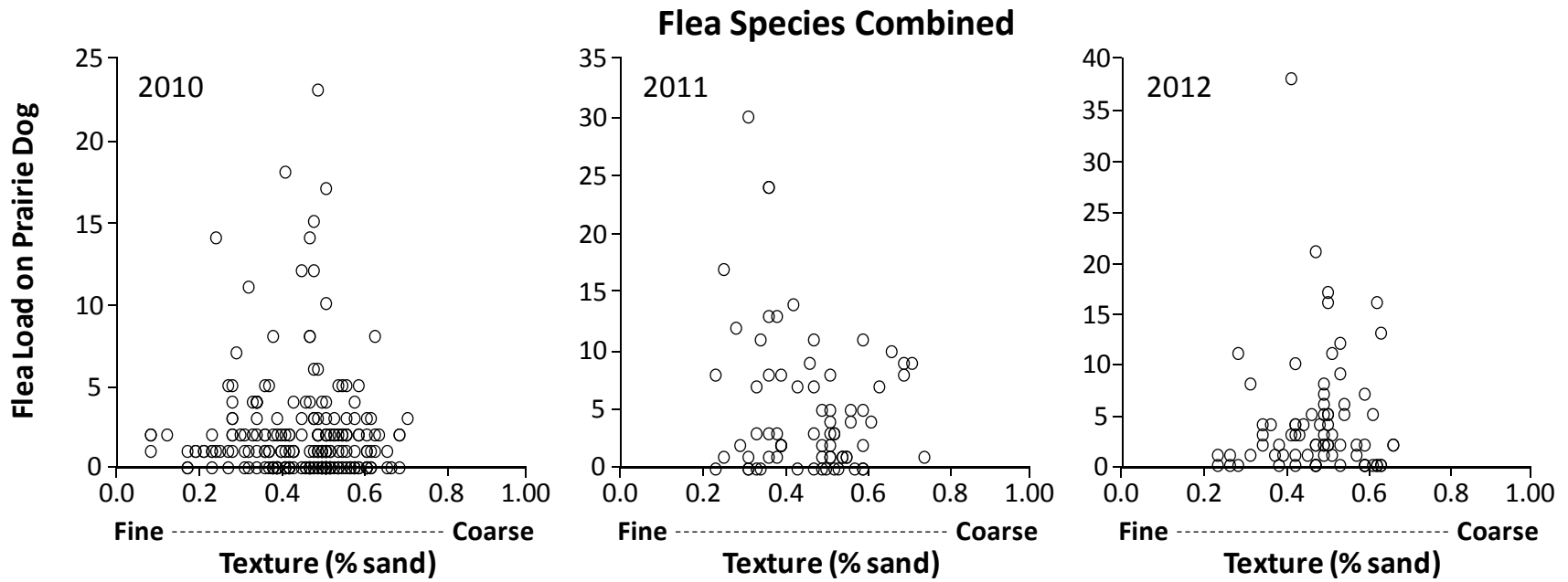
Figure 7 Abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012, in areas with subsurface-soils that differed in texture at the Vermejo Park Ranch, New Mexico, USA. Texture was expressed as the percentage of sand particles in soil samples.
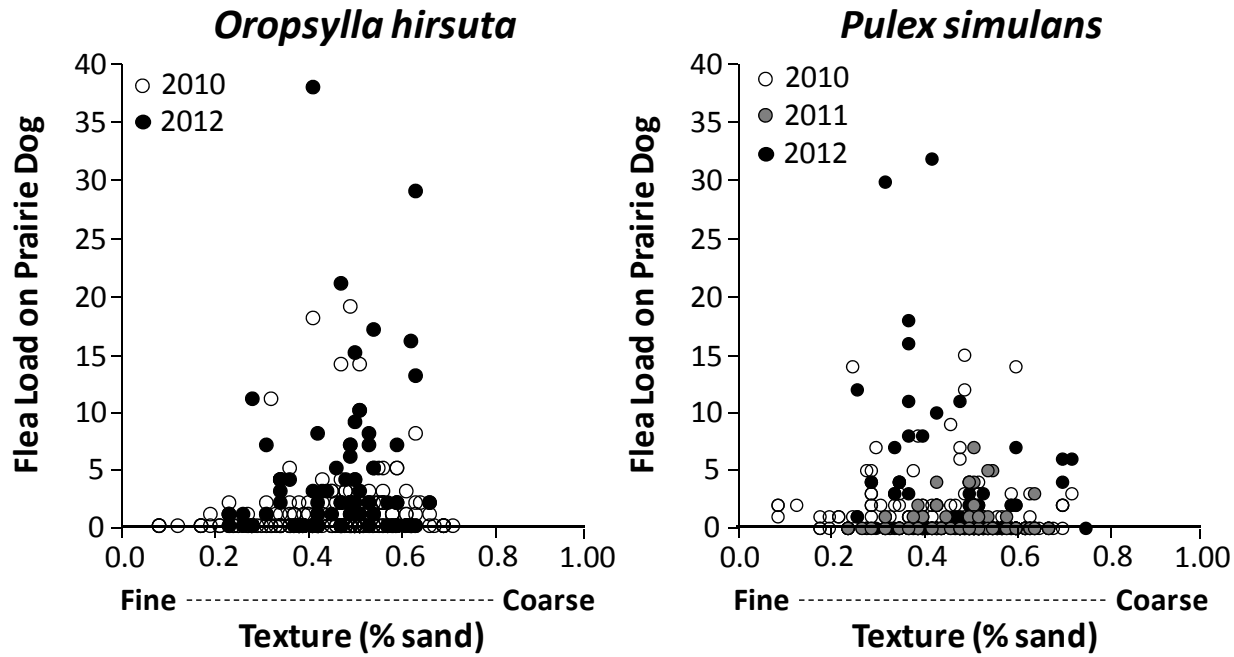
Figure 8 Abundance of adult *Oropsylla hirsuta* and *Pulex simulans* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012, in areas with subsurface-soils that differed in texture at the Vermejo Park Ranch, New Mexico, USA. Texture was expressed as the percentage of sand particles in soil samples.
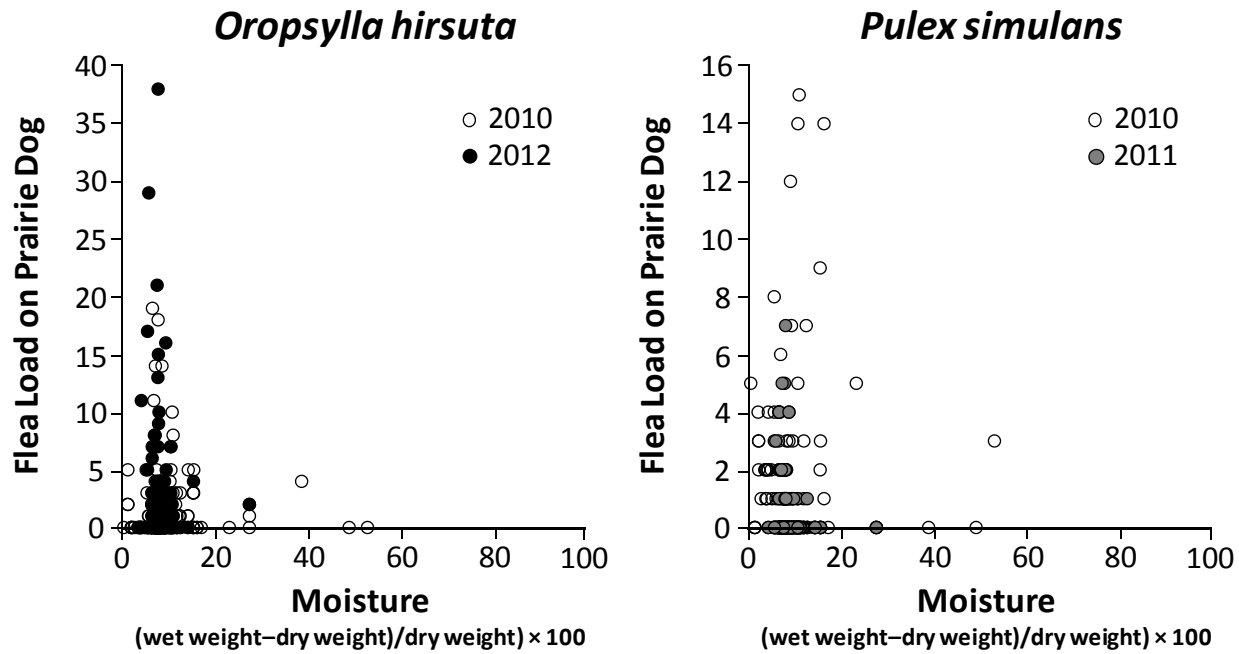
Figure 9 Abundance of adult *Oropsylla hirsuta* (2010 and 2012) and *Pulex simulans* (2010 and 2011) on black-tailed prairie dogs

(*Cynomys ludovicianus*) in areas with subsurface-soils that differed in moisture (water content) during June–August, 2010–2012, at

the Vermejo Park Ranch, New Mexico, USA.

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CHAPTER THREE

FLEAS ARE MOST ABUNDANT IN OLDER PRAIRIE DOG COLONIES:

EVIDENCE FROM BLACK-TAILED PRAIRIE DOGS IN NEW MEXICO

Plague is a rodent-associated disease caused by the primarily flea-borne bacterium *Yersinia pestis*. An increased understanding of flea ecology[1] is needed because rates of plague transmission can be heightened if fleas are abundant, and *Y. pestis* may persist in flea populations between outbreaks. We compared flea-loads on black-tailed prairie dogs (*Cynomys ludovicianus*) occupying old and young colonies in Vermejo Park Ranch, New Mexico, USA, June–August 2010–2012. Fleas were 110% more abundant in old colonies and their abundance was positively correlated with the number of years since a colony was established. Fleas may accumulate to high densities in old colonies because prairie dogs have created deep burrows there, and deep burrows provide ectothermic fleas with humid microclimates and stable temperatures. In addition, old burrows, long occupied by prairie dogs, presumably contain an accumulation of organic matter upon which flea larvae feed. Our results suggest plague may commonly degrade prairie dog populations in old colonies, thereby inhibiting these rodents from serving their historic, ecologic functions as keystone species. Protection of large, high-density prairie dog populations with insecticides, vaccines, and forthcoming mitigation tools may remedy this and other conservation dilemmas caused by plague.

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[1] Ecology: used herein as a general term for the study of factors that affect the abundance and dispersion of wildlife (*sensu* Krebs, C.J. 2009. Ecology: The Experimental Analysis of Distribution and Abundance, sixth edition. Benjamin Cummings, San Francisco).

Introduction

Animals are often highly susceptible to introduced pathogens with which they have no evolutionary history (Dobson and Hudson 1986) and, in some cases, introduced diseases can distort trophic relationships and negatively affect a plethora of species (Jones et al. 2008, Estes et al. 2011, Selakovic et al. 2014). There is an urgent need to acquire an increased understanding of introduced diseases so appropriate mitigation strategies can be identified and implemented in a proactive manner (Thomas et al. 2005).

Plague is a primarily flea-borne zoonosis caused by the gram-negative bacterium *Yersinia pestis*. The bacterium originated about 1,500–20,000 years ago in Asia but has been transported by humans to multiple additional continents and islands where it dramatically influences populations of a wide array of rodents (Achtman et al. 1999, Gage and Kosoy 2005, 2006). In turn, it alters competitive relationships and actively transforms ecosystems (Biggins and Kosoy 2001a).

In western North America, plague poses a significant risk to prairie dogs (*Cynomys* spp.), colonial rodents that structure grassland ecosystems as keystone species (Kotliar et al. 2006, Davidson et al. 2010). *Y. pestis* was introduced to US Pacific ports around the year 1900 and spread eastward >2,200 km in 40 years (Adjemian et al. 2007), reaching prairie dogs by the 1930s (Eskey and Haas 1940). Since, plague has converted what were once large, high-density prairie dog populations into metapopulations of reduced density that are incapable of serving their historic, ecologic functions (Biggins and Kosoy 2001a, b, Antolin et al. 2002, Cully et al. 2006, 2010).

In ravaging prairie dog populations, plague reduces the availability of habitat for, and directly kills black-footed ferrets (*Mustela nigripes*), endangered predators of prairie dogs (Godbey et al. 2006, Matchett et al. 2010). In addition, plague reduces the availability of habitat for birds that prefer to nest on prairie dog colonies, including burrowing owls (*Athene cunicularia*) and mountain plovers (*Charadrius montanus*), both of which are increasingly rare (Desmond et al. 2000, Dinsmore and Smith 2010). As a result, plague is receiving increased attention from the conservation community (Biggins and Kosoy 2001a, Biggins et al. 2010, Dinsmore and Smith 2010, Matchett et al. 2010).

There is an urgent need for research that identifies ways in which mitigation efforts can be strategized to manage plague (Jachowski et al. 2011). In particular, there is a need for studies on fleas, the arthropod vectors of *Y. pestis.* Vector efficiency is often <25% and, therefore, an abundance of fleas is usually needed to infect a host (Lorange et al. 2005, Eisen et al. 2009). If mitigation strategies are first targeted in areas where fleas are abundant, perhaps before fleas attain their highest densities (Wilder et al. 2008), it may be possible to reduce the ability of *Y. pestis* to persist and spread in prairie dog colonies.

Many factors can affect flea densities, but the ages of prairie dog colonies could be particularly important. For instance, at least initially, fleas should be scarce in newly established colonies because the founding prairie dogs may have brought few fleas with them (Jones and Britten 2010, Brinkerhoff et al. 2011). As a colony ages, however, flea populations may take advantage of improved habitats that could result from increased burrow depths and accumulations of organic matter, thus producing differences in flea densities among old and young prairie dog colonies.

Fleas can also exhibit seasonal differences in abundance and, in some cases, the incidence of plague increases during seasons when fleas attain their highest densities (Hirst 1927, Pauli et al. 2006, Tripp et al. 2009). Thus, in addition to evaluating spatial variation in the abundance of fleas among old and young prairie dog colonies, it is important to evaluate the phenology of flea abundance (Wilder et al. 2008, Eisen et al. 2009, Hubbart et al. 2011).

It is also important to determine if certain age-sex classes of hosts harbor the largest flea-loads, because those particular animals may play an important role in spreading infectious fleas to susceptible hosts. For instance, Tripp et al. (2009) found relatively large flea-loads on adult male prairie dogs and noted that when protecting their harem-territories, adult males commonly interact with intruders at contested territory boundaries (Hoogland 1995), perhaps facilitating the spread of infectious fleas in colonies.

With the above information in mind, we collected adult fleas from black-tailed prairie dogs (*C. ludovicianus*) in New Mexico, USA and posited the following:

(1) We hypothesized that fleas would be most abundant in old colonies, long occupied by prairie dogs. For colonies with known ages, we posited that flea-loads on prairie dogs would positively correlate with the number of years since the colonies were established.

(2) Within our sampling periods (June-August), we expected to find relatively large flea-loads on prairie dogs during July, a pattern noted in Colorado (Tripp et al. 2009).

(3) We expected to find larger flea-loads on adult prairie dogs, relative to juveniles, another pattern noted in Colorado (Tripp et al. 2009, Jachowski et al. 2011).

Materials and Methods

Study Species, Site, and Sampling Plots

Black-tailed prairie dogs create burrows that provide them, and many animals, with refuge from inclement weather, aerial predators, and terrestrial carnivores (King 1984). Nesting-chambers are often created 2 m underground, and fleas are thought to use the chambers as breeding and nesting sites (Sheets et al. 1971).[2]

We live-trapped black-tailed prairie dogs (hereafter "prairie dogs") during June-August, 2010-2012 in a complex of colonies situated in the southeastern portion of Vermejo Park Ranch, a site managed by Turner Enterprises Incorporated (hereafter "Vermejo"). The Vermejo short-grass steppe is characterized by gently rolling hills and arroyos, and is dominated by blue grama (*Bouteloua gracilis*) and dispersed patches of cholla cacti (*Cylindropuntia imbricata*).

Prairie dogs were captured in 13 colonies each established naturally by prairie dogs or via translocation by biologists (Fig. 10; *n* = 6 natural, 7 translocation). Translocations were initiated using deltamethrin, an insecticide that can kill fleas (DeltaDust®; Seery et al. 2003, Long et al. 2006). The natural colonies were never treated with an insecticide. We accounted for these differing histories when analyzing data.

Like Roach et al. (2001), Augustine et al. (2008), and Hartley et al. (2009), we classified each of the colonies as either old or young. Repeated surveys of Vermejo allowed us to categorize old colonies as those that originated 8 or more years before the start of our study (maximum = 11 years) and young colonies as those that originated 6 or fewer years before the

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[2] During excavations of 18 prairie dog burrows in South Dakota, Sheets et al. (1971) found immature stages of fleas in all nesting chambers, but did not report finding fleas elsewhere in the burrows.

study (minimum = 3 years; D.L. Long, Turner Endangered Species Fund, unpublished data). The exact age was known for five natural colonies, providing a subset of data that were used in a separate analysis ($n = 8$ plots in colonies 7–12 years-old at the start of our study; Fig. 10).

Sampling plots were 1.54 or 2.25 ha in size and each contained 25 or 37 live-traps ($16 \times \text{ha}^{-1}$) distributed among active burrows with fresh prairie dog scat (Tomahawk single-door live traps, Hazelhurst, Wisconsin, USA). Each year, we categorized the plots into five groups of 2–5 plots and sequentially sampled the groups in randomized order during 10-day work periods.

We did not observe rapid die-offs of prairie dogs, which are indicative of plague epizootics (Cully et al. 2006). Therefore, we suggest plague epizootics did not occur in prairie dogs within our study areas. Fieldwork was completed under Colorado State University Institutional Animal Care and Use Committee Protocol #10-1785A.

Trapping Prairie Dogs and Sampling Fleas

Trapping and sampling methods are described in Eads et al. (2013).[3] Briefly, we live-trapped prairie dogs; aged and sexed them; weighed them; anesthetized them and fleas on their bodies using isoflurane; combed them as thoroughly as possible for 45 seconds to collect and count fleas; marked their ears with unique tags for permanent identification; and measured the length of their right hind foot (Hoogland 1995, Biggins et al. 2010, Martin et al. 2013). Prairie dogs were categorized into four age-sex categories (Biggins et al. 2010). We released each prairie dog at its trapping location, where we recorded Universal Transverse Mercator (UTM) coordinates as spatial reference points.

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[3] Chapter 5 in this dissertation.

Fleas were identified to species using a Leica Microsystems (Buffalo Grove, Illinois) WILD M3Z stereo zoom microscope and keys in Stark (1958) and Hubbard (1968). Two flea species were most prevalent and their abundances are analyzed collectively and separately herein: *Oropsylla hirsuta* (a prairie dog specialist[4]) and *Pulex simulans* (a generalist). All male *Pulex* were identified as *P. simulans*, so all *Pulex* were assumed to be *P. simulans* (Tripp et al. 2009).

Data Analyses

We fit multivariable models that related the number of fleas including zero counts (abundance, Bush et al. 1997) to covariates. Results remained the same if flea-loads were expressed as the number of fleas per unit of a host's body surface to control for host size (mass$^{-0.67}$; Heusner 1985). Maximum likelihood estimates of the negative binomial parameter k for flea abundance were always below 1.00 ('theta.ml' package, Program R version 2.13.2, R Development Core Team 2011), so we used the negative binomial family for aggregated count data (Wilson et al. 2002).

Detection of fleas is likely to be imperfect (Chapters 5 and 6), especially when considering detection of individual fleas. Consequently, our data on flea abundance should be considered indices. Because we standardized our sampling methods, we assume the data can be used for relative comparisons of flea abundance between old and young colonies, and among months and age-sex classes of hosts.

We fit generalized linear mixed models (GLMMs) to account for the nested-nature of our data ('PROC GLIMMIX,' SAS® 9.3, SAS Institute Inc.). Inclusion of a random effect for

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[4] Results from this study should prove applicable not only to prairie dogs, but also to black-footed ferrets. These endangered carnivores commonly carry *O. hirsuta* (Harris et al. 2014).

"Prairie Dog ID" never increased fit, suggesting little repeatability of flea abundance within individual prairie dogs[5] (e.g., Krasnov et al. 2006). Therefore, we did not include "Prairie Dog ID" as a random effect. We included "Plot ID" as a random effect, however, to account for repeated samples from prairie dogs living in the same plots, where habitat characteristics may have affected flea densities.

During other analyses with our data, flea abundance correlated with the texture of surface-soils, densities of prairie dogs (minimum number alive, divided by plot area; Krebs 1996), and body condition indices for hosts (mass:foot ratio; Chapters 2 and 4, Eads et al. 2013). Thus, all GLMMs included these variables as controls.

We also included a binomial variable to account for the potential effect of insecticides in translocation colonies (distributed 4–11 years before the start of our study). The variable gained little support in the models for flea abundance (suggesting the effect of the insecticide had waned) but the variable was retained in all models as a precautionary measure (see Chapter 5 for a discussion of insecticides and flea prevalence/occupancy).

Year-to-year changes in flea densities were evident (e.g., Chapter 4), so we separated the GLMM analysis by year. Main-effects of interest included colony-age (COLONY-AGE), month-of-sampling (MONTH), and age-sex class of host (AGE-SEX). We ran all possible models for each yearly dataset, including a model with the control covariates alone ($n = 8$ models). We ranked the models by Akaike's Information Criterion adjusted for sample size (AICc) and calculated AICc differences (ΔAICc) and AICc weights (w_i; Burnham and Anderson 2002). Likelihood ratio tests ($\alpha = 0.100$; McCullagh and Nelder 1989) were used to compare

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[5] Similar results have been obtained during studies of black-tailed prairie dogs in Colorado (M.F. Antolin, personal communication).

nested submodels with more general models (differences would suggest the more complex model was useful).

We used a subset of data from the five natural colonies of known age (Fig. 10) to investigate the hypothesis that flea-loads would positively correlate with the number of years since a colony was established. In this case, instead than transforming colony-age into a binomial variable of old/young, colony-age was expressed in years (YEARS-SINCE-COLONIZATION). For each year separately, we fit a negative binomial GLMM with the YEARS-SINCE-COLONIZATION main-effect and control variables, and then used likelihood ratio tests to determine if YEARS-SINCE-COLONIZATION increased fit.

Moran's I analyses (Moran 1950) with residuals (Cliff and Ord 1981) from interpreted GLMMs suggested the "Plot ID" random effect accounted for spatial autocorrelation in the data. The 95% confidence interval of interpreted coefficients never encompassed zero. Goodness-of-fit X^2 statistics for the most general models were ~1.00, suggesting good model fit (Burnham and Anderson 2002).

Results

Flea Species Combined

The 2010 dataset included 603 captures of 311 prairie dogs. We collected 1,338 fleas ($\bar{x} = 2.22$, $SD = 3.00$) which were aggregated among hosts ($k = 0.79$). In the GLMM exercise, the most supported model (Table 6, Model 1) gained 100% of Akaike weight and included main-effects for MONTH ($X^2_2 = 19.59$, $P < 0.001$) and AGE-SEX ($X^2_3 = 17.95$, $P < 0.001$). Fleas

were least abundant in June, most abundant in July, and intermediately abundant in August (Fig. 11). Differences among AGE-SEX classes primarily reflected differences among adults and juveniles. Adults carried about one more flea than juveniles did, on average.

The 2011 dataset included 545 captures of 287 prairie dogs. We collected 4,493 fleas ($\bar{x} =$ 8.24, $SD = 10.88$, $k = 0.64$). The most supported model gained 100% of Akaike weight (Table 6, Model 3) and contained effects for COLONY-AGE ($X^2_1 = 35.93$, $P < 0.001$), MONTH ($X^2_2 = 105.47$, $P < 0.001$) and AGE-SEX ($X^2_3 = 18.95$, $P < 0.001$). Prairie dogs in the old colonies carried about 7.5 more fleas than prairie dogs in the young colonies (Fig. 12). Fleas were least abundant in June, increased to maximum abundance in July, and declined to intermediate abundance in August (Fig. 11). Differences in flea loads on adult males and females were slight; sample sizes were limited for juveniles.[6]

The 2012 dataset included 554 captures of 268 prairie dogs. We collected 2,529 fleas ($\bar{x} =$ 5.00, $SD = 6.55$, $k = 0.77$). The most supported model contained all main-effects, and each of the effects increased fit (Table 6, Model 4; COLONY-AGE: $X^2_1 = 4.23$, $P = 0.040$; MONTH: $X^2_2 = 10.42$, $P = 0.005$; AGE-SEX: $X^2_3 = 17.55$, $P = 0.001$). Prairie dogs in the old colonies carried about 2.5 more fleas than prairie dogs in the young colonies (Fig. 12). Fleas were least abundant in June, increased to maximum abundance in July, and declined to intermediate abundance in August (Fig. 11). Adult males carried about 1.5 more fleas than adult females, and adults carried about 2.5 more fleas than juveniles did.

Considering patterns for the young colonies, where prairie dogs may have been extending their burrows deeper belowground as our study progressed, flea abundance tended to increase year-by-year during 2010–2012 (Fig. 12). In contrast, in the old colonies that presumably contained deep burrows at the start of our study, flea abundance peaked in 2011 and declined in

[6] Very few juveniles were captured in 2011, the driest spring-summer on record for New Mexico.

2012, reflecting a temporal pattern of variation that may relate to weather conditions during our study (Chapter 4).

The subset of data from the five natural colonies of known age included 711 captures of 292 prairie dogs ($n = 4{,}924$, $\bar{x} = 6.93$, $SD = 10.17$, $k = 0.55$). The YEARS-SINCE-COLONIZATION variable increased fit for all 3 years of study (2010: $X^2_1 = 6.11$, $P = 0.013$; 2011: $X^2_1 = 9.11$, $P = 0.011$; 2012: $X^2_1 = 2.84$, $P = 0.092$). During each field season, flea abundance was positively correlated with YEARS-SINCE-COLONIZATION. Fleas were consistently most abundant in the oldest colonies (Fig. 13).

Analyses for Oropsylla hirsuta

In 2010, we collected 698 *O. hirusta* ($\bar{x} = 1.16$, $SD = 2.20$, $k = 0.41$). The most supported model (Table 7, Model 1) included main-effects for COLONY-AGE ($X^2_1 = 37.37$, $P < 0.001$) and AGE-SEX ($X^2_3 = 13.27$, $P = 0.004$). Inclusion of MONTH in Model 2 did not substantially increase fit ($X^2_2 = 3.16$, $P = 0.206$) so we interpreted Model 1 (Table 7). Prairie dogs in the new colonies carried about one more *O. hirsuta*, on average, than prairie dogs in the old colonies (Fig. 12). Differences among AGE-SEX classes primarily reflected differences among adults and juveniles. Adults carried about one more *O. hirsuta* than juveniles did.

