Effects of Climate Change on Plague Exposure Pathways and Resulting Disease Dynamics

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14. ABSTRACT
Sylvatic plague, a zoonotic flea-borne disease, caused by the bacterium Yersinia pestis, is relevant to the Department of Defense (DOD), because prairie dogs and other susceptible rodents are present on military installations in several western states. Arthropod-borne diseases, like plague, are thought to be particularly sensitive to local climate conditions. Expected changes in temperature and humidity over the next several decades will likely increase the geographical expansion of plague outbreaks in wildlife. Through a combination of field and laboratory work, along with data-driven modeling, we evaluated the potential effects of climate change on plague exposure pathways in prairie dogs and associated rodents to provide guidance to DOD partners regarding the potential for future outbreaks. Briefly, our specific objectives were to determine the relation between local climate conditions and the prevalence of plague and other pathogens while assessing the ecological roles of specific rodent hosts and vector species in plague dynamics, evaluate flea intensity on rodent hosts and in burrows in relation to local climate conditions, and develop models to predict the effects of climate change on plague dynamics.

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Climate change, Cynomys spp., fleas, non-target rodents, Oropsylla spp., Peromyscus, plague, sylvatic plague vaccine, prairie dogs, Yersinia pestis

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Table of Contents

List of Tables vi
List of Figures ..... **Error! Bookmark not defined.**
List of Appendices ..... **Error! Bookmark not defined.**
Acronyms vi
Keywords vi
Acknowledgements vii
Abstract 8
Introduction and Objectives 8
Technical Approach 8
Results 8
Benefits. 9
Executive Summary 9
Introduction 9
Objectives 9
Aim 1: Ecological studies. 9
Aim 2: Vector biology. 9
Aim 3: Modeling impacts of climate change on plague dynamics. 9
Technical Approach 10
Results and Discussion 13
Implications for Future Research and Benefits 18
Final Report 19
Objectives 19
Aim 1: Ecological studies. 19
Aim 2: Vector biology. 19
Aim 3: Modeling impacts of climate change on plague dynamics. 20
Background 20
Materials and Methods 21
Aim 1: Ecological studies. 21
Aim 2: Vector Biology. 24
Aim 3: Modeling impacts of climate change on plague dynamics. 26
Results and Discussion 29

Aim 1: Ecological studies. 29
Aim 2: Vector Biology. 33
Aim 3: Modeling impacts of climate change on plague dynamics. 40
Conclusions and Implications for Future Research/Implementation 44
Literature Cited 46
Appendices 52
Appendix 1. 52
Appendix 2. 53
Appendix 3. 55

List of Tables

Table 1. List of study areas and designations.
Table 2. Estimated flea replacement rates as a function of temperature.
Table 3. Flea switching among rodent hosts.
Table 4. Sample size and maximum likelihood estimate infection rate per 100 fleas collected from mice.
Table 5. Pathogens detected using PCR in flea pools collected from live prairie dogs
Table 6. Pathogens detected using PCR in flea pools collected from live prairie dogs on plague-positive sites.
Table 7. Species and collection locations of flea pools collected from live prairie dogs positive by PCR for pathogens.
Table 8. Pathogens detected using PCR in flea pools collected from prairie dog carcasses.
Table 9. Serology results of blood samples collected from live prairie dogs.
Table 10. General relations between climate variables and on-host flea prevalence.
Table 11. Fleas collected from prairie dog burrows.
Table 12. Effects of sampling time, temperature, and precipitation on the prevalence of flea-infested burrows and the abundance of fleas in burrows.
Table 13. Estimates and confidence intervals for variables related to mean burrow temperature.

List of Figures

Figure 1. Map of study pairs.
Figure 2. Map of field sites used for burrow swabbing.
Figure 3. Schematic of plague transmission routes.
Figure 4. Schematic of flea transmission.
Figure 5. Mean flea abundance in burrows during early and late sampling sessions.
Figure 6. Relationship of burrow flea abundance with monthly mean high temperature and winter precipitation.
Figure 7. Relationship of mean burrow temperatures with monthly mean high temperatures.
Figure 8. Flea development in relation to temperature and humidity.
Figure 9. Black-tailed prairie dog body condition in relation to climate factors.
Figure 10. White-tailed prairie dog body condition in relation to climate factors.
Figure 11. Utah prairie dog body condition in relation to climate factors.
Figure 12. The relationship between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog given a contact on the percentage of simulations that resulted in a plague outbreak.
Figure 13. The relationship between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog given a contact and the probability of transmission.
Figure 14. The relationship between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog given a contact and the time to extinction.

List of Appendices

Appendix 1. Parameters from models of flea dynamics
Appendix 2. Parameters used in plague models
Appendix 3. List of scientific publications associated with this study to date.
Appendix 4. Poje (2019) dissertation.

Acronyms

ABM	agent-based models
AZ	Arizona
BGSD	Buffalo Gap National Grassland, South Dakota
BTCO	black-tailed prairie dog site, Colorado
BTPD	black-tailed prairie dog
CBUT	Coyote Basin, Utah
CCUT	Cedar City, Utah
CI	confidence interval
CMR	Charles M. Russell National Wildlife Refuge, Montana
CO	Colorado
(g)DNA	(genomic) deoxyribonucleic acid acid
DOD	Department of Defense
DOI	Department of the Interior
ERAZ	Espee Ranch, Arizona
GPD	Gunnison's prairie dog
GUCO	Gunnison's prairie dog site, Colorado
HEUT	high elevation, Utah (Awapa Plateau)
LASSO	least absolute shrinkage and selection operator
LBSD	Lower Brule Sioux tribal lands, South Dakota
MT	Montana
ND	North Dakota
NDVI	normalized difference vegetation index
NM	New Mexico
NMDS	nonmetric multidimensional scaling
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PRWY	Pitchfork Ranch, Wyoming
RBTX	Rita Blanca National Grassland, Texas
RH	relative humidity
SD	standard deviation
SPV	sylvatic plague vaccine
UPD	Utah prairie dog
USGS	U.S. Geological Survey
WCSD	Wind Cave National Park, South Dakota
WTPD	white-tailed prairie dog

Keywords

Climate change, *Cynomys* spp., fleas, non-target rodents, *Oropsylla* spp., *Peromyscus*, plague, sylvatic plague vaccine, prairie dogs, *Yersinia pestis*

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Abstract

Introduction and Objectives: Sylvatic plague, a zoonotic flea-borne disease, caused by the bacterium *Yersinia pestis*, is relevant to the Department of Defense (DOD), because prairie dogs and other susceptible rodents are present on military installations in several western states. Arthropod-borne diseases, like plague, are thought to be particularly sensitive to local climate conditions. Expected changes in temperature and humidity over the next several decades will likely increase the geographical expansion of plague outbreaks in wildlife. Through a combination of field and laboratory work, along with data-driven modeling, we evaluated the potential effects of climate change on plague exposure pathways in prairie dogs and associated rodents to provide guidance to DOD partners regarding the potential for future outbreaks. Briefly, our specific objectives were to determine the relation between local climate conditions and the prevalence of plague and other pathogens while assessing the ecological roles of specific rodent hosts and vector species in plague dynamics, evaluate flea intensity on rodent hosts and in burrows in relation to local climate conditions, and develop models to predict the effects of climate change on plague dynamics.

Technical Approach: Using data and samples collected during a large field study on the effectiveness of vaccination to manage plague in prairie dogs, we assessed rodent/flea assemblages, pathogen prevalence in fleas, and determined how local climate conditions influence flea development rates and relative abundance. Live animals (prairie dogs and some small rodents) were trapped to collect fleas and other samples on 46 prairie dog plots in 6 western states, many sites near DOD lands. At seven additional locations on a latitudinal gradient, fleas were collected from burrows several times per year to assess seasonality and effects of local climate conditions on flea abundance. These data were then used to develop predictive models that could be used to test specific hypotheses.

Results: We determined that flea developmental rates, on-host flea abundance, species composition of the flea community, and burrow temperatures varied across a latitudinal gradient. Rodent and flea community composition and abundance differed geographically and were highly specialized. Flea-switching between prairie dogs and short-lived rodents was rare. Flea development rates, on-host flea abundance, and burrow temperatures increased with increasing ambient temperature. Although relative humidity can affect flea development, burrow humidity was uniformly high (~85%) across sampling sites and seasons. A large increase in the number of fleas found on a prairie dog colony, coupled with a greater number of infested burrows, could have substantial effects on plague dynamics in the western United States as the climate warms. In addition to affecting flea load, climate change may also influence body condition of prairie dogs by reducing the amount of forage. This may result in animals being more tolerant of high flea loads (less engaged in grooming behavior) and more vulnerable to disease.

Benefits: We have summarized information on flea communities and pathogen distribution in prairie dog populations across a broad range of species and geographic areas. We have developed a predictive model based on empirical data that can provide guidance regarding expected changes in plague frequency under different climate scenarios. These models can be used to estimate the potential effects of mitigation activities on the frequency and intensity of disease outbreaks, which will benefit DOD in planning military exercises in areas affected by plague.

Executive Summary

Introduction

Periodic outbreaks of sylvatic plague, a zoonotic flea-borne disease, caused by the bacterium *Yersinia pestis*, have had near catastrophic effects on some mammal populations, including prairie dogs and the endangered black-footed ferret (*Mustela nigripes*). Although human plague cases in the United States are relatively infrequent, the disease can be fatal, and its occurrence generates considerable public concern and media attention. A recent outbreak of plague in prairie dogs outside of Denver, Colorado, forced the closure of several state parks and restricted outdoor recreational activities. Sylvatic plague is relevant to the Department of Defense (DOD) because prairie dogs, ground squirrels, and other susceptible rodents are present on military installations in several western states, and the occurrence of plague has been known to curtail military exercises in the past. Other vector-borne zoonotic pathogens, such as *Bartonella* spp. and *Rickettsia* spp., are also associated with rodents and fleas. Arthropod-borne diseases, like plague, are thought to be particularly sensitive to local climate conditions, and expected changes in temperature and humidity over the next several decades will likely increase the northern expansion of plague outbreaks in wildlife. Through a combination of field and laboratory work, along with data-driven modeling, we evaluated the potential effects of climate change on plague exposure pathways in prairie dogs, ground squirrels, and other rodents to provide guidance to DOD partners regarding the potential for future outbreaks. As part of this project, we validated the use of an orally delivered sylvatic plague vaccine (SPV) for use as a management tool to prevent outbreaks.

Objectives

Aim 1: Ecological studies. Determine the relation between local climate conditions and the prevalence of plague and other flea and/or blood-borne pathogens (e.g. *Francisella tularensis*, *Bartonella* spp., *Rickettsia* spp.) on DOD and nearby lands while assessing the ecological roles of specific rodent hosts and vector species in plague dynamics.

Aim 2: Vector biology. Evaluate flea intensity on rodent hosts in relation to local climate conditions and the relative abundance of fleas in burrows in relation to burrow microclimate.

Aim 3: Modeling impacts of climate change on plague dynamics. Develop models to predict the effects of climate change on plague dynamics, including the frequency and intensity of outbreaks and the potential use of vaccination to reduce outbreaks.

Technical Approach

We use data and archived samples from a large-scale field study in western states to determine the composition of flea and small mammal communities, assess the prevalence of *Yersinia pestis* and other pathogens in hosts and vectors, and determine how local climate (including burrow microclimate) influences flea survival and relative abundance. These data were collected as part of a large-scale study recently completed by the U.S. Geological Survey (USGS) and several other Department of Interior (DOI) and state agencies to test sylvatic plague vaccine (SPV) as a potential management tool to reduce the risk of plague outbreaks in grassland ecosystems. Data were included from 23 paired study plots (placebo and vaccine deployed sites) at 10 locations in 6 western states, many sites near Department of Defense (DOD) lands, including pairs on black-tailed (*Cynomys ludovicianus*–BTPD), Gunnison's (*Cynomys gunnisoni*–GPD), white-tailed (*Cynomys leucurus*–WTPD), and Utah (C*ynomys parvidens*–UPD) prairie dog colonies (Figure 1; see details and data in Rocke et al. 2017, Russell et al. 2018, and Russell 2019).

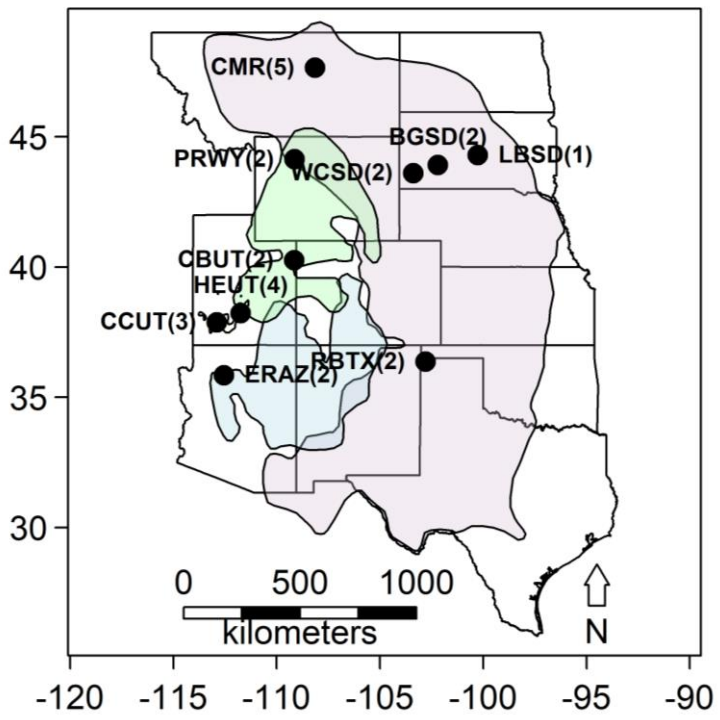

Figure 1. Map of study pairs. Polygons indicate the ranges of different prairie dog species; lavender for black-tailed prairie dogs (CMR: Charles M. Russell National Wildlife Refuge, Montana; WCSD: Wind Cave National Park, South Dakota; BGSD: Buffalo Gap National Grassland, South Dakota; LBSD: Lower Brule Sioux tribal lands, South Dakota; RBTX: Rita Blanca National Grassland, Texas, light green for white-tailed prairie dogs (PRWY: Pitchfork Ranch, Wyoming; CBUT: Coyote Basin, Utah), light yellow for Utah prairie dogs (CCUT: Cedar City, Utah; HEUT: High elevation, Utah), and blue for Gunnison's prairie dogs (ERAZ: Espee Ranch, Arizona. Numbers in parenthesis indicate the number of paired plots.

Vaccine or placebo was delivered through small flavored baits, distributed on prairie dog towns. At least 1 week and no more than 1 month post-bait distribution each year, local collaborators captured, marked, and sampled prairie dogs for a minimum of three trap days. Squirrels and other small rodents were also trapped for at least three consecutive nights on most plots (see Bron et al. 2018 for details). Sex, estimated age, and the identity of all current year and prior year recaptures were recorded for each captured animal.

Hair/whiskers, blood, and fleas were collected from captured prairie dogs and small mammals.

To determine the relation between ambient climate, burrow microclimate, and flea populations, we conducted additional field studies across a wide latitudinal gradient, which allowed us to survey a range of environmental conditions. We swabbed prairie dog burrows to collect fleas during two sessions, one in early summer and one in late summer at BTPD colonies at seven sites that spanned the entire BTPD range within the United States (Figure 2). Burrow and ambient temperature and relative humidity were measured by iButton Hygrochron data loggers placed in burrows and on the surface at each colony. See Poje (2019) for details (also available as Appendix 4).

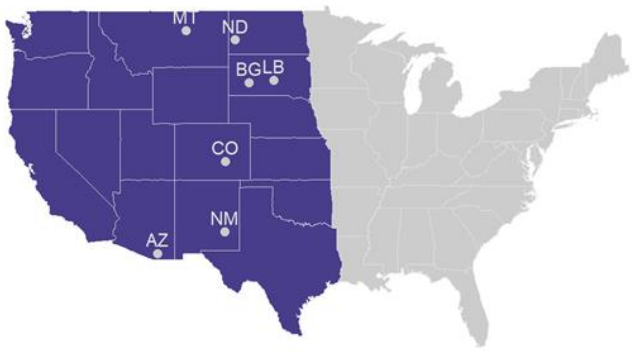

Figure 2. For burrow swabbing to collect questing fleas, seven field sites (labeled in gray) were chosen in six U.S. states (Montana (MT), North Dakota (ND), South Dakota (BG and LB), Colorado (CO), New Mexico (NM), and Arizona (AZ)). MT,CO and BG and LB sites in South Dakota were sampled during 2016 and 2017; AZ was sampled in 2017 only, and NM was sampled in 2016 only.

Fleas collected from live animals, carcasses, and burrows were counted and identified to species using published taxonomic references (Furman and Catts, 1982; Hubbard, 1947; Stark, 1970), and pooled by species and sex, up to 10 individuals per pool from a single animal or burrow. Flea pools were tested for the presence of *Yersinia pestis*, *Francisella tularensis*, *Rickettsia* spp., and *Bartonella* spp. using real-time polymerase chain reaction (PCR).

Lateral flow tests (Abbott et al. 2014) were used to detect antibodies against *Y. pestis* F1 and V antigens in blood samples collected from prairie dogs and small rodents.

We analyzed flea abundance and prevalence on prairie dogs. We used logistic regression and negative binomial regression with LASSO (least absolute shrinkage and selection operator; Tibshirani, 1996) priors in a Bayesian framework to assess factors related to total flea abundance, *Oropsylla hirsuta* abundance, and *Pulex simulans* abundance. This allowed us to examine factors related to both the number of fleas on a host (abundance) and the presence/absence of fleas on a host (prevalence). We evaluated factors associated with prevalence of fleas and overall flea abundance, including variables related to climate and seasonal weather patterns (see Russell et al. 2018 for details).

To assess the association between the rodent and flea communities on our different study areas, we used a study plot by flea species matrix and a study plot by rodent matrix with the

abundance of all years combined to create the most complete dataset. The rodent abundance matrix (plot by rodent species) contained the individual rodent species that were combed for fleas. These matrices were visualized in nonmetric multidimensional scaling (NMDS) plots. We compared Bray-Curtis dissimilarity matrices of flea species per plot and rodent species per plot. Host-flea associations were quantified and qualified. To assess the level of individual specialization of the flea species, we quantified host specificity (see Bron, 2017 for details).

We modeled the relative abundance of a given flea species and the prevalence of fleas in burrows in generalized linear models as a response to temperature and precipitation. We also modeled daily mean burrow temperature and daily mean relative humidity in relation to climate variables. See Poje (2019; Appendix 4) for details.

Poje (2019) used unpublished data from a dissertation (Metzger, 2000) in which *Oropsylla montana* fleas were reared on captive California ground squirrels (*Otospermophilus beecheyi*) at various temperatures and relative humidity levels. Using a generalized linear mixed model, Poje (2019) used these data to develop a degree-day model to predict flea development rates and potential generation times across a range of temperatures (see Poje 2019 [Appendix 4] for details).

We developed spatially explicit agent-based models (ABM) of plague dynamics based on previously developed models (Richgels et al. 2016 and Buhnerkempe et al. 2011). Our ABMs are parameterized from field data, laboratory studies and previously published literature. In our models, the probability that a prairie dog will interact with an infected flea, infected live prairie dog, or infected carcass is controlled by parameters estimated from field data. We used spatial-capture recapture (Royle et al. 2013) to estimate the size of the activity centers for each prairie dog and estimate a center of activity for both observed individuals and individuals estimated to be in the population.

Transmission of plague by fleas is a complex process that involves fleas proceeding from early to latent to late stages of infection (Figure 3). During the early and latent stages of infection, fleas may clear infection and return to the susceptible stage. The parameters in our models included estimated uncertainty. Therefore, we ran multiple scenarios encompassing the full range of the parameter estimates. Latin Hypercube sampling (Iman et al. 1981) was used to reduce the number of total simulations run. We then conducted a boosted regression tree on the response variable outbreak occurrence where outbreak occurrence was 1 if <20 prairie dogs survived the 150 days and outbreak occurrence was 0 if >20 prairie dogs survived the season, mean number of prairie dogs surviving the season, and time to extinction.

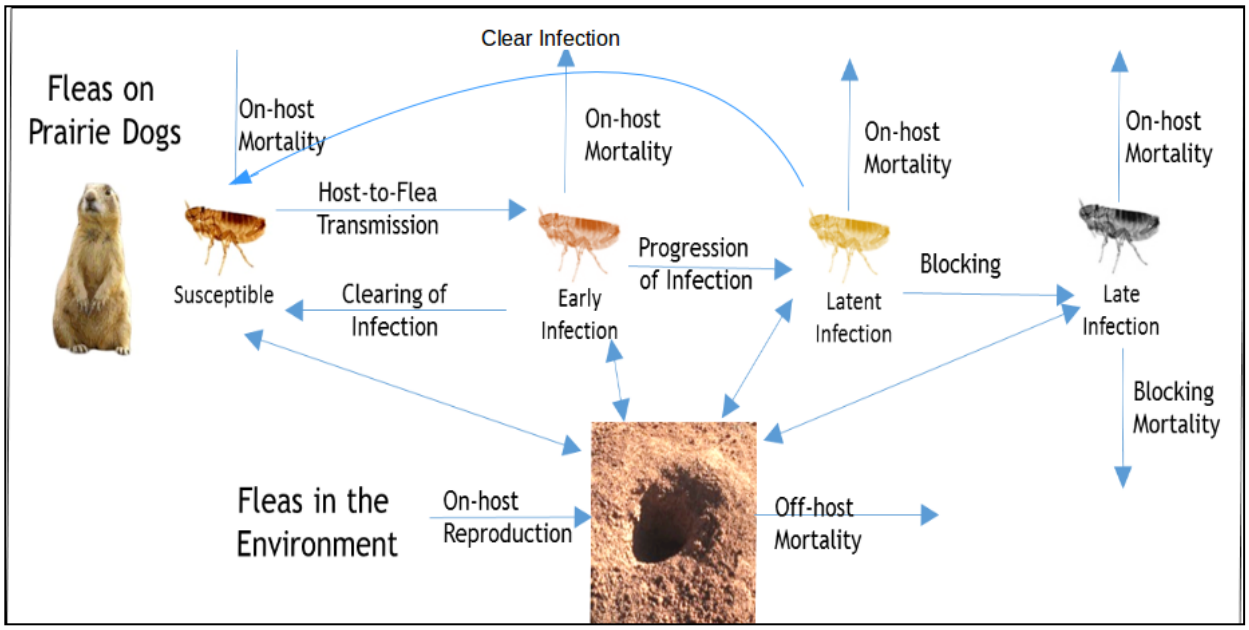

Figure 3. Schematic of plague transmission.

Results and Discussion

We identified host/flea relationships in small mammal communities on prairie dog colonies to assess the most critical plague exposure pathways. Briefly, we identified 20,041 fleas from 6,542 prairie dogs sampled between June and November over a 4-year period and identified 18 species of fleas. The prairie dog specialist flea *O. hirsuta* was the most common species detected, comprising 59% of all fleas sampled and occurring on all pairs except Utah prairie dog (UPD) pairs located at high elevation (HEUT) and both WTPD pairs in Pitchfork Ranch, Wyoming (PRWY). The second most commonly detected flea species was *Pulex simulans,* a generalist flea species, accounting for 23% of fleas identified. See Russell et al. 2018 for details.

We assessed the geographical variation in flea community composition and flea specificity on our study sites. Our results indicate rodent and flea species community composition and abundance per plot are related and differ geographically. Host specialization of individual flea species in each study area varied, but many fleas were specialists. Flea switching was rare; 0.3% of fleas collected from short-lived rodents were prairie dog fleas, and 0.05% of the fleas collected from prairie dogs were short-lived rodent fleas. This is additional evidence that plague transmission between short-lived rodents and prairie dogs by fleas is extremely rare, but not impossible.