In 2011, we collected 1,958 *O. hirusta* ($\bar{x} = 3.59$, $SD = 5.24$, $k = 0.65$). The most supported model (Table 7, Model 4) included main-effects for MONTH ($X^2_2 = 64.00$, $P < 0.001$) and AGE-SEX ($X^2_3 = 11.87$, $P = 0.008$). Though *O. hirsuta* tended to be more abundant in old colonies (Fig. 12), inclusion of COLONY-AGE (Table 7, Model 5) did not increase fit ($X^2_1 = 0.37$, $P = 0.543$) so we interpreted Model 4. *O. hirsuta* were least abundant in June, most abundant in July,

and intermediately abundant in August (Fig. 13). Differences in flea loads on adult males and females were slight.

In 2012, we collected 1,806 *O. hirusta* ($\bar{x} = 3.26$, $SD = 5.95$, $k = 0.58$). The most supported model (Table 7, Model 8) included main-effects for MONTH ($X^2_2 = 15.33$, $P < 0.001$) and AGE-SEX ($X^2_3 = 23.65$, $P < 0.001$). *O. hirsuta* tended to be more abundant in old colonies (Fig. 12) but inclusion of COLONY-AGE (Table 7; Model 9) did not increase fit ($X^2_1 = 0.08$, $P = 0.777$) so we interpreted Model 8. *O. hirsuta* were least abundant in June, most abundant in July, and intermediately abundant in August (Fig. 13). Adults carried about four more *O. hirsuta* than juveniles did, on average, and adult males carried about two more *O. hirsuta* than adult females.

With the subset of data for *O. hirsuta* from the five natural colonies of known age ($n = 2,457$, $\bar{x} = 3.46$, $SD = 5.89$, $k = 0.43$), the YEARS-SINCE-COLONIZATION variable increased fit for 2010 ($X^2_1 = 4.22$, $P = 0.040$) and 2011 ($X^2_1 = 3.43$, $P = 0.064$). The correlation was positive for 2012, but not supported by a likelihood ratio test ($X^2_1 = 0.17$, $P = 0.680$). During 2010 and 2011, abundance of *O. hirsuta* was positively correlated with YEARS-SINCE-COLONIZATION.

Analyses for Pulex simulans

We collected 640 *P. simulans* in 2010 ($\bar{x} = 1.06$, $SD = 2.21$, $k = 0.22$). The most supported model (Table 8, Model 1) included main-effects for COLONY-AGE ($X^2_1 = 88.87$, $P < 0.001$), MONTH ($X^2_2 = 25.66$, $P < 0.001$), and AGE-SEX ($X^2_3 = 14.55$, $P = 0.002$). Prairie dogs in the old colonies carried about one more *P. simulans* than prairie dogs in the new colonies (Fig. 12). *P. simulans* was intermediately abundant in June, most abundant in July, and least abundant in

August (Fig. 13). Differences among AGE-SEX classes primarily reflected differences among adults and juveniles. Adults carried about one more *P. simulans* than juveniles did.

In 2011, we collected 2,535 *P. simulans* ($\bar{x} = 4.65$, $SD = 7.83$, $k = 0.27$). The most supported model (Table 8, Model 3) included main-effects for COLONY-AGE ($X^2_1 = 92.54$, $P < 0.001$), MONTH ($X^2_2 = 91.15$, $P < 0.001$), and AGE-SEX ($X^2_3 = 7.43$, $P = 0.060$). Prairie dogs in the old colonies carried about six more *P. simulans* than prairie dogs in the new colonies (Fig. 12). *P. simulans* was intermediately abundant in June, most abundant in July, and least abundant in August (Fig. 13). Adults carried about four more *P. simulans* than juveniles did.

In 2012, we collected 571 *P. simulans* ($\bar{x} = 1.03$, $SD = 1.96$, $k = 0.35$). The most supported model (Table 8, Model 5) included main-effects for COLONY-AGE ($X^2_1 = 16.42$, $P < 0.001$) and MONTH ($X^2_2 = 45.71$, $P < 0.001$). Inclusion of AGE-SEX (Table 8, Model 6) did not increase fit ($X^2_3 = 3.37$, $P = 0.338$) so we interpreted Model 5. Prairie dogs in the old colonies carried about two more *P. simulans* than prairie dogs in the new colonies (Fig. 12). *P. simulans* was least abundant in June, most abundant in July, and intermediately abundant in August (Fig. 13).

With the subset of data for *P. simulans* from the five natural colonies of known age (2,467 fleas, $\bar{x} = 3.47$, $SD = 6.76$, $k = 0.25$), the YEARS-SINCE-COLONIZATION variable increased fit for 2010 ($X^2_1 = 4.69$, $P = 0.030$) and 2012 ($X^2_1 = 3.26$, $P = 0.071$), but not 2011 ($X^2_1 = 0.00$, $P = 1.000$). During 2010 and 2012, the abundance of *P. simulans* was positively correlated with YEARS-SINCE-COLONIZATION.

Discussion

Numbers of fleas found on the age-sex classes of prairie dogs were similar. Any differences were slight, often involving differences of only a few fleas, on average. Therefore, we refrain from discussing host age and sex. Potential explanations for differences in flea-loads on adult and juvenile rodents have been discussed elsewhere (Krasnov 2008; for a discussion on prairie dogs, see Brinkerhoff et al. 2006, Tripp et al. 2009).

O. hirsuta and *P. simulans* exhibited somewhat differing phonologies during June-August (Fig. 11). We suspect that the phenologies reflect seasonal patterns of reproduction by each species, and species-specific responses to temporal variation in temperature and humidity, for instance as suggested for fleas parasitizing California ground squirrels (*Otospermophilus beecheyi*; review in Hubbart et al. 2011). Additional study is needed on these topics, however.

P. simulans was most abundant in old colonies during all three years of study, and *O. hirsuta* was most abundant in old colonies during the first year (during the second and third year, *O. hirsuta* became more widely dispersed, which may have clouded differences between old and young colonies for that species; Chapter 4). Overall flea abundance tended to increase year-by-year in the young colonies. Moreover, with the data restricted to colonies of known age, the abundance of both flea species tended to correlate positively with the number of years since the sites were colonized by prairie dogs.

Fleas might be most abundant in old prairie dog colonies simply because they require time to migrate and establish populations in new areas. However, at least three additional, non-exclusive factors could help to explain why fleas tended to be most abundant in old colonies, including

temporal changes in (1) the depths of burrows, (2) the amount of organic matter in burrows, and (3) the composition of host species.

The depth of rodent burrows tends to increase over time, suggesting that, during our study, burrows in the old colonies would have been deeper than burrows in the young colonies (Longhurst 1944, Fitch 1948, Smith and Gardner 1985, Reichman and Smith 1990, Armitage 2003). Temperatures tend to be more stable, and humidity tends to be higher in deep burrows, which could benefit ectothermic fleas that are prone to desiccation (Schmidt-Nielsen 1964, Clark 1971, Smith 1982, Shenbrot et al. 2002, Krasnov 2008).

Burrows in old colonies, long occupied by prairie dogs, presumably contain a wealth of organic matter upon which flea larvae feed. Large amounts of prairie dog feces, flaked skin particles, and hair, as well as other organic matter such as dead insects or plant material (Wilcomb 1954, Sheets et al. 1971) of blood-containing feces from adult fleas (Sharif 1937, Silverman and Appel 1994) within burrows in old colonies might support considerable numbers of larval fleas, thereby boosting flea populations. Conversely, organic matter could be limited in young burrows within new colonies, thereby negatively affecting larvae due to food limitations and competition[7] (Krasnov 2008).

In reference to host composition, this topic seems most pertinent to the generalist *P. simulans*. The grazing and burrowing activities of prairie dogs create unique habitats that are attractive to many mammals, including rodents, lagomorphs, and carnivores (Kotliar et al. 2006, Lomolino and Smith 2004, Slobodchikoff et al. 2009). Many of these mammals are potential hosts for *P. simulans* (Hopla 1980, Brinkerhoff 2008). As prairie dog colonies age over time and

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[7] Larval fleas exhibit exploitative competition (for food) and interference competition (cannibalism), especially when organic matter is limited within host nests (Krasnov 2008).

various mammals use the sites, *P. simulans* may gain access to a plethora of hosts and, perhaps, increase in abundance.

The above hypothesis hinges on the assumption that host species diversity increases with the age of prairie dog colonies, thereby facilitating *P. simulans*. The diversity of mammals in prairie dog colonies might increase over time because (1) prairie dog densities could increase (up to some point), thus attracting carnivores, (3) burrow depths would increase, potentially attracting various rodents and lagomorphs that could benefit from inhabiting deep, complex burrows, and (3) vegetative communities in older colonies are characterized by forbs (Hartley et al. 2009) that are seemingly preferred forage (Hansen and Gold 1977) for *Sylvilagus* rabbits (that are commonly parasitized by *P. simulans*). Nonetheless, field studies are needed on this topic (experimentation will be required to account for the effects of plague on mammals).

Conservation Implications

Currently, the abundance of prairie dogs at many sites is so low that *Cynomys* cannot serve their historic, ecologic functions (Kotliar et al. 2006, McDonald et al. 2011, Miller and Reading 2012). Accumulating evidence suggests that preservation and restoration of prairie dog populations is impossible without plague management (Miller and Reading 2012).

Regarding preservation, biologists sometimes attempt to manage plague throughout large expanses of land, which is cost- and labor-intensive (Griebel 2009). Our results suggest preservation efforts could be strategized in relation to the ages of prairie dog colonies, for instance by targeting management actions in old colonies and then shifting to younger colonies. To do so, colonies must be monitored over time so their ages are known (e.g., Cully et al. 2010).

Regarding restoration, prairie dogs are sometimes translocated to inactive sites that contain old burrows. While the "best practical indicator of suitable habitat at potential release sites is visible evidence of previous occupancy by prairie dogs" (i.e., old burrows; Truett et al. 2001:865), our results suggest that old burrows provide ideal habitats for fleas and, thus, re-emergence of plague.

The above speculation may be supported by research from Rocky Mountain Arsenal, Colorado. Translocations of prairie dogs to plague-extirpated sites with old burrows were repeatedly unsuccessful unless insecticides were used to reduce flea densities (D.B. Seery, U.S. Fish and Wildlife Service, personal communication). Infectious fleas (Kartman et al. 1962, Bazanova and Maevskii 1996) and *Y. pestis*-contaminated organic matter (Baltazard 1964, Bazanova et al. 1997) might have persisted in the old, deep burrows, providing context for plague transmission (Eisen and Gage 2009, Wimsatt and Biggins 2009, Boegler et al. 2012).

The suitability of old, deep burrows for fleas (and perhaps *Y. pestis*) may also help to explain how plague transforms prairie dog populations into degraded metapopulations (Stapp et al. 2004, Antolin et al. 2006). When a colony is forced to extinction by plague or nearly so, prairie dogs sometimes recolonize or supplement the site, usually by re-excavating old burrows (Koford 1958:14).[8] Habitat patches with old, deep burrows may function as "population sinks," allowing fleas to attain high densities, thereby providing context for repeated epizootics.

Conversely, colonies or habitat patches with young burrows may function as "population sources." During early studies of plague, when small numbers of prairie dogs survived plague epizootics, survivors of a decimated colony were "individuals who pioneered new and less favorable habitat" away from the other prairie dogs (Barnes 1993:34). These peripheral areas

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[8] For instance, as observed in Pawnee National Grassland (M.F. Antolin, personal communication).

usually contain young burrows (Koford 1958). In some cases, perhaps prairie dogs survive in these areas because the burrows are young and, as a potential consequence, fleas are scarce.

Source-sink dynamics may allow for continued presence of *C. ludovicianus* in metapopulations, but the corresponding habitat fragmentation and continued oscillations in prairie dog numbers may inhibit these rodents from establishing long-term, high-density colonies (Hartley et al. 2009). This speculation suggests utility in translocations that help to establish new prairie dog colonies (see also Long et al. 2006).

Considering our results overall, the study suggests that plague may commonly degrade prairie dog populations in areas with old burrows, thereby inhibiting these rodents from reaching densities sufficient to serve their ecologic functions as keystone species and ecosystem engineers (see also McDonald et al. 2011). Insecticides (Seery et al. 2003), vaccines (Abott et al. 2012), translocations (Long et al. 2006), and forthcoming mitigation tools may remedy this and other conservation dilemmas caused by plague.

Table 6 Generalized linear mixed models investigating the abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012 at the Vermejo Park Ranch, New Mexico, USA. Main-effects included age-of-colony (COLONY-AGE), month-of-sampling (MONTH), and the age-sex class of prairie dogs (AGE-SEX). Each model contained four control variables (see text). A random effect was included in all models to account for repeated measurements from sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Only those models with $w_i > 0.00$ are included.

Year-of-Sampling	Model No.	Model	AICc	ΔAICc	w_i
2010	1	Controls + MONTH + AGE-SEX	2324.05	0	1.00
2011	3	Controls + COLONY-AGE + MONTH + AGE-SEX	3150.58	0	1.00
2012	4	Controls + COLONY-AGE + MONTH + AGE-SEX	2873.12	0	0.75
	5	Controls + MONTH + AGE-SEX	2875.86	2.74	0.19
	6	Controls + COLONY-AGE + AGE-SEX	2879.23	6.11	0.04
	7	Controls + AGE-SEX	2879.95	6.83	0.02

Table 7 Generalized linear mixed models investigating the abundance of adult *Oropsylla hirsuta* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012 at the Vermejo Park Ranch, New Mexico, USA. Main-effects included age-of-colony (COLONY-AGE), month-of-sampling (MONTH), and the age-sex class of prairie dogs (AGE-SEX). Each model contained four control variables (see text). A random effect was included in all models to account for repeated measurements from sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Only those models with $w_i > 0.00$ are included.

Year-of-Sampling	Model No.	Model	AICc	ΔAICc	w_i
2010	1	Controls + COLONY-AGE + AGE-SEX	1672.91	0	0.61
	2	Controls + COLONY-AGE + MONTH + AGE-SEX	1673.94	1.03	0.37
	3	Controls + COLONY-AGE	1679.95	7.04	0.02
2011	4	Controls + MONTH + AGE-SEX	2490.12	0	0.66
	5	Controls + COLONY-AGE + MONTH + AGE-SEX	2491.86	1.74	0.28
	6	Controls + MONTH	2495.71	5.59	0.04
	7	Controls + COLONY-AGE + MONTH	2497.12	7.00	0.02
2012	8	Controls + MONTH + AGE-SEX	2606.35	0	0.73
	9	Controls + COLONY-AGE + MONTH + AGE-SEX	2608.38	2.03	0.26

Table 8 Generalized linear mixed models investigating the abundance of adult *Pulex simulans* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012 at the Vermejo Park Ranch, New Mexico, USA. Main-effects included age-of-colony (COLONY-AGE), month-of-sampling (MONTH), and the age-sex class of prairie dogs (AGE-SEX). Each model contained four control variables (see text). A random effect was included in all models to account for repeated measurements from sampling plots. We ranked the models according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc) and model weights (w_i). Only those models with $w_i > 0.00$ are included.

Year-of-Sampling	Model No.	Model	AICc	ΔAICc	w_i
2010	1	Controls + COLONY-AGE + MONTH + AGE-SEX	1413.79	0	0.98
	2	Controls + COLONY-AGE + MONTH	1422.08	8.29	0.02
2011	3	Controls + COLONY-AGE + MONTH + AGE-SEX	2292.01	0	0.64
	4	Controls + COLONY-AGE + MONTH	2293.15	1.14	0.36
2012	5	Controls + COLONY-AGE + MONTH	1380.13	0	0.81
	6	Controls + COLONY-AGE + MONTH + AGE-SEX	1383.05	2.92	0.19
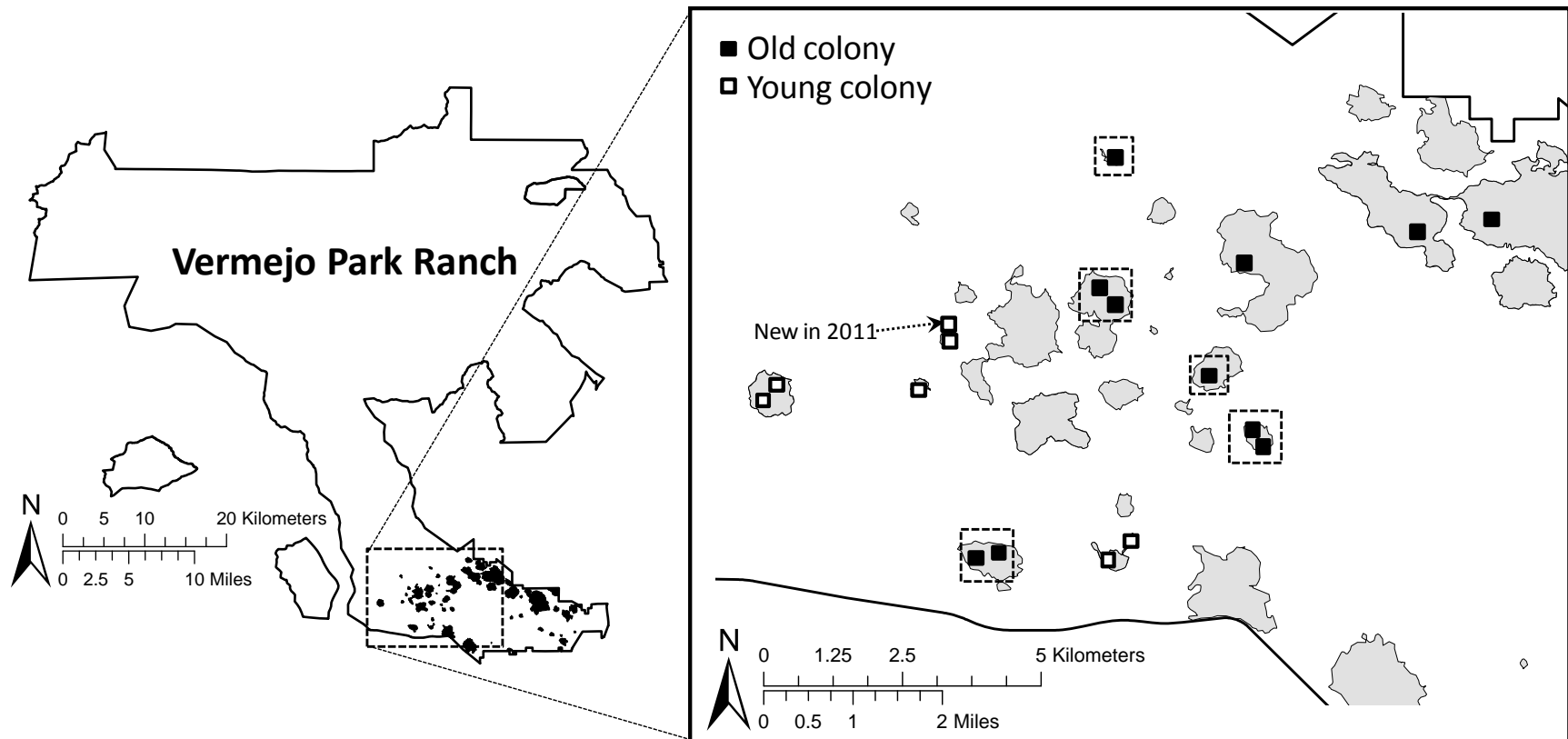
Figure 10 We trapped black-tailed prairie dogs (*Cynomys ludovicianus*) in old and young colonies in the Vermejo Park Ranch, New Mexico, USA during June–August, 2010–2012. On the right, prairie dog colonies are shaded gray. All of the plots were included in our assessment of flea-loads in old and young colonies. Plots encompassed by dashed squares correspond with natural colonies (with no history of insecticide treatment) of a known age in years; a subset of data from these eight plots was used in an analysis that excluded the other plots.
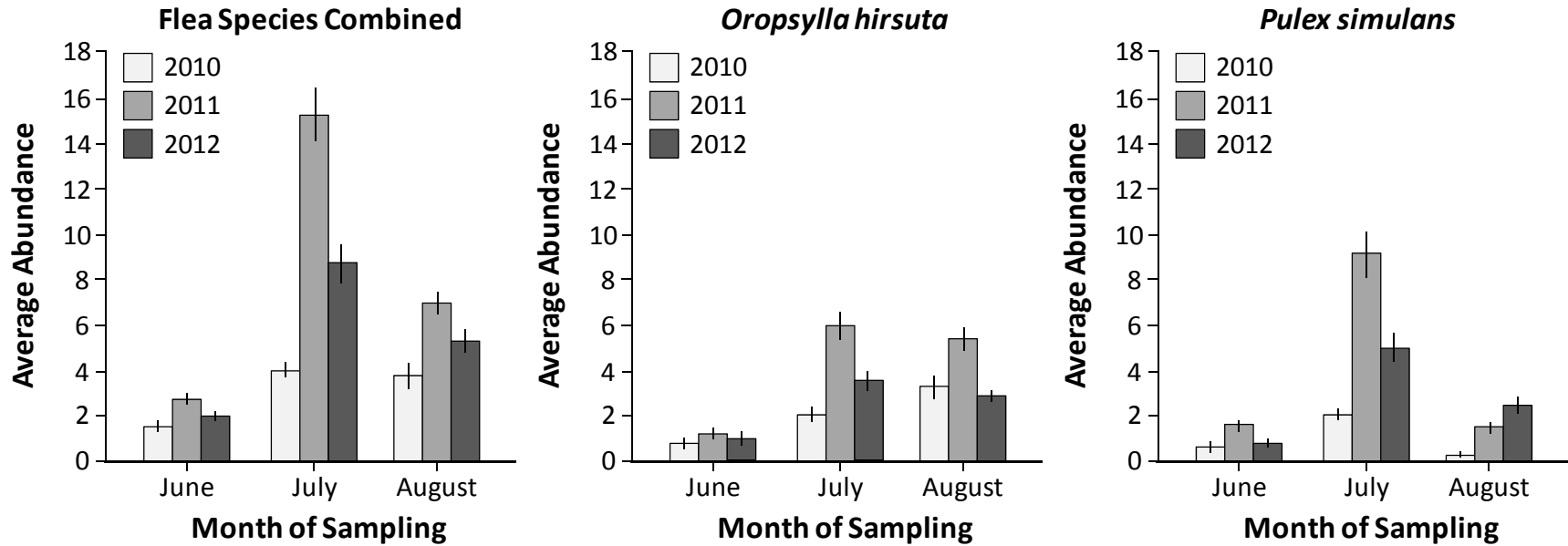
Figure 11 Average abundance of adult fleas (± 95% confidence intervals) on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. Data are presented for flea species combined, and *Oropsylla hirsuta* and *Pulex simulans* separately.
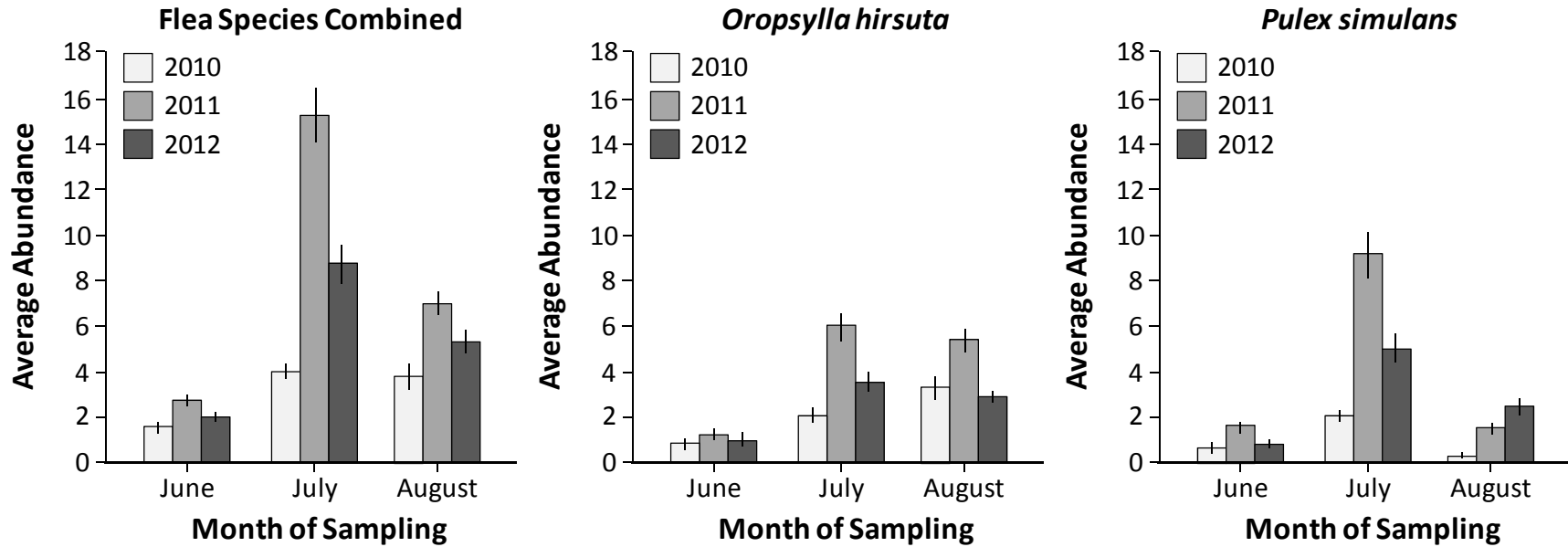
Figure 12 Average abundance of adult fleas (± 95% confidence intervals) on black-tailed prairie dogs (*Cynomys ludovicianus*) living in old and young colonies during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. Data are presented for flea species combined, and *Oropsylla hirsuta* and *Pulex simulans* separately.

Flea Species Combined
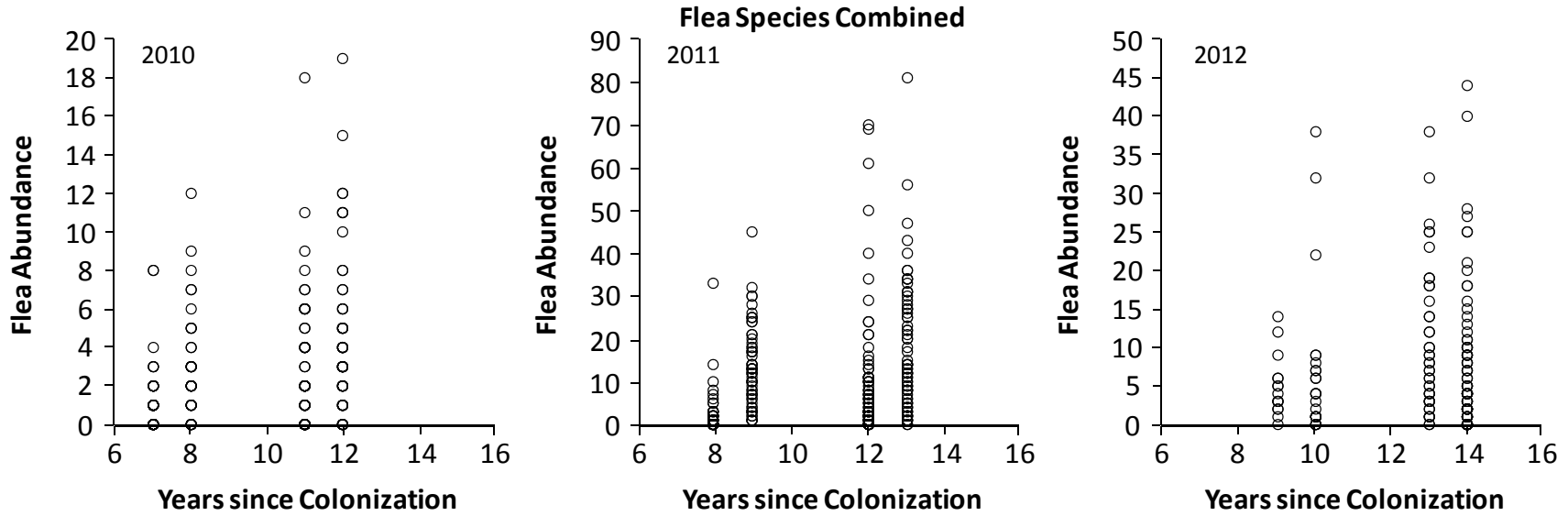
Figure 13 Adult flea abundance (species combined; ± 95% confidence intervals) on black-tailed prairie dogs (*Cynomys ludovicianus*) living in five natural colonies (with no history of insecticide treatment) during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. Two of the colonies were the same age (starting at 12 years-old in 2010).

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CHAPTER FOUR

DROUGHTS MAY INCREASE THE SUSCEPTABILITY OF PRAIRIE DOGS TO FLEAS

THAT VECTOR THE PLAGUE BACTERIUM *YERSINIA PESTIS*

Fleas are ectothermic arthropods that transmit the plague bacterium *Yersinia pestis* to mammalian hosts. An increased understanding of flea ecology[24] is needed because rates of plague transmission can be heightened if fleas are abundant. Fleas are highly susceptible to desiccation under hot-dry conditions and, as a result, it is commonly assumed their densities decline during droughts. We evaluated this assumption using data from black-tailed prairie dogs (*Cynomys ludovicianus*) at the Vermejo Park Ranch, New Mexico, USA, June–August 2010–2012. Precipitation was relatively plentiful in 2010 and 2012 but scarce during 2011, the driest spring-summer on record for New Mexico. Unexpectedly, fleas were 250% more abundant in 2011. During that dry 2011 field season, prairie dogs were in poor condition and devoted little time to grooming. In contrast, during 2010 and 2012, prairie dogs were in 27% better condition and, when controlling for month and observer variation, devoted 450% more time to grooming. Prairie dogs provided with supplemental food and water during March-May 2012 were in 18% better condition and carried 40% fewer fleas during June-August. Collectively, these results suggest an importance of food/water availability for the competency of prairie dog defenses against fleas. Increased flea densities during droughts may provide context for the maintenance and spread of plague.

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[24] Ecology: used here as a general term for the study of factors that affect the abundance and dispersion of wildlife (*sensu* Krebs, C.J. 2009. Ecology: The Experimental Analysis of Distribution and Abundance, sixth edition. Benjamin Cummings, San Francisco).

Introduction

Rates of reproduction and survival for arthropod vectors and disease-causing pathogens are often affected by precipitation, temperature, and humidity, and outbreaks of some vector-borne diseases are most commonly observed under particular weather scenarios (Harvell et al. 2002, Gage et al. 2008). Consequently, meteorological variables are increasingly used in an attempt to forecast disease outbreaks, in the hopes that mitigation actions can be implemented in a proactive manner (Jones et al. 2008, Rohr et al. 2011).

Plague is a rodent-associated zoonosis caused by the primarily flea-borne bacterium *Yersinia pestis*. The disease is arguably best known for causing devastating pandemics in humans, including the Black Death during the 14th century in Europe. More recently, it has gained the public's attention as a potential agent of warfare and bioterrorism (Orent 2004, Riedel 2005). Plague also gained attention from the conservation community, given the disease degrades populations of a wide array of rodents (Biggins and Kosoy 2001a, b).

Numerous factors affect the spread and maintenance of plague, but weather variables have been discussed for >100 years (Greenwood 1911, Martin 1911) and appear to be especially important (Eisen and Gage 2012). For instance, the Black Death erupted in Asia during the first half of the 14th century and spread westward to Europe and Africa (Achtman et al. 2004) as temperatures warmed and precipitation became increasingly abundant (Stenseth et al. 2006). Similarly, plague's Modern Pandemic may have been triggered by warmer, wetter climatic conditions in Central Asia during the late-1800s, providing context for the spread of plague to multiple, additional continents and islands (Stenseth et al. 2006).

Accumulating evidence suggests plague epizootics in the western United States are clustered during years with wetter springs and cooler summers, and become less prevalent during droughts (Ben Ari et al. 2011, Gage 2012, Eisen and Gage 2012). The scarcity of epizootics during droughts has been explained by lab studies in which fleas desiccated and died if temperatures were too hot and humidity too low (Krasnov 2008). Essentially, it is assumed that flea densities decline during droughts, thereby causing a reduction in rates of plague transmission (Enscore et al. 2002, Collinge et al. 2005, Ben Ari et al. 2008).

Herein, we evaluate this assumption with black-tailed prairie dogs, colonial rodents that amplify plague (Barnes 1982). We studied black-tailed prairie dogs during June–August, 2010–2012, at the Vermejo Park Ranch, Colfax County, New Mexico, USA (Fig. 14). Precipitation was relatively plentiful during 2010 (Fig. 15) when we collected small flea-loads from prairie dogs. The first seven months of 2011 were the driest start to any year on record for New Mexico (http://www.srh.noaa.gov/abq/?n=climonhigh2011julprecipsum). Under consideration of historical literature and our own discussions in May 2011, we posited that flea densities would be extremely low during 2011. Unexpectedly, fleas exhibited a nearly 3-fold increase in abundance.

As we collected large numbers of fleas from prairie dogs during June 2011, we recognized an alternative hypothesis that fleas might increase in abundance during droughts when hosts are food-limited and, as a potential consequence (Krasnov 2008), exhibit inefficient defenses against fleas. Herein, we evaluate this hypothesis by comparing flea-loads on prairie dogs during all three years of study (2010–2012) and quantifying the grooming behaviors of prairie dogs during 2011 and 2012. We also analyze data from a supplementation experiment in 2012 that allowed us to further evaluate the effect of food/water availability on the condition of prairie dogs and the flea-loads they carry.

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Study Site and Sampling Fleas from Prairie Dogs

The Vermejo Park Ranch short-grass prairie is situated east of the Sangre de Cristo Mountains (Fig. 14). Dominate vegetation includes blue grama (*Bouteloua gracilis*) and dispersed patches of tree cholla (*Cylindropuntia imbricata*). We did not observe rapid die-offs of prairie dogs, which are indicative of plague epizootics (Cully et al. 2006), and therefore suggest plague epizootics did not occur in prairie dogs occupying our study areas.

We established 1.54 or 2.25 ha sampling plots in 13 colonies of black-tailed prairie dogs (hereafter "prairie dogs"; Fig. 14). Each plot contained 25 or 37 traps ($16 \times ha^{-1}$) distributed among active burrows with fresh prairie dog scat (Tomahawk single-door live traps, Hazelhurst, Wisconsin, USA). Each year, we categorized the plots into five groups of 2–5 plots and sequentially sampled the groups in randomized order during 10-day work periods.

Flea abundance was indexed by combing adult fleas from live-trapped prairie dogs (Gage 1999, Eads et al. 2013). Body condition indices were calculated using mass:foot ratios (Martin et al. 2013). Hand-held global positioning units were used to acquire spatial reference points for capture events.

Fleas were identified to species using light microscopy and keys in Stark (1958) and Hubbard (1968). Two species were most prevalent and their abundances are analyzed herein: *Oropsylla hirsuta* (a prairie dog specialist[25]) and *Pulex simulans* (a generalist). All male *Pulex* were identified as *P. simulans*, so we assumed all *Pulex* were *P. simulans* (Tripp et al. 2009).

Detection of fleas is likely to be imperfect (Chapters 5 and 6), especially when considering detection of individual fleas. Consequently, our data on flea abundance should be considered

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[25] Results from this study should prove applicable not only to prairie dogs, but also black-footed ferrets (*Mustela nigripes*), because ferrets commonly carry *O. hirsuta* (Harris et al. 2014).

indices. Nonetheless, we standardized our sampling methods, suggesting the data could be used for comparisons of flea abundance among years and hosts.

Below, we present each of our study topics in sections. Hypotheses are presented in some cases and, when needed, additional field methods are described. Fieldwork was completed under Colorado State University Institutional Animal Care and Use Committee Protocol #10-1785A.

Flea-Loads Relative to Year-of-Sampling and Prairie Dog Body Condition

Predictions

Precipitation was relatively plentiful during 2010, scarce during 2011, and moderate in 2012 (Fig. 15). Partly as a result (Lauenroth 2008), mid-August aboveground primary production in the Vermejo short-grass prairie was 4-fold higher in 2010 and 2012, relative to 2011 (Turner Enterprises Incorporated, unpublished data). Using mass:foot ratios, prairie dogs were in 21% better condition during 2010 and 2012 relative to the dry 2011 field season (Fig. 16; see also Facka et al. 2010).

During June-August 2010, we collected small flea-loads from prairie dogs. In June 2011, we recognized a hypothesis that fleas might increase in abundance during droughts when prairie dogs are in poor condition. From another perspective, fleas might attain lower densities during wetter years when prairie dogs are in better condition and, perhaps, better prepared to combat fleas. During February-June 2012, we noted moderate amounts of precipitation at our study site (Fig. 15). We suggested the abundance of fleas might decline from 2011 (drier) to 2012 (wetter).

Within years, fleas might incur fitness benefits from parasitizing malnourished hosts (Krasnov 2008). For instance, during some lab studies, adult fleas produced more eggs, and eggs and emerging larvae exhibited increased survival rates if the associated mother flea had fed on malnourished rodents (Krasnov 2008). We hypothesized that during our study, fleas would attain their highest densities on prairie dogs that were in relatively poor condition.

Data Analyses

We restricted the data to 17 plots that were sampled during all years of study and ran generalized linear mixed effects models (GLMMs) for flea abundance (i.e., the number of fleas found on each prairie dog, including zero counts; Bush et al. 1992). Results remained the same if flea-loads were expressed as the number of fleas per unit of a host's body surface to control for host size (mass$^{-0.67}$; Heusner 1985).

The negative binomial parameter *k* for flea abundance was below 1.00 in all cases, indicating an aggregated distribution for flea abundance (Wilson et al. 2002; 'theta.ml' package, Program R version 2.13.2, R Development Core Team 2011). Therefore, we used the negative binomial distribution for overdispersed count data when running GLMMs.

GLMMs were fit using 'PROC GLIMMIX' in SAS® version 9.3 (SAS Institute Inc.). Inclusion of a random effect for "Prairie Dog ID" did not increase fit, suggesting little repeatability of flea abundance for individual prairie dogs[26] (see also Krasnov et al. 2006). Therefore, we did not include "Prairie Dog ID" as a random effect. We included "Plot ID" as a random effect, however, to account for repeated samples from the same plots.

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[26] Similar results have been found with black-tailed prairie dogs in Colorado (M.F. Antolin, personal communication).

We also included control variables that accounted for variation in flea abundance during other analyses, including month-of-sampling, age-of-prairie dog, sex-of-prairie dog, texture of surface-soils, and densities of prairie dogs in sampling plots (Chapters 2 and 3, Eads et al. 2013). We included an additional control for types of colonies to account for differing forms of colony establishment (see Eads et al. 2013). These six variables served as controls in all models and are not discussed herein.

We evaluated main-effects for year-of-sampling (YEAR) and host body condition (CONDITION). We also evaluated a CONDITION × YEAR interaction to determine if the magnitude or direction of the CONDITION effect differed among years.

We fit a control model and four other models with combinations of the YEAR, CONDITION, and CONDITION × YEAR effects and ranked the models by an adjusted Akaike's Information Criterion (AICc; Burnhman and Anderson 2002). Akaike weights (w_i) were calculated. Likelihood ratio tests ($\alpha = 0.100$; McCullagh and Nelder 1989) were used to compare nested submodels with more general models (differences may suggest the more complex model was useful).

Moran's I analyses (Moran 1950) with spatial reference points for capture locations and the residuals of any interpreted GLMM (Cliff and Ord 1981) suggested the "Plot ID" random effect accounted for spatial dependency in the data. The 95% confidence interval ($95\%_{CI}$) of interpreted coefficients did not encompass 0.00. Goodness-of-fit X^2 statistics for the most general GLMMs were ~1.00, suggesting good fit (Burnham and Anderson 2002).

Results

We collected 8,132 fleas from 687 unique prairie dogs ($n = 1,665$ combings, $k = 0.56$). In the GLMM exercise with flea species combined, no other model was within 118 AICc units of the most general model. Fleas were about 280% more abundant in 2011 than 2010 and 65% more abundant in 2011 than 2012[27] (YEAR effect; Fig. 17). Very few juvenile prairie dogs were captured in 2011. With the data limited to adult hosts, fleas were 220% more abundant in 2011 than 2010 and 40% more abundant in 2011 than 2012.

One could argue that adult fleas were more abundant on prairie dogs in 2011 simply because the adult fleas spent more time on hosts to acquire blood meals that would presumably increase their water content and reduce their susceptibility to desiccation. We used a swabbing technique to collect fleas from prairie dog burrows during June–August 2010 ($n = 2,201$ events) and 2011 ($n = 2,765$) and fleas were 240% more abundant on swabs in 2011 (Fig. 17). These results suggest an overall increase in flea densities (1) during the dry 2011 field season both (1) on prairie dogs and (2) in prairie dog burrows.

We separated the analysis by YEAR to investigate the CONDITION × YEAR interaction ($X^2_2 = 122.91$, $P < 0.001$). During 2010 ($n = 604$ combings, $k = 0.79$), there was little support for a correlation between CONDITION and flea abundance ($X^2_1 = 0.56$, $P = 0.454$). In contrast, during 2011 ($n = 528$ combings, $k = 0.66$), fleas were more abundant on prairie dogs in poorer CONDITION (Fig. 18; $X^2_1 = 149.86$, $P < 0.001$). A similar negative correlation between CONDITION and flea abundance gained support with the 2012 data (Fig. 18; $n = 533$ combings, $k = 0.79$, $X^2_1 = 6.90$, $P = 0.009$).

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[27] Lice (Anoplura) provided additional evidence of increased ectoparasite-loads during 2011. We found zero lice in 2010, 92 in 2011, and 32 in 2012.

With the data restricted to *O. hirsuta* ($k = 0.47$), the most general model gained 100% of Akaike weight (minimum ΔAICc = 35.69). *O. hirsuta* was 220% more abundant in 2011 than 2010 and 18% more abundant in 2011 than 2012 (Fig. 19). We separated the analysis by YEAR to investigate the CONDITION × YEAR interaction ($X^2_2 = 39.76$, $P < 0.001$). *O. hirsuta* was aggregated among hosts during all 3 years, but became less aggregated as the study progressed (2010 $k = 0.40$; 2011 $k = 0.65$; 2012 $k = 0.60$). *O. hirsuta* was most abundant on prairie dogs that were in relatively poor CONDITION during all 3 years of study (2010: $X^2_1 = 8.87$, $P = 0.003$; 2011: $X^2_1 = 13.76$, $P < 0.001$; 2012: $X^2_1 = 3.16$, $P = 0.075$).

With the data restricted to *P. simulans* ($k = 0.22$), the most general model gained 100% of Akaike weight (minimum ΔAICc = 132.00). *P. simulans* was 354% more abundant in 2011 than 2010 and 352% more abundant in 2011 than 2012 (Fig. 19). We separated the analysis by YEAR to investigate the CONDITION × YEAR interaction ($X^2_2 = 136.07$, $P < 0.001$). Like *O. hirsuta*, the generalist *P. simulans* became less aggregated as the study progressed (2010 $k = 0.22$; 2011 $k = 0.28$; 2012 $k = 0.35$). Though both of the correlations were negative, the CONDITION effect was not quite supported for 2010 ($X^2_1 = 1.63$, $P = 0.202$) or 2012 ($X^2_1 = 1.98$, $P = 0.159$). In 2011, *P. simulans* was most abundant on prairie dogs that were in relatively poor CONDITION ($X^2_1 = 164.47$, $P < 0.001$).

Prairie Dog Self-Grooming Behaviors

Predictions

Grooming can play a large role in mediating interactions between hosts and the fleas they harbor (Marshall 1981, Krasnov 2008). In a study of house cats hosting fleas, their grooming accounted for 50% or more of flea mortality during weekly intervals (Rust and Dryden 1997). In a multitude of studies ectoparasite loads increased on animals fitted with grooming-preventive harnesses, demonstrating that grooming is an important defense against ectoparasites (Bennett 1969, Mooring et al. 1996, Hinkle et al. 1998, Eckstein and Hart 2000).

We collected data on prairie dog grooming during the dry 2011 field season and the relatively wet 2012 season. We predicted that prairie dogs would spend less time self-grooming in 2011 because succulent vegetation was scarce and the prairie dogs may have spent most of their time searching for food and, consequently, less time grooming, given foraging and self-grooming are mutually exclusive behaviors for prairie dogs. In addition, the prairie dogs might have reduced energy expenditures associated with grooming during 2011, helping to explain why fleas were most abundant in 2011.

Study Design

Focal observations of adult prairie dogs were collected during July–August 2011 and June–August 2012 from locations at which disturbance was limited but observations of grooming behaviors were possible via 20x–60x spotting scopes (Altmann 1974). We collected

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observations of grooming behaviors at plots where trapping was not currently in progress, with sampling effort distributed equally as possible among regular sampling plots (Fig. 14). Repeated observation of the same animal during the same day was limited by sampling systematically across a plot, and refraining from observing animals in regions already sampled. We may have easily observed some prairie dogs two or more times during a given year, but sampling events at any plot were separated by at least 14 days.

We scored focal observations on laptop computers using JWatcher[TM] (Blumstein and Daniel 2007). Before each observation, an observer recorded their identity and the year of sampling, and then initiated a focal sample. Computer keys were stroked to record the start and end of self-grooming behaviors (oral and scratch) and changes in visibility of the animal (visible or "out-of-sight"). Later, we used JWatcher[TM] to extract from each sample the pre-observation data, focal sample duration (which excluded time "out-of-sight"), and durations of bouts of self-grooming. Allogrooming (social grooming) was rarely observed and is not considered herein.

Data Analyses

We intended to observe animals during 10-min sessions, but some of the sessions were truncated because the focal animal partially descended into a burrow or descended fully belowground, or was disturbed by a predator.[28] In analyses, we included focal sessions in which a prairie dog was visible and undisturbed for five or more minutes ($\bar{x} = 9.40$ minutes, $SD = 1.14$). Three observers collected data during 2011, and four observers collected data during 2012. One observer was a repeat-observer in both years. As a precautionary measure, we controlled for

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[28] In 2012, prairie rattlesnakes (*Crotalus viridis viridis*) commonly confronted prairie dogs, especially in areas where juvenile prairie dogs were abundant (D.A. Eads, personal observations).

observer identification during analyses using an OBSERVER covariate. We also included

MONTH as a control because flea-loads changed during June–August (Chapter 3).

We fit a GLMM with a YEAR effect to compare self-grooming behaviors during 2011 and

2012. Inclusion of a random effect for "Plot ID" did not increase fit, so the variable was not

retained. The response variable was percentage of time spent grooming, multiplied by 100 for

expression as a numbered variable from 0 to 100 (percent), thus allowing for use of the negative

binomial error distribution (the data were numbered and right-skewed). We used a likelihood

ratio test to determine if the YEAR effect increased fit (the $95\%_{CI}$ did not encompass 0.00). The

goodness-of-fit X^2 statistic for the most general GLMM was ~1.00, suggesting good fit.

Results

The dataset included 392 focal observations ($n = 114$ in 2011 and 278 in 2012). The YEAR

effect increased fit ($X^2_1 = 8.71$, $P = 0.003$). With control covariates included, prairie dogs

devoted 450% more time to grooming in 2012 (lower flea-loads) relative to 2011 (higher flea-

loads). Because we observed prairie dogs during July–August 2011 (2 months) and June–August

2012 (3 months), we ran an analysis with the data limited to July–August ($n = 66$ observations in

2011 and 89 in 2012). The trend remained; prairie dogs groomed for longer amounts of time in

2012 (Fig. 20; $X^2_1 = 35.46$, $P < 0.001$).

Food/Water Supplementation, Prairie Dog Body Condition, and Flea-Loads

Predictions

During an experiment in 2012, we provided animals in treatment plots with supplemental food and water (Fig. 14). We predicted that supplementation would cause an increase in the condition of prairie dogs and a decline in flea-loads. We collected observations of grooming by prairie dogs in treatment and control areas, but the data were too sparse for an analysis.

Experimental Design

Before the 2012 field season, we established three 2.25 ha treatment plots, each near a control plot but far enough away that prairie dogs would be unlikely to move between any plot. In the third pairing, the control plot was randomly selected from two regular sampling plots in the associated colony (Fig. 14). Prairie dogs in the treatment plots received supplemental food/water starting immediately after the adults may have bred in March (Hoogland 1995) and stopping as juveniles began to emerge from burrows (observed by crew observation).

In early March 2012, we distributed seven plastic water dispensers evenly in each treatment plot (1 gallon, Mobile Chicken Coops, Burnet Texas). We refilled the dispensers so water was available from late-March to 1 June 2012. During the same period, we provided supplemental food in the treatment plots every 5–7 days using 14% crude protein sweet feed (MannaPro[®] pelleted feed, St. Louis, Missouri).[29] When providing supplemental food at a station within a

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[29] Ingredients and nutritional information are available at: http://www.midwayfarmandranchsupply.com/super-horse-performance-14-textured-sweet-feed-14-protein/.

treatment plot, we placed 1 gallon of feed in two of the nearest burrow openings. The bait was inserted into burrow openings to discourage bird use.

Data Analyses

We trapped prairie dogs in treatment and control plots during June–August 2012; each of the two paired plots were trapped during the same day. We used GLMMs to evaluate the effect of supplementation (SUPPLEMENT) on body condition indices and flea-loads while controlling for month-of-sampling, and the age and sex of prairie dogs. Because the treatment and control plots were paired, we included an effect for PAIR-ID. The month (MONTH), age, sex, and PAIR-ID variables were included in all models. The SUPPLEMENT main-effect was of primary interest and could be included or not in a model. In addition, SUPPLEMENT × PAIR-ID and SUPPLEMENT × MONTH interactions might be included to determine if the SUPPLEMENT effect varied among pairs or months.

We fit all possible GLMMs ($n = 5$) and ranked the models according to AICc. A "Prairie Dog ID" random effect increased fit during the assessment of body condition and was included in that exercise (Gaussian family), whereas that random effect did not increase fit in the assessment of flea-loads and was therefore excluded (negative binomial model because $k < 0.00$). The 95%$_{CI}$ of each interpreted coefficients did not encompass 0.00 and goodness-of-fit X^2 statistic for the most general GLMMs was always ~1.00, suggesting good fit.

We did not include PD-DENSITY as a control variable because prairie dog densities were similar in treatment and control plots in the 2nd and 3rd experimental pairs, thus reducing the

chance of detecting an effect of PD-DENSITY (2nd pair: treatment and control = 9.8 prairie dogs

$\times$ ha^{-1}; 3rd pair: control = 9.8 $\times$ ha^{-1}, treatment = 8.4 $\times$ ha^{-1}).

Results

The assessment of body condition indices included data from 119 unique prairie dogs (55

control and 64 treatment; *n* captures = 93 control and 130 treatment). Two GLMMs competed;

one contained the SUPPLEMENT effect and the other contained the SUPPLEMENT $\times$ PAIR-ID

interaction (Table 9). The SUPPLEMENT $\times$ MONTH interaction did not increase fit (X^2_2 =

2.34, *P* = 0.310), but inclusion of the SUPPLEMENT $\times$ PAIR-ID interaction did (X^2_2 = 6.45, *P* =

0.040). Therefore, we separated the analysis by PAIR-ID (Table 9). Prairie dogs were in 27%

better condition in the treatment plot in the 1st experimental pair (X^2_1 = 10.78, *P* = 0.001), 10%

better condition in the treatment plot in the 2nd pair (X^2_1 = 3.01, *P* = 0.083), and 18% better

condition in the treatment plot in the 3rd pair (X^2_1 = 13.61, *P* < 0.001).

Flea abundance was assessed for the same 119 captured prairie dogs (*n* = 1,736 fleas, *k* =

0.67). The most supported GLMM included the SUPPLEMENT effect and SUPPLEMENT $\times$

PAIR-ID interaction (Table 10). The SUPPLEMENT $\times$ MONTH interaction in the next model

did not increase fit (X^2_2 = 1.59, *P* = 0.452). We separated the analysis by PAIR-ID due to the

SUPPLEMENT $\times$ PAIR-ID interaction (X^2_2 = 5.34, *P* = 0.069). Flea-loads were 51–53% lower

on prairie dogs in the treatment plots in the 1st and 3rd experimental pairs (Fig. 21; 1st pair: X^2_1 =

209.94, *P* < 0.001, *k* = 1.28; 3rd pair: X^2_1 = 6.72, *P* = 0.010, *k* = 1.03). Flea-loads were 16%

lower on prairie dogs in the treatment plot of the 2nd pair (Fig. 21; X^2_1 = 4.58, *P* = 0.032, *k* =

0.76).

With the flea data restricted to *O. hirsuta* ($n = 888$ fleas, $k = 0.63$), the most supported model included the SUPPLEMENT × MONTH interaction (Table 11). The interaction did not increase fit ($X^2_2 = 3.29$, $P = 0.193$), so we interpreted the second most supported model, which contained an effect of SUPPLEMENT ($X^2_1 = 9.23$, $P = 0.002$). Flea-loads for *O. hirsuta* were 31% lower on prairie dogs in the treatment plots.