The number of prairie dogs with *Y. pestis* detections in fleas was low (n = 64 prairie dogs with positive fleas out of 5,024 prairie dogs sampled). Twenty-five of the prairie dogs with *Y. pestis* positive flea pools were found on placebo plots and 39 on vaccine plots. *Y. pestis* positive fleas were found in flea pools of 7 different species including *P. simulans*, *O. hirsuta*, *Oropsylla tuberculata*, *Oropsylla labis*, *Oropsylla idahoensis*, *Thrassis francisi*, and *Neopsylla inopina*.

We did not detect plague-positive fleas on short-lived rodents during plague outbreaks in prairie dogs. However, plague-positive mouse fleas (*Aetheca wagneri*, *Pleochaetis exilis*, *Orchopeas leucopus*) were detected on mice prior to plague-induced declines in BTPD in Montana and WTPD in Wyoming. This finding led to the speculation that plague-positive mouse fleas might have fed on bacteremic prairie dogs, although no prairie dog DNA was detected during blood meal analysis of plague-positive mouse fleas. Mice may be involved in *Y. pestis* maintenance, introduction, and/or propagation on prairie dog colonies (Bron et al. 2019).

We used PCR to compare the prevalence of *Y. pestis*, *F. tularensis*, *Ricketsia* spp., and *Bartonella* spp. in rodent and flea species (Table 1). We will use these results to test how co-infection may affect plague prevalence in these communities.

Table 1. Pathogens detected using PCR in flea pools collected from live prairie dogs.

Pathogen	% positive (number of positives)	Number tested
Yersinia pestis	1.2% (58)	5024
Bartonella spp.	12.7% (103)	813
Rickettsia spp.	1.7% (12)	690

We quantified the relation between climate and flea populations by relating on-host flea relative abundance and intensity to local climate, burrow conditions, and rodent abundance across a latitudinal gradient. Multiple weather and climate-related variables were associated with on-host flea abundance and presence/absence on prairie dogs (see Russell et al. 2018 for details; Table 2.). Many factors were associated with flea abundance on prairie dogs, indicating the complexity of host-vector-disease dynamics in this system. Our results indicate that local weather factors influenced flea abundance, potentially by providing environmental conditions suitable for flea reproduction. As expected, however, environmental factors had different effects at different locations due to the wide range of environmental conditions and geographic locations of our study pairs. Differences in the direction of the relation of environmental covariates with flea abundance for different prairie dog species is likely the result of differences in local conditions and differences in the biology of the prairie dog and flea species in question. Abundance of fleas is likely more important for epizootic plague, versus the presence or absence of fleas on hosts at a location.

Table 2. General relations for non-zero parameter estimates between climate variables and on-host total flea populations and *Oropsylla hirsuta* populations only as analyzed in a Bayesian framework using LASSO (least absolute shrinkage and selection operator).

Climate Variables	**Total Fleas**	***O. hirsuta***
Winter precipitation	negative	no relation
Summer precipitation (prior year)	no relation	negative

Normalized difference vegetation index	negative	no relation
Days > 85ºF	positive	positive

We also quantified off-host fleas by swabbing 6,466 prairie dog burrows over 3-day sampling periods at each site during the summers of 2016 and 2017. From these, we collected 2,024 fleas from 629 burrows (see Poje 2019 [Appendix 4] for details). Patterns of flea abundance (total number of fleas collected) varied by region and sampling session. Flea abundance decreased from early sampling periods in the spring/early summer to late sampling sessions in late summer/early fall at southern sites, while flea abundance increased from early to late sampling at northern sites. For questing burrow fleas, our models showed that fleas were more abundant in the late session than the early session and in 2017 than 2016. Mean flea abundance/prairie dog burrow/sampling period increased with an increase in monthly mean high temperature. The effect of precipitation on mean flea abundance changed between the early and late session with flea abundance declining by 76% (early) or no significant change (2017) for every 1 cm increase in winter precipitation (Figure 5).

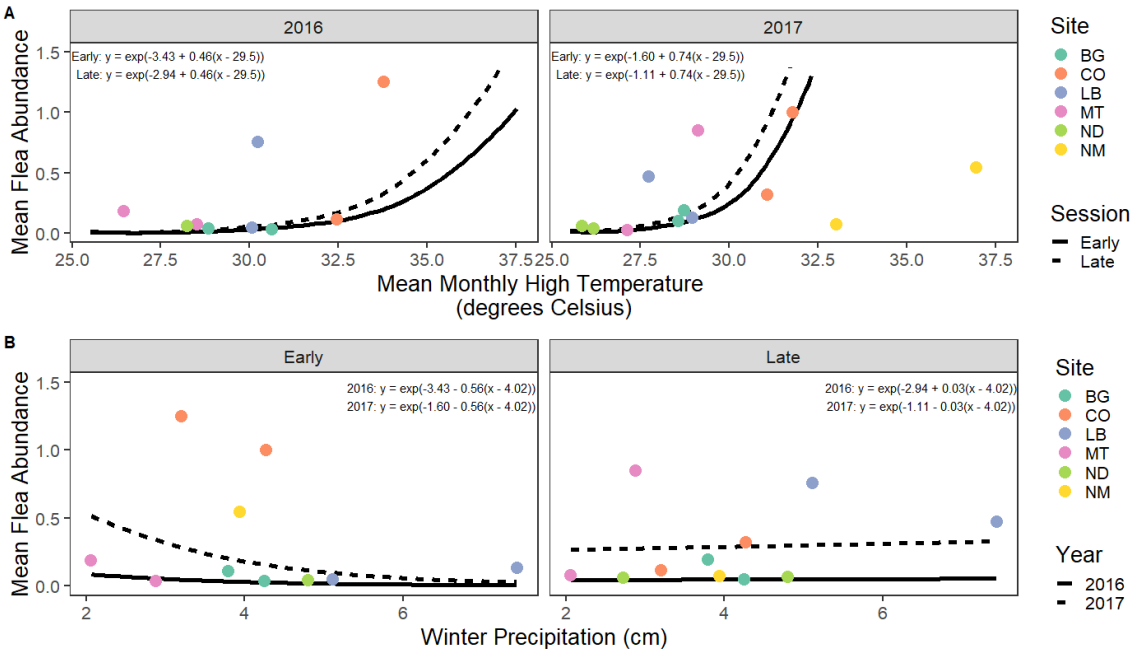

Figure 5. (A) Mean flea abundance per prairie dog burrow increased with monthly mean high temperature at different rates in 2016 and 2017 for sites shown in Figure 2. In 2016, mean flea abundance increased by a rate of 1.58 for every 1°C increase in temperature, while in 2017 abundance increased by a rate of 2.09. During both years, mean flea abundance was 1.63 times higher in the late session than the early session. (B) The relation between mean flea abundance and winter precipitation changed between the early and the late sampling sessions. In the early session, mean flea abundance declined at a rate of 0.43 for every additional cm increase in precipitation. In the late session, there was no

significant effect of winter precipitation on abundance. During both sessions, mean flea abundance was 6.23 times higher in 2017 than 2016.

O. hirsuta was the most abundant species of flea in the prairie dog burrows that we sampled, followed by *P. simulans*. There were different patterns of relative abundance among flea species: *O. tuberculata* relative abundance was higher in the early summer than the late summer, *P. simulans* was more abundant in the late summer than the early summer, and *O. hirsuta* relative abundance declined from the early to late summer in the southern plains and increased in the northern plains. Different flea species have different capacities as vectors, and therefore changes in flea abundance could affect plague dynamics. Because *O. tuberculata* is a more effective vector than *O. hirsuta*, epizootics may be more likely in early spring, when it is the most abundant. However, the larger overall abundance of *O. hirsuta* may make the difference in vector capacity irrelevant, as the larger number of flea bites could make up for the lower transmission rate. It is currently unclear what specific factors are driving these seasonal and geographic changes in flea abundance, and future work could focus on determining how environmental conditions contribute to these patterns in flea species abundance, especially regarding future climate changes.

Using modeling, we characterized burrow microclimates by determining the relation between burrow temperature and humidity and current and past local climate variables. Our linear mixed model showed that both monthly mean high temperature and sampling session were significant predictors of daily mean burrow temperature. Daily mean burrow temperature increased with increasing monthly mean high temperature (Figure 6). Daily mean burrow temperature during the late sampling session was higher than during the early sampling session.

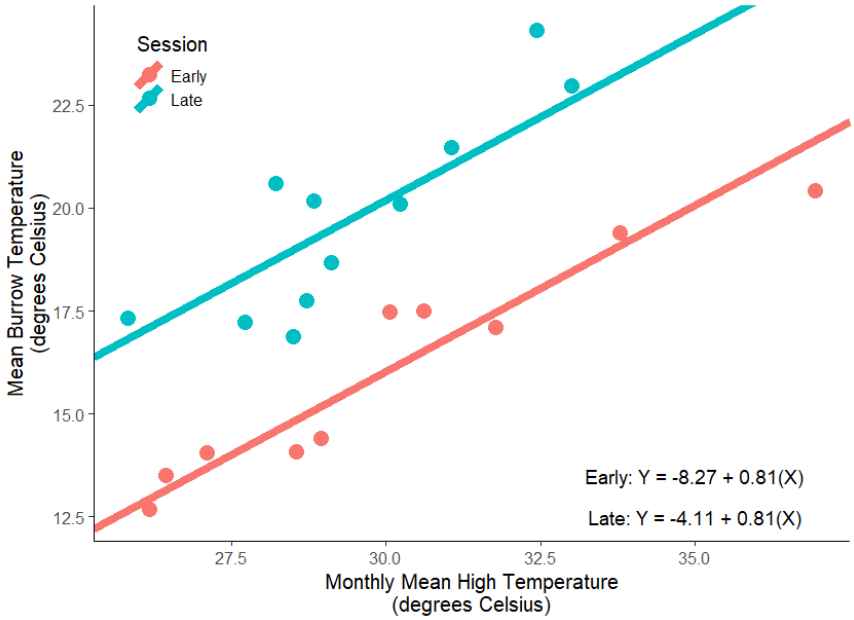

Figure 6. Daily mean burrow temperatures are correlated with monthly mean high temperatures. Each 1°C increase in monthly mean high temperature increases daily mean

burrow temperature by 0.81°C. Burrow temperatures were 4°C higher during late sampling sessions than during early sampling sessions.

The western Great Plains are projected to get hotter and drier through the next century, with temperatures projected to warm by 1.9-3.6°C by 2085 (Kunkel et al. 2013a, 2013b). As the climate warms, our results indicate prairie dog burrow temperatures will increase as well. Based on our models, flea abundance will increase between 450% and 540% under this projected level of warming, and the prevalence of infested burrows will increase by between 285% and 540%. Because fleabites are the primary method of plague transmission, a large increase in the number of fleas found on a colony coupled with a greater number of infested burrows could have substantial effects on plague dynamics.

Poje (2019) used data from controlled laboratory studies of *O. montana* (Metzger 2000) to determine the effect of temperature and humidity on flea development, including survival and reproduction. According to our model, flea development rates increased significantly ($p < 0.0001$) with temperature (Figure 4; see Poje 2019 [Appendix 4] for details) and slightly with higher humidity. Because fleas are ectothermic, changes in their environment can substantially affect their development. The results suggests that in both the northern and southern plains, prairie dog flea development rates are likely to increase with predicted warming temperatures. Together, faster development rates over longer growing seasons coupled with higher recruitment rates could increase flea abundance dramatically. However, further research is necessary to determine how *O. hirsuta* and *O. tuberculata* respond to warmer and longer periods of favorable temperatures. Flea bites are responsible for >70% of plague transmission during epizootic plague (Richgels et al. 2016). If fleas are present for longer periods of time at higher abundance, future plague outbreaks could potentially last longer and be more severe.

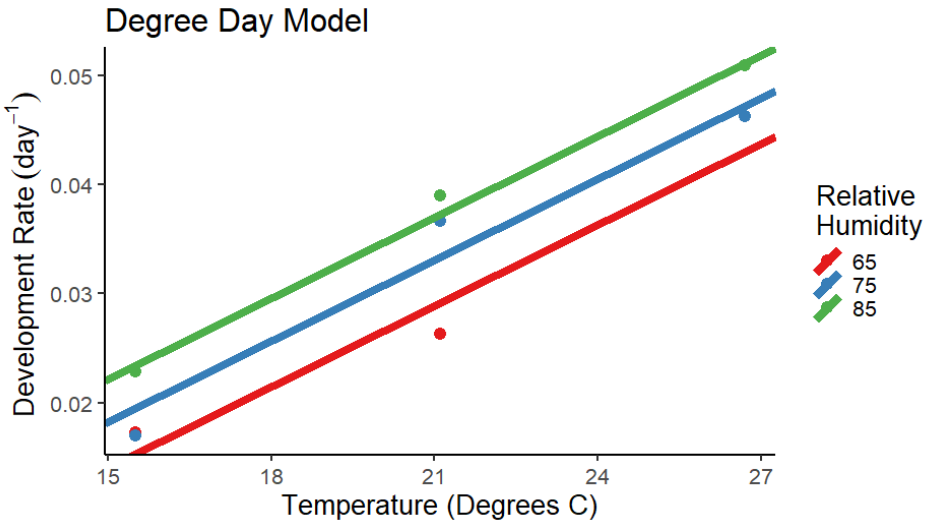

Figure 4. Flea development rate increases by 0.00247 day^{-1} ($t = 14.5$, $df = 12$, $p < 0.0001$, 95% confidence interval (CI) 0.0022 – 0.0028) for every 1°C increase in temperature. At the same temperature, flea development is 0.004 day^{-1} faster at 75% relative humidity than 65% relative humidity and 0.004 day^{-1} faster at 85% relative humidity than 75% relative humidity.

Changing climate conditions will likely affect aspects of both flea and host communities, including population densities and species composition, and lead to changes in plague dynamics. Our results support the hypothesis that local conditions, including host, vector, and environmental factors, influence the likelihood of plague outbreaks, and that predicting changes to plague dynamics under climate change scenarios will require consideration of both host and vector responses to local factors. We continue to develop models to predict the effects of climate change on plague dynamics, including the frequency and intensity of outbreaks and the potential use of vaccination to reduce outbreaks.

Implications for Future Research and Benefits

Although our findings strongly indicate local environmental conditions at the rodent burrow level influence plague outbreaks, more work is needed to determine specific factors that drive seasonal and geographic changes in flea abundance, especially regarding future climate changes. Knowledge derived from our models about the future potential spread of plague and incidence of outbreaks could help managers and others target and prioritize control and prevention activities, but additional work is needed to improve targeted interventions against plague in wildlife. Although dusting burrows with insecticides is still used extensively to control outbreaks once they have begun, fleas are developing resistance against commonly used products (Eads et al. 2018). Recent work by USGS National Wildlife Health Center indicates oral vaccination campaigns using vaccine-laden baits targeted at specific rodent species may be useful in preventing outbreaks (Rocke et al. 2017; Tripp et al. 2017), but additional work is needed to optimize and improve vaccines, baiting strategies, and delivery methods.

Final Report

Objectives

Aim 1: Ecological studies.

Several recent models of plague dynamics (Davis et al. 2008; Snäll et al. 2009; Salkeld et al. 2010; Buhnerkempe et al. 2011) have concluded that prairie dogs alone (i.e., single host-to-host transmissions) are unlikely to sustain plague in prairie ecosystems. However, considerable uncertainty exists regarding the mechanisms and environmental conditions that allow plague to spread between social groups, leading to large-scale disease outbreaks. Other small mammals may supply this link by transporting infectious fleas across prairie dog colonies (Salkeld et al. 2010). The availability of an oral plague vaccine (Rocke et al. 2014) that allows perturbation of the prairie dog/plague system provides an excellent opportunity to experimentally test how protection of one or more community members against plague affects disease transmission. *We hypothesized that a combination of factors, including climate and host, vector, and pathogen diversity, influence the occurrence, frequency, and intensity of plague outbreaks. Vaccine application may change this dynamic by providing protection in the host population, in the community at large, and by decreasing pathogen prevalence.*

Our objective was to determine the prevalence of *Yersinia pestis* and other flea-and/or blood-borne pathogens (e.g. *Francisella tularensis*, *Bartonella* spp., *Rickettsia* spp.) on DOD and/or nearby lands in relation to local climate (weather) conditions, assessing the ecological roles of specific host and vector species. To accomplish this aim, we conducted fieldwork to document the rodent/flea assemblages and collect blood and flea samples on DOD and nearby lands and then analyzed these and archived samples from our ongoing sylvatic plague vaccine (SPV) study by polymerase chain reaction (PCR) and serology. This aim directly addresses the first objective of the statement of need to determine ecology of potential pathogens and their vectors on DOD lands and will help guide future plague management actions.

Aim 2: Vector biology.

In the western United States, the response of rodent communities to climatic variations, especially as it relates to hantaviruses, has been fairly well studied (Yates et al. 2002; Calisher et al. 2005; Luis et al, 2010). In contrast, the effects of climate on flea vector demographics have been virtually unstudied (but see Salkeld and Stapp 2009), even though flea populations supposedly drive epizootic plague outbreaks and are sensitive to desiccation (Krasnov et al. 2001; Enscore et al. 2002; Eisen and Gage 2009). Fleas are thought to reproduce and mature within prairie dog burrows, with increases in host switching and numbers of questing fleas found in burrows indicative of imminent plague outbreaks. However, conditions within burrows and their relation to local climate are poorly understood. Burrow conditions are potentially more stable than local climatic variations. For example, Hoogland (1995) found that burrows at one site in South Dakota varied between 5 and 10 ºC in the winter and between 15 and 25 ºC in the summer with mean

humidity of 88%. Soil type and soil moisture were also positive predictors of flea intensities on prairie dogs (Eads 2014), indicating that the burrow microclimate is important for flea populations. However, the sensitivity of flea species to changes in temperature and humidity are similarly understudied, though preliminary evidence indicates even small variations in temperature and humidity had disparate effects on growth, survival, and *Y. pestis* transmission in co-occurring rat fleas, *Xenopsylla* spp. (Krasnov 2001; Schotthoefer et al. 2011). The two most common prairie dog fleas, *O. hirsuta* and *O. tuberculata*, peak in abundance at different times of the year (Tripp et al. 2009), indicating that perhaps they also vary in their temperature and humidity tolerances. Thus, to predict the effects of climate change on plague dynamics in the western United States, including DOD lands, we determined the relation between prairie dog flea prevalence and abundance, burrow conditions, and local climatic factors. *We hypothesized that flea survival and reproduction peak within an optimal temperature and humidity range, and that flea populations will increase when burrow conditions are within this thermal niche. We also hypothesized that surface temperature and humidity will affect questing behavior by off-host fleas, which has the potential to affect plague dynamics.*

Our objective was to evaluate flea intensity by species and relative abundance in relation to local climate, taking advantage of the latitudinal gradient of our study sites (from Texas to Montana). We also measured abundance of questing fleas in prairie dog burrows in relation to measurements of burrow temperature and humidity. Laboratory studies were attempted to determine basic reproductive and survival parameters of fleas found to be most relevant to plague ecology in Aim 1. This aim also relates to the first objective of the statement of need.

Aim 3: Modeling impacts of climate change on plague dynamics.

Changing climate regimes will influence the population dynamics (reproduction and survival) of all three components of sylvatic plague—prairie dogs, the environment, and fleas. We hypothesized that changes in temperature associated with climate change would result in increased probabilities of plague outbreaks, reduced numbers of prairie dogs alive at the end of a season, and shorter times to colony extirpation. Our objective was to develop models of plague dynamics to assess the effects of climate change on hosts, vectors, reservoirs, flea loads, and interactions between *Yersinia pestis* and other pathogens (co-infection rates). We also aimed to model potential changes in the distribution of plague in response to climate change. This aim addresses Objective 2 and 3 of the statement of need, providing plausible future scenarios that may affect plague exposure pathways and predictive modeling frameworks.

Background

Periodic outbreaks of sylvatic plague, a zoonotic flea-borne disease, caused by the bacterium *Yersinia pestis*, have had near catastrophic effects on some mammal populations (Gage and Kosoy 2005), including prairie dogs (*Cynomys* spp.) and the endangered black-footed ferret (*Mustela nigripes*). Although human plague cases in the United States are

relatively infrequent, the disease can be fatal, and its occurrence generates considerable public concern and media attention. A recent outbreak of plague in prairie dogs outside of Denver, Colorado, forced the closure of several state parks and interfered with outdoor recreational activities. Sylvatic plague is relevant to the Department of Defense (DOD), because prairie dogs, ground squirrels, and other susceptible rodents are present on military installations in several western states, and the occurrence of plague has been known to curtail military exercises in the past. Other vector-borne zoonotic pathogens, such as *Bartonella* spp. and *Rickettsia* spp., are also associated with rodents and fleas. Like other arthropod-borne diseases, the occurrence of plague.is sensitive to the influence of climate conditions, with warm, wet weather patterns linked to higher incidence in both humans (Parmenter et al. 1999; Enscore et al. 2002) and prairie dogs (Stapp et al. 2004). Because most climate change models predict summer temperatures over western and central United States will undergo a warming trend, some predict that the occurrence of plague will expand with increasing latitude and altitude (Ben Ari et al. 2008; Eisen et al. 2007). Indeed, recent analyses indicate that climate was the major driver of plague introductions in Europe in the Middle Ages (Schmid et al. 2015), and that climate change may be one of the causes of human plague re-emergence over the past 50 years (Nakazawa et al. 2007). Our objective was to use newly collected field data from DOD lands and previously collected data along with archived samples from a previous study testing an oral sylvatic plague vaccine (SPV) for prairie dogs (Rocke et al. 2017) to parameterize a model of plague transmission dynamics that can be used to predict the effects of climate change on the frequency and intensity of plague outbreaks. Data are available at https://doi.org/10.5066/f7tm79ck; Russell et al. 2019.

Materials and Methods

Aim 1: Ecological studies.

Study Areas and Design. We used data collected on flea and pathogen prevalence collected at 23 study pairs (46 prairie dog plots) at 10 locations in 6 states in western USA to evaluate environmental and climate factors related to flea abundance. These study sites included 11 pairs on black-tailed prairie dog colonies (*Cynomys ludovicianus*–BTPD), 1 on a Gunnison's prairie dog colony (*Cynomys gunnisoni*–GPD), 4 on white-tailed prairie dog colonies (*Cynomys leucurus*–WTPD), and 7 on Utah prairie dog colonies (*Cynomys parvidens*–UPD) that were part of a larger study testing vaccine effectiveness in prairie dogs (Table 1; Figure 1; Rocke et al. 2017; Russell et al. 2018).

Table 1. List of study areas and designations.