With the data restricted to *P. simulans* ($n = 848$ fleas, $k = 0.23$), the most supported model gained 74% of Akaike weight (Table 11) and included the SUPPLEMENT × PAIR-ID interaction ($X^2_2 = 18.18$, $P < 0.001$). The GLMM log-likelihood declined when the SUPPLEMENT × MONTH interaction was included, so we interpreted the most supported model and separated the analysis by PAIR-ID. Flea-loads for *P. simulans* were 197% lower in the treatment plot of the 1st experimental pair ($X^2_1 = 227.95$, $P < 0.001$) and 85% lower in the treatment plot of the 2nd pair ($X^2_1 = 7.21$, $P = 0.007$). The SUPPLEMENT effect was not quite supported with the 3rd pair, where only eight *P. simulans* were collected (7 in the control, 1 in the treatment; $X^2_1 = 1.96$, $P = 0.162$).

Discussion

Accumulating evidence suggests that plague epizootics are most prevalent during years with wetter springs and cooler summers, and less prevalent during droughts (Ben Ari et al. 2011, Gage 2012, Eisen and Gage 2012). As a result, it would be logical to assume that fleas attain their highest densities during years with wetter springs and cooler summers, perhaps thereby providing context for increased rates of plague transmission.

To our knowledge, one study has attempted to evaluate this assumption. Using data from Central Asia, Stenseth et al. (2006) fit models for the prevalence of plague epizootics in populations of great gerbils (*Rhombomys opimus*). Fleas were most prevalent during years with wetter springs and cooler summers, when the detected prevalence of plague increased in great gerbils.

While the study of Stenseth et al. (2006) is suggestive, fleas concentrate on dwindling numbers of hosts during plague epizootics (Poland and Barnes 1979, Tripp et al. 2009), thereby clouding comparisons of flea densities before and during outbreaks. It may appear as if fleas are most abundant or prevalent during years with wetter springs and cooler summers (when epizootics are commonly observed) because fleas become concentrated on the small number of surviving hosts during outbreaks (Brinkerhoff et al. 2010).

Our results, which are not confounded by the presence of plague epizootics, suggest fleas may attain their highest densities on prairie dogs during dry years. Below, we attempt to combine our results and previous studies into a unified framework for prairie dogs and, in doing so, develop a verbal and testable model of plague ecology. We conclude by discussing some conservation implications of plague.

A Conceptual Model of Flea and Plague Ecology in Prairie Dog Colonies

During dry years in arid grasslands, the availability of succulent plants is reduced (Lauenroth et al. 1994, Fay et al. 2003, Brown and Ernest 2002, Lauenroth 2008). Many studies demonstrate that herbivorous rodents suffer reductions in condition when primary production is

reduced (Chew and Butterworth 1964, van de Graaff and Balda 1973, Gillespie et al. 2008) and

our results (Fig. 16) and those of Facka et al. (2010) suggest the same for prairie dogs.

If prairie dogs are in poor condition during droughts, their defenses against fleas could be

compromised. Foraging and grooming are exclusive behaviors (Krasnov 2008:252-254), and

high quality food is limited during droughts. A reduction in grooming during a drought, as

observed during our research (Fig. 20), should increase survival rates for fleas (Krasnov 2008[30]).

Flea-infested domestic cats remove about 50% of fleas from their bodies during weekly intervals

(Rust and Dryden 1997). However, if cats in that study were fitted with grooming-preventive

harnesses, about 70% of fleas remained for 50 days or more (Rust and Dryden 1997).

In addition to grooming, rodents combat fleas using acquired immune responses (Krasnov

2008) but the responses are costly to maintain and can be suppressed when food is limited

(Sheldon and Verhulst 1996, Demas and Nelson 1998, Lochmiller and Deerenberg 2000). If

primary production is limited, as is the case during dry years in the short-grass prairie of North

America (Lauenroth 2008), prairie dogs may "surrender to fleas rather than spend much energy

on defense" (Krasnov et al. 2005, Krasnov 2008:266, Combes 2001). Our supplementation

experiment provides support for this contention—the food provided the prairie dogs with

vitamins A, C, and E, all of which might boost immunocompetence in mammals (Wobeser

2006).

When rodents are in poor condition during dry years, ectoparasitic fleas may cause a further

decline in host condition due to stress (St. Juliana et al. 2014), thereby creating a positive

feedback cycle that benefits fleas (Beldomenico and Begon 2010). Reduction in the

effectiveness of host defenses against fleas would (1) reduce sources of mortality for fleas, (2)

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[30] Krasnov (2008) presents a plethora of literature that suggests a reduction in grooming effort by hosts may
stimulate a substantial increase in flea-loads on hosts.

allow fleas to more easily acquire blood meals, and (3) facilitate offspring production by fleas (Hawlena et al. 2008, Krasnov 2008). Generations of reproduction could then overlap and adult fleas would increase in abundance. A greater number of co-occurring attackers could then "suppress the defense system of the host by a cumulative effect of factors contained in their saliva" (Krasnov 2008:176; see also Ribeiro and Francischetti 2003, Bosio et al. 2014).

We suggest like others that deep burrows can buffer fleas from droughts (Longanecker and Burroughs 1952, Rothschild and Clay 1952, Wilcomb 1954, Krasnov et al. 2001, Krasnov 2008, Tripp 2009, Ben-Ari et al. 2011). In the case of prairie dogs, these rodents tend to create their nests 2 m underground (Sheets et al. 1971) and studies suggest burrows deeper than about 0.5 m are relatively cool in summer and exhibit little daily variation in temperature[31] (Schmidt-Nielsen 1964, Clark 1971, Gettinger 1975, Chappell and Bartholomew 1981, Smith 1982, Reichman and Smith 1990, Shenbrot et al. 2002, Van Vuren and Ordeñana 2012).

Mild temperatures within prairie dog burrows may not only facilitate flea survival, but also increase the ability of adult fleas to successfully transmit *Y. pestis* to hosts (Schotthoefer et al. 2011a, Williams et al. 2013; but see Schotthoefer et al. 2011b). Usually, when a rodent host dies from plague, adult fleas jump from the carcass and "quest" to burrow openings at which they might encounter living hosts with warm blood (Gage and Kosoy 2005, Buhnerkempe et al. 2011). However, during droughts, dry conditions aboveground may encourage fleas to remain deep underground, thus reducing the rate at which infectious fleas encounter susceptible hosts (perhaps helping to explain why the prevalence of epizootics declines during droughts).[32]

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[31] Prairie dog burrows exhibit little daily variation in temperature (D.E. Biggins, unpublished data).
[32] We swabbed prairie dog burrows for fleas in 2010 and 2011. We collected more fleas on swabs that were inserted deeper belowground. However, this trend was most evident during the dry 2011 field season, when hotter temperatures may have encouraged fleas to seek refuge deeper belowground (D.A. Eads, unpublished data).

High flea densities during dry years may help *Y. pestis* to persist in prairie dog colonies at an enzootic level. Biggins et al. (2010) and Wilder et al. (2008:7) suggest plague might be "maintained at a surreptitious enzootic level" among patches of prairie dogs and their fleas, and such an outcome seems plausible in those patches of habitat where fleas are relatively abundant.

Our results suggest *O. hirsuta* and *P. simulans* may attain high densities in patches of malnourished prairie dogs. This hypothesis is supported by (1) the increased abundance of both species during the 2011 drought and (2) the increased abundance of *O. hirsuta* on malnourished prairie dogs during all three years of study, and *P. simulans* during 2011. More importantly, *O. hirsuta* and *P. simulans* tended to be more abundant on prairie dogs in the control plots during our experiment, suggesting an effect of host body condition on fleas (not vice versa).

As a drought subsides, fleas might already be abundant in some areas due to the preceding drought, and the availability of succulent forage would facilitate offspring production by prairie dogs (Davidson et al. 2010, Facka et al. 2010). The combination of increased host and flea densities might create connectivity among infectious fleas and susceptible hosts, thereby facilitating the spread of plague and, perhaps, sparking an epizootic (Davis et al. 2008, Heier et al. 2011, Samia et al. 2011, Gage 2012, Reijniers et al. 2012). Mild temperatures aboveground at burrow openings may help questing fleas to survive until they encounter new, plague-susceptible hosts, thus facilitating the spread of *Y. pestis*.

Oropsylla hirsuta and Pulex simulans

O. hirsuta increased in abundance during the 2011 drought and declined to intermediate abundance in 2012. Overall, *O. hirsuta* became more widely dispersed among prairie dogs as the

study progressed. *P. simulans* exploded during 2011 and returned to background levels of lower abundance the following year. Like *O. hirsuta*, the generalist *P. simulans* became more widely dispersed among prairie dogs as the study progressed.

The intimate association between *O. hirsuta* and prairie dogs suggests natural selection may have acted upon prairie dogs in a manner to select for efficient defenses against *O. hirsuta* (Møller et al. 2005). When prairie dogs are in poor condition, however, they may exhibit weakened defenses against fleas and, consequently, *O. hirusta*'s may quickly increase in abundance, allowing the species to become more widely dispersed among prairie dogs.

P. simulans may also be prone to increases in abundance during droughts because prairie dogs are in poor condition, but also because *P. simulans* is a generalist that can capitalize on multiple mammal species (Hopla 1980). During droughts, many of the mammals that associate with prairie dogs (reviewed by Reading 2009) would presumably suffer declines in condition due to a scarcity of vegetation, or reductions in the abundance of small mammalian prey (Holmgren et al. 2006), thereby providing *P. simulans* with increased opportunities to exploit malnourished hosts.

An explosion of the generalist *P. simulans* during a drought might facilitate continued transmission of *Y. pestis* within and among multiple mammal and flea species, thereby facilitating persistence of *Y. pestis* in flea-mammal communities within and near prairie dog colonies. Further, an abundance of *P. simulans* during droughts, in unison with higher densities of *O. hirsuta*, may allow *Y. pestis* to fester in patches of prairie dogs (Biggins et al. 2010).

When a plague epizootic ensues, *O. hirsuta* and *P. simulans* (and other flea species) may collectively facilitate the outbreak as they concentrate on the dwindling number of plague-susceptible hosts (Tripp et al. 2009), thereby heightening the rate of plague transmission

(Lorange et al. 2005, Eisen et al. 2009). In describing this process of vectors concentrating on smaller numbers of hosts during outbreaks, Hudson (2005:9) noted that a "disease could drive species to extinction." Local extirpation is sometimes the outcome of plague epizootics for prairie dogs (Cully et al. 2006). In other cases, fragmented, degraded metapopulations may survive, but suffer from chronic rates of plague-caused mortality during enzootic periods (Biggins et al. 2010) and occasional, repeated epizootics (Antolin et al. 2006). Either of these outcomes is concerning from a conservation perspective (Biggins et al. 2010, McDonald et al. 2011).

Conservation Implications

Prairie dogs are now restricted to a small fraction of their historic areas of occupancy, and their abundance at many sites is so cyclical and low that they cannot fulfill their ecological functions (Biggins and Kosoy 2001a, b, Antolin et al. 2002, Stapp et al. 2004, Cully et al. 2006). As a result, the grasslands of western North America have been transformed (Biggins and Kosoy 2001a, Chapter 7). Proposals have been submitted for more effective conservation of prairie dogs (Miller and Reading 2012) and we supplement those proposals by discussing some implications of our research.

First, our results suggest a correlation between habitat quality for prairie dogs and their risk to plague. Where precipitation and succulent forage are limited, prairie dog populations may be suppressed not only because food and water are limited (Facka et al. 2010, Davidson et al. 2014), but also because the prairie dogs are in poor condition and highly susceptible to fleas and plague. Without careful consideration, we might erroneously conclude the prairie dogs are reduced in n

number because the habitat is of low quality, when in fact the animals are also suffering from increased flea-loads and occasional cases of plague-caused mortality.

Second, climate change is projected to result in longer periods of more frequent drought in the prairie dog range (Knapp et al. 2008, http://nca2014.globalchange.gov/). Our results suggest that dry conditions not only reduce the availability of food and water to prairie dogs, but also increase their susceptibility to fleas, perhaps providing context for the maintenance and spread of plague.

Dry conditions aboveground during droughts may encourage fleas to remain deep within burrows, thus reducing the rate at which plague spreads among prairie dog families. However, plague could still affect pockets of prairie dog families that experience relatively low rainfall and aboveground production, thus increasing their susceptibility to plague. Such an outcome could be catastrophic for black-footed ferrets (*Mustela nigripes*), endangered carnivores that specialize on prairie dogs for prey. The "risk posed by even widely spaced carcasses could be serious for the relatively mobile foraging ferrets" that usually die upon consumption of even a single *Y. pestis*-infected carcass (Godbey et al. 2006:236).

Grasslands should be managed so greater amounts of habitat and succulent vegetation are available to prairie dogs. Such management may help prairie dogs to attain higher densities and defend themselves against flea-borne plague. As a precautionary measure, insecticides (Seery et al. 2003) and a recently developed bait plague-vaccine (Rocke et al. 2010, Abbott et al. 2012) could be distributed to increase survival rates. The prairie dogs may then reach higher densities and establish larger colonies, and once again function as ecosystem engineers.

While the use of bait-administered vaccines may protect prairie dogs from plague, it remains to be seen if a *Cynomys*-vaccine will indirectly protect black-footed ferrets. Insecticides can

protect ferrets, but fleas may evolve resistance to the insecticides (D.E. Biggins and D.A. Eads, unpublished data). In the long-term, an integrative approach likely will be needed to manage plague so prairie dogs, ferrets, and other mammals are protected. Vaccines for multiple species, varied use of insecticides and forthcoming mitigation tools may allow for effective management of plague.

Table 9 Generalized linear mixed models investigating body condition indices (mass:foot) for black-tailed prairie dogs (*Cynomys ludovicianus*) during an experiment in which we provided supplemental water and food to treatment plots, but not control plots. We related body condition indices to experimental treatment (SUPPLEMENT = treatment or control), identification of the experimental pair (PAIR-ID), and month of sampling (MONTH). Each model contained control variables (see text). A random effect for "Prairie Dog ID" was included in all models to account for repeated measurements from individual prairie dogs. Moreover, PAIR-ID was included in all models. The models are ranked according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc), and model weights (w_i). Only those models with $w_i > 0.00$ are presented. Data were collected during June–August, 2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID)	1939.62	0	0.15
Controls + PAIR-ID + SUPPLEMENT	1940.32	0.70	0.11
Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID) + (SUPPLEMENT × MONTH)	1941.85	2.23	0.05
Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × MONTH)	1943.74	4.12	0.02

Table 10 Generalized linear models investigating flea-loads on black-tailed prairie dogs (*Cynomys ludovicianus*) during an experiment in which we provided supplemental water and food to treatment plots, but not control plots. We related flea-loads to experimental treatment (SUPPLEMENT = treatment or control), identification of the experimental pairing (PAIR-ID), and month of sampling (MONTH). Each model contained control variables (see text). The PAIR-ID variable was included in all models. The models are ranked according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc), and model weights (w_i). Only those models with $w_i > 0.00$ are presented. Data were collected during June–August, 2012, at the Vermejo Park Ranch, New Mexico, USA.

Model	AICc	ΔAICc	w_i
Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID)	1251.63	0	0.66
Controls + PAIR-ID + SUPPLEMENT	1253.74	2.11	0.23
Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID) + (SUPPLEMENT × MONTH)	1255.91	4.28	0.08
Controls + PAIR-ID + TREATMENT + (SUPPLEMENT × MONTH)	1257.87	6.24	0.03

Table 11 Generalized linear models investigating flea-loads (*Oropsylla hirsuta* and *Pulex simulans*) on black-tailed prairie dogs (*Cynomys ludovicianus*) during an experiment in which we provided supplemental water and food to treatment plots, but not control plots. We related flea-loads to experimental treatment (SUPPLEMENT = treatment or control), identification of the experimental pairing (PAIR-ID), and month of sampling (MONTH). Each model contained control variables (see text). The PAIR-ID variable was included in all models. The models are ranked according to Akaike's Information Criterion (AICc). Also presented are AICc differences (ΔAICc), and model weights (w_i). Only those models with $w_i > 0.00$ are presented. Data were collected during June–August, 2012, at the Vermejo Park Ranch, New Mexico, USA.

Flea Species	Model	AICc	ΔAICc	w_i
Oropsylla hirsuta	Controls + PAIR-ID + TREATMENT + (SUPPLEMENT × MONTH)	1035.28	0	0.67
	Controls + PAIR-ID + SUPPLEMENT	1037.83	2.55	0.19
	Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID)	1039.20	3.92	0.09
	Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID) + (SUPPLEMENT × MONTH)	1040.50	5.22	0.05
	Controls + PAIR-ID	1044.84	9.56	0.01
Pulex simulans	Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID)	741.74	0	0.74
	Controls + PAIR-ID + SUPPLEMENT + (SUPPLEMENT × PAIR-ID) + (SUPPLEMENT × MONTH)	743.88	2.14	0.25
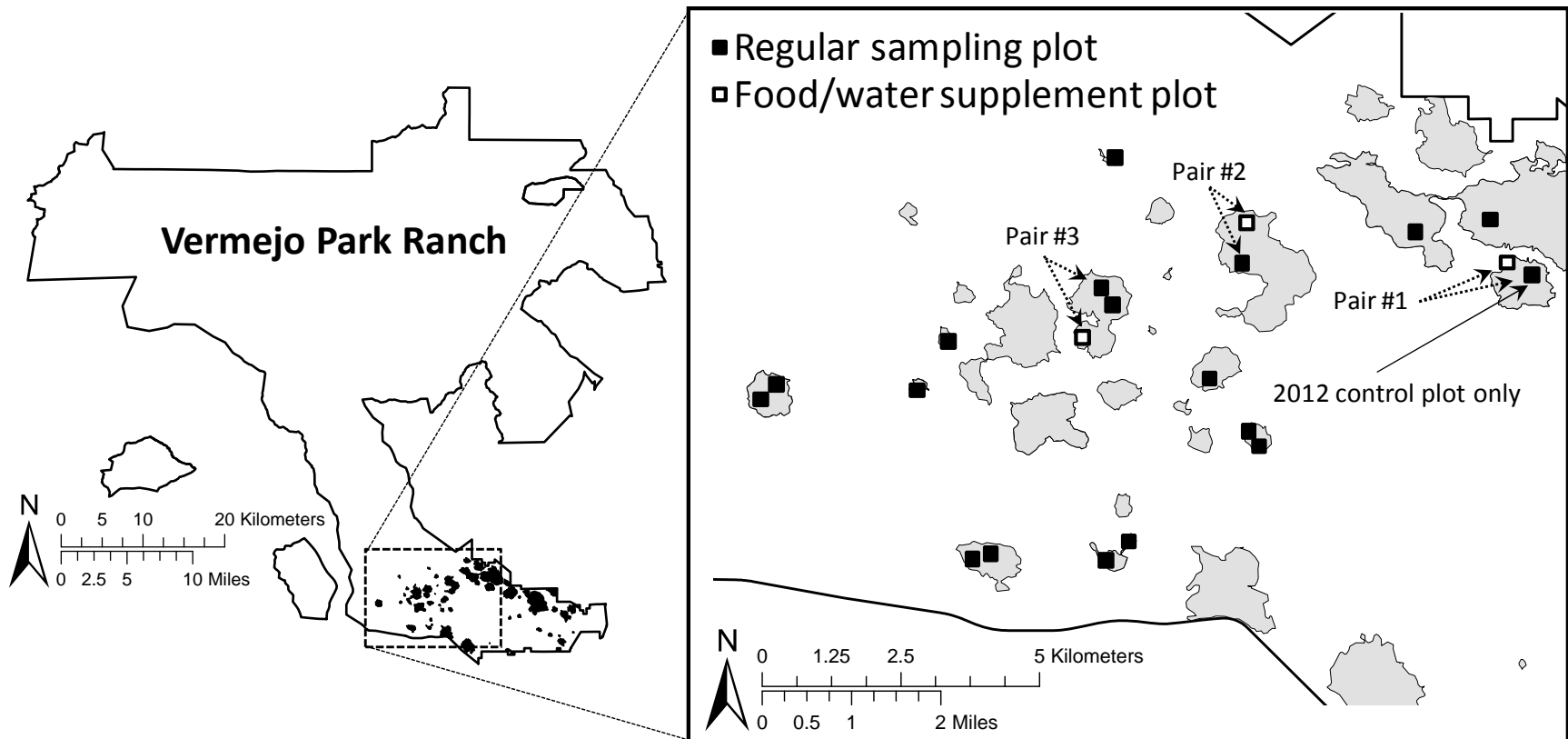
Figure 14 We studied black-tailed prairie dogs (*Cynomys ludovicianus*) in the Vermejo Park Ranch, Colfax County, New Mexico, USA, during June–August, 2010–2012. On the right, prairie dog colonies are shaded gray. We sampled prairie dogs and fleas in 17 sampling plots and three pairs of plots that were used during an experiment in which we provided prairie dogs with supplemental food/water.
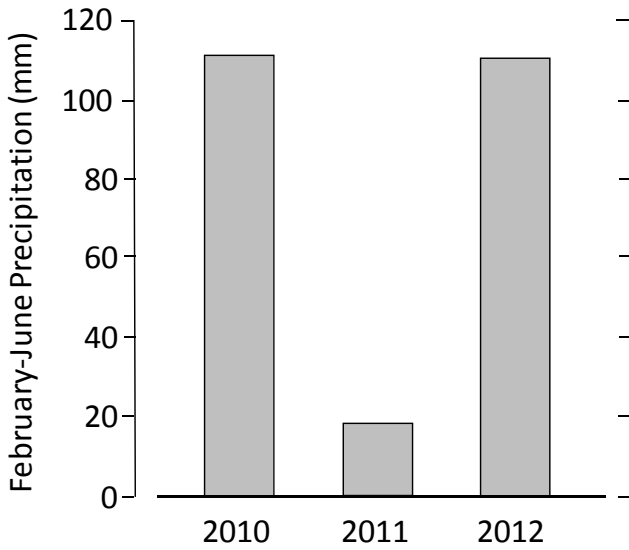
Figure 15 Cumulative precipitation (mm) during February–June, 2010–2012 at a weather station situated immediately north of our sampling plots, in the short-grass prairie of the Vermejo Park Ranch, New Mexico, USA (Turner Enterprises Incorporated, unpublished data).
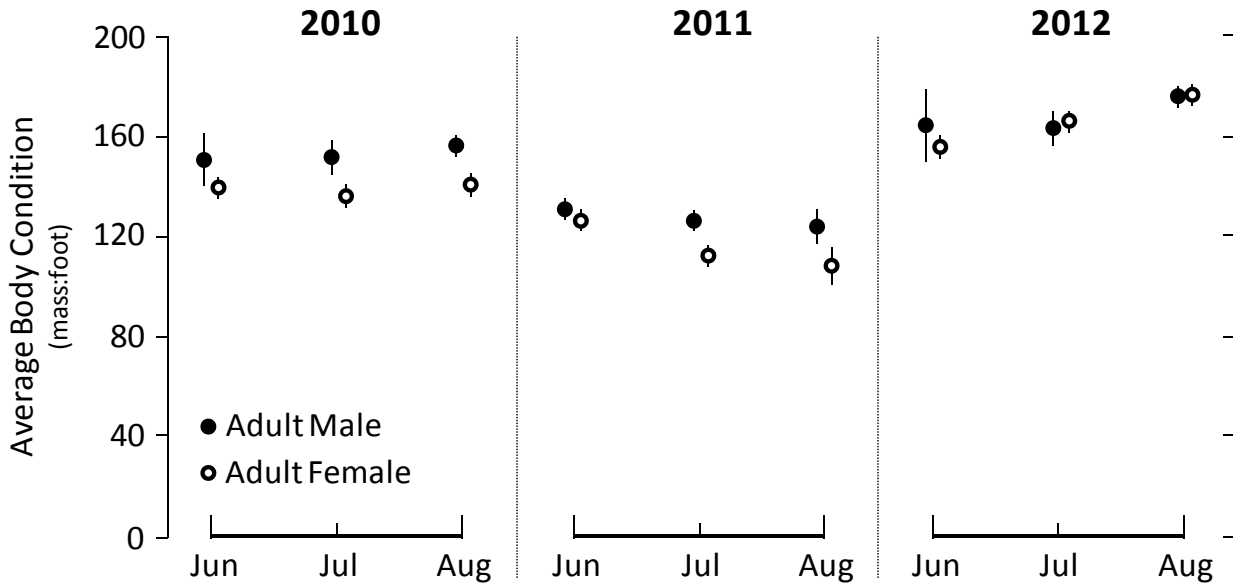
Figure 16 Average body condition indices (mass:foot ratios) for adult male and female black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. Vertical lines depict 95% confidence intervals. Comparisons are limited to adults because very few juveniles were captured in 2011.
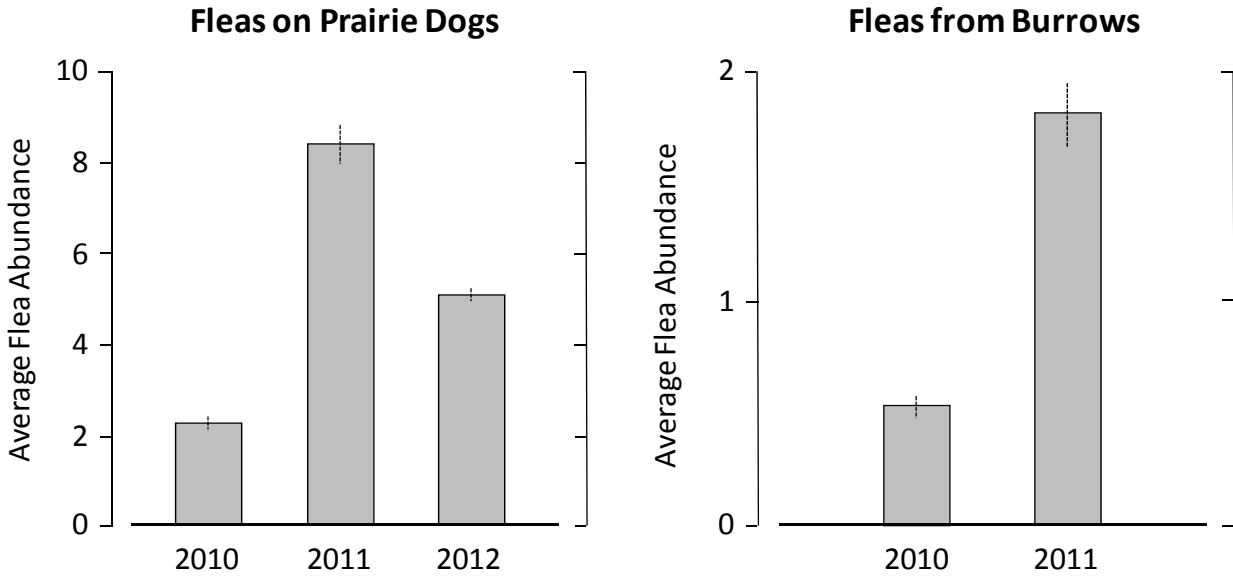
Figure 17 The left image depicts average adult flea abundance on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010–2012, at the Vermejo Park Ranch, New Mexico, USA. The right image depicts average flea abundance on swabs inserted into prairie dog burrows during the same months in 2010 and 2011; the swabbing data provide another indication that fleas were much more abundant in 2011 than 2010. Vertical lines depict 95% confidence intervals.
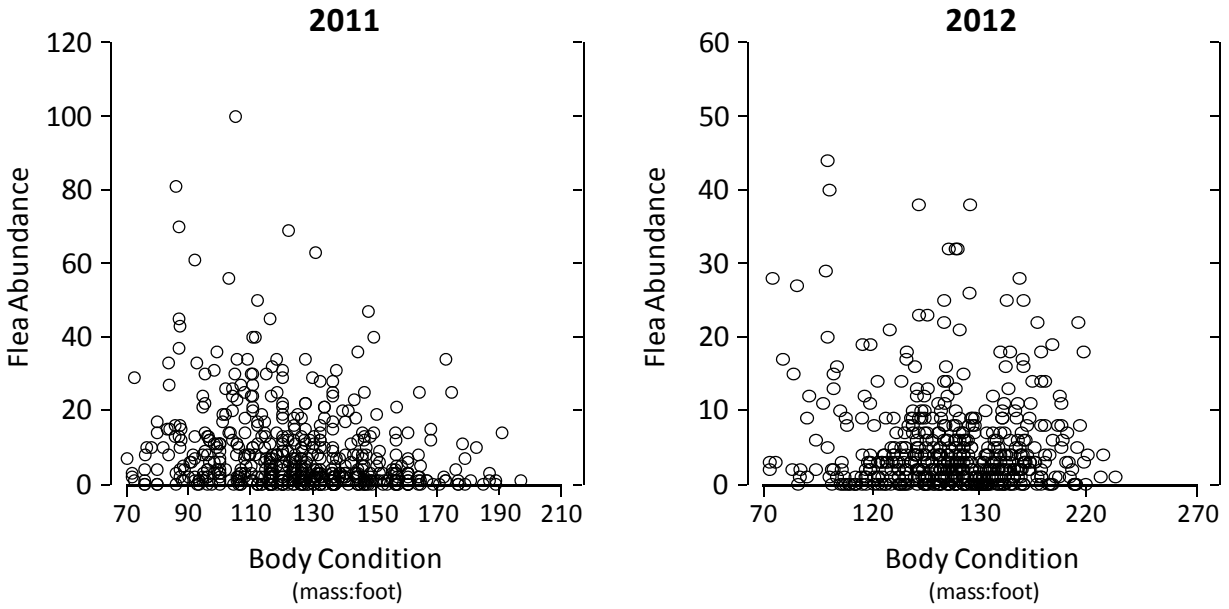
Figure 18 Abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) in differing body condition (mass:foot ratios) during June–August, 2011-2012, at the Vermejo Park Ranch, New Mexico, USA. Higher mass:foot ratios correspond with greater body mass and condition. The 2011 data include very few juveniles, whereas an abundance of juveniles were sampled in 2012. Analyses controlled for host age.

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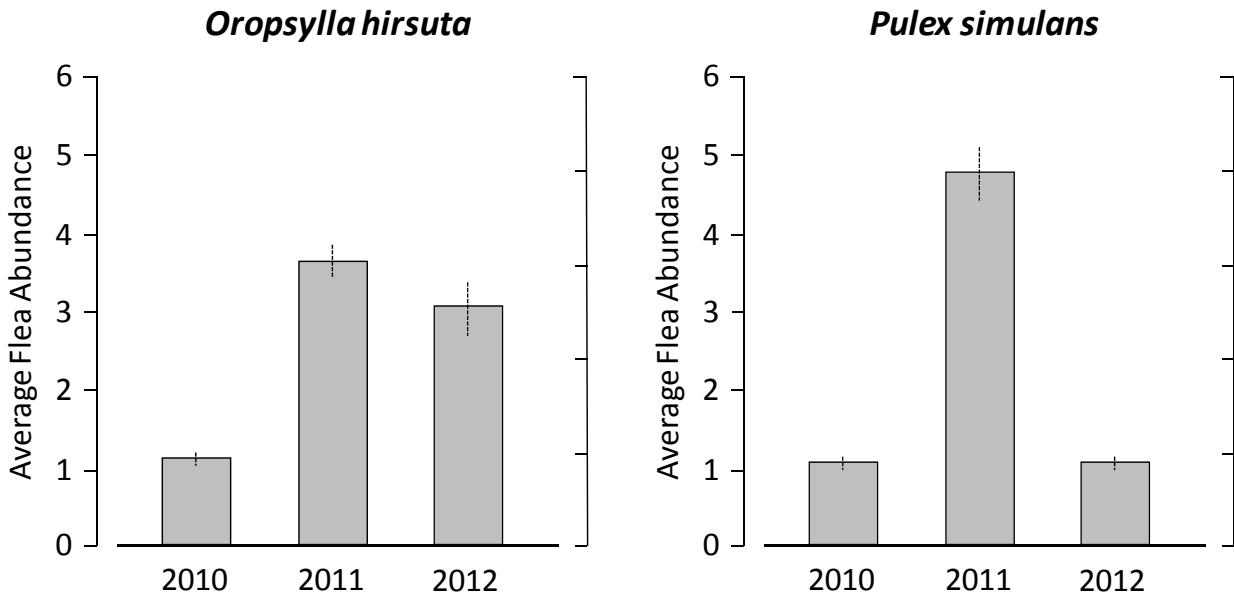
Figure 19 Average abundance of adult *Oropsylla hirsuta* and *Pulex simulans* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) during June–August, 2010-2012, at the Vermejo Park Ranch, New Mexico, USA. Vertical lines depict 95% confidence intervals.
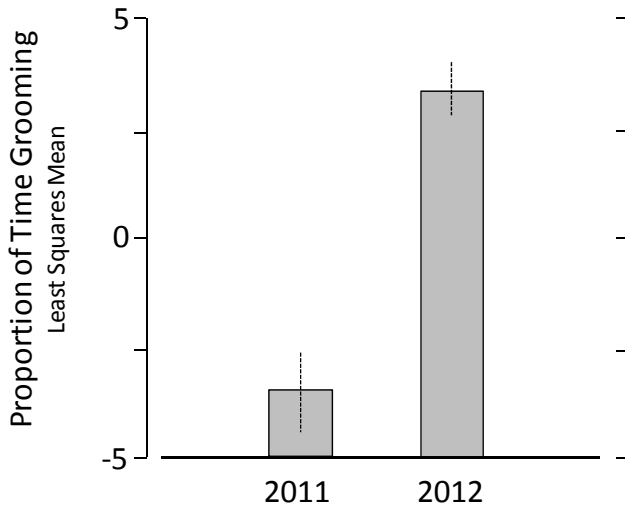
Figure 20 The proportionate amount of time black-tailed prairie dogs (*Cynomys ludovicianus*) spent grooming (duration) during focal observations in 2011 and 2012. Grooming duration is presented as least squares means to control for other variables in a generalized linear model. This figure is limited to July-August so the months of sampling are the same between years. Vertical lines depict 95% confidence intervals. Data were collected at the Vermejo Park Ranch, New Mexico, USA.
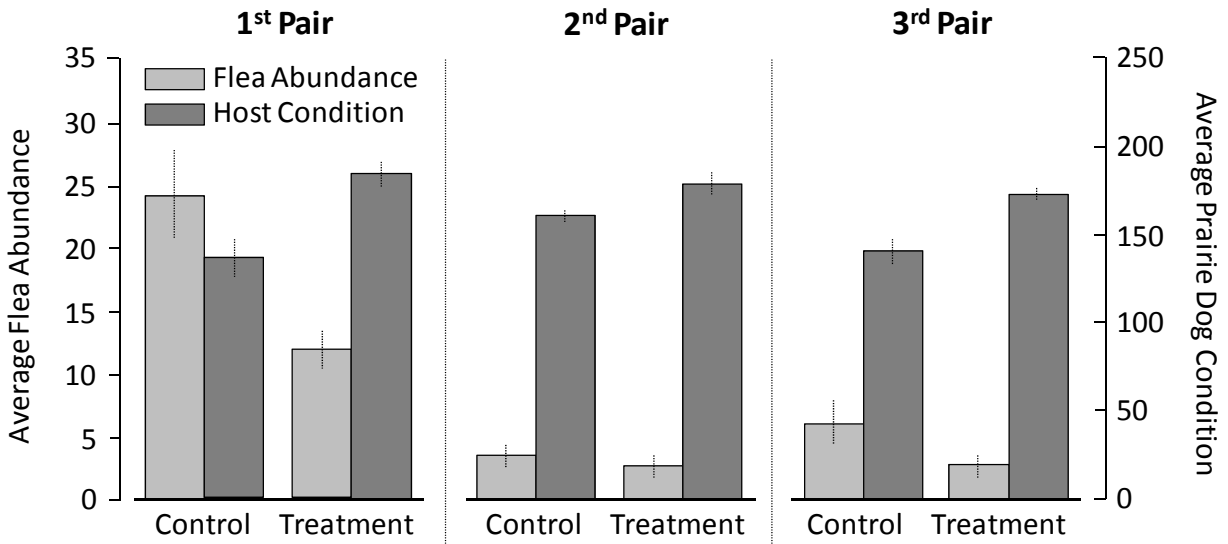
Figure 21 Average abundance of adult fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) and average condition (mass:foot) of prairie dogs during an experiment at the Vermejo Park Ranch, New Mexico, USA. Data were collected in three experimental pairs (control and treatment). Treatment plots received supplemental food and water during March–May, 2012, whereas the control plots did not. Fleas were collected from prairie dogs during June–August, 2012. Vertical lines depict 95% confidence intervals.

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CHAPTER FIVE[33]

USING OCCUPANCY MODELS TO INVESTIGATE THE PREVALENCE OF

ECTOPARASITIC VECTORS ON HOSTS:

AN EXAMPLE WITH FLEAS ON PRAIRIE DOGS

Ectoparasites are often difficult to detect in the field. We developed a method that can be

used with occupancy models to estimate the prevalence of ectoparasites on hosts, and to

investigate factors that influence rates of ectoparasite occupancy while accounting for imperfect

detection. We describe the approach using a study of fleas (Siphonaptera) on black-tailed prairie

dogs (*Cynomys ludovicianus*). During each primary occasion (monthly trapping event), we

combed a prairie dog three consecutive times to detect fleas (15 s/combing). We used robust

design occupancy modeling to evaluate hypotheses for factors that might correlate with the

occurrence of fleas on prairie dogs, and factors that might influence the rate at which prairie dogs

are colonized by fleas. Our combing method was highly effective; dislodged fleas fell into a tub

of water and could not escape, and there was an estimated 99.3% probability of detecting a flea

on an occupied host when using three combings. While overall detection was high, the

probability of detection was always <1.00 during each primary combing occasion, highlighting

the importance of considering imperfect detection. The combing method (removal of fleas)

caused a decline in detection during primary occasions, and we accounted for that decline to

avoid inflated estimates of occupancy. Regarding prairie dogs, flea occupancy was heightened

in old/natural colonies of prairie dogs, and on hosts that were in poor condition. Occupancy was initially low in plots with high densities of prairie dogs, but, as the study progressed, the rate of flea colonization increased in plots with high densities of prairie dogs in particular. Our methodology can be used to improve studies of ectoparasites, especially when the probability of detection is low. Moreover, the method can be modified to investigate the co-occurrence of ectoparasite species, and community level factors such as species richness and interspecific interactions.

Introduction

Several vector-borne diseases can compromise human and wildlife health, and are receiving increased attention from scientists (Jones et al., 2008). For example, plague, an infamous zoonotic disease caused by the primarily flea-borne bacterium *Yersinia pestis*, is estimated to have killed >200,000,000 humans. Moreover, and from a wildlife perspective, plague can negatively affect free-living mammals and distort trophic relationships (Biggins and Kosoy, 2001a,b; Gage, 2012). As a result, intensive effort is devoted to studying plague, as exemplified by reviews of historical literature on the topic, and a recent international symposium (Gage and Kosoy, 2005, 2006; Antolin et al., 2010; Eisen and Gage, 2012).

Currently, flea-control with insecticides is the primary method to mitigate plague-caused mortality, which highlights the relevance of flea ecology in plague management (Cully et al., 2006; Wimsatt and Biggins, 2009; Biggins et al., 2010). With an increased understanding of flea

ecology, insecticides could be distributed in a strategic fashion to control fleas in areas where they are most abundant.[34]

To implement such a strategy, however, we require methods that are effective in monitoring flea populations. In particular, researchers require methods that are effective in studying fleas that parasitize rodents, because rodents are especially susceptible to plague and, along with their fleas (Siphonaptera), are the primary hosts of *Y. pestis* (Barnes, 1982; Gage and Kosoy, 2005).

When studying fleas that parasitize rodents, observers use a comb to collect fleas from hosts and concentrate on two parasitological indices: the proportion of sampled hosts observed as parasitized by at least one flea (prevalence) and the number of fleas collected from each sampled host (abundance)[35] (Bush et al., 1997). Of these two indices, prevalence is more commonly used in studies of fleas (and other ecto- or macroparasites) because the index is straightforward to implement and because highly skewed distributions of abundance often hinder analyses and interpretation.

Imperfect detection of wildlife has received much attention in recent years (MacKenzie et al., 2006) but is rarely considered in studies of ectoparasites such as fleas (Jenelle et al., 2007; McClintock et al., 2010; Cooch et al., 2012; but see examples in Thompson, 2007; Abad-Franch et al., 2010; Adams et al., 2010; Gómez-Díaz et al., 2010). It seems that imperfect detection should be considered when studying fleas, however, because natural selection among these insects has favored anatomical and behavioral characteristics that facilitate movement within a host's pelage and resistance to disturbance, perhaps including combing by a biologist (Traub, 1972, 1980; Marshall, 1981; Krasnov, 2008).

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[34] Continued developments with vaccines may also provide an effective management approach (Abbott et al., 2012).
[35] Intensity is also sometimes considered (i.e., numbers of fleas on hosts with at least one flea).

We propose that detection of fleas on live-caught hosts is at least sometimes imperfect, which can result in underestimates of prevalence and biases in parameter estimates from multivariable models that link parasitism-status with host or environmental covariates (Nagelkerke et al., 1990; de Vlas et al., 1993; Jenelle et al., 2007; Thompson, 2007; McClintock et al., 2010; Cooch et al., 2012). Moreover, imperfect detection may affect experiments that aim to evaluate the effectiveness of insecticides in reducing flea prevalence on hosts (Jachowski et al., 2011).

Direct estimation of detection probabilities may improve inferences in studies of fleas and other ectoparasitic vectors. We use flea data from black-tailed prairie dogs (*Cynomys ludovicianus*) in a case study to present a method that can help to estimate the occurrence of fleas and other ectoparasites on hosts while accounting for imperfect detection. To date, studies of fleas on prairie dogs have used naïve indices from sampling events in which fleas are removed from each prairie dog's pelage during a single combing event, and imperfect detection has not been considered (e.g., Brinkerhoff et al., 2006, 2010; Pauli et al., 2006; Tripp et al., 2009; Biggins et al., 2010; Jachowski et al., 2011, 2012). We describe the use of three repeated 15-s combings to acquire data that can be used with occupancy models to account for imperfect detection. To our knowledge, our approach is the first extension of occupancy modeling to ectoparasites on hosts. The collective approach is equally applicable to many host-ectoparasite systems, including hosts parasitized by lice (Phthiraptera), and mites and ticks (Acariformes).

Materials and Methods

Study Subjects, Site, and Sampling Plots

Black-tailed prairie dogs are mid-sized, sciurid rodents that live in colonies of harem-polygynous families. These rodents are highly susceptible to plague (Hoogland, 1995; Cully et al., 2006).

We conducted our study during May–September, 2011, at the Vermejo Park Ranch, Colfax County, New Mexico (hereafter Vermejo). Vermejo is a 240,000 ha bison (*Bison bison*) ranch that is owned and operated by Turner Enterprises Incorporated. We studied black-tailed prairie dogs in a complex of colonies situated in the southeastern portion of Vermejo, in 24,300 ha of semi-arid short-grass prairie dominated by blue grama (*Bouteloua gracilis*). Precipitation was limited during spring and summer, 2011. Consequently, above ground vegetation was sparse just before and during our study, and most of the prairie dogs were in poor condition and appeared malnourished (D.A. Eads, unpublished data).[36]

The prairie dog colonies differed in the length of time they had been inhabited and the manner in which they were established (D.H. Long, 1996–2013, unpublished data). Like Augustine et al. (2008) and Hartley et al. (2009), we classified the colonies as either "old" or "young." We defined old colonies as those that originated 9 or more years before the study, and young colonies as those that originated 7 or fewer years before the study (there were no 8-year-old colonies at the start of our study). Some colonies were established naturally by prairie dogs (type = natural) and others were established when biologists translocated prairie dogs during 1999–2006 (type = translocation; Long et al., 2006). Before translocating the prairie dogs,

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[36] Also see Chapter 4.

biologists used a deltamethrin-containing insecticide to remove fleas from the prairie dogs and from burrows in the translocation areas (DeltaDust®, Bayer Environmental Science, Research Triangle Park, NC, USA). Fleas had colonized the translocation colonies by the time of our study, but the insecticide treatment may have created differences in flea ecology between the translocation and natural colonies (a hypothesis that we investigated).

We captured prairie dogs in 20 plots distributed among old or young, and natural or translocation colonies. Thus, the scale of sampling related to plots, each within a colony (Fig. 22). Plots were established at random locations in the colonies. We categorized the plots into five groups of 2–3 plots each and sequentially sampled these groups in randomized order during 10-day work periods. Field research was completed under Colorado State University Institutional Animal Care and Use Committee Protocol #10-1785A.

Trapping Prairie Dogs and Combing Them to Collect Fleas

We distributed 25 or 37 single-door live-traps throughout each plot, with the density of traps standardized at $16.3 \times$ ha^{-1} (Tomahawk Live Trap, Hazelhurst, WI, USA). During a field day, we set traps in a group of plots using 11% sweet feed grains (MannaPro®, St. Louis, MO, USA) laced with peanut butter, and returned to check the traps immediately after the early-morning peak activity by prairie dogs. We placed prairie dogs in the shade of a truck to protect them from direct sunlight. Because fleas can leap ~30–40 cm during one jump (Krasnov, 2008), we placed the trapped animals ≥ 50 cm apart to reduce the probability of fleas jumping from one prairie dog to another before we sampled them. We successively processed each animal near or inside the bed of the truck to reduce wind disturbance.

We moved each prairie dog from the trap into a pre-weighed pillowcase and then weighed

the prairie dog to the nearest gram using a Pesola® spring-scale (Kapuskasing, ON, Canada) that

was calibrated with a digital scale. We visually confirmed the prairie dog's sex (Hoogland,

1995) and measured its right hind foot using a tape measure (nearest 0.25 cm). The weight[37] and

skeletal measurements allowed us to calculate an index of each animal's body condition,

expressed as the ratio between its weight and hind-foot length; higher values of weight:foot

ratios indicate greater body condition (Krebs and Singleton, 1993). Some prairie dogs were

sampled in multiple months; for these animals, we used their average weight:foot ratio in

analyses.

We transferred each trapped prairie dog to an induction chamber containing isoflurane to

anesthetize it and the fleas it might be carrying. After the prairie dog was in the induction

chamber for 20 s, we removed it and flea collection was initiated. We combed each prairie dog

as thoroughly as possible during three 15-s combings (timed with a digital clock), each

conducted over a unique tub containing about 4 cm of water. Two people combed prairie dogs;

intensive training helped to standardize the methods and reduce heterogeneity between

observers. During combing, the person held the prairie dog vertically by the nape, started a 15-s

timer on the digital watch, and started to comb using firm, repeated downward strokes, each the

length of the prairie dog's body. Combing was started on the dorsal surface, and the observer

turned the prairie dog clockwise to comb the right lateral, ventral, and left lateral surfaces of the

prairie dog. The combing was then repeated in reverse order by turning the prairie dog counter

clockwise, and the 15-s period ended after the dorsal surface was recombed. The observer

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[37] Technically, this term should be "mass" throughout the paper (thus, the body condition indices are mass:foot ratios).

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quickly shifted the prairie dog to the next tub and initiated the next combing, and so forth until all three combings were completed.

Dislodged fleas fell into the tubs and floated in the water, leaving them unable to jump away as we collected them. We counted fleas from each of the three tubs separately, which resulted in an encounter history comprising three consecutive attempts at detecting fleas on a prairie dog. For example, an encounter history of '1-1-0' indicates that at least one flea was found during the 1st and 2nd combing occasions, but no flea was found on the 3rd occasion. All fleas from a unique host were placed in one vial.

After combing each prairie dog, we marked each of its ears with a #1 monel fingerling fish tag for permanent identification (National Band and Tag Company, Newport, KY, USA) (Fagerstone and Biggins, 1986; Hoogland, 1995; Biggins et al., 2010). The tags allowed us to consistently identify each individual throughout the field season (Hoogland, 1995). We released each prairie dog at its trapping location.

If a prairie dog was trapped within a certain month (May–September), we used its encounter history for that month. In some cases an animal was captured two times in one month (0 prairie dogs captured twice in May, 58 in June, 34 in July, 16 in August, and 1 in September). In such cases, we randomly selected one of the sampling occasions for the month. If a prairie dog was not trapped during a certain month, its sampling history contained a blank entry for that month (i.e., indicated by periods, '.-.-.'). Thus, the data were collected using a "robust design," with each month serving as a primary sampling occasion (Fig. 23). We assumed that a prairie dog was "closed" to changes in flea occupancy during a primary occasion, but could be colonized by fleas, or lose fleas, between primary occasions (Fig. 23; MacKenzie et al., 2006).

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We applied multiple-season occupancy models to investigate detection of fleas ($p =$ probability of detection, given a flea is present), and patterns of flea prevalence ($\Psi =$ probability of occupancy), colonization ($\gamma =$ probability of a previously unoccupied host becoming occupied), and extinction ($\varepsilon =$ probability of a previously occupied host becoming unoccupied) (Fig. 23; MacKenzie et al., 2003, 2006). The definition of detection in occupancy modeling differs from the definition in mark-recapture studies. In occupancy modeling, it refers to the probability of detecting at least one animal (regardless of its unique identity) from a population of N animals, whereas in mark-recapture studies it relates to the probability of detecting a unique animal. Flea colonization is similar to the parasitological index "incidence," the proportion of previously unoccupied hosts that become occupied over a particular time interval (Bush et al., 1997).

We were interested in potential variation in flea prevalence among plots with differing densities of prairie dogs (as noted in the *a priori* hypotheses below). We indexed densities of prairie dogs in trapping plots by dividing the total number of individuals trapped in a plot by the area of that plot (minimum number alive converted to naïve density estimates; Krebs,1966; Otis et al., 1978; White et al., 1982; Pocock et al., 2004; Jones et al., 2012). Effort was similar among plots (given the sampling approach described above) suggesting that the density indices are useful as relative values.

A priori Hypotheses

We used occupancy models to investigate hypotheses for factors that may correlate with the occurrence of fleas on black-tailed prairie dogs and colonization of prairie dogs by fleas. The

prairie dogs were parasitized primarily by two flea species: *Oropsylla hirsuta* and *Pulex simulans* (D.A. Eads, unpublished data). Little is known about the comparative efficiency of these species as plague vectors (Eisen and Gage, 2012), so we concentrated on the occurrence of fleas in general.

Flea occupancy and colonization were related to monthly patterns (season), characteristics of prairie dogs (host), and characteristics of prairie dog colonies (habitat). The hypotheses chosen for evaluation are listed below:

(1) Flea prevalence can differ among months due to the seasonality of flea life cycles and influences of temperature and humidity on flea development and survival (Krasnov, 2008). Thus, we hypothesized that flea occupancy and colonization would vary during our field season (May–September).

(2) In some rodents, flea prevalence differs between female and male hosts (e.g., due to behavioral or immunological differences) and males typically harbor more fleas than females (Krasnov, 2008). Thus, we hypothesized that flea occupancy and colonization would be higher for male prairie dogs.

(3) Fleas are sometimes more prevalent on hosts that are in relatively poor body condition, because such hosts tend to exhibit weakened defenses against fleas (Krasnov, 2008). Thus, we hypothesized that flea occupancy and colonization would be greater for prairie dogs in relatively poor condition.

(4) Fleas are sometimes more prevalent in areas where hosts are abundant, because an abundance of hosts can provide fleas with many feeding opportunities, and behavioral interactions between hosts provide opportunities for fleas to disperse among hosts (Krasnov, 2008). In other cases, however, fleas can be less prevalent in areas with an

abundance of hosts because the fleas are concentrated on particular hosts, and not others (Krasnov, 2008). We evaluated these competing hypotheses and predicted that flea occupancy and colonization would vary among plots with differing densities of prairie dogs.

(5) Flea ecology might also vary among colonies of prairie dogs, for instance between old and young colonies, or natural and translocation colonies. Regarding colony ages at our study site, prairie dogs had occupied old colonies for at least 9 years and young colonies for 7 or fewer years. Perhaps fleas are more prevalent in old colonies that have been occupied by prairie dogs for many years, relative to younger colonies, because the old colonies might contain relatively deep burrows that provide stable microclimates for ectothermic fleas. We hypothesized that flea occupancy and colonization would be greater in the old colonies.

(6) Lastly, at our study site, biologists used DeltaDust[®] to establish translocation colonies, but had never used any insecticide at the natural colonies. Although the effectiveness of DeltaDust[®] wanes over time, initial use of an insecticide would have hampered flea populations in the translocation colonies (Seery et al., 2003; Biggins et al., 2010) and that effect on fleas might have persisted into the period of our research, 11–12 years (old colonies) and 5–7 years (young colonies) after the translocation events. We hypothesized that flea occupancy and colonization would be greater in the natural colonies with no history of insecticide treatment.

We used multiple-season (essentially multi-month) robust design occupancy models in Program MARK to investigate the prevalence of fleas on prairie dogs, and to relate predictor variables to flea prevalence and colonization (White and Burnham, 1999). Predictor variables included MONTH (May–September), SEX of prairie dog, CONDITION of prairie dog (weight:foot), COLONYAGE (old or young), COLONYTYPE (natural or translocation), and PD-DENSITY (density of prairie dogs in a plot). Only six juvenile prairie dogs were captured and those data were removed from the dataset (juveniles contributed to the indices of PD-DENSITY, however). We assumed that detection was the same for all prairie dogs, given we standardized the combing method among hosts, and therefore did not relate detection to the predictor variables.