Study area	Pair designation	Species	# pairs	# plots
Buffalo Gap National Grassland, South Dakota	BGSD	BTPD	2	4
Charles M. Russell NWR, Montana	CMR	BTPD	5	10
Lower Brule Sioux tribal lands, South Dakota	LBSD	BTPD	1	2
Rita Blanca National Grassland, Texas	RBTX	BTPD	2	4

Wind Cave National Park, South Dakota	WCSD	BTPD	1	2
Espee Ranch, Arizona	ERAZ	GPD	1	2
Coyote Basin, Utah	CBUT	WTPD	2	4
Pitchfork Ranch, Wyoming	PRWY	WTPD	2	4
Cedar City, Utah	CCUT	UPD	3	6
Awapa Plateau, Utah	HEUT	UPD	4	8

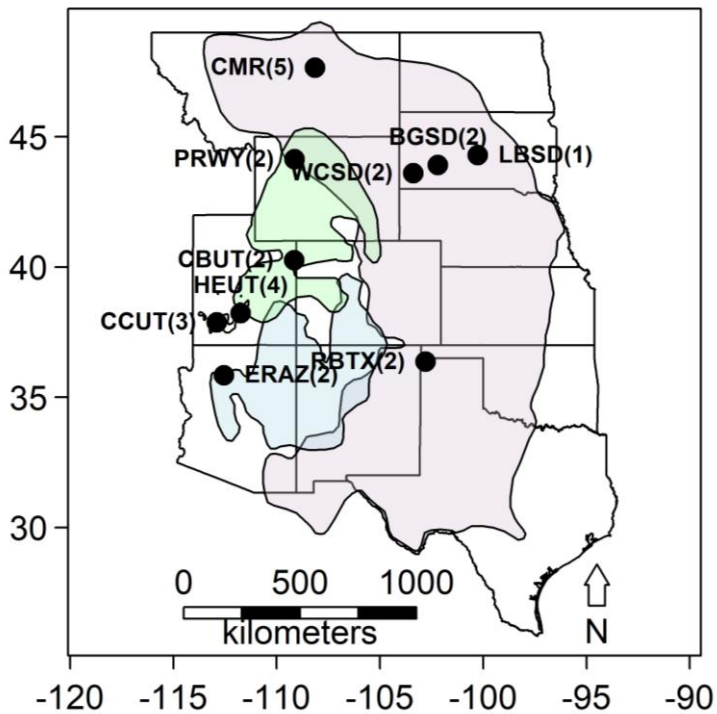

Figure 1. Map of study pairs. Polygons indicate the ranges of different prairie dog species; lavendar for black-tailed prairie dogs (CMR: Charles M. Russell National Wildlife Refug, Montana; WCSD: Wind Cave National Park, South Dakota; BGSD: Buffalo Gap National Grassland, South Dakota; LBSD: Lower Brule Sioux tribal lands, South Dakota; RBTX: Rita Blanca National Grassland, Texas, light green for white-tailed prairie dogs (PRWY: Pitchfork Ranch, Wyoming; CBUT: Coyote Basin, Utah), light yellow for Utah prairie dogs (CCUT: Cedar City, Utah; HEUT: High elevation, Utah), and blue for Gunnison's prairie dogs (ERAZ: Espee Ranch, Arizona. Numbers in parenthesis indicate the number of paired plots.

As described in Rocke et al. (2017), SPV and placebo baits were distributed by hand on paired prairie dog colonies each year from 2013-2015 (100-125 baits/hectare). At least 1 week and no more than 2 months post-baiting each year, local collaborators captured, marked, and sampled prairie dogs for a minimum of three trap days. Both plots in a pair were trapped on the same day, and, with few exceptions, trap effort (number of traps, number of days, area trapped) between plots of the same pair was similar. The goal was to capture and mark a minimum of 50 unique prairie dogs from each plot each year as previously described (Tripp et al. 2009, 2014). Sex, estimated age, and the identity of all current year and prior year recaptures were recorded for each captured animal. Squirrels and other small rodents were trapped with Sherman live traps for at least three consecutive nights on selected plots (Bron et al. 2018). Hair/whiskers, blood, and fleas were collected

from captured prairie dogs and small mammals. After sampling, all animals were released at the location of capture; trap coordinates and individual numbers were noted.

Flea identification and analyses. Fleas collected from live animals and carcasses were stored at −20°C in saline or ethanol until identification (see Russell et al. 2018 and Bron 2017 for details). Fleas were counted and identified to species using published taxonomic references (Furman and Catts 1982; Hubbard 1947; Stark 1970), and pooled by species and sex, up to 10 individuals per pool from a single animal. Flea pools were tested for the presence of *Yersinia pestis*, *Francisella tularensis*, *Rickettsia* spp., and *Bartonella* spp. using real-time PCR. Flea DNA was extracted using the Zymo Quick gDNA Miniprep Kit (>1 flea) or Micro Kit (1 flea) (Zymo Research; Irvine, California) depending on the number of fleas in each pool.

For *Y. pestis*, DNA samples were screened for the *pim* gene, which resides on the pPCP1 plasmid of *Y. pestis*, using real-time PCR, as we found this gene to be more sensitive than the *pla* gene for fleas. Suspect positive fleas were then confirmed using *caf1* gene as described in Rocke et al. 2017. *Y. pestis* strain CO92 genomic DNA (BEI Resources, #NR-2717) was used as our positive control and was used to determine the cut-off value for identifying positive vs. negative results.

For *F. tularensis*, DNA samples were screened for the 23kDa gene of *F. tularensis* using a SYBR Green real-time PCR assay using primers described by Versage et al. 2003. *F. tularensis* genomic DNA (BEI Resources, Manassas, Virginia; #NR-3015 and #NR-3014) was used as our positive control and was used to determine the cut-off value for identifying positive vs. negative results. Samples that tested positive for *F. tularensis* were further identified as either Type A or Type B using primers specific to each type (World Health Organization 2007).

For *Rickettsia* spp., DNA samples were screened for the 17 kDa gene of *Rickettsia* spp. using a SYBR Green real-time PCR assay using primers described by Watthanaworawit et al. 2013. *Rickettsia rickettsia* Strain Smith (149) (BEI Resources, Manassas, Virginia; #NR-48825) was used as our positive control and was used to determine the cut-off value for identifying positive vs. negative results.

For *Bartonella* spp., DNA samples were screened for the 16S citrate synthase (gltA) gene of *Bartonella washoensis* using a SYBR Green real-time PCR assay. *Bartonella doshiae* genomic DNA (Michael Kosoy, Centers for Disease Control (CDC), Fort Collins, Colorado) was used as our positive control and was used to determine the cut-off value for identifying positive vs. negative results.

Serology. Lateral flow tests (Abbott et al. 2014) were used to detect antibodies against *Y. pestis* F1 and V antigens. Blood-filled Nobuto strips were stored at -20°C until elution and testing. Strips were cut into 5–6 pieces, placed into 400 µl of phosphate-buffered saline (PBS), and shaken overnight at 4°C. Eluates were transferred to a clean tube and stored at -20°C until testing. We combined 5 µl of eluate or 1 µl serum with 100 µl diluent for the protein G-based lateral flow test for F1 and V. Optical scores of the test ranged from 0 (negative) to 4 (strong positive).

Statistical analyses. We used logistic regression and negative binomial regression with LASSO (least absolute shrinkage and selection operator; Tibshirani, 1996) priors in a Bayesian framework to assess factors related to total flea abundance and *Oropsylla hirsuta* abundance (Russell et al. 2018). In this framework, parameter estimates for factors that do not influence the response variable shrink to zero; we report effects for non-zero parameter estimates.

We included variables related to climate and seasonal weather patterns (NDVI [normalized difference vegetation index—a measure of vegetation greenness, see Table 1], number of days with a temperature over 85°F [Collinge et al. 2005], amount of precipitation during the prior year's growing season, winter precipitation amount, and day of sampling), plot-level variables (catch per unit effort as an index of relative abundance of prairie dogs [see Rocke et al. 2017 for details], treatment [placebo versus vaccine], plague status [plague detected versus not detected], and elevation), and individual covariates (age, sex, body condition [=weight/foot length]) (Table 1). To incorporate effects of plot, we used a random effect for BTPD (n=22 plots), UPD (n=14), and WTPD (n=8). We used the negative binomial regression to examine factors related to the number of fleas on a host (abundance) and the logistic regression to look at presence/absence of fleas on a host (prevalence), related to the factors listed above. The LASSO allows for model selection and regularization to take place automatically, while improving predictive accuracy and providing interpretable parameter estimates (Tibshirani, 1996). Parameter estimates for variables that are not important predictors shrink to zero.

All models were run in R (R Core Team 2017) using rjags (Plummer 2013). Models were run for a total of 60,000 iterations with a burn in of 20,000. We used gelman-rubin diagnostics to assess convergence (Gelman & Rubin 1992). Finally, we used area under the curve statistics in the "pROC" package (Xavier et al. 2011) to evaluate goodness of fit for our logistic regression models. For negative binomial models, we used posterior predictive checks on the parameters of the negative binomial distribution by simulating 1,000 datasets from the estimated model parameters and comparing to the observed data (Gelman et al. 2004).

Aim 2: Vector Biology.

To assess the association between the rodent and on-host flea community described in Aim 1 on our different study areas, we used a study plot by flea species matrix and a study plot by rodent matrix with the abundance of all years combined to create the most complete dataset. The rodent abundance matrix (plot by rodent species) contained the individual

rodent species that were combed for fleas. These matrices were visualized in nonmetric multidimensional scaling (NMDS) plots. We compared Bray-Curtis dissimilarity matrices of flea species per plot and rodent species per plot. Host-flea associations were quantified and qualified. To assess the level of individual specialization of the flea species, we quantified host specificity (see Bron, 2017 for details).

Additional Field Studies: To determine the relation between ambient climate, burrow microclimate, and flea populations, we conducted additional field studies across a wide latitudinal gradient, which allowed us to survey a range of environmental conditions. We studied burrow fleas on prairie dog colonies at seven sites that spanned the entire black-tailed prairie dog range (BTPD) within the United States (Figure 2). Each colony was sampled during two sessions, one in early summer during May or June (early session) and once in August (late session) in 2016 and 2017. We swabbed 100 conveniently selected burrows per colony to collect fleas and identified fleas to species as described in Aim 1.

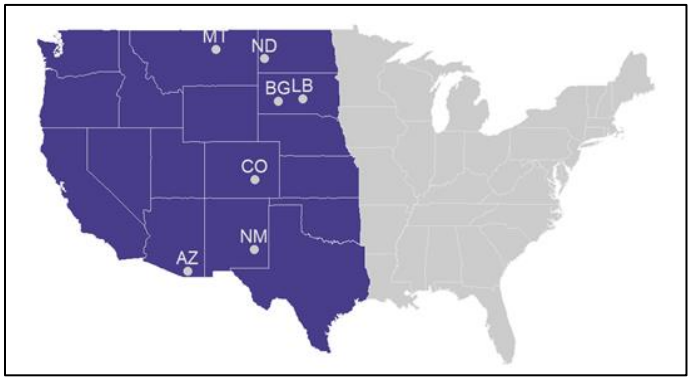

Figure 2. For burrow swabbing to collect questing fleas, seven field sites (labeled in gray) were chosen in six U.S. states (Montana (MT), North Dakota (ND), South Dakota (BG and LB), Colorado (CO), New Mexico (NM), and Arizona (AZ)). MT, CO, and BG and LB, sites in South Dakota were sampled during 2016 and 2017; AZ was sampled in 2017 only, and NM was sampled in 2016 only.

Burrow and ambient temperature and relative humidity were measured by iButton Hygrochron data loggers (DS1923, Maxim Integrated). We placed loggers in burrows at least 1-m deep to track burrow microclimate conditions and placed one on the surface to track ambient conditions at each colony. Data were recorded every 30 minutes for the duration of swabbing (3-4 days), once during the early session (May-June) and once during the late session (July-August). The loggers were synchronized to take simultaneous readings. We calculated the mean burrow temperature and mean relative humidity over the swabbing session by taking the mean of the data loggers at each timestep, and then taking the mean of the timesteps for each site over the sampling session. See Poje (2019; Appendix 4) for details.

We modeled the relative abundance of a given flea species and the prevalence of fleas in burrows in generalized linear models as a response to temperature and precipitation. We also modeled daily mean burrow temperature, and daily mean relative humidity as response to climate variables. See Poje (2019; Appendix 4) for details.

Laboratory Studies: Of the *Oropsylla* species, only *O. montana*, the primary vector of plague in California ground squirrels, has been successfully reared in captivity. Therefore, we used it as a proxy for *O. hirsuta* and *O. tuberculata*. Initially, we attempted to raise *O. montana* fleas in the laboratory to track development under conditions mimicking burrow

microclimates. Unfortunately, rearing fleas in the laboratory is difficult and we were unsuccessful in obtaining our desired data. As an alternative, Poje (2019) used unpublished data from a dissertation (Metzger 2000) in which *O. montana* fleas were reared on captive California ground squirrels (*Otospermophilus beecheyi*) at various temperatures and relative humidity levels. Hatch date, date of pupation, and date of adult emergence were tracked. Using a generalized linear mixed model, Poje (2019) used these data to determine the temperature dependent development rate of *O. montana* by including temperature as a fixed effect and relative humidity as a random effect. This allowed Poje (2019) to develop a degree-day model to predict flea development rates and potential generation times across a range of temperatures (see Poje 2019 [Appendix 4] for details).

Aim 3: Modeling impacts of climate change on plague dynamics.

We developed spatially explicit agent-based models (ABM) of plague dynamics based on previous developed models (Richgels et al. 2016 and Buhnerkempe et al. 2011). Our ABMs are parameterized from field data, laboratory studies, and previously published literature.

In general, prairie dogs are infected through three possible routes; flea transmission, direct transmission between prairie dogs, or transmission from a carcass (prairie dogs will consume the carcasses of conspecifics) (Figure 3). In our models the probability that a prairie dog will interact with an infected flea, infected live prairie dog, or infected carcass is controlled by parameters estimated from field data. We used spatial-capture recapture (Royle et al. 2013) to estimate the size of the activity centers for each prairie dog and estimate a center of activity for both observed individuals and individuals estimated to be in the population. In other words, capture-recapture methods estimate the number of individuals in the population that were never detected. Spatial capture recapture estimates the spatial distribution of those individuals. We used these data to estimate the probabilities of overlap between individual activity centers and therefore the likelihood the prairie dog will encounter an infected flea from an adjacent prairie dog or an infected carcass in an adjacent activity center. In addition to death from plague, prairie dogs are subject to a natural mortality rate.

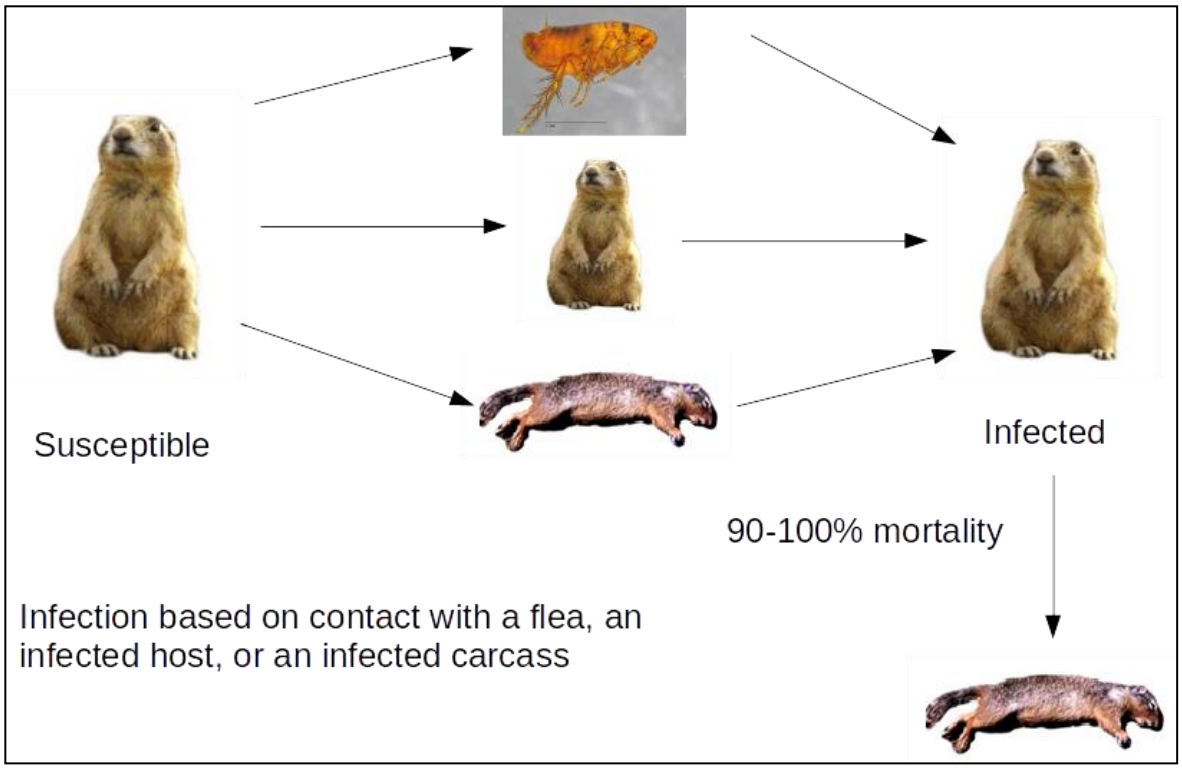

Figure 3. Schematic of plague transmission routes.

Transmission of plague by fleas is a complex process that involves fleas proceeding from early to latent to late stages of infection, during the early and latent stages of infection fleas may clear infection and return to the susceptible stage (Figure 4). There is much debate in the literature regarding the relative importance of early phase transmission versus late phase transmission and whether *O. hirsuta* fleas "block" (Eisen et al. 2006; Wilder et al. 2008a and b; Hinnebusch et al. 2017). Blocking is a mechanism by which *Y. pestis* bacteria become lodged in the gut of the flea and prevent the flea from feeding. This causes them to repeatedly bite host animals in an attempt to get a blood meal, which increases the likelihood of plague transmission.

The parameters in our models included estimated uncertainty (Appendix 1). We accounted for this uncertainty in a variety of ways including (1) adding stochasticity to the models, (2) conducting aleatory analyses to determine the number of simulations per parameter "set" necessary to capture the variation in the response variable, (3) running multiple parameter sets that spanned the range of possible values for each parameter, and (4) conducting boosted regression tree analyses on the response variables (mean on host fleas and plague outbreak occurrence). Stochasticity was added to two parameters (direct transmission and carcass transmission) by drawing from a random normal distribution with a mean and standard deviation that captured our assumptions regarding the distribution of the parameter.

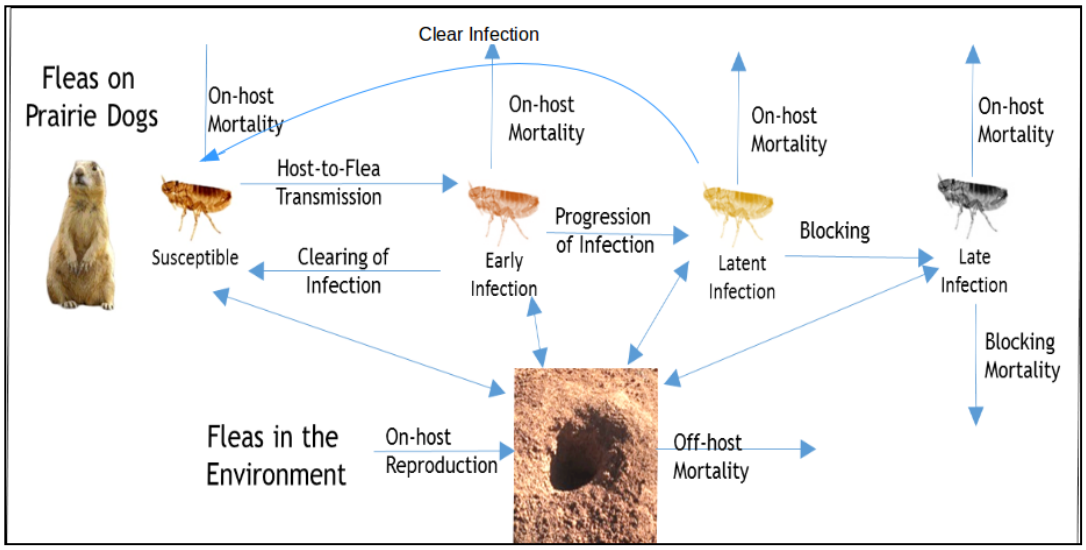

Figure 4. Schematic of flea transmission.

We began by running the non-plague model of flea and prairie dog dynamics, to assess whether our model could recreate realistic flea numbers across the course of a season (150 days). An aleatory analysis indicated that 100 simulations per parameter "set" was necessary to capture the variability in the response variable "mean on-host flea number". Therefore we ran 100 simulations of each of 30,000 parameter "sets" and determined which parameter sets resulted in mean on-host flea distributions that were <=15 flea per host (Tripp et al. 2009, Russell et al. 2018), which reflected our field-based observations. We subset our parameter sets to the 1,827 parameter sets that produced realistic estimates of on-host flea abundance. We conducted a boosted regression tree to estimate the relative influence of the parameters on the mean on-host flea abundance and mean flea abundance in the activity center.

For our plague models, we then used Latin Hypercube sampling (Iman et al. 1981) to generate 18,270 parameter sets (each of the 1,827 parameter sets were repeated 10 times in the dataset) which included 16 new parameters (Appendix 2). Of those 16 parameters, 10 were included in the Latin Hypercube and 6 were included as fixed parameters. Each of the 18,270 parameter sets were run 100 times. We then conducted a boosted regression tree on the response variable outbreak occurrence, mean number of prairie dogs remaining, and mean time to extinction where outbreak occurrence was 1 if <20 prairie dogs survived the 150 days and outbreak occurrence was 0 if >20 prairie dogs survived the season.

To address the effects of climate change we used the estimated development rate of fleas from Poje (2019; Appendix 4) across an increasing ambient temperature gradient from 28C (the lowest ambient temperature recorded) to 36C (four degrees above the higher ambient temperature recorded) and calculated a replacement rate based on the expected number of fleas per clutch of eggs (Table 2). Development rate * number of eggs per clutch expected to reach adulthood=replacement rate per day. For our purposes, we generated scenarios assuming 5, 10 or 15 fleas were produced per egg clutch. By varying the replacement rate between 0.1 and 0.4, we produced a variety of scenarios to reflect the expected increase in temperature.

Table 2. Estimated flea replacement rates as a function of temperature.

Temperature (°C)	Development rate	Number of fleas	Replacement rate
28	0.021	5	0.11
	0.021	10	0.21
30	0.025	5	0.13
	0.025	10	0.25
32	0.029	5	0.15
	0.029	10	0.29
34	0.033	5	0.17
	0.033	10	0.33
36	0.041	5	0.20
	0.041	10	0.41

Results and Discussion

Aim 1: Ecological studies.

We identified host/flea relationships in small mammal communities on prairie dog colonies to assess the most critical plague exposure pathways. Results of flea collection from live prairie dogs were published in Russell et al. (2018). Briefly, we identified 20,041 fleas from 6,542 prairie dogs sampled between June and November over a 3-year period and identified 18 species of fleas. The prairie dog specialist flea *O. hirsuta* was the most common species detected, comprising 59% of all fleas sampled. The second most commonly detected flea species was *Pulex simulans*, a generalist flea species, accounting for 23% of fleas identified. The ground squirrel flea, *Thrassis francisi*, comprised ~10% of detections. Three other *Oropsylla* species, *O. tuberculata*, *O. labis*, and *O. idahoensis*, comprised less than 10% of detections and were most commonly found on pairs where *O. hirsuta* was not detected. UPD pairs located at high elevation in Utah (HEUT) and WTPD pairs at Pitchfork Ranch, Wyoming (PRWY) had the most diverse flea populations, with 10 flea species detected at HEUT and 6 flea species at PRWY (see Russell et al. 2018).

We assessed the geographical variation in flea community composition and flea specificity on our study sites. Our results indicate rodent and flea species community composition and abundance per plot are related and differ geographically. The communities of small rodents and fleas were similar on BTPD colonies in Montana and South Dakota, but different on WTPD and UPD colonies at high and low elevation. High elevation Utah prairie dog colonies had the highest flea species richness, with *Aetheca wagneri*, *O. tuberculata* and *Thrassis francisi* dominating the community. Specialization of individual flea species in each study area varied, but many fleas were specialists. Most specialized was *O. hirsuta*, followed by the Ord's kangaroo rat (*Dipodomys ordii*) flea, *Meringis parkeri*, and the chipmunk flea, *Eumolpianus eumolpi*. The rodent-flea networks on prairie dog colonies were highly specialized, and flea switching was rare; 0.3% of fleas collected from short-lived rodents were prairie dog fleas, and 0.05% of the fleas collected from prairie dogs were short-lived rodent fleas. This is additional evidence that plague transmission between short-

lived rodents and prairie dogs by fleas is extremely rare, but not impossible (Table 3; see Bron 2017 for details).