In the modeling exercise, we included main-effects only (i.e., no interactions). All hypotheses were plausible, so we ran all possible subsets of models with the following restrictions:

(1) In organizing the data, we noted that if a prairie dog was occupied by at least one flea during a primary occasion (monthly combing), it was occupied by at least one flea during all subsequent primary occasions. That is, once a flea occupied a prairie dog (e.g., in July), at least one flea occupied the prairie dog during subsequent primary occasions (in August and September). Thus, extinction necessarily equaled zero (Fig. 23) and we fixed extinction to zero because it was useful to fix that parameter and concentrate on estimating other parameters. Two points are important to note. First, all instances of '0-0-0' encounter histories corresponded with the first sampling

occasion for a prairie dog (i.e., the first month in which certain prairie dogs were captured and processed). The occupancy models considered the possibility that hosts with '0-0-0' encounter histories were simply "unoccupied." Second, while the removal of fleas from prairie dogs during primary occasions could conceivably result in "user-induced extinction" between primary occasions (e.g., months), we emphasize that between-month extinction events did not occur during our study.

(2) Multiple-season occupancy models assume that a sampling unit is closed to immigration (colonization), emigration (extinction), and ectoparasite population extinction during a primary occasion (MacKenzie et al., 2006). During combings, immigration of fleas on to a prairie dog was unlikely because we sampled hosts while holding them in hand. Emigration of fleas from a prairie dog was highly probable because the combing method is designed to remove fleas from prairie dogs. However, we might not have been able to remove all fleas given difficulties associated with removing fleas from hosts, suggesting 'population extinction' was unlikely to have occurred during a primary occasion. Indeed, in many cases, we found fleas on prairie dogs after we had finished the 3rd combing. The assumption of 'no emigration' can be relaxed with the use of covariates that account for changes in animal abundance during primary occasions (MacKenzie et al., 2006; Riddle et al., 2010). We assumed that if fleas were found during a secondary occasion within a primary occasion, then the probability of detecting a flea during subsequent combings within that same primary occasion should be reduced because fleas were already removed from the host (see also Riddle et al., 2010). Thus, we included covariates (REMOVAL) for detection that denoted whether or not fleas were combed from a

host (i.e., removed) on the 1st or 2nd secondary occasions (Fig. 23). For instance, if

fleas were not found on the 1st occasion but were found on the 2nd, then

$REMOVAL_1 = 0$ for the 2nd occasion and $REMOVAL_2 = 1$ for the 3rd occasion.

The $REMOVAL_1$ and $REMOVAL_2$ effects for detection were included in all models,

except during a bootstrap assessment of model fit that is described below.

(3) Occupancy and colonization could either vary or remain constant by prairie dog SEX,

prairie dog CONDITION, PD-DENSITY, COLONYAGE, and COLONYTYPE.

(4) Colonization could either vary or remain constant by MONTH. We knew that

occupancy varied to some degree among months, and monthly variation in occupancy

was incorporated into the models.

(5) We assumed that if occupancy varied by PD-DENSITY, then colonization would also

vary by PD-DENSITY. In addition, we assumed that if colonization varied by PD-

DENSITY, then occupancy would also vary by PD-DENSITY. Thus, if an effect of

PD-DENSITY for occupancy or colonization was included in a model, then an effect

of PD-DENSITY was included for the other parameter.

To test for overdispersion (Burnham and Anderson, 2002), we ran a model that included all

independent variables except individual covariates (SEX, CONDITION, PD-DENSITY, and

$REMOVAL_1$ and $REMOVAL_2$) and assessed goodness-of-fit using a parametric bootstrap

(10,000 simulations; MacKenzie and Bailey, 2004). We could not include individual covariates

in this assessment because the simulations homogenize animals into cohorts if they have similar

covariate values. Many of the covariate values differed among individual prairie dogs, leading to

a very large number of cohorts, and the data would have been too sparse for a meaningful

bootstrap analysis (MacKenzie and Bailey, 2004).

We ran all possible models with the restrictions above ($n = 1{,}024$ models) and ranked the models by Akaike's Information Criterion adjusted for small sample size (AICc). We calculated differences between AICc for the most supported model and the other models (Δ AICc) and calculated AICc weights (w) for each model (Burnham and Anderson, 2002; Anderson, 2008). Then, we calculated cumulative weights for each main-effect by summing w's from all models containing the effect (maximum weight = 1.00; Burnham and Anderson, 2002, Anderson, 2008). In the results, we investigate main-effects with cumulative weights > 0.50 (Barbieri and Berger, 2004). We used model-averaged parameter estimates, with 95% confidence intervals, to plot categorical main-effects (Burnham and Anderson, 2002; Anderson, 2008). For each continuous effect (CONDITION and PD-DENSITY), we interpreted figures derived from the highest ranked model containing the effect.

Results

We sampled 299 adult prairie dogs, including 156 females and 143 males. Of the 299 adults examined, 201 were from old, and 98 from young colonies, and 166 from natural, and 133 from translocation colonies. Effective sample sizes were 299 for occupancy and 494 for detection ($\bar{x}$ primary occasions per prairie dog = 1.65, range = 1–4). Naïve densities of prairie dogs in trapping plots ranged from 3.90 to $18.21 \times \text{ha}^{-1}$ ($\bar{x} = 10.93 \times \text{ha}^{-1}$). Body condition indices (weight:foot) ranged from 72.00 to 196.00 ($\bar{x} = 126.81$).

We detected at least one flea on a prairie dog during 396 of the 494 primary occasions. Detection was imperfect and, consequently, naïve indices of flea prevalence were often biased low relative to estimates of prevalence from the occupancy models (Fig. 24). Moreover,

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estimates of flea occupancy tended to be more precise than the naïve indices. For instance, during July-September, the model-averaged estimates of prevalence from occupancy modeling were characterized by smaller confidence intervals than the naïve indices of prevalence (Fig. 24). Confidence intervals were wider for the model-averaged estimates in May, but relatively few prairie dogs were sampled in that month. The occupancy models allowed us to acknowledge uncertainty in estimating occupancy for May, whereas the naïve indices suggested there was greater confidence (Fig. 24).

Among months, and on average, the detection probability during primary occasions was 91.7% for the 1st combing, 85.4% for the 2nd, and 81.1% for the 3rd. Thus, detection declined during consecutive combings, which highlights the utility of the REMOVAL covariates (Fig. 25). On average, if a flea was not detected during the 1st combing, detection was 94.6% for the 2nd combing, suggesting 5.4% error in prevalence if only one 15-s combing was used. If a flea was not detected during the 1st or 2nd combings, detection was 99.3% for the 3rd, suggesting an error of 0.7% if two combings were used, and that we rarely failed to detect a flea on an occupied prairie dog when using three combings (99.3% ~ 100%).

The goodness-of-fit simulation suggested little overdispersion in the data (all $P \geq 0.59$) and, therefore, we did not adjust parameter estimates or AICc values with a dispersion parameter. Ranking of models via AICc indicated model selection uncertainty (see Supplementary material). Eight variables received cumulative weights > 0.50 (Table 12). Cumulative weights for the remaining variables were ≤ 0.45.

Flea occupancy increased from May into July, and peaked in August and September (Fig. 26). In September, at least one flea was collected from every prairie dog; detection was

imperfect, however, because fleas were not always collected during the first combing. The rate

of flea colonization increased from June into July, and declined thereafter (Fig. 26).

Flea occupancy was consistently higher in the old colonies (Fig. 26). Flea occupancy was

higher in the translocation colonies in May, but both colonization and occupancy were higher in

the natural colonies during June–September (Fig. 26). For the old and natural colonies,

previously unoccupied prairie dogs were almost always colonized by fleas by July–August,

resulting in very high rates of occupancy in those colonies during the latter portions of our study

(Fig. 26). In contrast, for the young and translocation colonies, rates of colonization were lower

in July and August, and some prairie dogs in those colonies remained unoccupied by fleas during

August. Although all prairie dogs harbored fleas in September, our occupancy estimates suggest

that some of the non-sampled prairie dogs in the young and translocation colonies were

unoccupied by fleas in September (Fig. 26).

Flea occupancy was lower in plots with higher densities of prairie dogs but, as occupancy

increased during our study, rates of flea colonization were higher in plots with higher densities of

prairie dogs (Fig. 27). Lastly, flea occupancy was higher for prairie dogs that were in relatively

poor condition (Fig. 28).

Discussion

The use of occupancy models in parasitology has increased in recent years (Jennelle et al.,

2007; McClintock et al., 2010; Cooch et al., 2012; Lachish et al., 2012; Miller et al., 2012). At

least one study has used such models to investigate the prevalence of disease vectors. Abad-

Franch et al. (2010) sampled palm trees for hemipteran vectors of the parasite *Trypanosoma*

cruzi, and then used occupancy models to estimate rates of tree-occupancy. To our knowledge, occupancy models have not been used to study the prevalence of ectoparasites or vectors on hosts and, consequently, our approach is a novel extension of the use of occupancy models.

Assumptions of Ectoparasite Occupancy Models

Occupancy models make numerous assumptions, some of which can be relaxed (MacKenzie et al., 2003, 2006). First, the models assume that the population of interest may or may not be detected during a survey, and is not falsely detected when absent. This assumption was well met in our study, because we detected fleas during some sampling occasions but not others, and the fleas were easily distinguishable from other ectoparasites, such as lice, mites and ticks. In future studies that utilize our methodology, if identification of the ectoparasite is difficult, care should be taken to confirm its identity.

Second, the models assume that detection histories of individual sampling units are independent (i.e., detection histories for different prairie dogs are independent). This assumption seems well met with our methodology because the sampling method was standardized, and we processed each prairie dog separately.

Third, the models assume occupancy and abundance do not change within primary occasions (in our case, a monthly sampling occasion). We suspect that occupancy rarely changed during primary occasions, because we often found fleas on prairie dogs after the 3rd combing during a primary occasion. In relation to abundance, we relaxed the assumption of constant abundance by using the REMOVAL covariates that accounted for removal of fleas. We address the REMOVAL covariates below (Section 4.3).

Fourth, the models assume no non-modeled heterogeneity remains in any of the parameters. This assumption seems difficult to meet, given that it is difficult or simply impossible to collect data on all factors that influence rates of occupancy, colonization, extinction, and detection. Careful pre-study brainstorming can help to increase the odds of meeting this assumption, but we suspect the assumption is at least partly violated *sensu stricto* in virtually all wildlife studies that rely on model-based inference.

Lastly, our combing method requires a sufficient sample size for use with occupancy models, and assumes that the sampling design is effective for parasitized and unparasitized hosts alike. If few hosts are sampled, and/or if host detection probability varies between parasitized and unparasitized hosts, estimates of vector occupancy may suffer from low sample sizes or biases (Jennelle et al., 2007; Cooch et al., 2012). For instance, if parasitized hosts are less likely to be sampled than unparasitized animals, the resulting estimates of vector occupancy will be biased low. This potential bias might not apply to our study because flea prevalence was generally high overall, but future studies of ectoparasites may need to account for variability in host detection.

The New Combing Method

Traditionally, fleas are combed from a prairie dog into an empty tub and collected using forceps, which is difficult. Fleas seem to succumb to anesthesia more slowly and awake from anesthesia more quickly than prairie dogs, and can jump back on to the prairie dog, thereby reducing the chances of collecting fleas (D.A. Eads and D.E. Biggins, personal observations). Moreover, if a flea remains in the tub and is visible, then it is available for counting, but fleas are small and difficult to collect from empty tubs.

Our combing method is effective in removing fleas from prairie dogs. Dislodged fleas fell from the prairie dog into a pool of water that lined a tub, and the viscosity of water is low enough that the fleas could not escape from the surface, but instead either floated in the water or sank to the bottom of the tub. This allowed us to use forceps and a vial to easily collect and count each flea, which likely increased detection. Indeed, although imperfect, estimates of flea detection from our occupancy models were always well above 0.50, a detection probability that is considered "high" (MacKenzie and Royle, 2005).

The high probability of detection during primary occasions in our study (99.3%) could suggest that there is little need to account for imperfect detection when a prairie dog is combed for at least 45 seconds and a water-lined tub is used to collect fleas. In fact, one could argue that error is relatively small when using one (5.4%) or two combings (0.7%) and, consequently, only one or two combings are needed to study the prevalence of fleas on prairie dogs. In future studies, if time or logistical constraints limit the amount of time that can be devoted to combing hosts, or if trapping success is extremely high and a surplus of animals await sampling, then one or two combings might suffice when studying flea prevalence.

Nonetheless, we suggest that it is useful to account for imperfect detection for at least four reasons. First, indices of ectoparasite prevalence and their confidence intervals are assumed to represent true variation in nature, and this assumption is violated when detection is at least somewhat imperfect (Jennelle et al., 2007). Thus, in general, investigators should account for imperfect detection when possible, and acknowledge when they are unable to do so (MacKenzie et al., 2003, 2006).

Second, the use of three combings is useful because occupancy models allow for an evaluation of flea colonization and extinction, and the dynamics of flea parasitism among hosts.

In contrast, if only one combing is used, then only naïve indices of prevalence are obtainable, and colonization/extinction dynamics are difficult to study.

Third, we tended to gain precision in our estimates of occupancy by accounting for the small degree of imperfect detection (Fig. 24). In addition, it seems that the estimates of occupancy would be more accurate because they accounted for the small degree of imperfect detection.

Lastly, consideration of imperfect detection, and the use of three combings is warranted because the probability of detection is likely to vary among host species, and perhaps among individuals within a species, and will vary according to the ectoparasite of interest. For example, if mammalian hosts are of interest, the probability of detecting ectoparasites could vary due to differences in the density and thickness of guard hairs and under fur, or the phase of molting. Detection could also vary due to differences among ectoparasite species in their ability to remain on the host, or in their preferences for feeding locations on a host's body. Indeed, some ectoparasites are especially difficult to detect. For example, Mize (2009) sampled *Peromyscus* mice for ectoparasites, and 91.2% of lice were missed in the field. In such cases, our sampling approach could help to account for imperfect detection.

Accounting for the Removal of Ectoparasites

Royle and Nichols (2003) noted that in studies of animal occupancy, an important source of heterogeneity in detection probabilities is variation in animal abundance among sampling units or sampling occasions. In fact, this might be the most important source of heterogeneity because animals are easier to detect if they are abundant (Royle and Nichols, 2003). In our study, on average, detection of fleas was highest during the 1st combing (92%) and then declined during

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the 2nd (85%) and 3rd (81%) combings. This trend was expected because as fleas are removed during a combing, fewer are available for detection during subsequent combings. Moreover, the first combing disturbs fleas, and if these insects are not fully anesthetized (which is sometimes the case) they begin to exhibit evasive behaviors that may reduce detection. A similar trend is sometimes observed in studies of animals that seek refuge after detecting human observers, and such avoidance responses can reduce rates of detection during repeated surveys (Riddle et al., 2010).

We accounted for reductions in detection during consecutive combings by using covariates that denoted whether or not fleas were detected (removed) during the 1st and/or 2nd combing in a primary occasion (REMOVAL$_1$ and REMOVAL$_2$; see Riddle et al., 2010 for a similar example). This approach proved useful because if the REMOVAL covariates were excluded, the probability of detection was reduced and, consequently, the estimates of occupancy were inflated. Indeed, when we excluded the REMOVAL covariates from the most supported model in our analysis and allowed for variation in detection during a primary occasion, the probabilities of detection were estimated at 94.3%, 52.3%, and 30.1% (values that are much lower than those in Fig. 25). These negative biases in the rates of detection caused inflation in the estimates of flea occupancy by about 7%.

Thus, it is important to account for reductions in flea densities caused by removal during combing. Otherwise, estimates for the probability of detection can be underestimated and estimates of occupancy/colonization become inflated. In future studies, if some hosts lose fleas between primary occasions (i.e., if extinction events occur), a failure to use the REMOVAL covariates could reduce the estimates for rates of extinction because the models would assume

that some cases of extinction should be attributed to a failure to detect at least one flea on an occupied host.

The above line of thinking indicates that the REMOVAL covariates can help to account for removal that is induced by our combing method and, thereby, help to relax the assumption of closure between combings during a primary occasion. Moreover, this approach should allow researchers to reduce bias and acquire more accurate estimates of ectoparasite occupancy, colonization, and extinction. Therefore, we encourage the use of REMOVAL covariates when implementing our methods or similar methodology in the future.

We caution, however, that our method is not a panacea, and studies are needed to compare our approach to other methods that account for "abundance-induced heterogeneity" in detection. For instance, Royle and Nichols (2003) describe a class of occupancy models that specifically deal with variation in detectability induced by the abundance of individuals. We suspect that at least some of the models proposed by Royle and Nichols (2003) would be useful in studies of ectoparasitic vectors. In particular, the negative binomial model for abundance may help to account for the aggregated distribution of ectoparasites among hosts (see also Lachish et al., 2012).

We also caution that if all ectoparasites are removed from a host during a primary occasion, and attributes of the host and/or ectoparasite prevent the host from acquiring new ectoparasites during the interval between primary occasions, then our sampling procedure causes a "user-induced extinction." In such cases, the assumption that hosts are "open" to ectoparasite colonization/extinction between primary occasions would be violated (Fig. 23). Thus, studies should be designed such that the interval between primary occasions is of sufficient duration for

hosts to acquire new ectopararsites. This seems to have been the case in our study because we removed fleas from hosts but did not observe extinctions between primary occasions.

Potential Extensions of Our Methodology

Our methodology could be modified in the future to accommodate additional study objectives. For example, if hosts are sampled during one season (e.g., 1 month) then single-season occupancy models can be used (MacKenzie et al., 2006). Also, instead of collecting ectoparasites in the same vial, as we did, researchers could use separate vials for each tub and later identify the ectoparasites to the species level, resulting in separate detection histories for each species. This approach would permit use of multi-species occupancy models, which could prove highly useful in studies of ectoparasites, including those that serve as vectors of infectious disease agents. Indeed, multi-species occupancy models can be used to investigate relationships between species (e.g., co-occurrence or lack thereof) and community level factors (e.g., species richness and species interactions) (MacKenzie et al., 2006).

Laboratory methods could also complement field sampling to account for imperfect detection of pathogens in ectoparasitic vectors. For example, if vectors are tested for the presence of a pathogen of interest, laboratory work could include three or more tests of each set of vectors collected from hosts, allowing researchers to also account for imperfect detection of the pathogen in different vector species (McClintock et al., 2010). This approach proved useful in estimating the prevalence of *Borrelia burgdorferi* bacterial spirochetes in different species of ticks, and provided insight into which species might contribute most to the dynamics of Lyme disease (Gómez-Díaz et al., 2010; see also Thompson, 2007; Kendall, 2009; Adams et al., 2010). By

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accounting for imperfect detection, we can increase understanding of factors that influence the prevalence of ectoparasites and blood-borne pathogens in vectors, and increase our ability to manage vector-borne diseases (McClintock et al., 2010), including plague within colonies of prairie dogs.

Implications of the Case Study

As predicted, the probabilities of flea occupancy and colonization were higher for prairie dogs in the old colonies. At least two factors could explain these trends: differences between old and young colonies in (1) burrow depths and (2) the amount of organic matter in burrows. Burrows in the old colonies ($\geq$9 years old) might have been deeper and contained more organic debris than burrows in the young colonies ($\leq$7 years old). Deep burrows provide more stable microclimates than shallow burrows, and a stable microclimate would presumably benefit fleas that are ectothermic and prone to desiccation (Clark, 1971; Smith, 1982; Shenbrot et al., 2002; Krasnov, 2008). Moreover, large amounts of prairie dog feces and hair, and accumulations of organic nesting materials inside burrows in the old colonies might have provided sufficient resources to support large numbers of flea larvae that could grow to the adult life stage that parasitizes prairie dogs.

We predicted that flea occupancy would be higher for prairie dogs in the natural colonies with no history of insecticide treatment, relative to prairie dogs in the translocation colonies that were once treated with insecticides. Flea occupancy appeared to be higher in the translocation colonies during May, which differs from our predictions. However, the sample size was low for the natural colonies in that month. Fleas increased in abundance as the field season progressed

(Chapter 3) and rates of flea colonization increased in the natural colonies in particular, which supported our hypothesis. These results may suggest that the effect of the insecticide persisted into our study, and reduced the rates of flea colonization in the translocation colonies.

Flea occupancy and colonization also related to the densities of prairie dogs in our sampling plots. The proportion of hosts infected by fleas was initially lower in plots with higher densities of prairie dogs, but as the field season progressed, and fleas increased in prevalence and abundance (Chapter 3), the rates of flea colonization and occupancy increased in plots with high densities of prairie dogs in particular. When fleas are not abundant, as found during May in our study, fleas might be less prevalent on prairie dogs in high density plots because the small numbers of fleas are concentrated on particular hosts. As fleas increase in abundance, however, there are more fleas to parasitize the large number of hosts, and fleas might colonize a large proportion of hosts.

Many factors could facilitate the rate at which fleas colonize prairie dogs in areas where these rodents are abundant. For example, traffic within prairie dog burrows is likely high in areas with an abundance of prairie dogs, and many of the prairie dogs might acquire fleas while moving within the burrow systems, especially during months in which fleas are abundant. Moreover, behavioral interactions and physical contact between prairie dogs might be more common in areas where they are abundant, creating connectivity that increases rates of flea transfer among hosts (Krasnov, 2008), which can increase the probability of fleas colonizing new prairie dogs.

Flea occupancy was higher for prairie dogs in relatively poor body condition, perhaps because these hosts were immunocompromised (Demas and Nelson, 1998) and fleas feed better on hosts with compromised immune systems (Krasnov, 2008). In addition, if in poor condition,

a host might increase its foraging efforts and, in doing so, reduce its grooming efforts because these two behaviors are mutually exclusive (Krasnov, 2008). A reduction in host grooming would benefit fleas because grooming is the primary behavioral defense used by hosts to disrupt and kill fleas (Krasnov, 2008). Thus, compromised immunity, reduced grooming effort, or both of these factors might help to explain why fleas were more prevalent on prairie dogs in poor body condition (Chapter 5).

At least one additional factor could help to explain why flea occupancy was higher for prairie dogs in relatively poor condition: perhaps these animals lived with other prairie dogs that were in poor condition (e.g., due to food limitations), and flea prevalence was greater on hosts in poor condition simply because these hosts acquired fleas from malnourished prairie dogs that died nearby. Indeed, rates of mortality are higher for prairie dogs in poor body condition (Hoogland, 1995), and when a host dies, fleas abandon the carcass to find a living-host from which warm blood can be acquired (Krasnov, 2008).

Our results may provide insight to methods for managing plague. By protecting prairie dogs from fleas and blood-borne transmission of *Y. pestis*, we can facilitate conservation efforts for prairie dogs and the many species that associate with these rodents, thereby helping to facilitate and restore grassland ecosystems in western North America. Our results suggest that flea occupancy and plague risk might each be relatively high in old/natural colonies of prairie dogs and in areas with an abundance of prairie dogs, especially if the prairie dogs are in poor condition. When managing plague in complexes of prairie dog colonies, it might be beneficial to distribute insecticides in old colonies with no history of insecticide treatment first, especially in portions of colonies with an abundance of prairie dogs. Moreover, it might be beneficial to distribute insecticides during periods when prairie dogs are in poor condition, such as when

above ground vegetation is limited, given that fleas can benefit from parasitizing malnourished hosts.

In conclusion, our combing method is highly effective in removing fleas from prairie dogs, and provides data that can be analyzed with occupancy models to account for imperfect detection. This approach will be most useful in studies of ectoparasites when the probability of detection is low.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijppaw.2013.09.002.

Table 12 Hypothesis numbers (*2.3* A priori *hypotheses*) and main-effects with cumulative weights > 0.50. Main-effects related to detection of fleas (*p*), flea occupancy (Ψ), and flea colonization (γ): month of sampling (MONTH), age of black-tailed prairie dog (*Cynomys ludovicianus*) (AGE), body condition of prairie dog (weight:foot, CONDITION), density of prairie dogs in a sampling plot (PD-DENSITY), type of prairie dog colony (natural or translocation, TYPE), and age of prairie dog colony (COLONYAGE).

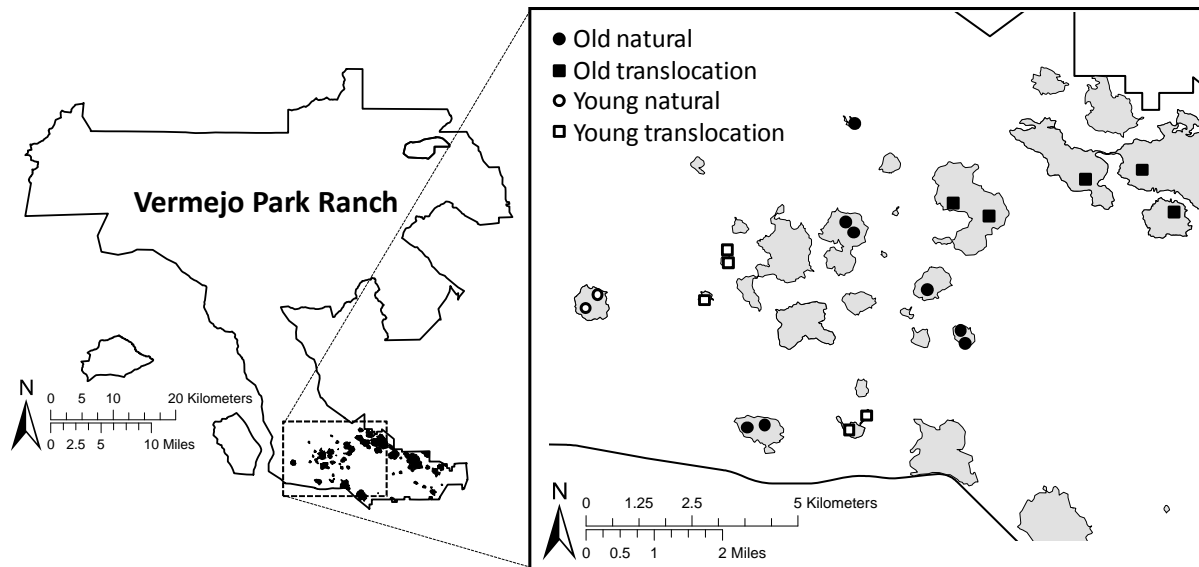| Hypothesis Number	Main-effect	Cumulative weight
1	γ MONTH	0.99
5	γ COLONYAGE	0.98
4	Ψ PD-DENSITY	0.68
4	γ PD-DENSITY	0.68
6	Ψ COLONYTYPE	0.67
6	γ COLONYTYPE	0.61
3	Ψ CONDITION	0.57
5	Ψ COLONYAGE	0.54

Figure 22 Map of the study area within the Vermejo Park Ranch, Colfax County, New Mexico,

showing old and young, and natural and translocation colonies of black-tailed prairie dogs

(*Cynomys ludovicianus*). Gray areas indicate extent of prairie dog colonies in 2009.

Study Design and Example Encounter Histories

Primary Sampling Occasions
Month-of-Year

May	June	July	August	September
3 Combings Secondary Occasions	**3 Combings** Secondary Occasions	**3 Combings** Secondary Occasions	**Not sampled**	**3 Combings** Secondary Occasions
p_1 p_2 p_3	p_1 p_2 p_3	p_1 p_2 p_3		p_1 p_2 p_3
REMOVAL$_1$ REMOVAL$_2$	REMOVAL$_1$ REMOVAL$_2$	REMOVAL$_1$ REMOVAL$_2$		REMOVAL$_1$ REMOVAL$_2$
Closure	Closure	Closure		Closure

Encounter Histories

0-0-0 $\xrightarrow{\text{Open}}$ 0-0-0 $\xrightarrow{\text{Open}}$ 1-0-0 $\xrightarrow{\varepsilon = 0}$.-.-. $\xrightarrow{\varepsilon = 0}$ 1-0-0

No detection — No detection — Detection (colonized) — Not sampled — Detection

Figure 23 The robust design for occupancy models of flea prevalence on black-tailed prairie dogs (*Cynomys ludovicianus*). Prairie dogs were sampled during primary occasions in different months of the year (May–September 2011). Each primary occasion comprised 3 secondary occasions (combings) during which fleas might be detected (p = probability of detection, given presence). A prairie dog was "open" to colonization by fleas between primary occasions. Once a prairie dog was colonized, it was occupied by fleas during all subsequent primary occasions (thus, the extinction probability, ε, was fixed at zero, once a prairie dog was occupied by fleas). Closure was assumed during the secondary occasions, but we used behavioral covariates to account for removal of fleas from hosts during each secondary combing (REMOVAL$_1$ and REMOVAL$_2$, see text). In the example encounter history, a '1' indicates that at least 1 flea was detected during a combing event, and a '0' indicates that no fleas were detected.
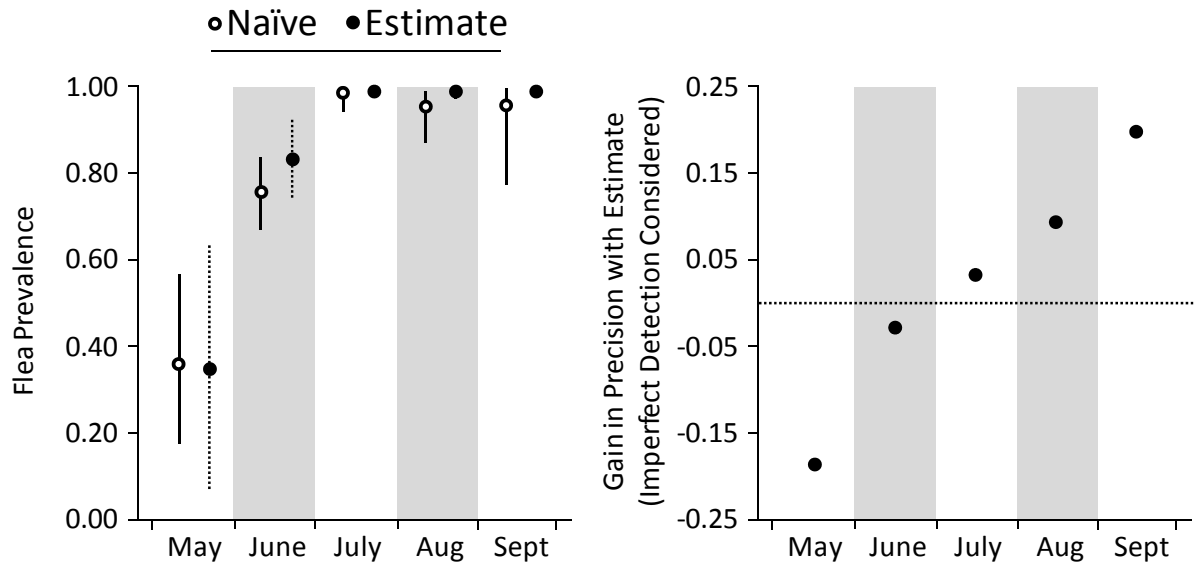
Figure 24 Indices for and estimates of flea prevalence on prairie dogs inside old colonies. The

estimates are model-averaged values from occupancy models that accounted for imperfect

detection of fleas. The naïve indices do not consider imperfect detection. Gains in precision

(95% confidence interval) when estimating prevalence are depicted on the right. Confidence

intervals for the estimates of prevalence during July–September are very small.
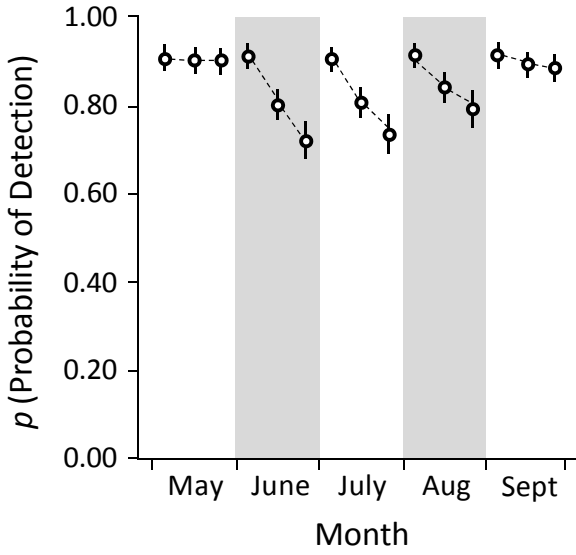
Figure 25 Model-averaged probabilities for detecting fleas (*p)* on a black-tailed prairie dog

(*Cynomys ludovicianus*) during May–September 2011, at the Vermejo Park Ranch, New Mexico.

Bars depict 95% confidence intervals.
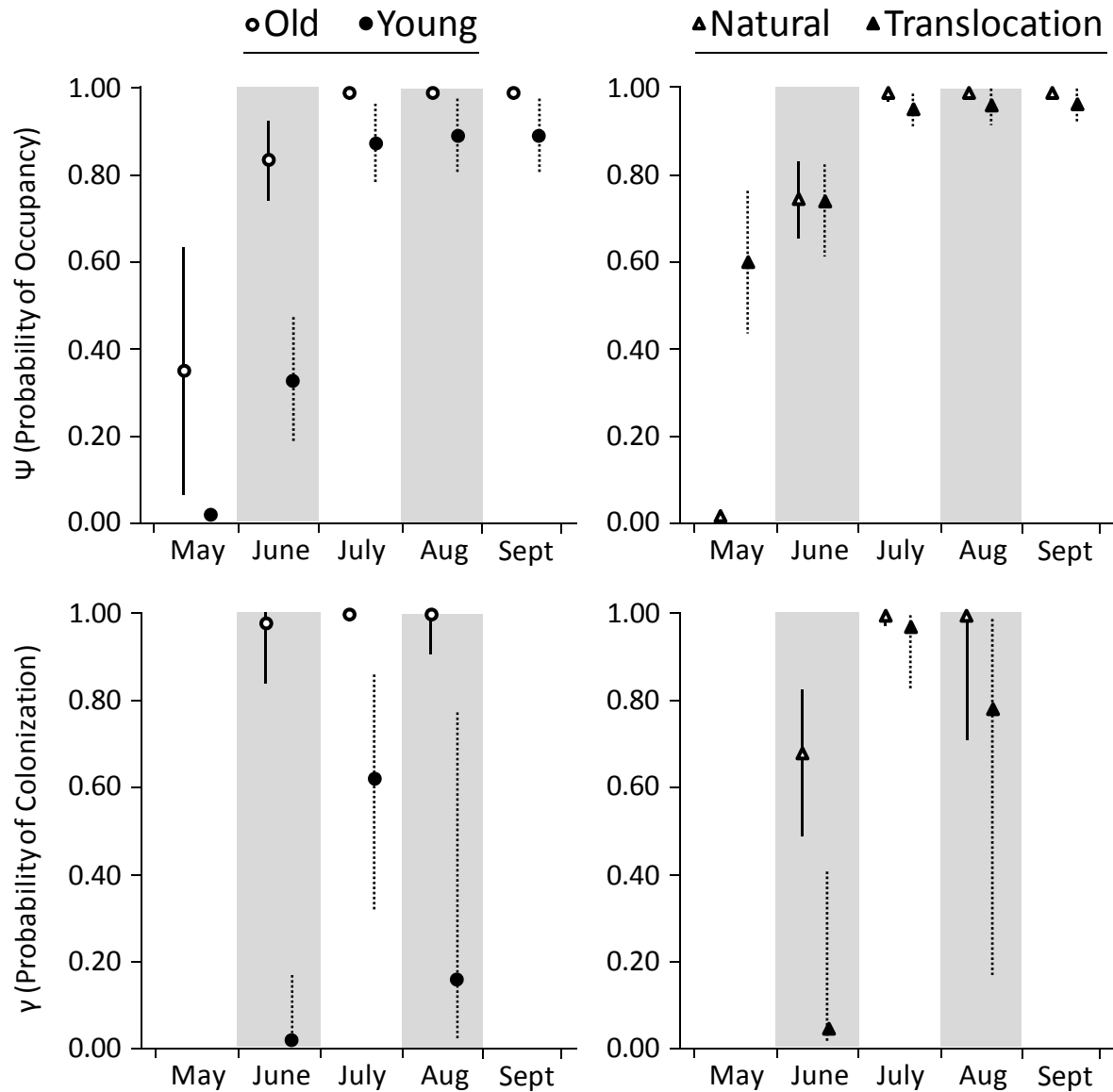
Figure 26 Model-averaged probabilities of flea occupancy (Ψ) and flea colonization (γ) for black-tailed prairie dogs (*Cynomys ludovicianus*) in old and young colonies, and natural and translocation colonies during May–September 2011, at the Vermejo Park Ranch, New Mexico (see Fig. 22 and text for colony descriptions). Bars depict 95% confidence intervals. We do not report estimates of colonization for September, because few prairie dogs were sampled in that month.
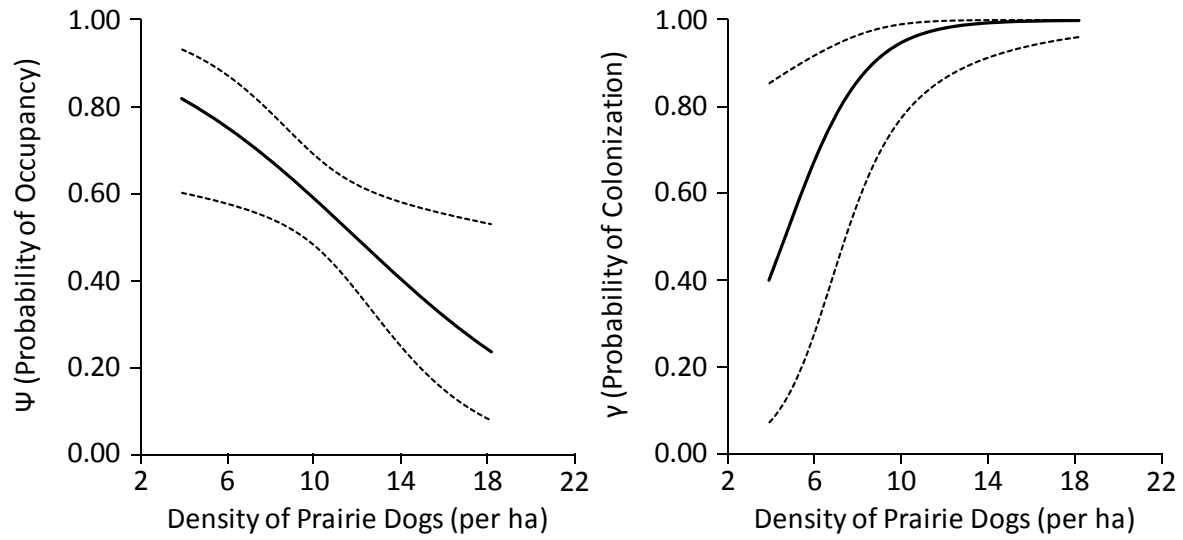
Figure 27 Probabilities of flea occupancy (Ψ) and flea colonization (γ) for black-tailed prairie

dogs (*Cynomys ludovicianus*) in plots with differing densities of prairie dogs during May–

September 2011, at the Vermejo Park Ranch, New Mexico. Solid lines depict estimates and

dotted lines depict 95% confidence intervals.
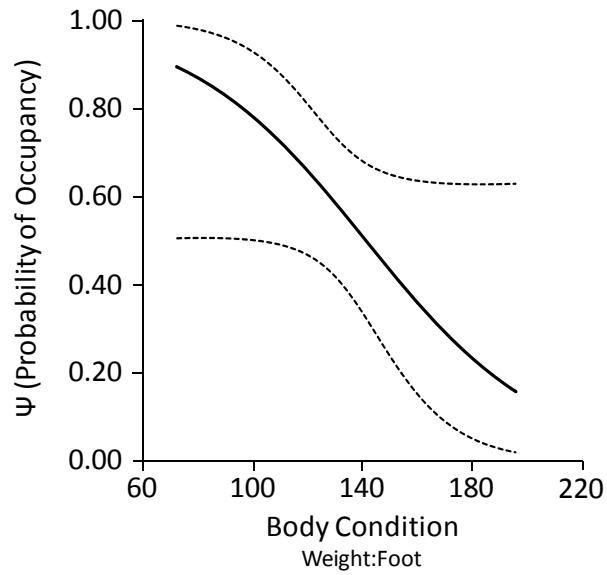
Figure 28 Probabilities of flea occupancy (Ψ) for black-tailed prairie dogs (*Cynomys ludovicianus*) in differing body condition during May–September 2011, at the Vermejo Park Ranch, New Mexico. The solid line depicts estimates of occupancy and dotted lines depict 95% confidence intervals.

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CHAPTER SIX[38]

PREVALENCE OF THE GENERALIST FLEA *PULEX SIMULANS* ON

BLACK-TAILED PRAIRIE DOGS IN NEW MEXICO:

THE IMPORTANCE OF CONSIDERING IMPERFECT DETECTION

If a parasite is not detected during a survey, one of two outcomes is possible: the parasite was

truly absent or present but not detected. We fit occupancy models to account for imperfect

detection when combing fleas (Siphonaptera) from black-tailed prairie dogs (*Cynomys

ludovicianus*) during June–August, 2012 in the Vermejo Park Ranch, New Mexico. Using

detection histories from combing events during monthly trapping sessions, we fit occupancy

models for two flea species: *Oropsylla hirusta* (a prairie dog specialist) and *Pulex simulans* (a

generalist). Detection probability was less than 100% for both species and about 21% lower for

P. simulans. *P. simulans* may be especially difficult to detect because it is about 2-times smaller

than *O. hirusta*. Monthly occupancy (prevalence) for *P. simulans* was estimated at 24% (June,

95% CI=19–30), 39% (July, 32–47), and 56% (August, 49–64) in new prairie dog colonies, and

43% (32–54), 61% (49–71), and 79% (70–87) in old colonies. These results suggest *P. simulans*

can attain high prevalence on prairie dogs, especially in old colonies. If *P. simulans* is highly

prevalent on prairie dogs, it may serve as a "bridge vector" between *Cynomys* and other

mammalian hosts of the plague bacterium *Yersinia pestis*, and even function as a reservoir of

plague between outbreaks.[39]

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[38] This chapter is formatted for, and has been accepted by Journal of Wildlife Diseases.
[39] The body of the paper is included below.

The importance of imperfect detection has received increased attention from ecologists interested in estimating occupancy rates for wildlife (MacKenzie et al. 2006; Bailey et al. 2013). In particular, disease ecologists and parasitologists increasingly acknowledge that cases of non-detection are ambiguous because parasites are notoriously difficult to collect. If detection is imperfect, then indices of parasite occupancy (prevalence) are biased low and inferences from such data are compromised (McClintock et al. 2010; Cooch et al. 2012).

In this study, we estimated prevalence for the generalist flea *Pulex simulans* on black-tailed prairie dogs (*Cynomys ludovicianus*). During past studies with substantial sample sizes (>100 animals), fewer than 40% of prairie dogs harbored *P. simulans* (Brinkerhoff 2008; Tripp et al. 2009). A collection of studies with smaller sample sizes suggest *P. simulans* may sometimes attain high prevalence on prairie dogs (Pfaffenberger et al. 1984; Nascarella et al. 2005; Holmes et al. 2006). For comparison, we also estimated rates of prevalence for the flea *Oropsylla hirsuta*, a prairie dog specialist. *O. hirsuta* is often found on >70% of prairie dogs (Brinkerhoff 2008; Tripp et al. 2009) and is about 2-times larger than *P. simulans*, suggesting it might be easier to detect. Imperfect detection of *P. simulans* or *O. hirsuta* would be important because both of these fleas may transmit the plague bacterium *Yersinia pestis* to prairie dogs and the endangered black-footed ferret (*Mustela nigripes*; Tripp et al. 2009; Harris et al. 2014). Moreover, *P. simulans* is a generalist (Hopla 1980) that may spread *Y. pestis* among multiple mammal species (Brinkerhoff 2008).

We live-trapped prairie dogs during June–August 2012, in the Vermejo Park Ranch, New Mexico. Data were collected in 19 plots (1.54 or 2.25 ha). We categorized the plots into five groups of 2–3 plots (clustered geographically for ease of sampling) and sequentially sampled the groups in randomized order during 10-day work periods.

We anesthetized each prairie dog using isoflurane in an induction chamber, marked its ears with tags, and then combed it three times, for 15 sec each, over unique tubs containing water. We collected fleas from each tub separately and stored them in vials (Tripp et al. 2009). Eads et al. (2013) describe the combing procedure and discuss assumptions inherent to the approach.

We identified flea species using light microscopy and keys presented in Stark (1958) and Hubbard (1968). All male *Pulex* were identified as *P. simulans*, so we assumed all *Pulex* were *P. simulans* (Tripp et al. 2009). We collected 1,486 *P. simulans* and 1,278 *O. hirsuta* from 270 individual prairie dogs during 578 processing events.

We developed flea encounter histories using detections from combings. If a prairie dog was captured multiple times in one month, we randomly selected one of the captures ($n = 19$ prairie dogs captured 2 or more times in June, 74 in July, and 77 in August). As an example, an encounter history of '110-100-…' denoted that a flea species was detected during the 1st and 2nd combing in June and the 1st combing in July, but the prairie dog was not captured in August. Of the 270 prairie dogs, 60% were sampled in one month, 32% in two months, and 8% during all three months. For those prairie dogs captured during consecutive months, an average of 31 days (SD=11.19) elapsed between sampling events.

We fit multi-season (monthly) occupancy models for each species in Program PRESENCE 6.2 (Hines 2006) to estimate unconditional detection rates (p=probabilities of detecting at least one flea on an occupied host) and flea prevalence (Ψ=probability that a prairie dog will host a particular flea species; MacKenzie et al. 2006). The models also estimated probabilities of colonization (γ= probability that a prairie dog did not host a particular flea species in month t but did in month t+1).

We modeled detection probability as varying among the three combings within each monthly session. In addition, binomial variables were used to denote if a flea species was removed from a prairie dog during the first and/or second combing within each monthly session. Removals reduce detection rates during subsequent combings within a monthly occasion and, thus, cause an inflation in estimates of flea prevalence (Eads et al. 2013:253-254). We aimed to avoid this bias.

We modeled occupancy and colonization as both a constant variable across months and as time varying with separate estimates for each month. Occupancy was allowed to vary or remain similar between new colonies of prairie dogs (≤8 years prairie dog occupancy, $n=7$ plots) and old colonies (≥10 years, $n=12$ plots; Eads et al. 2013).

We fit all possible models ($n=8$) for each flea species and ranked them by Akaike's Information Criterion (AIC; Burnham and Anderson 2002). Models within two AIC units of the most supported model were considered competing. We used likelihood ratio tests to identify parsimonious models (McCullagh and Nelder 1989).

For *P. simulans* (Table 13), prevalence varied among months ($X^2_2=21.100$, $P<0.001$) and old and young colonies ($X^2_1=14.090$, $P<0.001$). There was no evidence for monthly variation in colonization ($X^2_1=0.360$, $P=0.549$). Thus, we interpreted the most supported model (Table 13, Model 1).

For *O. hirsuta* (Table 13), there was evidence for monthly variation in prevalence ($X^2_2=14.360$, $P=0.001$). There was little evidence for variation in flea prevalence between old and young colonies ($X^2_1=1.290$, $P=0.256$) or monthly variation in colonization ($X^2_1=0.060$, $P=0.806$). Therefore, we interpreted the most supported model (Table 13, Model 9).

Goodness-of-fit tests with the most supported model for each flea species (10,000 simulations each) suggested good fit (chi-square P-values ≥ 0.67; package 'unmarked' Program R 3.1.1; Fiske and Chandler 2011). Therefore, we did not adjust the parameter estimates with a dipersion parameter (Burnham and Anderson 2002).

The overall probabilities of detecting at least one flea on an occupied host were 67% for *P. simulans* and 88% for *O. hirsuta*. As expected with a combing method that removes fleas from hosts, detection declined during consecutive combings (*P. simulans* first combing=43%, 95% CI=36–51; second=25%, 19–32; third=22%, 13–35; *O. hirusta* first=64%, 58–70; second=45%, 40–51; third=39%, 31–48).

Observed prevalence for *P. simulans* was 18% in June, 29% in July, and 43% in August for young colonies, and 32%, 51% and 70% for old colonies. When accounting for imperfect detection, monthly prevalence for *P. simulans* was estimated at 24% (19–30), 39% (32–47), and 56% (49–64) for young colonies, and 43% (32–54), 61% (49–71), and 79% (70–87) for old colonies. Observed monthly prevalence for *O. hirsuta* was 72%, 80%, and 62%. When accounting for imperfect detection, monthly estimates of prevalence for *O. hirsuta* were 74% (62–84), 83% (75–88), and 65% (57–73).

It is important to note that fleas occasionally fell into the induction chamber before we combed a prairie dog. During 60 of 578 sampling sessions (10%), fleas were not combed from the prairie dog but, upon inspection, at least one flea was found in the induction chamber. Thus, our estimates of prevalence are underestimates.

Despite this, our prevalence estimates for *P. simulans* during July–August are >30% higher than indices from other studies with substantial sample sizes for prairie dogs (Brinkerhoff 2008; Tripp et al. 2009). Thus, despite some limitations, our data suggest *P. simulans* can reach high

prevalence on prairie dogs, especially in old colonies (see Eads et al. 2013 for discussion on the importance of colony ages).

If *P. simulans* is highly prevalent, as we found in old prairie dog colonies, it may play an important role in spreading plague among prairie dogs (Tripp et al. 2009), perhaps even during periods between epizootics (Biggins et al. 2010). Moreover, *P. simulans* may function as a "bridge vector" between prairie dogs and other mammals, including lagomorphs, carnivores, and other rodents (Brinkerhoff 2008).

Perhaps *P. simulans* provides routes for *Y. pestis* to move among multiple host and flea species, thereby facilitating persistence of the plague bacterium in mammal-flea communities. In this way, *P. simulans* may function, along with its hosts, as a reservoir of plague, allowing *Y. pestis* to infect fleas and mammals during enzootic periods and then explode in epizootic form when conditions are suitable for an outbreak (Wimsatt and Biggins 2009).

Studies are needed to determine how efficient *P. simulans* is at transmitting *Y. pestis*. Many flea species are able to transmit *Y. pestis* efficiently for at least a few days after feeding on an infectious host (Eisen et al. 2009), suggesting *P. simulans* may do so. Even if *P. simulans* is an inefficient vector, its role in plague ecology should not be discounted because it could play an important role in maintaining *Y. pestis* in flea-mammal communities.

Table 13 Occupancy models for the prevalence of *Pulex simulans* and *Oropsylla hirsuta* fleas on black-tailed prairie dogs (*Cynomys ludovicianus*) in Vermejo Park Ranch, New Mexico, USA. Main-effects relate to detection of fleas (*p*), flea prevalence (Ψ), and flea colonization (γ). Sampling was completed during June-August (Month), 2012, and we evaluated potential differences in flea prevalence between old and yound prairie dog colonies (Colony-age). We used covariates for *p* that accounted for removal of fleas from hosts during sampling events (Eads et al. 2013). Included here are Akaike's Information Criterion (AIC), AICc differences (ΔAIC), numbers of parameters within models (No. parameters), and model log-likelihoods (-2 log-likelihood).

Flea Species	Model No.	Model structure	AIC	ΔAIC	No. parameters
Pulex simulans	1	Ψ(Month+Colony-age)+γ(.)+*p*(removal)	1147.59	0	8
	2	Ψ(Month+Colony-age)+γ(Month)+*p*(removal)	1149.95	2.36	9
	3	Ψ(Month)+γ(.)+*p*(removal)	1159.68	12.09	7
	4	Ψ(Month)+γ(Month)+*p*(removal)	1161.55	13.96	8
	5	Ψ(Colony-age)+γ(.)+*p*(removal)	1164.69	17.10	6
	6	Ψ(Colony-age)+γ(Month)+*p*(removal)	1166.17	18.58	7
	7	Ψ(.)+γ(.)+*p*(removal)	1180.82	33.23	5
	8	Ψ(.)+γ(Month)+*p*(removal)	1182.73	35.14	6
Oropsylla hirsuta	9	Ψ(Month)+γ(.)+*p*(removal)	1566.05	0	7
	10	Ψ(Month+Colony-age)+γ(.)+*p*(removal)	1566.76	0.71	8
	11	Ψ(Month)+γ(Month)+*p*(removal)	1567.76	1.71	8

12	Ψ(Month+Colony-age)+γ(Month)+p(removal)	1568.72	2.67	9
13	Ψ(.)+γ(Month)+p(removal)	1575.10	9.05	6
14	Ψ(Colony-age)+γ(Month)+p(removal)	1575.81	9.76	7
15	Ψ(.)+γ(.)+p(removal)	1576.41	10.36	5
16	Ψ(Colony-age)+γ(.)+p(removal)	1576.92	10.87	6

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CHAPTER SEVEN[40]

THE PLAGUE BACTERIUM *YERSINIA PESTIS* IS A TRANSFORMER SPECIES:

A CASE STUDY OF PRAIRIE DOGS AND THE GRASSLANDS OF

WESTERN NORTH AMERICA

Invasive "transformer species" change the character, condition, form, or nature of ecosystems and deserve considerable attention from conservation biologists (Richardson et al. 2000). Here, we extend the transformer species concept to the plague bacterium *Yersinia pestis* in western North America, where the pathogen was introduced around the year 1900. We present a review that describes how *Y. pestis* can transform grassland ecosystems by devastating populations of prairie dogs (*Cynomys* spp.), thereby causing 1) declines in native species abundance and diversity, 2) alterations in food web connections, 3) alterations in the import/export of nutrients, 4) loss of ecosystem resilience to encroaching invasive plants, and 5) modifications of prairie dog burrows. Existing information demonstrates that *Y. pestis* poses an important challenge to conservation biologists, but we have only a rudimentary understanding of its effects on ecosystems, thus highlighting the acute need for continued research on this transformer species.