Table 3. Flea switching among rodent hosts. The number of fleas in each flea species group per 1,000 fleas collected from the host (95% confidence interval). We did not target squirrels and lagomorphs in our trapping efforts. Cumulative, 1.8-4.7 fleas per 1,000 fleas on prairie dogs are short-lived rodent, chipmunk, or rat fleas. On short-lived rodents, 7.8 – 17.4 fleas of 1,000 are shared with prairie dogs or squirrels.

Flea group	**Prairie dog host**	**Short-lived rodent host**
Prairie dog fleas	970 (967.5, 972.3)	3.1 (1.7, 5.4)
Squirrel fleas	26.8 (24.7, 29.2)	8.6 (6.1, 12.0)
Lagomorph fleas	0.5 (0.3, 0.9)	0 (0, 1.1)
Rat and others fleas	1.9 (1.4, 2.7)	0 (0, 1.1)
Chipmunk fleas	0.3 (0.2, 0.7)	0 (0, 1.1)
Short-lived rodent fleas	0.5 (0.2, 1.3)	988.3 (984.4, 991.2)

We compared the number of prairie dogs and small rodents with plague-positive fleas between paired sites and quantified host switching by identifying flea species and source DNA in blood meals. The incidence of prairie dogs with *Y. pestis* detections in fleas was low (*n* = 64 prairie dogs with positive fleas out of 5,024 prairie dogs sampled from 4,218 individual prairie dogs). Fleas positive for *Y. pestis* were found on BTPD plots in Montana (2016) and Texas (2014 and 2015), WTPD plots in Wyoming (2015 and 2016) and Utah (2015 and 2016), high elevation UPD plots (2014 and 2015), and the one GPD plot in Arizona (2014). Twenty-five of the prairie dogs with *Y. pestis* positive flea pools were found on placebo plots and 39 on vaccine plots, which represent less than 2% of all prairie dogs tested. *Y. pestis* positive fleas were found on juvenile and adult prairie dogs of both sexes and in flea pools of seven different species including *P. simulans*, *O. hirsuta*, *O. tuberculata*, *O. labis*, *O. idahoensis*, *Thrassis francisi*, and *Neopsylla inopina* (*Y. pestis* was not detected in any *A. wagneri* pools collected from prairie dogs).

The presence of plague in fleas collected from prairie dogs was positively associated with flea abundance for all species except GPD. In addition, *O. hirsuta* and *P. simulans* abundance was associated with plague detection for BTPD. Flea abundance was higher on animals with PCR-positive flea pools (mean = 11.7, SD = 14.1) than animals without PCR-positive fleas (mean = 3.9, SD = 5.1). Plague detections in *P. simulans* only occurred at plots where plague was also detected in *O. hirsuta*. Plague-positive fleas (*T. francisi*, *O. labis*, *O. idahoensis*, and *O. tuberculata*) were found on PRWY and HEUT plots, but no *O. hirsuta* were detected on these plots. The chipmunk flea, *E. eumolpi*, was more often collected from prairie dogs than expected. In contrast, *P. simulans* was never collected from short-lived rodents, even though it was the predominant flea species in Montana. This illustrates that interspecific transmission of plague might vary between different regions in the United States.

We did not detect plague-positive fleas on short-lived rodents during plague outbreaks in prairie dogs (Table 4). However, plague-positive mouse fleas (*Aetheca wagneri*, *Pleochaetis exilis*, *Orchopeas leucopus*) were detected on mice prior to plague-induced

declines in BTPD in Montana and WTPD in Wyoming. This finding led to the speculation that plague-positive mouse fleas might have fed on bacteremic prairie dogs, although no prairie dog DNA was detected during blood meal analysis of plague-positive mouse fleas. Mice may be involved in *Y. pestis* maintenance, introduction and/or propagation on prairie dog colonies. Deer mouse abundance did not predict plague outbreaks, but we observed high abundance of deer mice in study areas the season prior to plague outbreaks on individual colonies within that study area, indicating mice could aid in the overall force of infection (see Bron 2017 and Bron et al. 2019 for details).

Table 4. Sample size and maximum likelihood estimate infection rate per 100 fleas collected from mice. Flea species shown are the most prevalent mouse flea species, plague positive species in bold and flea species shared with prairie dogs (*) on our study plots.

Flea species	Infection Rate (95% CI)	# Pools	# Positive pools	# Individuals
*Aetheca wagneri**	**0.05 (0.01 -0.16)**	1652	2	4197
*Catallagia decipiens**	0.00 (0-2.95)	94	0	125
*Eumolpianus eumolpi**	0.00 (0-1.12)	115	0	334
Malaraeus telchium	0.00 (0-1.24)	188	0	302
Meringis parkeri	0.00 (0 -1.35)	109	0	274
Opisodasys keeni	0.00 (0 – 0.91)	245	0	414
Orchopeas leucopus	**1.12 (0.06 – 5.35)**	60	1	90
*Oropsylla hirsuta**	0.00 (0-14.98)	10	0	19
*Oropsylla idahoensis**	0.00 (0-32.59)	1	0	4
*Oropsylla labis**	0.00 (0-65.76)	2	0	2
Peromyscopsylla hesperomys	0.00 (0-1.74)	106	0	212
Pleochaetis exilis	**4.72 (0.26-22.40)**	15	1	23
*Thrassis francisi**	0.00 (0 – 79.35)	1	0	1

We used PCR to compare the prevalence of *Y. pestis*, *F. tularensis*, *Rickettsia* spp., and *Bartonella* spp. in rodent and flea species. We will use these results to test how co-infection may affect plague prevalence in these communities. Flea samples from live prairie dogs were tested for the presence of pathogens *Y. pestis*, *Rickettsia spp*., and *Bartonella* spp. (Tables 5-7). Fleas collected from prairie dog carcasses were tested for *Y. pestis* and *F. tularensis* (Table 8).

Table 5. Pathogens detected using PCR in flea pools collected from live prairie dogs.

Pathogen	% positive (number of positives)	Number tested
Yersinia pestis	1.2% (58)	5024
Bartonella spp.	12.7% (103)	813
Rickettsia spp.	1.7% (12)	690

Table 6. Pathogens detected using PCR in flea pools collected from live prairie dogs on plague-positive sites.

***Yersinia pestis* PCR result**	**% positive for *Bartonella* spp. (number)**	**% positive for *Rickettsia* spp. (number)**
Y. pestis positive	18.6% (8)	2.1% (1)
Y. pestis negative	11.9% (92)	1.7% (11)
Number tested	813	690

Table 7. Species and collection locations of flea pools collected from live prairie dogs positive by PCR for pathogens.

Pathogen	**Positive flea species**	**Positive sites**
Yersinia pestis	*Oropsylla hirsuta* *O. idahoensis* *O. labis* *O. tuberculata* *Pulex simulans* *Thrassis francisi*	CBUT CMR ERAZ HEUT PRWY RBTX
Bartonella spp.	*O. hirsuta* *O. idahoensis* *O. labis* *O. tuberculata* *P. simulans* *T. francisi*	CBUT CMR ERAZ HEUT PRWY RBTX
Rickettsia spp.	*O. hirsuta* *P. simulans*	CMR

Table 8. Pathogens detected using PCR in flea pools collected from prairie dog carcasses.

	Flea pools collected from prairie dog carcasses			
Tissues from carcasses with fleas	***Yersinia pestis* positive**	***Y. pestis* negative**	***Francisella tularensis* positive**	***F. tularensis* negative**
Culture positive for *Y. pestis* (n=11)	4	7	0	11
PCR positive for *Y. pestis* (n=3)	2	1	0	3
Negative for *Y. pestis (n=4)*	1	3	0	4

Evaluation of the prevalence of flea and/or blood-borne pathogens in our study sites in relation to local climate conditions is planned.

Determination of the effect of SPV treatment on the number of seropositive and potential plague-resistant individuals compared to placebo treatment has been delayed by the limited supply of the lateral flow test strips. Preliminary serology results appear in Table 9. Analysis of the serology results is planned.

Table 9. Serology results of blood samples collected from live prairie dogs.

Treatment	F1 positive	V positive	F1/V positive	F1/V negative
Vaccine	210	653	348	2928
Placebo	103	52	70	2929

Vaccine sites

	Number	F1 positive	V positive	F1/V positive	F1/V negative
Bait uptake positiv	3233	124	604	300	1802
Bait uptake negative	1543	82	39	23	1087

Placebo sites

	Number	F1 positive	V positive	F1/V positive	F1/V negative
Bait uptake positive	3050	66	42	51	2104
Bait uptake negative	1325	36	10	18	812

Aim 2: Vector Biology.

On-host flea abundance. We analyzed the relation of on-host flea relative abundance and intensity to local climate, vegetation (as measured by normalized difference vegetation index –NDVI), and rodent abundance across a latitudinal gradient. Multiple weather and climate-related variables were associated with on-host flea abundance and presence/absence on prairie dogs (Table 10; see Russell et al. 2018 for details). Total on-host flea abundance increased 92% (95% Credible Interval [C.I.] 9–217%) on UPD pairs over every 10-day period. The odds of a prairie dog having at least one flea increased by 1.10 (95% C.I. 1.03–1.17) for BTPD and 1.16 (95% C.I. 1.09–1.26) for UPD as days in the season increased. In addition, on BTPD pairs, *O. hirsuta* abundance increased a mean of 214% (95% C.I. 88–392) with every 10 days later in the year, while *P. simulans* abundance decreased 66% (95% C.I. 50–78%). On UPD pairs, day of sampling was associated with an increase in odds of *O. hirsuta* presence (1.01, 95% C.I. 1.12–1.23) but not *P. simulans*.

Table 10. General relations for non-zero parameter estimates between climate variables and total on-host flea populations and *Oropsylla hirsuta* populations only as analyzed in a Bayesian framework using LASSO (least absolute shrinkage and selection operator).

Climate Variables	**Total Fleas**	***O. hirsuta***
Winter precipitation	negative	no relation
Summer precipitation (prior year)	no relation	negative
Normalized difference vegetation index	negative	no relation
Days > 85ºF	positive	positive

With a 10% increase in NDVI, total on-host flea abundance decreased by 89% (95% C.I. 80–95%) on BTPD pairs, but these differences were not significant for individual flea species. For every 10 days with temperatures over 85°F, total flea abundance increased with the exception of WTPD pairs, which decreased by 80% (95% C.I. 55–92%); the odds of flea presence also decreased by 0.06 (95% C.I. 0.02–0.16) for WTPD; however, these results were not evident for individual flea species. For the same change in number of degree days, total flea abundance increased by 5% (95% C.I. 0–11%) and the odds of flea presence increased by 1.19 (95% C.I. 1.08–1.31) for BTPD; these differences were driven largely by changes in *O. hirsuta* with mean increases in abundance of 21% (95% C.I. 14–20%) and increases in odds of *O. hirsuta* presence of 1.23 (95% C.I. 1.13–1.34).

Precipitation amounts also had varying effects on on-host flea abundance. For every 1 cm increase in winter precipitation on WTPD pairs, total flea abundance increased by 83% (95% C.I. 33–165%) with odds of flea presence increasing by 2.99 (95% C.I. 2.00–4.80). However, odds of *P. simulans* presence declined with increasing precipitation (0.44, 95% C. I. 0.12–0.89) on these plots. On BTPD plots, flea abundance decreased by 3% (95% C.I. 2–5%) and odds of flea presence decreased by 0.98 (95% C.I. 0.95–1.00) with increasing winter precipitation. These results were largely driven by declines in *P. simulans* with a mean decline of 7% (95% C.I. 4–10%) with increasing winter precipitation.

Many factors were associated with flea abundance on prairie dogs, indicating the complexity of host-vector-disease dynamics in this system. Our results indicate that local weather factors influenced flea abundance, potentially by providing environmental conditions suitable for flea reproduction. As expected, however, environmental factors had different effects at different locations due to the wide range of environmental conditions and geographic locations of our study pairs. Differences in the direction of the relation of environmental covariates with flea abundance for different prairie dog species is likely the result of differences in local conditions and differences in the biology of the prairie dog and flea species in question. Abundance of fleas is likely more important for epizootic plague, versus the presence or absence of fleas on hosts at a location.

We determined the effects of plague on flea populations by comparing vaccinated and unvaccinated paired sites (see Russell et al. 2018 for details). The presence of plague was positively associated with flea abundance for all species except GPD. In addition, *O. hirsuta*

and *P. simulans* abundance was associated with plague detection for BTPD. On average, the odds of flea presence was lower on vaccine versus placebo plots for WTPD (0.74, 95% C.I. 0.56–0.97) only, and these results were reflected in declines in the odds of *P. simulans* presence (0.36, 95% C.I. 0.16–0.83). Increases in odds of *O. hirsuta* presence were detected on UPD vaccine versus placebo plots (2.65, 95% C.I. 1.41–4.65); however, *O. hirsuta* were only found on Cedar City, Utah (CCUT) sites and not HEUT. The CCUT sites were small, <4 hectares in size, therefore the effects of the vaccine may have been swamped by movement on and off the site.

Flea abundance in burrows. We swabbed 6,466 prairie dog burrows during the summers of 2016 and 2017. From these, we collected 2,024 fleas from 629 burrows (see Poje 2019 [Appendix 4] for details; Table 11).

Table 11. Fleas collected from prairie dog burrows.

Flea species	Number of fleas	Proportion
Oropsylla hirsuta	1832	0.91
Pulex simulans	148	0.07
Oropsylla tuberculata	22	0.01
Thrassis fotus	11	0.01
Epitedia wenmanni	1	0.001
Polygenis gwyni	2	0.001
Unknown	8	0.004

Patterns of flea abundance in burrows (total number of fleas collected) varied by region and sampling session. In both 2016 and 2017, flea abundance decreased from the early to the late sampling session at southern sites, while flea abundance increased from early to late sampling at northern sites (Figure 5). *O. hirsuta* was the most common species of flea found during our study, comprising 91% of fleas collected. It was the dominant species at all sites during all sampling sessions, which is consistent with previous studies of flea communities in BTPD burrows (Cully et al. 2000; Stevenson et al. 2003; Holmes et al. 2006; Salkeld and Stapp 2008; Mize and Britten 2016; Seery et al. 2003). The relative abundance of *O. hirsuta* increased from the early to the late sampling session at sites in Montana, North Dakota, South Dakota, and declined significantly at sites in Colorado and New Mexico It is currently unclear what drives the difference between the northern and southern sites. Fleas, like many species of insects, are sensitive to changes in the temperature and relative humidity of their surroundings. Our sites cover a wide range of latitudes, and southern Colorado and New Mexico are considerably hotter and drier than the northern plains states of Montana and the Dakotas.

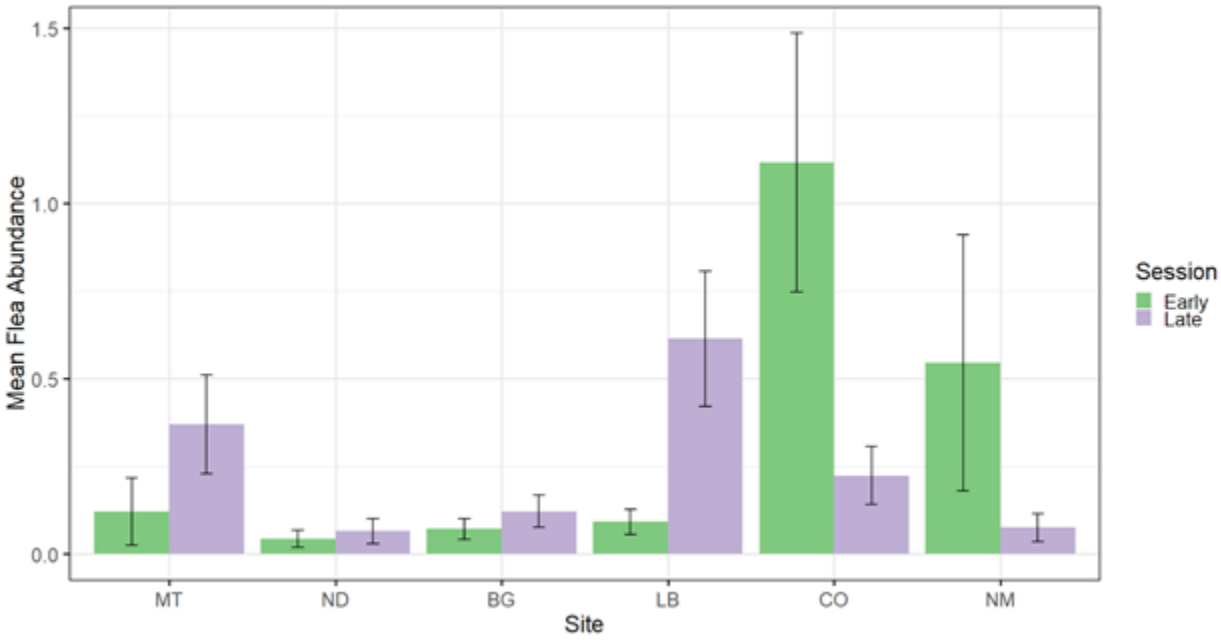

Figure 5. Mean flea abundance (total number of fleas collected / number of burrows sampled) with error bars representing 95% confidence intervals at six sites (shown in Figure 2) in Montana (MT), North Dakota (ND), South Dakota (BG and LB), Colorado (CO), and New Mexico (NM) during an early sampling session (late May – June) and a late sampling session (August) in the summers of 2016 and 2017. Mean flea abundance increased from the early sampling session to the late sampling session at the northern sites (MT – LB) and declined at the southern sites (CO and NM).

We found that flea abundance in burrows increased with summer temperature, declined with winter precipitation, and increased from the early to the late session (Table 12). The odds of finding an infested burrow were higher in 2017 and the late session than in 2016 and the early session. Higher temperatures increased the odds of a burrow being infested by 50% for every 1°C increase. During the early session, increased winter precipitation caused the prevalence of infested burrows to decline by 74% for every additional cm of precipitation, but in the late session increased winter precipitation had no significant effect on the prevalence of infested burrows for every additional cm of precipitation.

Table 12. Effects of sampling time, temperature, and precipitation on the prevalence of flea-infested burrows and the abundance of fleas in burrows. + indicates a positive effect; -|0 indicates a negative effect or no effect.

Factor	Prevalence	Abundance
Year (2016 to 2017)	+	+
Session (early to late)	+	+
Mean monthly high temperature	+	+
Winter precipitation	-|0	-|0

Our models showed that fleas were more abundant in the late session than the early session and in 2017 than 2016. Flea abundance increased by 58% (2016) or 112% (2017) for every

1°C increase in mean monthly high temperature. The effect of precipitation on flea abundance changed between the early and late session with flea abundance declining by 76% (early) or no significant change (2017) for every 1 cm increase in winter precipitation (Figure 6).

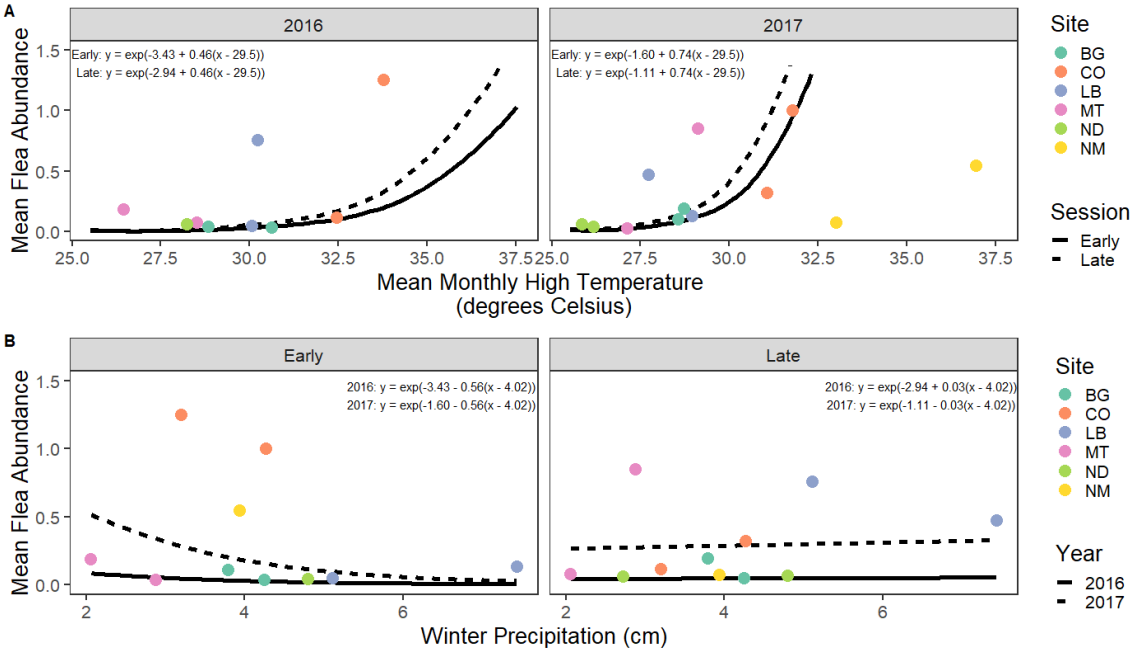

Figure 6. (A) Flea abundance increased with monthly mean high temperature at different rates in 2016 and 2017 for sites shown in Figure 2 based on model coefficients from generalized linear mixed modeling. In 2016, flea abundance increased by a 58% for every 1°C increase in temperature, while in 2017 abundance increased by 109%. During both years, mean flea abundance was 1.63 times higher in the late session than the early session. **(B)** The relation between flea abundance and winter precipitation changed between the early and the late sampling sessions. In the early session, flea abundance declined at a rate of 0.43 for every additional cm increase in precipitation. In the late session, there was no significant effect of winter precipitation on abundance. During both sessions, mean flea abundance was 6.23 times higher in 2017 than 2016.

Burrow microclimate. Data on temperature and relative humidity of burrows was collected at the same time they were swabbed. Using modeling, we characterized burrow microclimates by determining the relation between burrow temperature and humidity and current and past local climate variables. Our linear mixed model showed that both monthly mean high temperature and sampling session were significant predictors of daily mean burrow temperature (Figure 7). Mean burrow temperature increased with monthly mean high temperature, and was higher in the late sampling session than during the early sampling session (Table 11). Daily mean relative humidity was uniformly high (>85%) across sites, sampling sessions, and years. There was no relation with monthly precipitation ($R^2 = 0.05$).

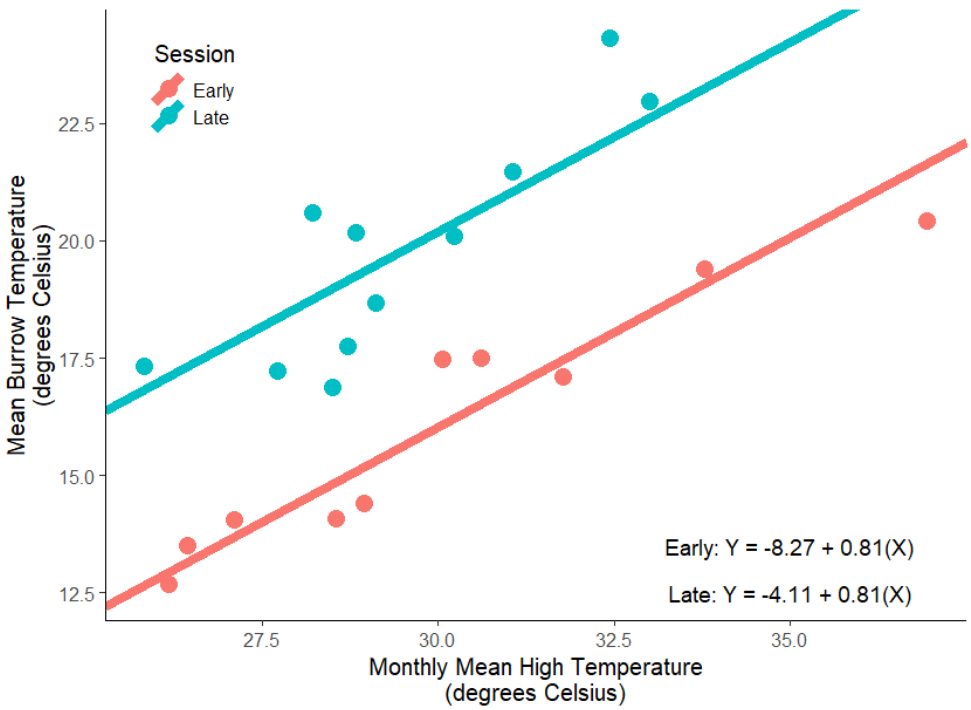

Figure 7. Daily mean burrow temperatures are correlated with monthly mean high ambient temperatures. Each 1°C increase in monthly mean high temperature increases mean burrow temperature by 0.81°C. Burrow temperatures were 4°C higher during late sampling sessions than during early sampling sessions.

Table 13. Estimates and confidence intervals for variables related to mean burrow temperature using generalized linear mixed modeling.

Explanatory Variable	Estimate	95% Confidence Interval	t - value
Monthly Mean High Temperature	0.81	0.64 - 0.98	9.54
Session (Late)	4.16	3.28 - 5.04	9.1

As we hypothesized, burrow microclimates remained stable throughout the day when compared to ambient conditions. The stability of burrow temperatures is generally attributed to the buffering capacity of the soil. Because soil has a higher thermal capacity than the atmosphere, it takes longer to heat up than the outside air, creating a refuge within the burrow to allow respite from outside conditions. Although burrows showed short-term stability, burrow temperatures showed seasonal changes, increasing by a mean of 4°C from early to late sampling sessions. These longer-term patterns follow seasonal climatic conditions driven by solar radiation (Bennett et al. 1988). We found that daily mean burrow temperature is driven by monthly mean high temperature and varied by site (longitudinal patterns). Daily mean burrow temperatures and monthly mean high temperature both decline with latitude, indicating that solar radiation is driving both phenomena.

The climate in the Great Plains is projected to warm 1.9-3.6°C by 2085 (Kunkel et al. 2013a and b). As the climate warms, our results indicate prairie dog burrow temperatures will increase as well. Based on our models, flea abundance could increase between 450% and 540% under this projected level of warming, and the prevalence of infested burrows will increase by between 285% and 540%.

Climate effects on flea development. Data from controlled laboratory studies of *O. montana* were used as a proxy for other common *Oropsylla* flea species to determine life history parameters, including the effect of temperature and humidity on flea development. According to our model, flea development rates increased significantly with temperature (Figure 8; see Poje 2019 [Appendix 4] for details). Although flea development was higher at higher relative humidity, it was not as important as changes in temperature Because the field sites in the northern plains (Montana, North Dakota, and South Dakota) have a cooler, wetter climate than those in the southern plains (Colorado and New Mexico), we estimated development rates for each region separately. If current monthly mean high temperatures rise by 4°C, burrow temperatures in the northern plains are predicted to increase to 17.9°C in June. This will increase the development rate to 0.029, and adults will emerge 34.2 days after eggs are laid, 13 days (28%) faster than current rates. In the southern plains, burrow temperatures will increase to 22.7°C in June, and the development rate will increase to 0.041. Adult fleas will emerge 24.2 days after eggs are laid, 6 days (20%) faster than current rates.

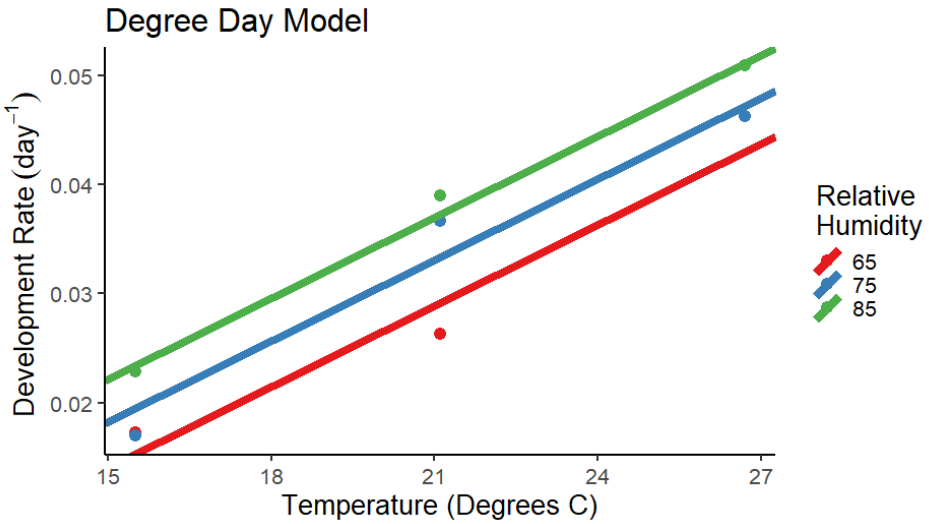

Figure 8. Flea development rate increases by 0.00247 day^{-1} ($t = 14.5$, $df = 12$, $p < 0.0001$, 95% confidence interval (CI) 0.0022 – 0.0028) for every 1°C increase in temperature. At the same temperature, flea development is 0.004 day^{-1} faster at 75% relative humidity than 65% relative humidity, and 0.004 day^{-1} faster at 85% relative humidity than 75% relative humidity.

Because fleas are ectothermic, it is not surprising their development is sensitive to environmental conditions. Higher relative humidity and temperature lead to faster flea development, and that likely leads to higher flea abundance. As the climate warms, prairie dog flea development rates are expected to increase, allowing fleas to produce more

generations in a single growing season and leading to a population increase. As winters become shorter, there will be a longer period of favorable temperatures for fleas to survive and reproduce.

By combining field and laboratory results, we identified the climatic niche of fleas most relevant to plague ecology (see Poje 2019 [Appendix 4] for details). In both the northern and southern plains, prairie dog flea development rates are projected to increase with warming temperatures. Currently, *O. hirsuta* are most abundant for about 150 days from June to October (Salkeld and Stapp 2008), and monthly mean high temperatures in the northern plains are lower than the minimum threshold necessary (6°C) for flea development to occur for 7 months, thus limiting the season of active flea recruitment. However, if temperatures increase as expected (Kunkel et al. 2013a and b), the number of days required to complete development will decrease, leading the number of generations produced over 150 days to increase from 3.2 to 4.4 in the northern plains, and 5.0 to 6.2 in the southern plains by 2100. These changes correspond to a potential flea population increase of 138% and 124%.

Together, higher development rates over longer growing seasons will produce higher recruitment rates, which could increase flea abundance dramatically. However, further research is necessary to determine how *O. hirstua* and *O. tuberculata* respond to warmer and longer periods of favorable temperatures. Flea bites are responsible for >70% of plague transmission during epizootic plague (Richgels et al. 2016). If fleas are present for longer periods of time at higher abundance in the future, it is possible that plague outbreaks could last longer and be more severe, but that remains to be determined.

Changing climate conditions will likely affect aspects of both flea and host communities, including population densities and species composition, and lead to changes in plague dynamics. Our results support the hypothesis that local conditions, including host, vector, and environmental factors, influence the likelihood of plague outbreaks, and that predicting changes to plague dynamics under climate change scenarios will require consideration of both host and vector responses to local factors.

Aim 3: Modeling impacts of climate change on plague dynamics.

Models were constructed to determine the relations between prairie dog body condition and climate factors (degree days= the number of days above 85 degrees Fahrenheit, prior year precip= the amount of prior year precipitation in cm, winter precip= the amount of winter precipitation in cm, NDVI= a measure of "greenness", and body condition= the weight of the prairie dog in grams divided by the foot length), controlling for age and sex. The strength of the association increases with an increasing absolute value of the coefficient. For BTPD on sites without plague, more precipitation in both winter and summer is related to poorer body condition and higher NDVI values (which in turn are negatively correlated with body condition). Increased summer temperatures (which are negatively related to NDVI) are associated with better body condition (Figure 9).

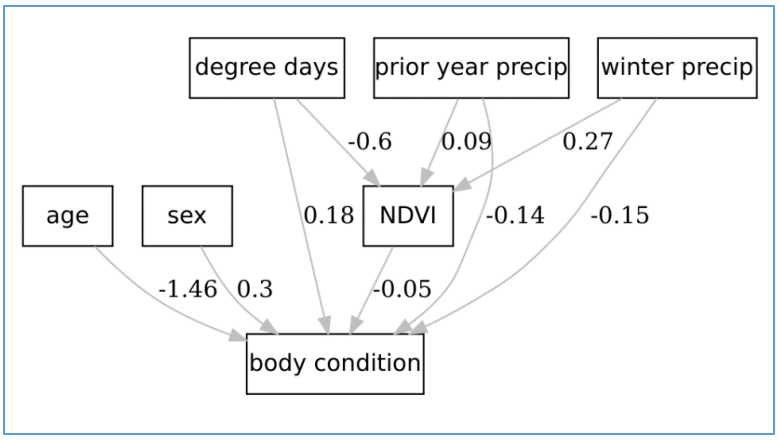

Figure 9. Black-tailed prairie dog body condition in relation to climate factors (controlling for age and sex). Numbers indicate coefficients from path analyses; the strength of the association increases with an increasing absolute value of the coefficient. Age is juvenile versus adult and sex is male versus female. All relations are statistically significant at $p<0.05$.

For WTPD on sites without plague (controlling for age and sex), more precipitation and hotter temperatures are related to better body condition. NDVI is negatively related to winter precipitation and summer temperatures but positively related to prior year summer precipitation (Figure 10).

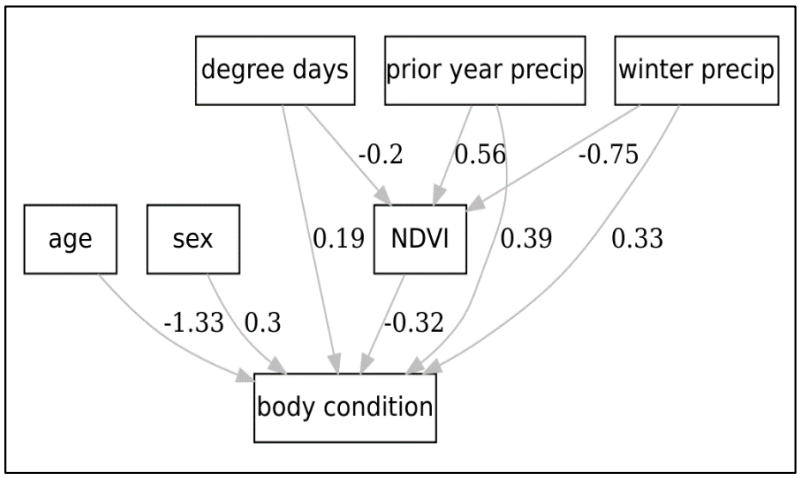

Figure 10. White-tailed prairie dog body condition in relation to climate factors (controlling for age and sex). Numbers indicate coefficients from path analyses; the strength of the association increases with an increasing absolute value of the coefficient. Age is juvenile versus adult and sex is male versus female. All relations are statistically significant at $p<0.05$.

For UPD on sites without plague (controlling for age and sex), prior year summer precipitation is positively related to body condition and NDVI (which is negatively related to body condition). Degree days is not significantly related to NDVI or body condition,

however winter precipitation is negatively related to NDVI. Winter precipitation and body condition relationships are not statistically significant (Figure 11).

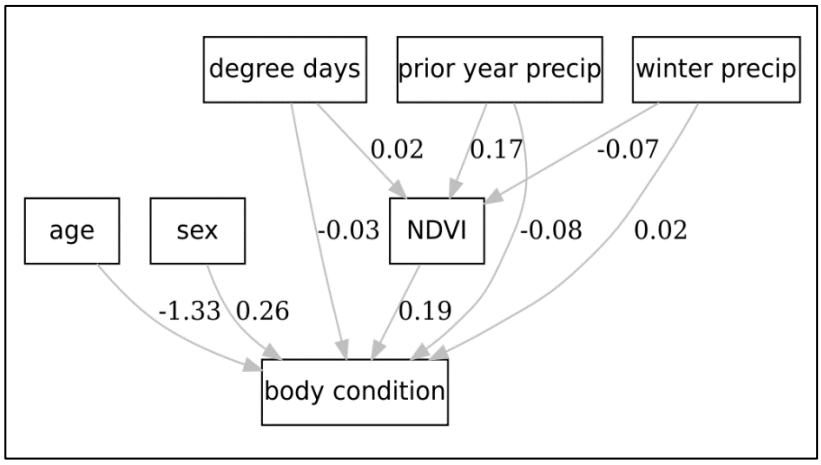

Figure 11. Utah prairie dog body condition in relation to climate factors (controlling for age and sex). Numbers indicate coefficients from path analyses; the strength of the association increases with an increasing absolute value of the coefficient. Age is juvenile versus adult and sex is male versus female. All relations are statistically significant at $p<0.05$.

Plague dynamics and climate

The probability of direct transmission from an infected to a susceptible prairie dog, given a contact, was the most influential variable on the probability of an outbreak, the mean number of prairie dog remaining at the end of 150 days, and the time to extinction (<20 prairie dogs) (Figures 12-14). No significant trends were evident in the proportion of simulations resulting in outbreaks, the mean number of prairie dogs surviving to 150 days, and the time to extinction for changes in the replacement rates of fleas (i.e., increasing development rates did not result in an increased number of outbreaks). Approximately 83-85% of simulations with a particular reproductive rate resulted in an outbreak. Across all simulations with a replacement rate of 0.1, the percentage of simulations resulting in plague outbreaks was roughly the same as it was for all simulations with a replacement rate of 0.2. Significant trends were not evident in the mean number of prairie dogs surviving until the end of the simulations (150 days) with changing flea reproductive rates. On average, 16-19 prairie dogs survived until the end of the simulations regardless of replacement rate, and the mean time to extinction was 43 days.

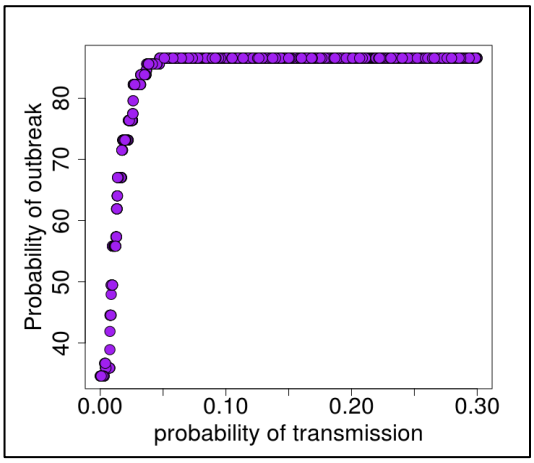

Figure 12. The relation between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog (given a contact) on the percentage of simulations (out of 100) that resulted in a plague outbreak (where a plague outbreak = less than 20 prairie dogs remaining at the end of a season).

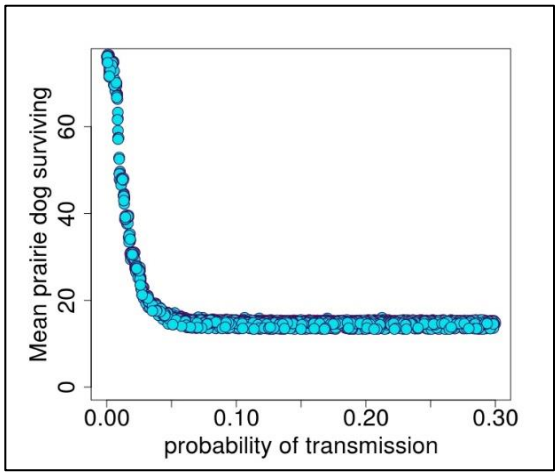

Figure 13. The relation between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog (given a contact), and the mean number of animals surviving until the end of the simulation.

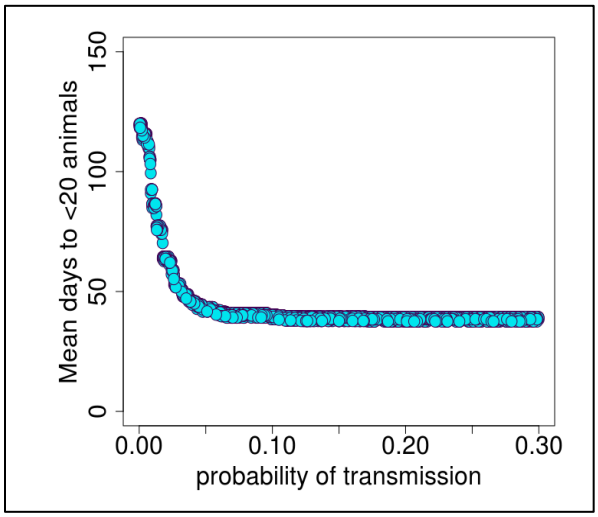

Figure 14. The relation between the probability of direct transmission from an infected prairie dog to a neighboring prairie dog (given a contact) and the time to extinction (i.e the number of days until the number of animals was <20).

Conclusions and Implications for Future Research/Implementation

We detected *Y. pestis* (plague), *Bartonella* spp., and *Rickettsia* spp. in fleas from live prairie dogs. *Yersinia pestis* was rarely detected (1.2% of 5024 flea pools tested), even in locations where plague morbidity or mortality was confirmed in prairie dogs (5.8%). Fleas were found to carry a higher prevalence of *Bartonella* spp. (12.7% of 813 flea pools), although only fleas from plague-positive locations were tested.

O. hirsuta was the most abundant species of flea on the prairie dog colonies that we sampled, followed by *Pulex simulans*. There were different patterns of relative abundance among flea species: *O. tuberculata* relative abundance was higher in the early summer than the late summer, *P. simulans* was more abundant in the late summer than the early summer, and *O. hirsuta* relative abundance declined from the early to late summer in the southern plains and increased in the northern plains. Different flea species have different capacities as vectors, and therefore changes in flea abundance could affect plague dynamics. Because *O. tuberculata* is a more effective vector than *O. hirsuta*, epizootics may be more likely in early spring, when it is the most abundant. However, the larger overall abundance of *O. hirsuta* may make the difference in vector capacity irrelevant, as the larger number of flea bites could make up for the lower transmission rate. It is currently unclear what specific factors are driving these seasonal and geographic changes in flea abundance, and future work should focus on determining how environmental conditions contribute to these patterns in flea species abundance, especially regarding future climate changes.

Key components of the flea life cycle occur within prairie dog burrows, but prior to our study, environmental conditions within burrows were rarely measured. We found that daily burrow temperature and humidity were highly stable and buffered against environmental extremes. However, burrow temperatures increased seasonally in relation to monthly mean ambient temperatures. Although we originally intended to conduct laboratory trials to measure flea survival and fecundity in relation to temperature and relative humidity, these experiments failed due to difficulties in maintaining a flea colony. Instead, we found and analyzed existing data to meet this objective. Our findings indicate temperature has the greatest effect on flea development. Under a scenario of warming temperatures, the number of days required to complete flea development will decrease, leading to significant increases in flea populations.

Our models indicated that once threshold levels of fleas are reached that are sufficient to drive a plague outbreak, further increases in the development rate do not affect the outcomes of plague emergence. Further exploration of the models is necessary to determine if eliminating or reducing the amount of direct transmission changes the relative influence of flea development rate on model outcomes. Additionally, we have not yet evaluated the effects of climate on the host. Increasing susceptibility due to poor body condition will likely result in shorter time to extinction and fewer prairie dogs surviving. Finally, it may be that local climate conditions will have little effect on plague dynamics due to stable burrow conditions and flexibility in the flea life cycle. Regional climate patterns may instead influence the geographic distribution of plague allowing it to spread eastward and

northward as climates change and create environments able to sustain threshold flea populations.

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Appendices

Appendix 1. Parameters from models of flea dynamics (probabilities are on a daily time step).

Parameter	Description	Probability Density Function Distribution Characteristics	Source
N	Size of prairie dog population	150-250 (increments of 50)	Sylvatic plague vaccine trials
σ	Sigma (scaling of probability of interactions with distance)	10-25 (increments of 5)	Spatial captur recpture data estimates from black-tailed prairie dog sites
μ	Background host mortality	0.0019	Hoogland 1995
λ_{host}	Mean number of fleas per animal	Mean 2 or 4 (number per animal is drawn from the negative binomial distribution with a mean of 2 or 4)	Russell et al. 2018
$\lambda_{activity\ center}$	Mean number of fleas per activity center	10-30 (number per activity center is drawn from the negative binomial distribution with a mean of 10 or 30 in increments of 10)	Assumption is that the number of fleas in an activity center is greater than the number on the hosts by a magnitude of 1-3.
p.leave	Probability that a flea jumps off a host	0.2 to 0.7 (increments of 0.1)	Assumed to be fairly high.
p.find	probability that a flea finds a resident prairie dog	0.1-0.3 (increments of 0.1)as the number of non- resident prairie dogs increases, the probability of finding a host increase	Assumed to be lower than jumping off.
μ_{HF}	Flea on-host mortality rate	0.02 to 0.1 (increments of 0.02)	Metzger 2000; Metzger and Rust 2002
μ_{EF}	Flea off-host mortality rate	0.1 to 0.2 (increments of 0.05)	Poje 2019; Appendix 4
r	Replacement Rate of fleas	0.05 to 0.4 (increments of 0.01)	Poje 2019; Appendix 4

Appendix 2. Parameters used in plague models (probabilities are on a daily time step) (LHS indicates that the exact parameter values were selected by Latin Hypercube sampling [LHS; Iman et al. 1981])

Parameters	Description	Probability Density Function Distribution Characteristics	Source
β_D	Maximum probability of transmission from an infected host given an interaction	mean=0.0-0.3 (LHS) var=0.001 Stochastic node: for each simulation and each individual prairie dog, the parameter is drawn from a random beta distribution. This parameter represents the maximum probability of transmission which is dependent on time since infection (the maximum value is reached just prior to death of the animal).	Agar et al. 2009
β_c	Probability of transmission from a plague carcass given an interaction between a live prairie dog and carcass.	mean=0.0-0.3 (LHS) var=0.001 Stochastic node: for each simulation and each individual prairie dog, the parameter is drawn from a random beta distribution.	Assumption that it is low. There is a general lack of data.
ξ	Probability of dying below ground	0.8 (single point)	Hoogland 1995
ν_{AG}	Probability of removal of a plague-infected carcass above ground	0.5 (single point)	Boone et al. 2009
ν_{BG}	Probability of removal of a plague-infected carcass below ground	0.06 (single point)	Godbey et al, 2006
β_{HF}	Maximum probability of logistically increasing infection from a host to a flea=probability a flea will bite a host.	Time dependent variable, as days post-infection increases probability of infection from the host increases.	Wilder et al. 2008a and b: daily probability of a bite 0.82 for *Oropslla tuberculata* and 0.84 for *Oropsylla hirsuta*
γ	Probability that non-blocked infected fleas clear infection.	0.0 -0.05 (LHS)	Hinnebusch et al. 2017; Engelthaler 2000; Burroughs 1956

β_{EF}	Probability of infection from an early stage infected flea (given the flea bites the host)	0 to 0.2 (LHS)	Wilder et al. 2008a
δ_{E}	Probability a flea will progress from early stage of infection to latent (non-infectious stage).	0.75 to 0.95 (LHS)	Wilder et al. 2008a
δ_{L}	Probability a flea will progress from latent to late stage.	0.0 to 0.01(LHS)	Hinnebusch et al. 2017
B	Probability that a late stage infected flea will block.	0.01-0.25 (LHS)	Eisen et al. 2009; Burroughs 1947; Kartman 1956; Hinnebusch et al. 2017
β_{IF}	Probability of infection from an unblocked late stage infected flea (given the flea bites the host).	0.00 to 0.2	Engelthaler et al. 2000
β_{BF}	Overall probability of infection from an infected blocked flea (includes probability of a bite).	0.3 –1 (LHS)	Lorange et al. 2005; Kartman, 1956
μ_{BF}	Blocked flea mortality.	0.1-0.25	Hinnebusch et al. 2017

Appendix 3. List of scientific publications associated with this study to date.