Introduction

Management and control of invasive species are some of the most important challenges facing conservation biologists today (Ehrenfeld 2010; Simberloff & Rejmánek 2011). In particular, some invasive species change the character, condition, form, or nature of ecosystems

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[40] This chapter is formatted for submission to Conservation Biology.

over a substantial area of the ecosystems they affect. Instead of denoting these species simply as invasive, Richardson et al. (2000) proposed the term "transformer species" (in relation to plants) and suggested these species deserve considerable attention from conservation biologists.

The transformer species concept was recently extended from plants to other taxa (Ehrenfeld 2011) and opportunities exist for further expansion. For example, although not yet discussed in relation to the transformer species concept, disease-causing pathogens can invade and, once established, influence ecosystems via direct-effects on susceptible native species and indirect-effects on trophic relationships (Selakovic et al. 2014), as demonstrated by the introduction of rinderpest to Africa (Thomas et al. 2005; Dobson et al. 2011).

In this article, we extend the transformer species concept to *Yersinia pestis*, an exceptionally lethal, primarily flea-borne bacterium that causes plague in mammals. It has received much attention as a human pathogen, an emerging disease, and a potential agent of bioterrorism (Orent 2004). However, *Y. pestis* has received little attention in recent reviews of invasive species (Lowe et al. 2000) or the effects of invasive species on ecosystems (Pimentel et al. 2000, 2001, 2005; Simberloff & Rejmánek 2011).

Y. pestis likely originated 1,500–20,000 years ago in Asia (Achtman et al. 1999) but has been transported by humans to multiple additional continents and islands (Stenseth et al. 2008) where it degrades populations of a wide array of rodents (Biggins and Kosoy 2001a, b). We concentrate on plague's invasion of North America.

Y. pestis was introduced to North America in 1900 when steamships from Asia ferried flea-infested rats to the US Pacific coast (Orent 2004). In the next few years, epidemiologists quickly noted that plague was resident in populations of peridomestic rats and free-ranging rodents in California (Eskey & Haas 1940; Pollitzer 1954). Efforts were made to eradicate the disease but

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the attempts were futile and *Y. pestis* spread to a broad range of mammalian hosts, especially

rodents of the families Cricetidae, Muridae, and Sciuridae (and their fleas; Gage & Kosoy 2005).

As early as the 1920s, ecologists recognized plague as a disease that can spread explosively

across vast areas, and the implications of plague for wildlife were discussed. For example, in his

seminal papers on disease ecology, Charles Elton (1925, 1931) emphasized plague as a disease

that could regulate rodent populations. Further, Elton discussed plague in his classic book on the

ecology of animals in 1933, and later in his book on invasive species in 1958.

By the time Elton's book on invasive species was published, less than 60 years after *Y. pestis*

was introduced to North America, the plague bacterium was entrenched in populations of

mammals in 17 western United States, where the pathogen is now resident and occasionally

spills over to humans (Barnes 1993).

Concepts of Invasive and Transformer Species in Relation to *Yersinia pestis* in North America

The process of biological invasion is often categorized into three phases (Richardson et al.

2000, Davis 2011). During the "introduction" phase (#1), humans help an organism to overcome

a major geographical barrier that is insurmountable without human assistance. In phase #2,

without barriers to population establishment, replication, and continued persistence, an

introduced organism is "naturalized." In phase # 3, the actual "invasion" occurs when the

introduced species spreads geographically as it overcomes "barriers to dispersal within the new

region" and copes "with the abiotic environment and biota in the general area" (Richardson et al.

2000:99).

For *Y. pestis* to be categorized as a transformer species, it must first be categorized as an invasive species. As noted above in relation to North America, *Y. pestis* overcame the Pacific Ocean with human assistance and (1) was <u>introduced</u> to San Francisco (among other places), (2) <u>naturalized</u> itself in the site(s) of introduction and (3) <u>invaded</u> western North America, causing dramatic declines in some mammal populations. In fact, *Y. pestis* invaded North America very quickly, spreading more than 2,245 km in approximately 40 years (Adjemian et al. 2007)

To be categorized as a transformer species, *Y. pestis* must affect a "substantial area" of ecosystems. This outcome seems most plausible in those cases when plague reduces populations of a keystone species whose ecological impact is disproportionately unique relative to its abundance (Power et al. 1996), as is the case with various species of prairie dogs (*Cynomys* spp.; Kotliar et al. 2006).

Prairie Dogs, Plague, and the Grasslands of Western North America

Prairie dogs (*Cynomys* spp.) are colonial, burrowing rodents that serve as prey for an abundance of predators, create subterranean burrows used by many species, and clip and forage on vegetation in ways that facilitate various forbs while reducing shrub encroachment. Due to these and other ecosystem services, prairie dogs are considered ecosystem engineers and keystone species (Kotliar et al. 2006). Prairie dogs are also foundation species (Dayton 1972) that, by their historical abundance (Proctor et al. 2006), defined much of the structure of grassland communities and, in doing so, created suitable conditions for other species.

Y. pestis was first detected in prairie dogs during the 1930s and all species of *Cynomys* are particularly susceptible to plague (Eskey & Haas 1940). In an area of a plague epizootic, 95–

100% of prairie dogs die (Cully et al. 2006), and plague-caused mortality persists to some degree between outbreaks (Biggins et al. 2010). Currently, prairie dog species are restricted to <5% of their historic areas of occupancy, plague is entrenched in most populations, and the disease continues to spread into their eastern-most colonies (Barnes 1993; Antolin et al. 2002; Cully et al. 2006; Proctor et al. 2006).

It has long been recognized that plague poses serious conservation challenges for prairie dogs and some of the species dependent on them (Torres 1973). We hope that considering the effects of plague within the relatively new transformer species concept will provide a framework that both formalizes and energizes the conservation discussion.

The effects of plague on prairie dogs are often drastic in space and time, and several examples demonstrate that plague can influence populations of prairie dogs throughout a substantial area of grassland ecosystems, a prerequisite for classification as a transformer species (Richardson et al. 2000). Considering first the white-tailed group of prairie dogs, Collier and Spillett (1972, 1973) suggested there were about 95,000 Utah prairie dogs (*C. parvidens*) in the early 1900s, and estimated that only 3,300 remained in the early 1970s, a 97% reduction. Although Utah prairie dogs were killed during poisoning campaigns in 1933, 1950, and 1960 (Collier & Spillett 1973), the poisoning campaigns have ceased. *Y. pestis* was detected in this species by the mid-1930s. Plague is a persistent cause of mortality for this federally-listed species (Biggins et al. 2010) and is the top-tier threat to its recovery (U.S. Fish and Wildlife Service 2012).

During 1941 in South Park, Colorado, Gunnison's prairie dogs (*C. gunnisoni*) occupied more than 370,000 ha, but plague removed them from 97,000 ha, a 26% reduction. Between 1947 and 1949, plague had restricted the Gunnison's prairie dogs to about 18,500 ha, which represented

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only 5% of the area occupied in South Park only 8 years earlier (Ecke & Johnson 1952). Eighteen years later, in 1977, no prairie dog colonies were known to exist in South Park (Fitzgerald 1993).

During 1985–1987 in the Moreno Valley, New Mexico, plague spread through a 10,000 ha complex of Gunnison's prairie dog colonies. In late-1987, only small, isolated pockets of surviving prairie dogs remained (Cully et al. 1997). Wagner et al. (2006) documented similar changes for 270 colonies of Gunnison's prairie dogs in Arizona. All of the colonies were actively occupied by prairie dogs during 1998. However, 70% of the colonies were inactive by 2000–2001. The total area occupied by prairie dogs declined by 67% from about 13,500 to 4,500 ha. Several lines of evidence indicate that plague was the cause of the decline (Wagner et al. 2006).

The third member of the white-tail group, the white-tailed prairie dog (*C. leucurus*), also has undergone substantial declines due to plague. Populations in large complexes of colonies near Shirley Basin and Meeteetse, Wyoming, were dramatically reduced by plague and held at chronically low levels during 10 years of monitoring (Biggins & Kosoy 2001b).

Examples of plague-caused disruptions to populations of black-tailed prairie dogs (*C. ludovicianus*) are even more abundant than for the white-tail group. During a 12-year period between 1986 and 1998 in Montana, plague caused a 50% reduction in the area occupied by black-tailed prairie dogs (*C. ludovicianus*), from about 72,900 to 36,450 ha (Montana Prairie Dog Working Group 2002; Luce et al. 2006). Similarly, from 1989 to 1998 in Cimarron County, Oklahoma, plague contributed to a 75% decline in the total area occupied by black-tailed prairie dogs, from about 4,300 to 900 ha. The average size of colonies declined by about 70% (Lomolino & Smith 2001). Cully et al. (2010b) monitored 815 colonies of black-tailed prairie

dogs during 2001–2005 in areas of Colorado, Kansas, New Mexico, Oklahoma, South Dakota, Texas, and Wyoming. Colonies were smaller, distances between neighboring colonies were greater, and the proportion of potential habitat actively occupied by prairie dogs was smaller at the sites known to be affected by plague.

During a six-year study, Augustine et al. (2008) found that 78% of black-tailed prairie dog colonies remained active at a site without evidence of plague (Oglala National Grassland, Nebraska), compared to rates of 3% (Comanche National Grassland, Colorado) and 25% (Phillips County, Montana) for sites known to be affected by plague. Plague reduced the area of prairie dog colonies in Thunder Basin National Grassland, Wyoming by greater than 7,500 ha within a single year, causing an 81% reduction in area of occupancy (Cully et al. 2010b).

Hoogland (1995) characterized an apparently plague-free population of prairie dogs in Wind Cave National Park, South Dakota as having significant annual variation in abundance during a 14-year period (coefficient of variation = 13%), but that variation is minor when compared to variation during 14-years in the population at the Rocky Mountain Arsenal, Colorado (CV = 96%), a site with recurrent epizootics (Cully et al. 2006). Black-tailed prairie dog colonies in northeastern Colorado provide another example of how plague can cause dramatic population oscillations in a keystone species (Antolin et al. 2002).

A recent dataset from the Conata Basin, South Dakota, demonstrates the impact of a new invasion of plague on black-tailed prairie dogs. In 2007, about 73 prairie dog colonies existed with a mean area of 174 ha (Griebel 2009). Plague ravaged the prairie dog population during 2008 and by November 2009, there were 143 colonies (more colonies) but their mean area had declined by 74% to 45 ha (Griebel 2009). Overall, the total area occupied by prairie dogs was reduced by about 50%, from 12,695 ha to 6,445 ha (Griebel 2009). The remaining, contiguous

habitat has been treated annually with insecticides to kill fleas. Prairie dogs have disappeared from almost all habitat that could not be treated.

This selection of examples covering the four US species of prairie dogs in 11 states, inhabiting northern mixed grass prairie, shrub-grassland, southern mixed grass prairie, shortgrass prairie, and intermountain grasslands, collectively demonstrate that plague can (1) dramatically reduce the abundance of prairie dogs throughout large expanses of land, (2) induce oscillations in prairie dog densities, (3) restrict prairie dogs to chronically low densities, and (4) rapidly accelerate the rate of colony turnover and movement.

These direct effects of plague on prairie dogs have been emphasized historically. In contrast, researchers have only rarely evaluated the indirect effects of plague on grassland ecosystems (Biggins and Kosoy 2001b, Antolin et al. 2002). Yet, accumulating evidence demonstrates that the loss of prairie dogs to *Y. pestis* indirectly changes the character, condition, form, or nature of ecosystems, another prerequisite for classification as a transformer species (Table 14). In particular, plague transforms grassland ecosystems by causing "structural or compositional modifications, alterations in the import or export of nutrients, loss of resilience to disturbance" and decreases in native species abundance and diversity (Soulé et al. 2003:1239).

Prairie Dog Grazing and Clipping–Effects on Plant Communities and Visibility

Black-tailed prairie dogs graze in ways that reduce the height of vegetation and facilitate an abundance of forbs and genetically distinct, more prostrate grasses (Detling 2006). Vegetative communities change when prairie dogs are removed. In Wind Cave National Park, active exclusion of black-tailed prairie dogs from a colony, which simulates a plague epizootic, resulted

in an approximate 35% increase in plant biomass, with reduced forb abundance and a concomitant increase in the abundance of tall graminoids (Cid et al. 1991). Four years after prairie dogs were poisoned in areas of the Conata Basin, biomass production by buffalograss (*Bouteloua dactyloides*) increased by about 83% (Uresk 1985). During another study in Conata Basin, the Forest Service detected a >2-fold increase in vegetation height in an area where prairie dogs were removed via poisoning two years previous (Griebel 2009). In Colorado, scarlet globemallow (a forb; *Sphaeralcea coccinea*) suffered dramatic declines on colonies extirpated by plague, and grasses increased in abundance (Hartley et al. 2009). In the same area of Colorado, and on sites occupied by black-tailed prairie dogs for 1-4 years but currently unoccupied due to recent plague epizootics, plant cover and composition transformed to conditions similar to unoccupied sites (more grasses) within a single growing season (Augustine et al. in press). These ecosystem changes relate to structural/compositional modifications and alterations in native species abundance.

Shrubs and woody plants continue to encroach upon the grasslands of western North America (Van Auken 2000), in some areas because black-tailed prairie dogs are increasingly rare. During an experiment in Texas, *Prosopis* mesquite trees were planted in areas occupied by black-tailed prairie dogs. The prairie dogs removed all of the mesquite within just 2 days (Weltzin et al. 1997:751). At the same site, and within 23 years of the removal of prairie dogs due to poisoning, *Prosopis* spread to 61% of ground cover (Weltzin et al. 1997). Similarly, when black-tailed prairie dogs were poisoned at the boundaries of a colony in Chihuahua, Mexico, mesquite became established 1450 m into the poisoned area, a 43% advance (List 1997; Ceballos et al. 2010).

Y. pestis might also facilitate the expansion of Russian thistle (*Kali tragus*), an annual forb that was introduced to North America in the late 1800s. In the Janos-Casas Grandes region of northwestern Chihuahua, Mexico, biologists removed black-tailed prairie dogs from treatment sites, but prairie dogs were left in other "control" sites (Davidson et al. 2010). About 1.5 years later, Russian thistle was 2-times taller in the treatment than control plots and was observably more abundant. Thus, by decimating populations of black-tailed prairie dogs, plague is likely to increase the rate at which some woody plants and noxious weeds encroach on grasslands, thereby causing an ecological loss of resilience to disturbance (Soulé et al. 2003; but see Seastedt et al. 2008).

Prairie dogs also influence the nitrogen content and digestibility of vegetation (Detling 2006). Over a single growing season in South Dakota, nitrogen concentration and digestability of graminoids were about 10% greater on a black-tailed prairie dog colony than an off-colony site (Coppock et al. 1983). The nitrogen concentration of forbs was about 40% greater on old colonies, suggesting a long-term benefit of prairie dog occupancy (Coppock et al. 1983). During another study in South Dakota, the nitrogen concentration of grasses and forbs was about 10-30% greater, and net mineralization of nitrogen was greater on colonies (Fahnestock & Detling 2002). The nitrogen content of plants declines when prairie dogs are removed or excluded from areas (Cid et al. 1991). This ecosystem change relates to alteration in the import or export of nutrients (Soulé et al. 2003) and the attractiveness of prairie dog colonies to at least some ungulates (Whicker & Detling 1988).

The mountain plover (*Charadrius montanus*), a ground-nesting bird of conservation concern (Dinsmore et al. 2005), benefits from vegetative alterations caused by prairie dogs. These plovers commonly nest on colonies of black-tailed and Mexican prairie dogs where vegetation is

short, and patches of bare ground are common (Knowles et al. 1982). Plover numbers decline, sometimes dramatically, when plague erupts in prairie dogs (Dinsmore et al. 2001; Augustine et al. 2008; Dinsmore & Smith 2010).

Prairie Dog Burrowing Activities–Effects on Soils and Physical Characteristics of Colonies

Prairie dogs can remove about 225 kg of soil when creating a burrow system (Munn 1993) and the densities of their burrows can be 20-40 ha^{-1} in some areas. These numbers translate into the turnover of about 4,500–9,000 kg of soil ha^{-1}, demonstrating that prairie dogs contribute greatly to soil turnover in grasslands (Whicker & Detling 1993). The burrowing activities of prairie dogs "change soil chemistry, increase microporosity of soil to allow deep penetration of precipitation, and increase the incorporation of organic materials into the soil" (Miller et al. 2000:320).

In the grasslands of northwestern Chihuahua, Mexico, soils hardened, water infiltration was reduced by 21%, and soil carbon storage was reduced by 54% when black-tailed prairie dogs were removed (Martínez-Estévez et al. 2013). This change relates to the import or export of nutrients in soils (Soulé et al. 2003).

The construction of burrows adds an important structural feature to prairie dog colonies. These burrows provide escape cover, shelter from inclement weather, nesting habitat, and foraging habitat for numerous vertebrate and invertebrate species (Slobodchikoff et al. 2009). Burrows collapsed only 3 years after prairie dogs were removed from colonies in Oklahoma (Butts & Lewis 1982). Wilcomb (1954) suggests burrows can collapse within 2 years of prairie dog removals. This change relates to structural modifications in ecosystems (Soulé et al. 2003).

The western burrowing owl (*Athene cunicularia*) commonly inhabits colonies of prairie dogs where they usurp burrows as nesting sites. Predator detection is enhanced for burrowing owls living in prairie dog colonies because *Cynomys* emit alarm vocalizations when a predator is present, thereby alerting owls to predators (Bryan & Wunder 2013). Also, vegetation on black-tailed prairie dog colonies is short, creating open viewsheds for prairie dogs and their associates (Butts & Lewis 1982). In Nebraska, burrowing owl populations declined by 63% when humans poisoned colonies of black-tailed prairie dogs (Desmond et al. 2000). During 1998, surveys of burrowing owls on the 14 National Grasslands from Texas to North Dakota detected owls on 69% of active black-tailed prairie dog colonies, but only 11 % of colonies that had experienced plague epizootics (Sidle et al. 2001). In Colorado, burrowing owls were nearly absent from black-tailed prairie dog colonies that were extirpated by plague (Conrey 2010). This effect of plague is especially germane in conservation biology because the western burrowing owl is a species of concern in the United States, Canada, and Mexico (Sheffield 1997).

Prairie dogs affect the structure of arthropod communities via their effects on vegetation, soils, and physical structures such as mounds and tunnels (Bangert and Slobodchikoff et al. 2006). Various arthropods are attracted to prairie dog colonies, including anthophilous insects that commonly visit flowering forbs, for instance as found in colonies of black-tailed prairie dogs in northeastern Colorado (Hardwicke 2006). Some of these insects use loose soils around burrow mounds as nesting habitat (Hardwicke 2006). Plague alters arthropod communities in grasslands via its direct effects on prairie dogs and indirect effects on vegetation and soils (Bangert and Slobodchikoff et al. 2006, Hardwicke 2006). Moreover, by affecting anthophilous insects, plague may indirectly affect flowering plants that rely on arthropod pollinators for dispersal and fertilization (Hardwicke 2006).

Prairie Dogs as Prey

The historic abundance of prairie dogs as prey contributed to their classification as a foundation species. Prairie dog colonies are favored hunting habitats for multiple species of raptors and mammalian predators. Ferruginous hawks (*Buteo regalis*), a species of conservation concern, commonly suffer when plague erupts into epizootic form. Females produce one brood per year, with the number of chicks per brood being closely related to the abundance of food (U.S. Fish and Wildlife Service 1996). In two sections of Moreno Valley, New Mexico, ferruginous hawks declined in number by >75% after a plague epizootic decimated the local population of Gunnison's prairie dogs (Cully 1991). In a separate study in Colorado, a population of ferruginous hawks (1) declined by about 89% subsequent to a plague epizootic in black-tailed prairie dogs during 1988-1989, (2) increased as the prairie dog population recovered, and (3) declined by about 63% subsequent to another epizootic during 1994-1995 (Seery & Matiatos 2000; see also Plumpton and Andersen 1998).

Black-footed ferrets (*Mustela nigripes*) are endangered carnivores that selectively prey on prairie dogs and use prairie dog burrows as den sites (Miller et al. 1996; Lockhart et al. 2006; Brickner et al. 2014). Ferrets suffer from the indirect _and_ direct effects of plague. Indirectly, ferrets suffer when plague decimates a prairie dog population because less food and fewer burrows are available. Directly, ferrets are highly susceptible to plague infection and mortality (Godbey et al. 2006). In a complex of white-tailed prairie dog colonies near Meeteetse, Wyoming, an epizootic of plague (and canine distemper) reduced the abundance of ferrets from 129 in fall 1984 to 16 in fall 1985, and the population ultimately went extinct (though some ferrets were captured to initiate a captive breeding program; Forrest et al. 1988). At the Conata

Basin, South Dakota, plague reduced a population of ferrets from 335 in 2007 to 32 in 2013, and the population continues to decline despite the use of insecticides (T.M. Livieri, Prairie Wildlife Research, unpublished data). In Montana, enzootic plague (between epizootics) reduced ferret survival by 240% (Matchett et al. 2010).

Conclusion and Looking Ahead

This non-exhaustive sample of literature demonstrates that *Y. pestis* affects the nature of grasslands over a substantial area, a prerequisite for classification as a transformer species (Richardson et al. 2000). In addition to affecting the nature of ecosystems over a substantial area, transformer species should cause "substantial" changes in the structure or function of ecosystems (Richardson et al. 2000). In a recent treatise on transformer species, Ehrenfeld (2011:668) noted that "While there are no generally accepted quantitative criteria for determining when a change in structure or function is "substantial," the concept implies that more than one, and usually several, of the components of an ecosystem, such as species abundances, nutrient flux rates or pool sizes, food web connections, or physical structure, are significantly altered by the presence of the transforming species." Our review demonstrates that, by reducing densities and distribution of prairie dogs in the grasslands of western North America, *Y. pestis* can cause (1) declines in native species abundance (mortality) and diversity (extirpations), including threatened and endangered forms, (2) alterations in food web connections, (3) alterations in the import or export of nutrients, (4) loss of resilience to encroaching woody plants, and (5) modifications of physical structures such as burrows. Thus, we conclude that *Y. pestis* is a transformer species in the grasslands of western North America.

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The plague bacterium was introduced to North America about 114 years ago and we have yet to fully understand the array of implications for wildlife (Biggins & Kosoy 2001a). We hope this essay serves as a reminder that *Y. pestis* is transforming grassland ecosystems in western North America. We also hope to highlight that the ecological implications of plague extend beyond grasslands.

Indeed, *Y. pestis* has been detected in various ecoregions inside 17 western United States, including Marine West Coast Forests, Northwestern Forested Mountains, North American Deserts (except the hottest and driest areas), Mediterranean California, Southern Semi-Arid Highlands, and the Great Plains (Commission of Environmental Cooperation 1997). In these ecoregions, pathogenic effects of plague have been noted in mice, voles, rats, squirrels, marmots, and prairie dogs (Perry & Fetherston 1997), and over half the rodent species of conservation concern in the U.S. occur within the range of plague (Hafner et al. 1998). Lagomorphs are also infected under natural conditions and infections have been noted in various Carnivora, including canids, felids, and mustelids (Gage et al. 1994; Gage & Kosoy 2005; Salkeld & Stapp 2006). Further, >230 North American flea species have been found to be infected by *Y. pestis* in the wild (Eisen et al. 2009).

The widespread distribution of plague at multiple trophic levels in North America and it's lethality in various mammals suggest the disease may be affecting ecological relationships throughout the western half of the US, thus encouraging studies that seek to (1) acquire a more complete understanding of the disease's effects on ecosystems and (2) develop tools for its control. In particular, the following topics are in need of urgent attention from conservation biologists.

First, little is known about how *Y. pestis* persists during enzootic periods. The disease festers in host and flea populations between epizootic outbreaks (Matchett et al. 2010) but the mechanism(s) of persistence remain elusive. Various hypotheses deserve investigation (reviews in Eisen & Gage 2009; Wimsatt & Biggins 2009; Gage 2012).

Second, there is an acute need to rapidly expand data collection across host species to better understand the effects of enzootic plague. We suspect that plague is impacting host populations by chronically dampening the density of small mammals, thereby reducing prey biomass and altering competitive relationships. These phenomena may be difficult to detect (Biggins et al. 2010; Matchett et al. 2010).

Third, studies are needed to investigate the effectiveness of tools that are used to manage plague. Research is underway with prairie dogs to evaluate the efficacy of a recently developed bait-delivered vaccine that can be distributed across vast landscapes (Abbott et al. 2012). The vaccine may not be a panacea, however, and research is needed to develop new mitigation tools and a more integrated approach to management.

Lastly, research is needed to evaluate how weather affects plague, so future scenarios can be considered under projections of climate change, and interventions can be most efficiently applied. The best approach may be to evaluate the effects of temperature, humidity, and precipitation on fleas, *Y. pestis*, and mammals at various ecological scales (Ben Ari et al. 2011; Eisen & Gage 2012). We suggest increased use of controlled experiments rather than undue reliance on conceptual and mathematical models.

Overall, it should be remembered that in western North America and elsewhere in the introduced range of *Y. pestis*, we may be observing disrupted ecosystems on much of the landscape. Because of the early invasion of this lethal microbe in the western US, there is a

strong possibility that we have unknowingly succumbed to shifting baselines that distort our

definitions of functional ecosystems in the region (McDonald et al. 2011).

Table 14 Summary of ecosystem transformations caused by the plague bacterium *Yersinia pestis* when it is present in populations of prairie dogs (*Cynomys* spp.) in western North America.

Ecosystem Factor	Transformation Caused by Plague's Effects on Prairie Dogs
Structural or compositional modifications	Burrows collapse
	Increased height of vegetation (grasses and shrubs)
Import or export of nutrients	Reduced rates of water infiltration into soils
	Reduced amounts of organic matter in soil
	Reduced plant nitrogen content
	Reduced digestibility of vegetation
Resilience to disturbance	Increased shrub encroachment on grasslands
Native species abundance	Reduced forb abundance; grasses and shrubs favored
	Arthropod communities altered due to changes in vegetation and soils
	Burrows collapse and burrowing owls lose nesting habitats
	Tall vegetation reduces visibility
	Tall vegetation reduces the availability of nesting sites for mountain plovers
	Plague moves among prairie dogs and small mammals
	Various predators suffer prey limitations (e.g., ferruginous hawks, eagles)

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Predators switch to alternative prey, increasing mortality rates on them

Black-footed ferrets lose habitat/prey and die from plague infections

Species diversity and abundance change; competitive relationships altered

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