Bron GM. 2017. The role of short-lived rodents and their fleas in plague ecology on prairie dog colonies. Dissertation, University of Wisconsin-Madison, Madison, WI.

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Appendix 4. Poje (2019) Dissertation.

THE IMPACTS OF ENVIRONMENTAL CONDITIONS ON PRAIRIE DOG FLEAS: IMPLICATIONS FOR SYLVATIC PLAGUE

by

Julia E. Poje

A thesis submitted in partial fulfillment of

Master of Science

at the

UNIVERSITY OF WISCONSIN-MADISON 2019

APPROVED

_____ _____

Michael Samuel Date

Professor Emeritus, Department of Forest and Wildlife Ecology

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Jonathan Pauli Date

Associate Professor, Department of Forest and Wildlife Ecology

Thesis Abstract

Sylvatic plague, caused by the bacterium *Yersinia pestis*, is endemic in ground squirrels (family Sciuridae) and prairie dogs (*Cynomys spp.*) of the western United States. Plague epizootics are devastating to prairie dog communities, often leading to 100% mortality, and are considered a major factor in the decline of prairie dog populations that have occurred since the start of the 20th century. Infection can spillover to humans, and there are multiple plague cases in the United States every year. Prairie dogs are keystone species in the US Great Plains, and their loss negatively impacts many other grassland species, including the highly endangered black-footed ferret. Prairie dog fleas (*Oropsylla* species) drive plague transmission during epizootics; however, little is known about the life history of these species. Survival and development of other flea species is impacted by environmental factors such as temperature and relative humidity, and these factors should also be important for prairie dog fleas. Because prairie dog fleas spend most of their time in burrows, burrow microclimates are probably the most important factor in flea development. The goal of this thesis was to determine if environmental conditions impact flea development

To determine if abiotic conditions influence prairie dog fleas, we measured flea abundance by swabbing 300 black -tailed prairie dog (*Cynomys ludovicianus*) burrows at 6 sites across a wide latitudinal gradient while simultaneously measuring burrow and ambient temperature and relative humidity. Sites were sampled in both early (May-June) and late (August) summer in 2016 and 2017. Chapter 1 desribes differences in relative abundance of the 3 most common flea species found on prairie dog colonies, *O. hirsuta, O. tuberculata,* and *Pulex simulans*. We found the relative abundance of *O. hirsuta* was

highest, *O. tuberculata* relative abundance was lowest, and *P. simulans* relative abundance was intermediate where it occurred. There were differences in relative abundance of each species between the sites, and the relative abundance of two of the species, *O. tuberculata* and *P. simulans*, varied from the early to the late sampling session.

The geographic and seasonal patterns in relative abundance found in Chapter 1 suggest that abiotic factors influence flea abundance. In Chapter 2, we analyze the temperature and relative humidity in prairie dog burrows to determine if burrow microclimates can explain variation in flea populations. Burrow temperatures were more stable than outside temperatures, increased with mean monthly high temperature and between the early and late sampling sessions. The relative humidity in burrows was uniformly high and did not differ between sites or month of sampling. Flea abundance increased with temperature, declined with winter precipitation, and increased from the early to the late session.

In Chapter 3, we use laboratory studies of flea development (egg to adult) rates to help understand the correlation between flea abundance and temperature seen in Chapter 2. *O. hirsuta* and *O. tuberculata* have never been successfully reared in captivity, so we used data collected by Metzger (2000) to determine the effect of temperature and relative humidity on the development rates of *O. montana*, a closely related ground squirrel flea. *O. montana* were reared in a laboratory at different combinations of temperature and relative humidity, and the time each individual took to complete development was measured. We created and used models of development rates as a function of temperature, in conjunction with burrow temperature models developed in Chapter 2, to

estimate flea development rates in prairie dog burrows now and in response to future climate warming. The projected increases in temperature lead to faster flea development. Faster development could lead to more generations of fleas emerging during the summer period of favorable temperatures, likely leading to an increase in abundance.

Understanding environmental drivers of flea populations is very important for understanding the dynamics of sylvatic plague on prairie dog colonies. The work in this thesis indicates that temperature plays an important role in flea abundance and development rates. Currently, the tools available to land managers to limit the spread of plague include an oral vaccine and spraying burrows with insecticides to kill fleas. Future research is necessary to determine how *O. hirsuta* and *O. tuberculata* respond to changes in temperatures, as well as how the measured changed in a laboratory setting translate to burrows.

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Table of Contents

Thesis Abstract..... i

Acknowledgements iv

Relative abundance of flea species found in black tailed prairie dog burrows..... 1

Prairie dog burrow microclimates and the effect of climate variables on flea abundance and prevalence 21

The impacts of environmental conditions on flea development rates.....48

Chapter 1 – Relative abundance of flea species found in black tailed prairie dog burrows

Introduction

Fleas, obligate hematophages, are common ectoparasites of vertebrates worldwide (Krasnov 2005). Many species of fleas form close relationships with a specific host and are rarely encountered on different species (Krasnov 2005) and if forced to feed on a non-preferred host exhibit reduced fitness (Prasad 1969, Krasnov et al. 2007). Flea infestations can be harmful, even for their co-evolved hosts. For an individual host, flea bites trigger immune responses, causing dermatitis and hair loss from excessive scratching (Scheidt 1988). Extreme infestations can cause anemia in the host (Yeruham et al. 1989, Araújo et al. 1998). Most importantly, fleas are vectors of many diseases, such as murine typhus (Rickettsia typhi) (Azad 2002), the myxomatosis virus (Rothschild 1965), cat scratch disease (*Bartonella henselae*) (Chomel et al. 1996), spotted fever (Rickettsia felis) (Yazid Abdad et al. 2011), and plague (*Yersinia pestis*) (Eskey 1938).

Plague, caused by the bacterium *Y. pestis*, is endemic in ground squirrels and prairie dogs of the western United States (Gage and Kosoy 2005). Epizootics of plague can cause up to 90% mortality in prairie dogs (*Cynomys* spp.) (Hoogland 1995, Cully and Williams 2001), and these outbreaks can spill over into human populations. Fleas are the primary vector of plague worldwide. After taking a bloodmeal from an infected host, fleas can spread the infection by two different mechanisms. The first, “early-phase transmission,” occurs when the flea passes *Y. pestis* to a novel host 1-4 days after being

infected, without the formation of a biofilm (Eisen et al. 2006). In the second, called "blocking", occurs when the ingested *Y. pestis* form a biofilm in the flea's proventriculus. When the flea attempts to feed, the biofilm blocks blood from passing out of the esophagus, causing the flea to regurgitate the now bacteria laden blood back into the host (Bacot and Martin 1914). Given that flea bites are responsible for 70% of the transmission events that occur during plague epizootics (Richgels et al. 2016), understanding flea populations is an important step towards understanding plague epizootiology and how to control plague outbreaks.

Prairie dogs are most commonly infested with fleas of the genus *Oropsylla* (Cully et al. 2000, Stevenson et al. 2003, Holmes et al. 2006, Salkeld and Stapp 2008, Mize and Britten 2016), which are usually host species specific and are rarely found on other host species (Lewis 2002). There are two primary species of *Oropsylla* found on prairie dogs: *O. hirsuta* and *O. tuberculata*. The two species have different seasonal peaks in abundance; *O. hirsuta* was most abundant in black-tailed prairie dog (*Cynomys ludovicianus*) burrows in the late summer and early fall, while *O. tuberculata* was most common in the early spring (Salkeld and Stapp 2008, Mize and Britten 2016). *Pulex simulans*, a generalist flea, was also commonly found in prairie dog burrows, though its abundance does not appear to vary seasonally (Salkeld and Stapp 2008). *Oropsylla* and *Pulex* species are competent vectors of plague, but the level of competency can vary among vectors (Eskey and Haas 1939, Burroughs 1947). *Oropsylla tuberculata* is the more effective of the two *Oropsylla* species, with exposed fleas transmitting infection 17% of the time (Wilder, Eisen, Bearden, Montenieri, Tripp, et al. 2008), followed by *O.*

hirsuta, which transmit 5.2% of the time (Wilder, Eisen, Bearden, Montenieri, Gage, et al. 2008). Plague positive *P. simulans* have been found in prairie dog burrows (Cully et al. 2000) and on prairie dogs and other wildlife (Fagerlund et al. 2001, Salkeld et al. 2007), but there are no laboratory studies measuring its transmission rate.

Black tailed prairie dogs range across the Great Plains of North America from southern Canada to northern Mexico. Fleas are highly sensitive to environmental conditions, such as temperature and relative humidity, and adverse conditions can lead to decreased survival of juvenile stages and slower development rates (Krasnov et al. 2001a, 2001b, Kreppel et al. 2016). Flea communities have been documented on black tailed prairie dog colonies in Kansas (Cully et al. 2000), Colorado (Seery et al. 2003, Stevenson et al. 2003, Salkeld and Stapp 2008), Nebraska, South Dakota, North Dakota, Wyoming (Mize and Britten 2016), and Montana (Holmes et al. 2006). Given the vast difference between the climate of the northern and southern plains, the abundance and prevalence of flea species could also vary with latitude as different flea species are best adapted to different environmental conditions.

We aimed to measure patterns of flea abundance in prairie dog burrows by burrow swabbing. We sampled sites across the range of the black tailed prairie dog at two time points to assess how patterns may change both geographically and temporally. We predicted that *O. tuberculata* would be more common earlier in the year, while *O. hirsuta* would dominate later in the summer, as suggested by previous studies (Salkeld and Stapp 2008). Because the range of black tailed prairie dogs covers such a wide range of latitudes, we tested the hypotheses that 1) there will be differences between the sites in

the timing of these patterns, and 2) there will be latitudinal differences in the relative abundance of the different species.

Methods

We studied seven sites that spanned the entire black tailed prairie dog range (BTPD) within the United States: Charles M. Russell National Wildlife Refuge (MT) in Phillips County MT, Theodore Roosevelt National Park (ND) in Billings County ND, Buffalo Gap National Grassland (BG) in Pennington County SD, Lower Brule Indian Reservation (LB) in Lyman County SD, Pueblo Chemical Depot (CO) in Pueblo County CO, Las Cienegas National Conservation Area (AZ) in Santa Cruz and Pima Counties AZ during 2016 only, and public lands surrounding Roswell (NM) in Chaves County NM during 2017 only (Figure 1). We selected three prairie dog colonies at each site. Each colony was sampled during two sessions, once during May or June (early session), and once in August (late session) in 2016 and 2017 (except where noted). After sampling in 2016, we learned that the AZ prairie dog colonies had been treated with the insecticide deltamethrin during the translocations that established them. As a result, we replaced this site with NM in 2017 and excluded AZ from our analysis. ND was added to the study during the summer of 2016, and so was only sampled during the late session that year.

We swabbed one hundred burrows per colony to collect fleas. Burrows were swabbed by attaching a 15 cm by 15 cm piece of white flannel to a 4.5 m plumbing snake and pushing the snake as far as possible down the burrow. We left swabs in the burrow for 30 seconds before slowly pulling them out and placing them in a zip-lock bag. Each bag was labeled with the GPS coordinates of the burrow, burrow status (active or

inactive), burrow orientation, and the depth of the swab in the burrow. Swabs were placed in a cooler, and dead fleas were then placed in tubes filled with 1 mL of 70% ethanol until later laboratory identification. We identified fleas to species using a dissecting microscope according to keys described by Hubbard (1947) and Furman and Catts (1982), with revisions by Stark (1970) and Lewis (2002). If a flea could not be identified to species, it was labeled as “Unknown” (8 fleas).

We modeled the relative abundance of a given flea species (# of that species/ total fleas collected) in a generalized linear model with a binomial distribution using the glm function from package stats in R (R Core Team 2018). Relative abundance was modeled as a response to site, sampling session, and year. We used backwards selection based on AIC to determine the most parsimonious model. We used a Cochran-Mantel-Haenszel test from the R package “stats” to determine if there was a consistent relationship between flea species prevalence and session across sites. To compare the relative abundance of each flea species between sites, we calculated a 95% confidence interval using the Clopper – Pearson exact interval.

Results

We swabbed 6466 burrows during the summers of 2016 and 2017. From these, we collected 2024 fleas from 629 burrows. *Oropsylla hirsuta* accounted for 91% (95% CI 89% - 92%) of fleas collected, followed by *P. simulans* (7%, 95% CI 6% - 8%) and *O. tuberculata* (1%, 95% CI 0.6% - 1.5%) (Table 1). Patterns of flea abundance (total number of fleas collected) varied by region and sampling session. In both 2016 and 2017, flea abundance decreased from the early to the late sampling session at southern

sites, while flea abundance increased from early to late sampling at northern sites (Figure 2).

Patterns of relative abundance varied among the different flea species. The relative abundance of *O. hirsuta* was higher than other species at every site, though there were differences in relative abundance between sites (Table 2). There was no significant change between sessions (Odds Ratio = 0.70, 95% CI 0.46 – 1.08) or years (Odds Ratio = 1.02, 95% CI 0.72 – 1.45). Oropsyllla tuberculata was found at every site except NM and was less common than other flea species at all of them (Table 3). Its relative abundance declined from the early to the late season across the sites (Cochran-Mantel-Haenszel common odds ratio = 0.102, 95% CI 0.03 – 0.31), and there was no significant effect of year (Odds ratio = 0.47, 95% CI 0.13 – 1.42) on *O. tuberculata* abundance. *Pulex simulans* was only found at MT, CO, and NM, and was less common than *O. hirsuta* but more common than *O. tuberculata* (Table 4). Its relative abundance increased from the early to the late session (Cochran-Mantel-Haenszel common odds ratio = 2.21, 95% CI 1.36 – 3.58).

The remaining flea species (*Thrassis fotus*, *Polygenis gwyni*, and *Epitedia wenmanni*) made up <1% of the collected fleas (Table 1) and were considered incidental and not analyzed separately.

Discussion

Oropsylla hirsuta was the most common species of flea found during our study, comprising 91% of fleas collected. It was the dominant species at all sites during all sampling sessions, which is consistent with previous studies of flea communities in

black-tailed prairie dog burrows (Cully et al. 2000, Seery et al. 2003, Stevenson et al. 2003, Holmes et al. 2006, Salkeld and Stapp 2008, Mize and Britten 2016). Though the relative abundance of *O. hirsuta* does not change between sessions, the total abundance of fleas increased from the early to the late session at MT, ND, LB and BG, and declined at CO and NM. It is currently unclear what drives the difference between the northern and southern sites. Fleas, like many species of insect, are sensitive to changes in the temperature and relative humidity of their surroundings (Krasnov 2005). Our sites cover a wide range of latitudes, and southern Colorado and New Mexico are considerably hotter and drier than the northern plains states of Montana and the Dakotas. It is possible, then, that patterns in *O. hirsuta* abundance may be driven by environmental factors which depend on geographic location. Future work should focus on how these conditions vary throughout the range of *O. hirsuta*, and whether these differences affect flea population dynamics.

We observed few *Oropsylla tuberculata* cynomuris but found their relative abundance was higher in the early summer than late summer. This is consistent with previous work in Colorado that found *O. tuberculata* was most abundant in March and April, and then declined in abundance through the summer (Salkeld and Stapp 2008). Like *O. hirsuta*, *O. tuberculata* could be sensitive to environmental conditions. If it prefers the cooler temperatures of early spring, it is likely that even our early sampling session (May or June) was too late to catch their seasonal peak in abundance. *Pulex simulans* was the second most abundant flea species overall, even though they were only found at 3 (MT, CO, and NM) of the 6 sites we sampled. Their relative abundance

increased through the summer at all 3 sites. *Pulex simulans* is a generalist flea with a wide distribution throughout North America (Wilson and Bishop 1966), so may be better adapted to a wide range of temperatures than the *Oropsylla* species.

Differences in the relative abundance of flea species in burrows could be an important factor for plague dynamics on prairie dog colonies. Flea species have different levels of competence as plague vectors (Eskey 1938; Burroughs 1947; Wilder, Eisen, Bearden, Montenieri, Tripp, et al. 2008) , and so the timing and severity of an epizootic could be influenced by the species of flea that is most prevalent. *Oropsylla tuberculata* is roughly 3 times more effective at transmitting plague than *O. hirsuta*, so differences in their relative abundance could impact the rate at which plague can spread through a prairie dog colony. Though its exact transmission efficiency remains unknown, the relative abundance of *P. simulans* could similarly impact plague dynamics. *Pulex simulans* abundance could have an additional impact, because they are host generalists while *O. hirsuta* and *O. tuberculata* are prairie dog specialists. Therefore, while outbreaks where *P. simulans* is absent may remain contained within prairie dog populations, outbreaks where *P. simulans* is present could spill over and involve other species as well.

Conclusion

O. hirsuta was the most abundant species of flea on the prairie dog colonies that we sampled, followed by *Pulex simulans*. There were different patterns of relative abundance among flea species: the relative abundance of *O. tuberculata* was higher in the early summer than the late summer, *P. simulans* was more abundant in the late summer

than the early summer, and *O. hirsuta* relative abundance remained high at both sampling sessions. Different flea species have different levels of competence as vectors, and therefore changes in flea abundance could impact plague dynamics. Because *O. tuberculata* is a more effective vector than *O. hirsuta*, epizootics may be more likely in early spring, when it is the most abundant. However, the larger overall abundance of *O. hirsuta* may make the difference in vector capacity irrelevant, as the larger number of flea bites could make up for the lower transmission rate. It is currently unclear what specific factors are driving these seasonal and geographic changes in flea abundance, and future work should focus on determining how environmental conditions contribute to these patterns in flea species abundance, especially regarding future climate changes.

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Site	Year	Session	Oropsylla hirsuta	Oropsylla tuberculata	Pulex simulans	Thrassis fotus	Epitedia wenmanni	Polygenis gwyni	Unknown	Session Totals	Yearly Totals
MT	2016	Early	33	13	5	0	0	0	0	51	
		Late	84	0	4	0	1	0	0	89	140
	2017	Early	9	1	0	0	0	0	0	10	
		Late	183	0	70	0	0	0	0	253	263
ND	2016	Late	18	0	0	0	0	0	0	18	18
	2017	Early	9	3	0	1	0	0	0	13	
		Late	19	0	0	0	0	0	0	19	32
BG	2016	Early	10	0	0	0	0	0	0	10	
		Late	1	0	0	0	0	0	0	1	11
	2017	Early	30	2	0	0	0	0	0	32	
		Late	80	0	0	1	0	0	1	82	114
LB	2016	Early	13	0	0	0	0	0	0	13	
		Late	236	0	0	0	0	0	0	236	249
	2017	Early	38	1	0	0	0	0	0	39	
		Late	146	0	0	0	0	0	0	146	185
CO	2016	Early	384	0	34	1	0	0	1	420	
		Late	28	2	6	0	0	0	5	41	461
	2017	Early	279	0	11	0	0	0	0	290	
		Late	87	0	6	5	0	0	1	99	389
NM	2017	Early	130	0	7	2	0	2	0	141	

Table 1. Abundance of flea species collected at study sites during early and late sampling sessions in 2016 and 2017 at the Charles M. Russell National Wildlife Refuge, Montana (MT), Theodore Roosevelt National Park, North Dakota (ND), Buffalo Gap National Grassland, South Dakota (BG), Lower Brule Sioux Reservation, South Dakota (LB), Pueblo Chemical Depot, Colorado (CO), and BLM land near Roswell, New Mexico, (NM).

15

Site	Proportion of *O. hirsuta*	95% Confidence Interval	Group
MT	0.767	0.722 - 0.807	A
ND	0.920	0.808 - 0.978	B
BG	0.968	0.920 - 0.991	B
LB	0.998	0.987 - 0.9999	C
CO	0.915	0.896 - 0.933	B
NM	0.895	0.837 - 0.938	B

Table 2. Relative abundance of *O. hirsuta* was higher than other species at every site sampled. The proportion of *O. hirsuta* and 95% confidence interval around the estimate at each site are shown here. Sites are grouped based on overlapping 95% confidence intervals, indicating that the relative abundance is similar between sites. *Oropsylla hirsuta* relative abundance is lower at MT (group A) compared to the other sites, similar at ND, BG, CO and NM (group B), and highest at LB (group C).

Site	Proportion of *O. tuberculata*	95% Confidence Interval	Group
MT	0.035	0.019-0.058	A
ND	0.060	0.012 - 0.165	A
BG	0.016	0.002 - 0.057	A, B
LB	0.002	0.00006 - 0.013	B
CO	0.002	0.0003 - 0.008	B
NM	0.000	0 - 0.023	B

Table 3. Relative abundance of *O. tuberculata* was lower than other species at every site sampled. The proportion of *O. tuberculata* and 95% confidence interval around the estimate at each site are shown here. Sites are grouped based on overlapping 95% confidence intervals, indicating that the relative abundance is similar between sites. *Oropsylla tuberculata* abundance is highest at MT and ND (group A) and lower at LB, CO, and NM (group B). The 95% confidence interval for BG is wide and overlaps with both groups.

Site	Proportion of *P. simulans*	95% Conifdence Interval	Group
MT	0.20	0.158 - 0.238	A
ND	0.00	0 - 0.071	B, C
BG	0.00	0 - 0.029	B
LB	0.00	0 - 0.008	B
CO	0.07	0.051 - 0.086	C
NM	0.07	0.039 - 0.126	C

Table 4. *Pulex simulans* was only detected at MT, CO and NM during our sampling periods. At these sites, relative abundance of *P. simulans* was lower than *O. hirsuta* and higher than *O. tuberculata*. The proportion of *P. simulans* and 95% confidence interval around the estimate at each site are shown here. Sites are grouped based on overlapping 95% confidence intervals, indicating that the relative abundance is similar between sites. Estimated *P. simulans* abundance was highest at MT (group A) and lower at CO and NM (group C), and lowest at BG and LB (group B). The 95% confidence interval for ND overlapped with both group B and C.

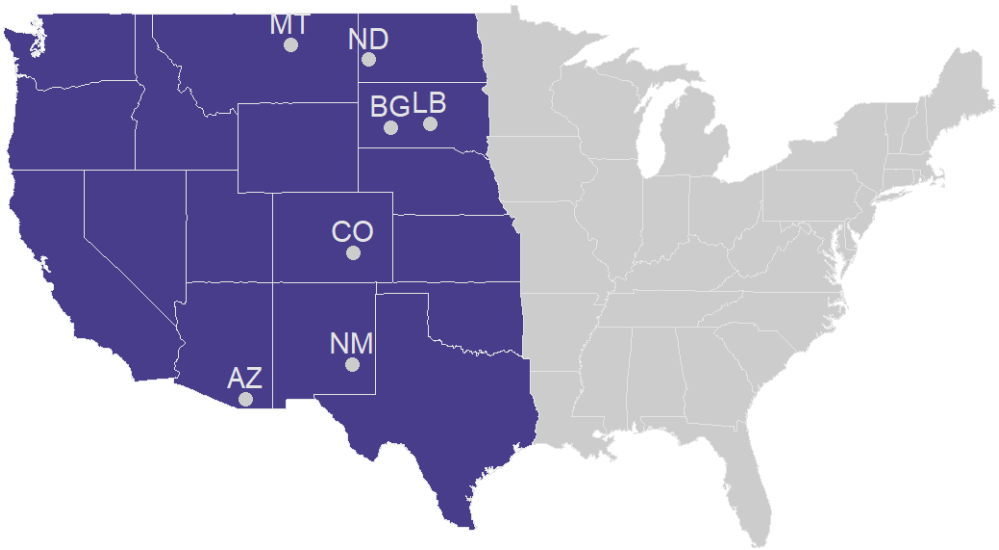

Figure 1. Plague is endemic in the western United States (purple states). Seven field sites (labeled in gray) were chosen in 6 US states (Montana, North Dakota, South Dakota, Colorado, New Mexico, and Arizona). Sites in Montana, North Dakota, and Colorado were sampled during 2016 and 2017. Sites in Arizona were sampled only in 2017, and sites in New Mexico were sampled during 2016 only.

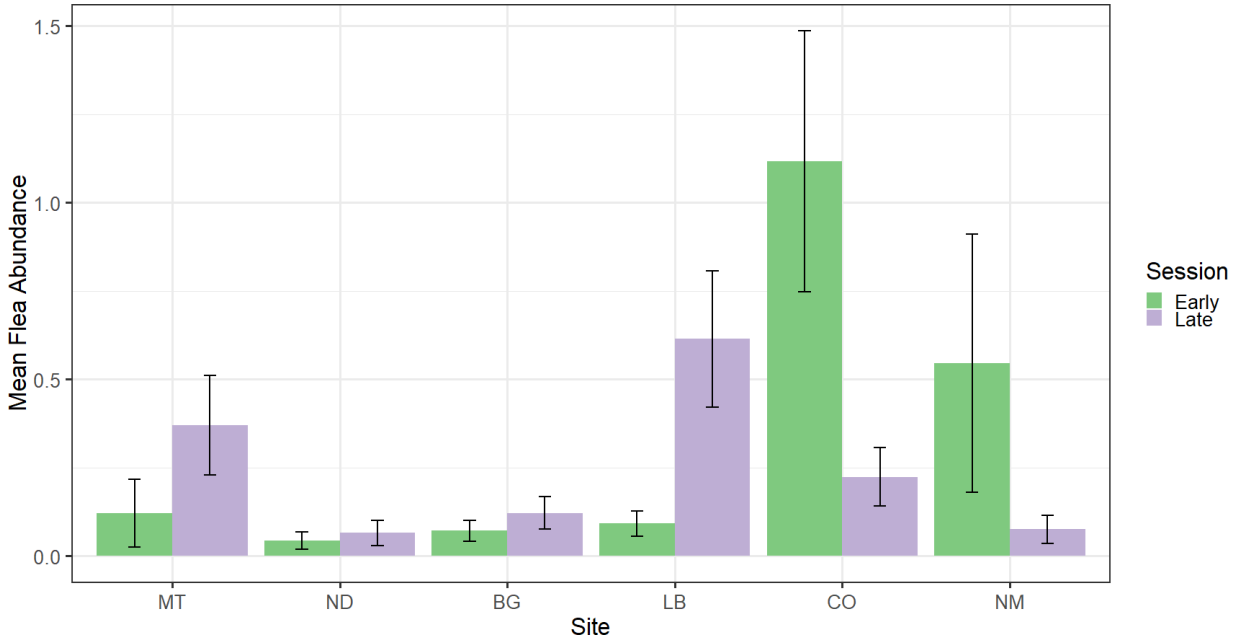

Figure 2. Mean flea abundance (total number of fleas collected / number of burrows sampled) at six sites in the US states of Montana (MT), North Dakota (ND), South Dakota (BG and LB), Colorado (CO) and New Mexico (NM) during an early sampling session (late May – June) and a late sampling session (August) in the summers of 2016 and 2017. Mean flea abundance increases from the early sampling session to the late sampling session at the northern sites (MT – LB) and declines at the southern sites (CO and NM).

Chapter 2 – Prairie Dog Burrow Microclimates and the Effect of Climate Variables on Flea Abundance and Prevalence

Introduction

Plague, a zoonotic disease of rodents caused by the bacteria *Yersinia pestis*, was introduced into the United States in the early 20th century and has since become endemic in the United States Great Plains (Cully et al. 2000, Gage and Kosoy 2005, Mize and Britten 2016). Outbreaks of plague are particularly lethal to prairie dogs (*Cynomys* spp), causing >90% mortality on a colony, and often leading to local extirpation (Pauli et al. 2006). Prairie dogs are keystone species in the Great Plains, directly and indirectly shaping their environment (Kotliar et al. 2006). Their burrowing, grazing, and clipping of tall grasses alter the plant communities on their colonies, creating areas that are preferentially grazed by large ungulates (Krueger 1986). Their burrows are an important source of shelter for many species such as burrowing owls (*Athene cunicularia*), prairie rattlesnakes (*Crotalus viridis*), and critically endangered black footed ferrets (*Mustela nigripes*). Prairie dogs are also an important prey species for many carnivores (Miller et al. 1994). In particular, the critically endangered black footed ferret is a prairie dog specialist which depends on prairie dogs which constitute ~90% of their prey (Campbell III et al. 1987). Ferrets cannot survive without large prairie dog colonies, but ferrets are also highly susceptible to plague (Williams et al. 1994), so controlling plague outbreaks is an important goal in black footed ferret recovery efforts (USFWS 2013).

Plague is transmitted among rodents by parasitic fleas from the order

Siphonaptera. Fleas can transmit *Y. pestis* to naive hosts after taking a blood meal from an infected host via one of two mechanisms: “early phase transmission” and “blocking”. Blocking occurs when *Y. pestis* forms a biofilm at the base of the esophagus that causes the flea to regurgitate blood and bacteria into a new host (Bacot and Martin 1914). In early phase transmission fleas are able to transmit bacteria to a novel host without the formation of a blockage (Eisen et al. 2006). Though there is potential for transmission from alternate pathways, including direct contact with infected animals, modeling studies indicate that flea bites are likely responsible for up to 70% of transmission events during plague epizootics (Richgels et al. 2016). Treating prairie dog burrows with the deltamethrin insecticide is effective at reducing flea abundance and plague mortality of prairie dogs compared to untreated colonies (Seery et al. 2003, Tripp et al. 2016), supporting the idea that fleas are critical for spreading plague during epizootics.

The most common fleas found on prairie dogs are *Oropsylla hirsuta* and *O. tuberculata* (Salkeld and Stapp 2008, Chapter 1). Field observations suggest that *O. hirsuta* and *O. tuberculata* have highly seasonal patterns of abundance (Salkeld and Stapp 2008, Hubbart et al. 2011), indicating that environmental factors may play an important role in flea development and abundance. In addition, laboratory studies have demonstrated that fleas are sensitive to environmental conditions (Krasnov et al. 2001a, 2001b, Kreppel et al. 2016), and that burrow dwelling fleas are especially sensitive to fluctuations in temperature and relative humidity (Cooke and Skewes 1988, Metzger and Rust 1999). Rearing *Oropsylla montana*, a flea of California ground squirrels (*Otospermophilus beecheyi*), under different temperatures and relative humidity affects

the number of eggs that hatch, larval survival, and the rate at which fleas complete development (Metzger and Rust 1999). Low temperatures can buffer against the negative impacts of low relative humidity, perhaps by reducing evapotranspiration and thus reducing desiccation. Because climate has a potentially significant effect on *O. montana* development, it is likely that it can also influence the development of the closely related *O. hirsuta* and *O. tuberculata*.

Modeling studies have suggested that climate change will impact the distribution and dynamics of plague outbreaks in the United States. Nakazawa et al. (2007) predicted that plague outbreaks in wildlife will generally shift northward, perhaps in response to increasingly unfavorably high temperatures in the Southern Great Plains. Similarly, Snäll et al. (2009) predicted that the future incidence of plague in prairie dog populations should drop, with the largest decrease corresponding to the greatest increase in temperature. Because flea borne transmission likely drives plague epizootics, the predicted decline in plague outbreaks as temperatures increase could be caused by conditions becoming inhospitable for fleas.

Unfavorable weather conditions do not always lead to declines in flea abundance, however. The number of fleas on black-tailed prairie dogs (*Cynomys* ludovicanus) in South Dakota was higher in summers that were preceded by abnormally dry years and low levels of winter and spring precipitation (Eads and Hoogland 2017). Eads et al. (2016) found that flea loads on black-tailed prairie dogs in New Mexico were most abundant in the driest year of their study, and plague outbreaks in eastern Colorado occurred with similar frequency during dry (48%) and wet (52%) years over a course of

23 years, although outbreaks were 3 times more likely in wet years preceded by a very dry year (Eads and Biggins 2017).

Prairie dog fleas spend considerable time in their host's burrow, so burrow microclimates are an important consideration when assessing the impact of weather patterns on flea demographics. Temperatures in burrows of several other mammal species are more stable than ambient temperatures, with daily fluctuations within burrows often less than 1.5°C (Kay and Whitford 1978, Hall and Myers 1978, Bennett et al. 1988, Shenbrot et al. 2002). Seasonal changes in burrow conditions also affected the relative abundance of flea species on European rabbits (*Oryctolagus cuniculus*) and California ground squirrels (Longanecker and Burroughs 1952, Osacar-Jimenez et al. 2001), because different flea species are better adapted to hot and dry conditions vs. cool and wet conditions. If burrows buffer against ambient weather, then future changes in flea abundance, and therefore plague dynamics, might not be as extreme as suggested by models based on climate change. However, little is known about the microclimate of prairie dog burrows and its relationship to ambient weather conditions. Therefore, we sought to determine the environmental conditions to which prairie dog fleas are exposed, and how those conditions impact flea abundance in prairie dog burrows. To determine the relationship between ambient climate, burrow microclimate, and flea populations, we conducted field studies across a wide latitudinal gradient, which allowed us to survey a range of environmental conditions. We hypothesized that burrows at the southern edge of the prairie dog range will be hotter and dryer than burrows at the northern edge of the range, following regional weather patterns. We would also expect that temperatures are

more stable inside burrows than they are aboveground. Because fleas are sensitive to desiccation, flea survival should be lower when burrows are hotter and relative humidity is low, and so we expect flea abundance will be lower at the southern-most sites.

Methods

Field Methods

We selected field sites at Charles M. Russell National Wildlife Refuge (MT, Phillips County MT), Theodore Roosevelt National Park (ND, Billings County ND), Buffalo Gap National Grassland (BG, Pennington County SD), Lower Brule Indian Reservation (LB, Lyman County SD), Pueblo Chemical Depot (CO, Pueblo County CO), Las Cienegas National Conservation Area (AZ, Santa Cruz and Pima Counties AZ; 2016 only), and BLM-managed land surrounding Roswell, NM (NM, Chaves County NM; 2017 only) (Figure 1). We selected three prairie dog colonies at each site to be a part of the study. Each colony was between 5 and 65 acres and had no history of using deltamethrin or other pesticides for vector control. Each colony was sampled during two sessions, once in early summer during May or June (Early session) and once in August (Late session) in 2016 and 2017; except sites AZ and NM as indicated in Chapter 1.

During each sampling session we swabbed one hundred arbitrarily selected burrows per colony to collect fleas. Burrows were swabbed by attaching a 15 cm by 15 cm piece of white flannel to a 4.5 m plumbing snake and pushing the snake as far as possible down the burrow. We left swabs in the burrow for 30 seconds before slowly pulling them out and placing them in a zip-lock bag. Each bag was labeled with the GPS

coordinates of the burrow, burrow status (active or inactive), orientation of the burrow entrance, and the depth of the swab in the burrow. Swabs were frozen for at least 24 hours to kill fleas, which we then collected and counted. Fleas were identified to species following published keys (Hubbard 1947, Furman and Catts 1982) and relevant revisions (Stark 1970, Lewis and Lewis 1985, Lewis 2002).

Burrow and ambient temperature and relative humidity were measured by iButton Hygrochron data loggers (DS1923, Maxim Integrated). In 2016 we placed nine loggers in burrows at least 1m deep to track burrow microclimate conditions and placed three on the surface to track ambient conditions at each site (n = 32 burrows). Data were recorded every 30 minutes for the duration of swabbing (3-4 days), once during the early session and once during the late session. In 2017, we placed three loggers at each site at the start of the early session and left them in burrows though the summer until the end of sampling during the late session (n = 28 burrows). The loggers were synchronized to take simultaneous readings. We calculated the average burrow temperature and average relative humidity over the swabbing session by taking the mean of the data loggers at each timestep, and then taking the mean of the timesteps for each site over the sampling session.

Data Analysis

We modeled average monthly burrow temperatures from 2017 in a linear mixed model using the lmer function from the lme4 package (Bates et al. 2015) in R (R Core Team 2018). Burrow temperatures were modeled as a response to the fixed effects of depth below the surface and sampling session, with site included as a random effect.

We modeled average burrow temperature with a linear mixed model using the lme function from the nlme package (Pinheiro et al. 2019) in R. Average burrow temperature was modeled as a response to mean monthly high temperature, and sampling session, with site included as a random effect. Mean monthly high for each site was obtained from NOAA's National Centers for Environmental Information for the nearest weather station with complete data for the time period sampled. The correlation between mean monthly high and average burrow temperatures was strong and mean monthly high was a better predictor of average burrow temperature than the data from ambient loggers, perhaps because the longer-term average better captured the atmospheric conditions driving temperatures than our short-term data.

We modeled average relative humidity in burrows with a linear mixed model using the lme function from the nlme package in R. Average relative humidity was modeled as a response to monthly precipitation, and session as fixed effects with site included as a random effect. Monthly precipitation was obtained from the same NOAA weather stations as mean monthly high temperature.

We modeled flea abundance (number of fleas found in a burrow) in a generalized linear mixed model with a negative binomial distribution using the glmer.nb function from package lme4 in R. Flea abundance was modeled as a response to mean monthly high temperature, winter precipitation, sampling session (Early or Late), and year. Colony was nested within site to account for the lack of independence between colonies at the same site, and both were included as random effects. We used backwards selection to determine which parameters were unnecessary to include in the final model.

We also modeled the prevalence of infested burrows (burrows with at least 1 flea = 1, burrows with 0 fleas = 0) in a generalized linear mixed model with a binomial distribution using the glmer function from package lme4 in R. We used the same explanatory variables and assessments for these models as we did when modeling flea abundance.

We used the ΔAIC to compare different models for abundance and prevalence of fleas in burrows. If ΔAIC for a given model is <2 from the lowest scoring model, we will consider it well supported by the data in addition to the lowest scoring model (Burnham and Anderson 1998). We evaluated the model goodness of fit by calculating the ratio of the residual deviance divided by the model degrees of freedom. We considered a model well-fitting if that value was <2.

Results

Burrow Microclimate

Burrow temperatures were stable throughout the day despite wide fluctuations in outside temperatures (Figure 2). Depth and sampling session were significant predictors of burrow temperature (AIC = 251, Without session ΔAIC = 38, Without Depth ΔAIC = 21). Burrow temperatures declined -2.0°C (95% CI -2.53 - -1.19, $t = -5.33$, $p < 0.001$, df = 45) with each meter in depth. Burrow temperatures increased by 4.46°C from the early to the late sampling session (95%CI = 3.31 – 5.59, $t = 7.69$, $p < 0.001$, df = 45) (Figure 3). Burrow temperatures varied widely across the sites, with the highest temperature (24.3°C) recorded in Colorado and the coolest temperature (12.7°C) in North Dakota.

The best fitting model for average burrow temperature included the fixed effects sampling session and mean monthly high temperature, and site as a random effect (AIC = 74, k = 5, GOF = 4.9). The model that used just sampling session (ΔAIC = 29, k = 4) or mean monthly high temperature (ΔAIC = 31, k = 4) as fixed effect did not fit the data as well as the full model. Our linear mixed model showed that both mean monthly high temperature and sampling session were significant predictors of average burrow temperature (Figure 4). Average burrow temperature increased 0.81 (95% CI = 0.64 – 0.98, t = 9.54, p < 0.001, df = 17) for every 1°C increase in mean monthly high temperature. Average burrow temperature during the late sampling session was 4.16°C (95% CI = 3.28 – 5.04, t = 9.10, p < 0.001, df = 17) higher than during the early sampling session. Average relative humidity was uniformly high (>89%) across sites, sampling sessions, and years (Table 1). Average relative humidity increased by 0.37% for every 1 cm increase in monthly precipitation (95% CI -0.004 – 0.62, t = 1.91, p = 0.08, df = 13), but this increase was not significant.

The best model for flea abundance included the fixed effects session, year, mean monthly high temperature, winter precipitation, the interaction between session and winter precipitation and the interaction between year and mean monthly high temperature, as well as the random effects of colony nested within site (AIC = 5923, K = 9). The next best model included the additional interactions between session and mean monthly high temperature and year and winter precipitation (AIC = 5925, K = 11). We selected the first model, with fewer interaction terms because it is more parsimonious. Fleas were more abundant in the late session than the early session (Risk Ratio = 1.63,

95% CI 1.29 – 2.07) and in 2017 than 2016 (RR = 6.22, 95% CI 4.15 – 9.34). Flea abundance increased by 58% (RR = 1.58, 95% CI 1.36 – 1.82) for every 1°C increase in mean monthly high temperature in 2016, and by 112% (RR = 2.12 95% CI 1.58 – 2.76) for every 1°C increase in mean monthly high temperature in 2017 (Figure 5a). The effect of precipitation on flea abundance changed between the early and late session. In the early session, abundance decreased by 76% (RR = 0.24, 95% CI 0.13 – 0.46) for every 1 cm increase in winter precipitation. In the late session, flea abundance increased at a rate of 11% (RR = 1.11, 95% CI 0.37 – 3.25) for every centimeter increase in winter precipitation (Figure 5b), however this increase was not significant.

Flea Abundance and Prevalence

The best model for the prevalence of flea infested burrows included the fixed effects session, year, mean monthly high temperature, winter precipitation, and the interaction between session and winter precipitation, as well as the random effects of colony nested within site (AIC = 3894, K = 7). The next best model included the same fixed and random effects, as well as the interactions between session and mean monthly high temperature, year and mean monthly high temperature, and year and winter precipitation (AIC = 3897, K = 10). We selected the first model, with fewer interaction terms because it was more parsimonious. The odds of finding an infested burrow were higher in 2017 (OR = 4.04, 95% CI 2.78 – 5.57) and the late session (OR = 1.35, 95% CI 1.13 – 1.62) than 2016 and the early session, respectively. Higher mean monthly high temperatures increased the odds of a burrow being infested by 50% (OR = 1.50, 95% CI 1.35 – 1.67) for every 1°C increase (Figure 6a). During the early session, increased

winter precipitation caused the prevalence of infested burrows to decline by 74% (OR = 0.26, 95% CI 0.15 – 0.46) for every additional cm of precipitation, but in the late session increased winter precipitation had no significant effect on the prevalence of infested burrows (OR = 0.77, 95% CI 0.30 – 1.77) (Figure 6b).

Discussion

As we hypothesized, burrow microclimates remained stable throughout the day when compared to ambient conditions. The pattern is most obvious at our southern site in the Chihuahuan desert near Roswell, NM where daily temperatures measured at the surface of burrows spanned 40°C. Despite this huge swing, temperatures measured in burrows fluctuated by less than 1°C. Our results are consistent with published measurements from burrow systems of many different terrestrial species that indicate stability of burrow temperatures compared to ambient temperature cycling (Kay and Whitford 1978, Hall and Myers 1978, Reichman and Smith 1990, Shenbrot et al. 2002). The stability of burrow temperatures is generally attributed to the buffering capacity of the soil. Because soil has a higher thermal capacity than the atmosphere, it takes longer to heat up than the outside air, creating a refuge within the burrow to allow respite from fluctuating ambient conditions.

Although burrows showed short-term stability, burrow temperatures showed seasonal changes, increasing by an average of 4°C from early to late sampling sessions. These longer-term patterns follow seasonal climatic conditions driven by solar radiation (Bennett et al. 1988). We found that average burrow temperature was driven by mean

monthly high temperature and varied by site (latitudinal patterns). Burrow temperatures and mean monthly high temperature both declined with latitude, indicating that solar radiation is driving both phenomena.

The western great plains are projected to get hotter and drier through the next century. As the climate warms, our results indicate prairie dog burrow temperatures will increase as well. Under conservative estimates, temperatures in the Plains states are likely to increase by 1.9 - 3.6°C by 2085 (Kunkel et al. 2013b, 2013a). Based on our models, flea abundance will increase between 110% and 226% under a change of that magnitude, and the prevalence of infested burrows will increase by between 95% and 180%. Because flea bites are the primary method of plague transmission (Richgels et al. 2016), a large increase in the number of fleas found on a colony coupled with a greater number of infested burrows could have meaningful impacts on plague dynamics. Previous modeling has demonstrated that the future distribution of plague outbreaks is likely to shift northward with a warming climate (Nakazawa et al. 2007), and that the areas with the greatest decline in projected plague outbreaks corresponds to the areas where the climate is predicted to warm the most. A possible mechanism to explain these predicted shifts is that temperatures become more favorable to flea populations in the northern parts of the US, while crossing an optimal temperature threshold and becoming unfavorable to flea populations in the south. However, burrows buffer against the effects of outside temperature, and so burrow temperatures may remain favorable to fleas even as outside temperatures exceed their thermal tolerance. If this is the case, we would expect flea abundance to increase with increased burrow temperatures, and for plague

outbreaks to remain a concern across the range of black-tailed prairie dogs.

Future work should focus on quantifying what specific factors drive the patterns of flea abundance we have observed. If temperature plays a significant role, knowing where the upper threshold lies for *O. hirsuta* and *O. tuberculata* could be important for predicting future plague epizootics and management decisions to control outbreaks. Currently, one of the most effective methods of preventing plague epizootics is by controlling flea populations with insecticides (Seery et al. 2003, Tripp et al. 2016). Being able to predict the abundance of fleas based on regional temperature could indicate if it is worth treating burrows on colonies that have not experienced a plague epizootic.

Conclusion

Prairie dog burrows provide an effective buffer against daily fluctuations in external environmental conditions. However, burrow temperatures change seasonally and are strongly correlated with the local mean monthly high temperature. Our results show higher temperature and lower winter precipitation are associated with significant increases in both flea abundance and the prevalence of infested burrows in the Great Plains. Changing flea populations could impact plague dynamics across the range of the black-tailed prairie dog, potentially leading to an increased likelihood of outbreaks as future climate becomes warmer and dryer. Future work focused on the impacts of higher temperatures on flea development and the survival of immature stages could help clarify whether seasonal declines of fleas in southern sites might be due to effects of temperature on flea demographics, or due to normal cycling in the flea population.

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Site	Year	Session	Relative Humidity
BG	2017	Early	99
BG	2017	Late	94.98
BG	2016	Early	99.24
BG	2016	Late	99.23
CO	2017	Early	98.65
CO	2017	Late	97.03
CO	2016	Early	98.86
CO	2016	Late	90.01
LB	2017	Early	97.9
LB	2017	Late	100
LB	2016	Early	98.98
LB	2016	Late	98.2
MT	2017	Early	97.86
MT	2017	Late	94.877
MT	2016	Early	98.77
MT	2016	Late	98.64
ND	2017	Early	99.98
ND	2017	Late	100
ND	2016	Early	*
ND	2016	Late	98.75
NM	2017	Early	97.51
NM	2017	Late	100

Table 1. A summary of relative humidity values for every sampling session. A star indicates that a site was not sampled at the given timepoint.

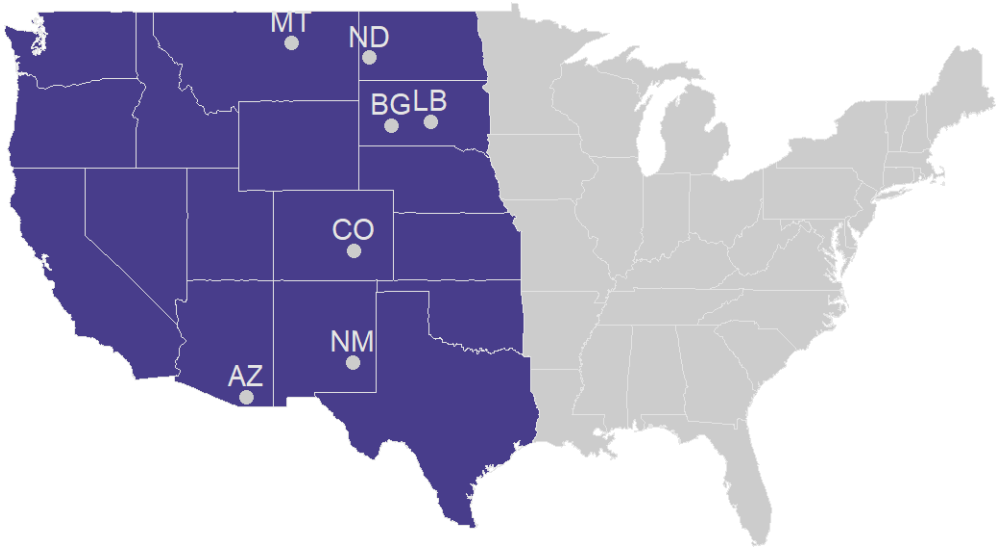

Figure 1. Plague is endemic in the western United States (purple states). Seven field sites (labeled in gray) were chosen in 6 US states (Montana, North Dakota, South Dakota, Colorado, New Mexico, and Arizona). Sites in Montana, North Dakota, and Colorado were sampled during 2016 and 2017. Sites in Arizona were sampled only in 2017, and sites in New Mexico were sampled during 2016 only.

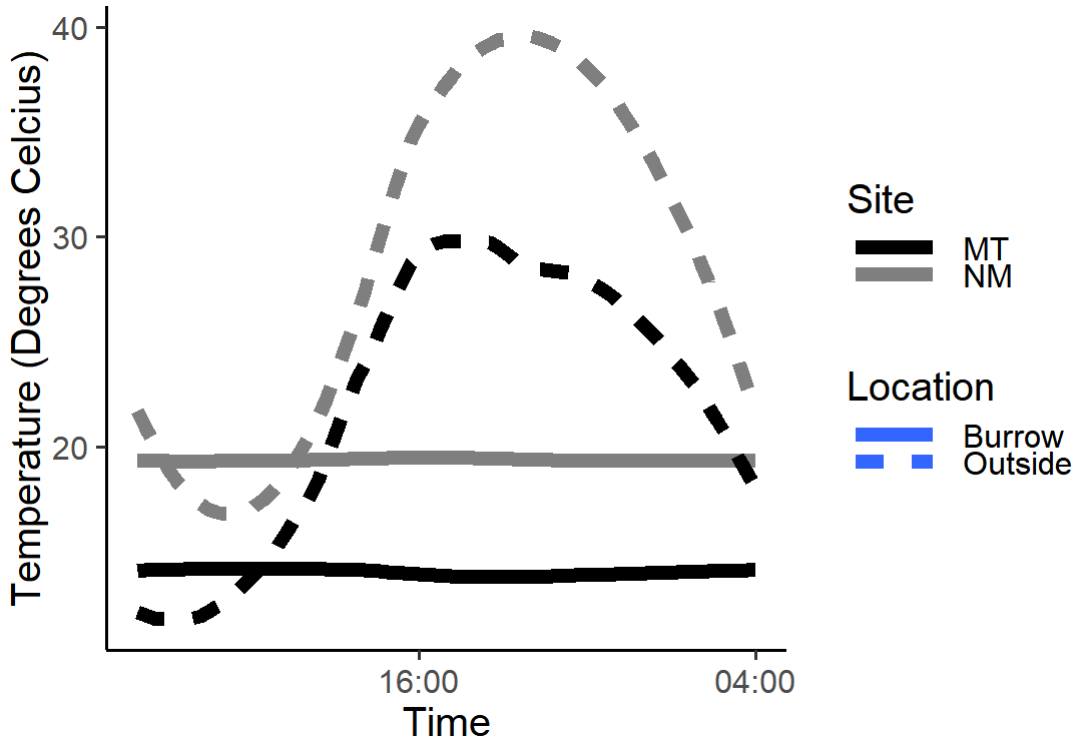

Figure 2. Burrow temperatures remain stable despite daily cycles in ambient temperature. Burrows at northern sites (ie. The Charles M. Russell National Wildlife Refuge in Montana (MT)) had lower temperatures than burrows at southern sites (ie. Colonies around Roswell, New Mexico (NM).

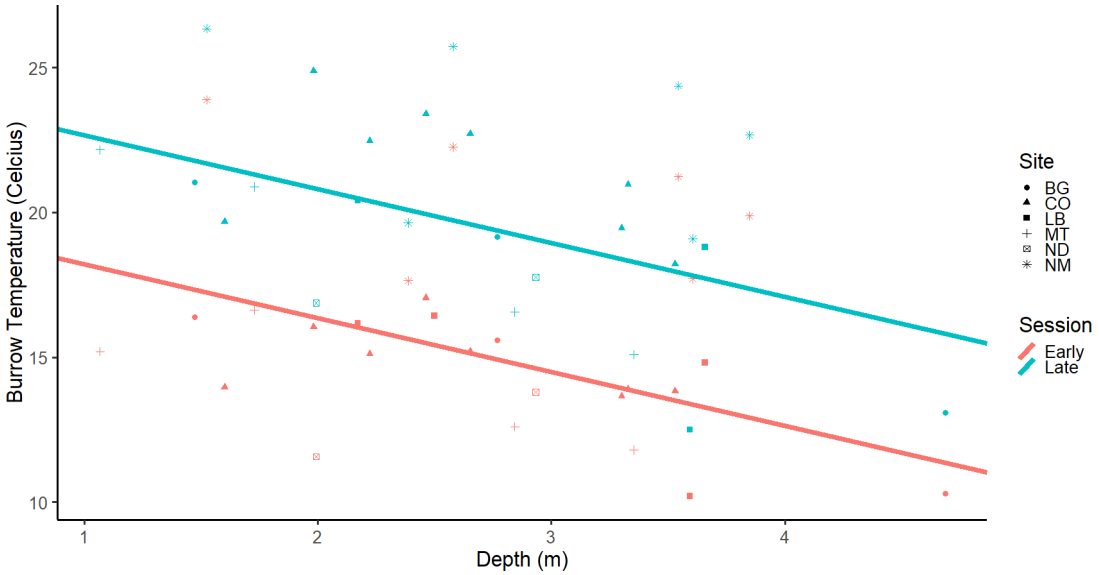

Figure 3. Temperatures declined with burrow depth. Burrow temperatures declined by 2°C for every meter of depth. Burrows were coolest in the early session, and temperatures increased by 4°C from the early to the late sampling session.

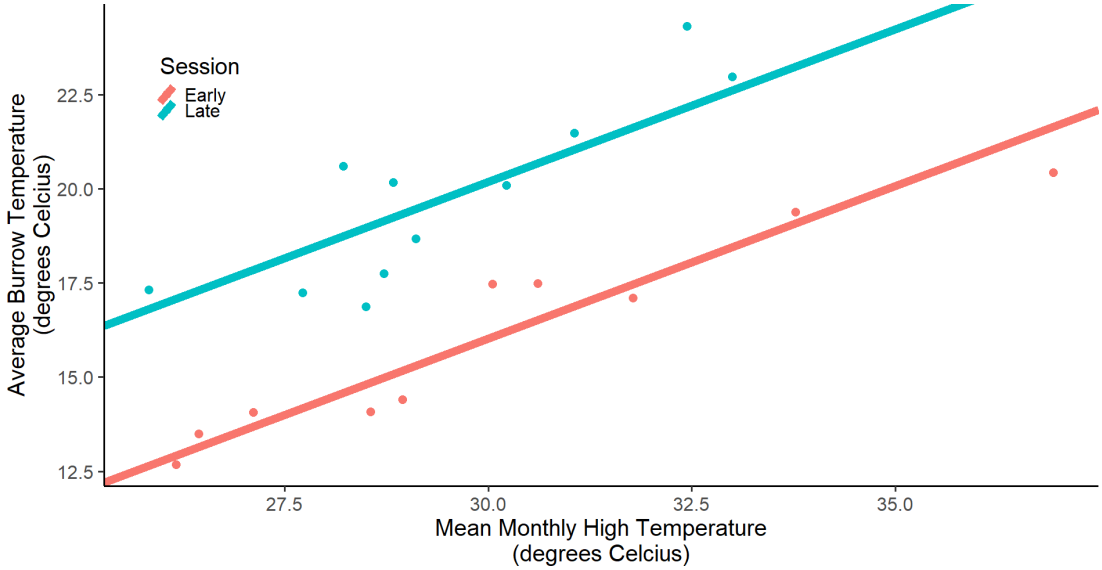

Figure 4. Average burrow temperatures are correlated with mean monthly high temperatures. Each 1°C increase in mean monthly high temperature increases average burrow temperature by 0.81°C. Burrow temperatures were 4°C higher during late sampling sessions than during.

46

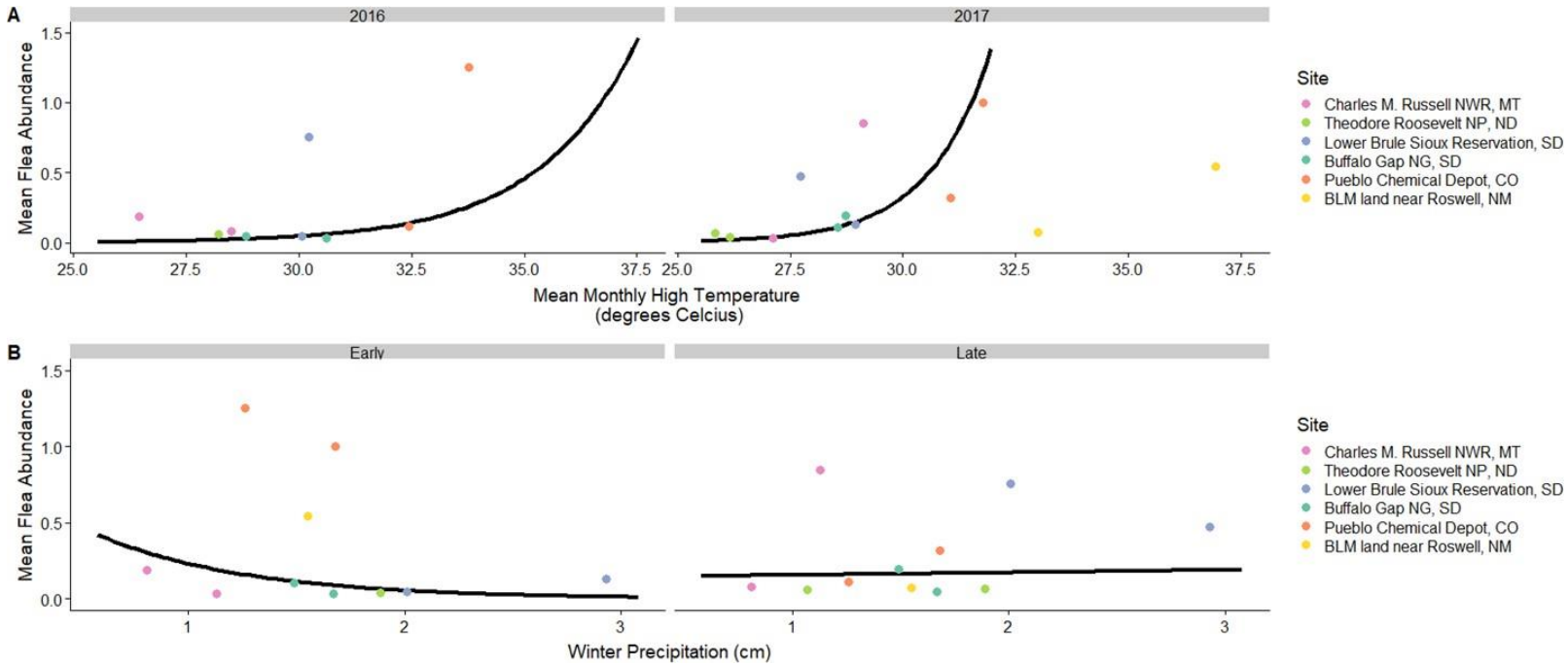

Figure 5. A) Flea abundance increases with mean monthly high temperature at different rates in 2016 and 2017. In 2016, flea abundance increases by a rate of 1.58 for every 1°C increase in temperature, while in 2017 abundance increases by a rate of 2.12 **B)** The relationship between flea abundance and winter precipitation changes between the early and the late sampling sessions. In the early session, flea abundance declines at a rate of 0.24 for every additional cm increase in precipitation. In the late session, there is no significant effect of winter precipitation on abundance.

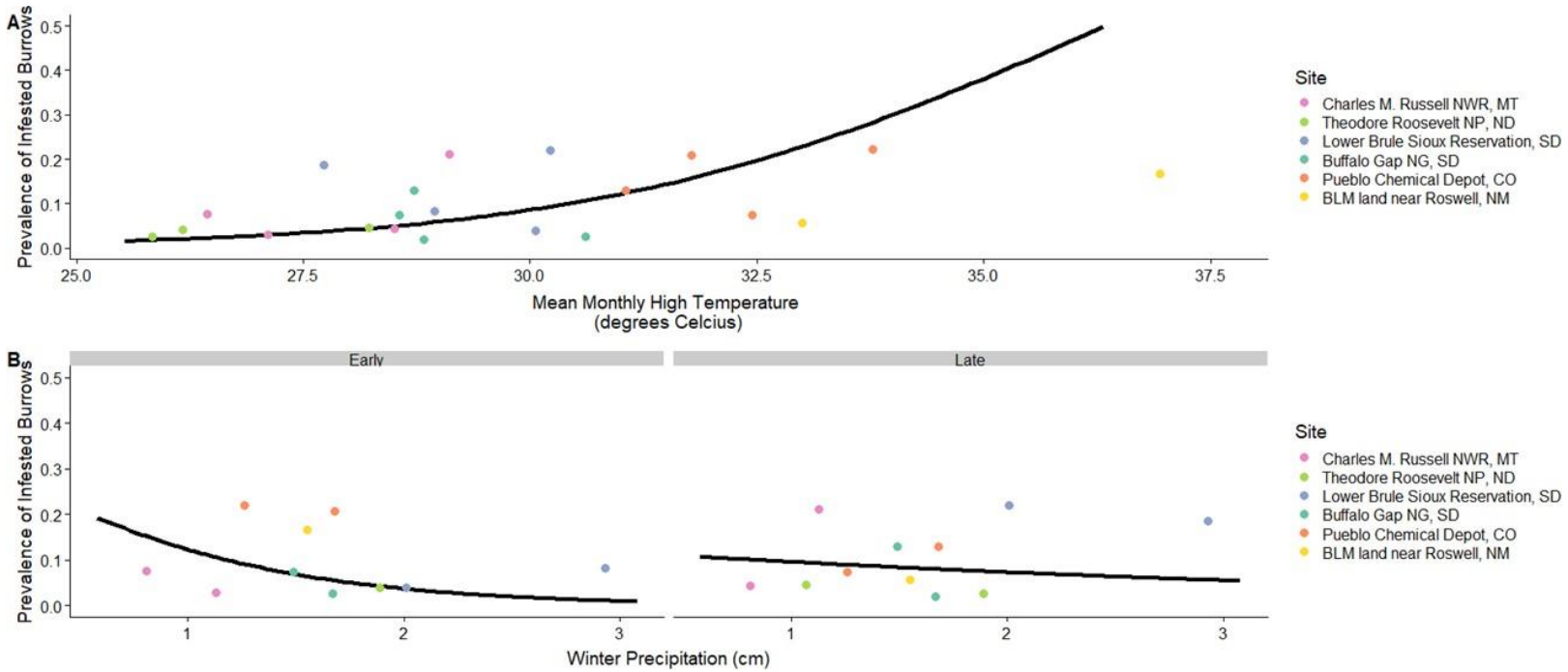

Figure 6. A) The likelihood of a burrow being infested increases by 1.50 with every additional 1°C increase in mean monthly high temperature. **B)** In the early session, the likelihood of a burrow being infested declines by 77% for every additional cm of winter precipitation. In the late session, there is no significant change in the prevalence of infested burrows with increased winter precipitation.

47

Chapter 3 – The impacts of environmental conditions on flea development rates

Introduction

Insects are ectotherms, and therefore are highly sensitive to the temperature of the environments they live in. Development only occurs within a specific range of temperatures, bounded by a lower threshold below which the insect cannot develop, and an upper threshold above which development rates decline and the insects die (Gilbert and Raworth 1996). To complete development, insects need to accumulate a certain amount of heat energy, which can be measured in degree-days (Wilson and Barnett 1983). As a result, development rates are slower at low temperatures and faster at high temperatures (Damos and Savopoulou-Soultani 2012). For a given species, development rate generally increases linearly with temperature from the lower threshold until the upper limit (Gilbert and Raworth 1996). Temperature thresholds and heat requirements vary from species to species and play a key role in determining species ranges and abundance (Andrewartha and Birch 1986), and there is a well-documented history of insect ranges expanding or contracting as the local climate cooled or warmed (Parmesan et al. 1999).

Earth's climate has been warming since the middle of the 20th century, and warming driven by human emissions of CO_2 and other greenhouse gasses is likely to continue (IPCC, 2014). By the end of the 21st century, the Great Plains could be up to 4.4°C warmer than the end of the 20th century (Kunkel et al. 2013a, 2013b). Insects have already exhibited phenological shifts due to climate warming. Shorter winters have led to earlier spring emergence of the first generation of adults (Robinet and Roques 2010). Insects are often classified as being uni-, bi-, or multi-voltine, indicating the

number of generations that occur in one year. If the date of first emergence becomes early enough, insects can increase their voltinism (Altermatt 2010), leading to a larger number of individuals that are present on the landscape for a longer period. Additionally, fecundity of some insect species increases with temperature, resulting in higher numbers of young per female (Kersting et al. 1999, Krasnov 2005). When coupled with faster development times, this could lead to an even more rapid increase in insect abundance.

Population and distribution changes are particularly notable for insect vectors of human and animal diseases. The distribution of vector borne diseases like Lyme and bluetongue provide two distinct examples. Black-legged ticks (*Ixodes scapularis*), which carry Lyme disease, have expanded northward, leading to the emergence of Lyme disease in previously unimpacted areas in the northern US and southern Canada (Clow et al. 2017). This expansion is predicted to continue as climatic conditions become increasingly favorable for tick populations along their northern border (Khatchikian et al. 2015, Clow et al. 2017). Bluetongue virus is transmitted to ruminants by biting *Culicoides* midges. The ranges of midge species are constrained by their habitat specificity more than temperatures, so bluetongue is not expanding northwards via an invasion of its vectors. Rather, warmer temperatures and shorter winters have led to an increase in voltinism of the midges; some species in northern Europe now complete five or six generations compared to one to four generations in the latter half of the 20th century (Elbers et al. 2015). Higher temperatures also increase the rate at which bluetongue virus replicates in *Culicoides* midges; when coupled with a longer active season for midges, transmission of bluetongue is more likely.

Fleas (order Siphonaptera) are common parasites of vertebrates worldwide (Krasnov 2005), and are vectors of several zoonotic diseases, including sylvatic plague (Eisen and Gage 2012). Like other insects, flea development is regulated by environmental conditions, especially temperature and humidity. Development rates increase with temperature across species; however, immature fleas are highly sensitive to desiccation and if relative humidity is too low, fleas will not complete development even if the temperature is favorable (Krasnov et al. 2001a, 2001b, Kreppel et al. 2016). In the US, the most common flea vectors of plague belong to the genus *Oropsylla* (Burroughs 1947): *O. montana* is a common parasite of California ground squirrels (*Otospermophilus beecheyi*) west of the rocky mountains, and *O. hirsuta*, *O. tuberculata*, and *O. labis* are common parasites of prairie dogs in the intermountain west and the Great Plains (Lewis 2002). These species have different seasonal and geographic patterns (Salkeld and Stapp 2008, Tripp et al. 2009), indicating that environmental conditions may play an important role in their distribution and abundance (Chapter 1 and 2). Because environmental conditions are a significant factor for flea development, climate change could impact their future distribution and abundance. High flea populations can have adverse impacts on their hosts, both through direct costs associated with parasitism, such as dermatitis and anemia (Scheidt 1988, Yeruham et al. 1989, Araújo et al. 1998, Bond et al. 2007), and increased risk of plague transmission (Tripp et al. 2009).

All life stages of *Oropsylla* fleas are commonly found in the nests of their host species (Eskey and Haas 1940, Sheets et al. 1971). California ground squirrels and prairie dogs both dig burrows that serve as their primary shelters and provide a buffer from the weather outside (Fitch 1948, Longanecker and Burroughs 1952, Hoogland 1995,

Chapter 2). Therefore, development from egg to adult emergence is mostly impacted by burrow microclimate, rather than ambient conditions. Because higher flea abundance could contribute to increased transmission of sylvatic plague, understanding the impacts of temperature on flea development rates is important for predicting how flea populations may change in the future. Here, we aim to demonstrate how climate change could impact flea development rates, while accounting for the buffering ability of the burrow systems where hosts and fleas live.

Methods

Of the *Oropsylla* species, only *O. montana*, the primary vector of plague in California ground squirrels, has been successfully reared in captivity. We used *O. montana* as a proxy for *O. hirsuta* and *O. tuberculata* because the microclimates governing their development and the life history of their hosts are similar. Like prairie dogs, California ground squirrels dig extensive burrow systems that they use for sleeping, rearing young and shelter (Fitch 1948, Hoogland 1995). The microclimate of these burrows is similar to what we measured in prairie dog burrows (high humidity, temperatures increasing from 15.5°C in May to 20°C in August) (Longanecker and Burroughs 1952), and *O. montana* are more common in burrows than on hosts (Eskey and Haas 1940). Laboratory studies of development rates of *O. montana* were conducted by Metzger (2000). Adult fleas were reared on ground squirrels housed in a dedicated nest box, and flea eggs were periodically collected to measure their development under different temperature and humidity conditions. Individual eggs were placed in separate wells of a 96 well ELISA plate, which were then placed into environmental chambers at all combinations of 55%, 65%, 75% and 85% relative humidity and 15.5, 21.1 and

26.7°C. Hatch date, date of pupation, and date of adult emergence were recorded for each egg. From these results we then determined the development rate for fleas at a given combination of environmental conditions by calculating the reciprocal of days to adult emergence.

We used a generalized linear mixed model with the lmer function in the lme4 package in R to determine the temperature dependent development rate of O. montanta by including temperature as a fixed effect and relative humidity as a random effect. This allowed us to develop a degree-day model to predict flea development rates and potential generation times across a range of temperatures.

Results

Oropsylla montana development

Low relative humidity inhibited *O. montana* development, as no fleas completed development at 55% relative humidity, and at 65% relative humidity, fleas only completed development at 15.5°C and 21.1°C. According to our model, flea development rates increased significantly with temperature. For every 1°C increase in daily temperature (i.e., degree-day), the development rate increased by 0.00247 ($t = 14.5$, $df = 12$, $p < 0.0001$, 95% CI 0.0022 – 0.0028) from an intercept of -0.019 (95% CI -0.027 - -0.011) (Figure 1). At 85% relative humidity, the development rate is 0 at 6.0°C, but for development to occur in <100 days, the temperature needs to be >10.6°C.

Predicted flea development rates

Because *Oropsylla* fleas develop within the buffered environment of host

burrows, burrow conditions must be considered when making predictions of flea development rates in their natural habitats. In previous work, we measured temperature and relative humidity in black-tailed prairie dog burrows at six sites in five US states (see Chapter 2 for detailed methods). Burrow temperatures were modeled in a generalized linear mixed model with mean monthly high temperature and session (month of sampling) as fixed effects, and year and site as random effects. We used this model to estimate burrow temperature (T_b) from the ambient mean monthly high temperature (T_a):

$$T_b = -8.05 + 0.81 * T_a \quad (1)$$

We used the early summer sampling session for this analysis, as it results in a lower estimated burrow temperature and therefore provides a conservative estimate of the development rate. We then used the development rate model for temperature (above) to estimate flea development rates (r) from T_b.

$$r = -0.0149 + 0.00247 * T_b \quad (2)$$

Since the measured relative humidity in burrows was uniformly high (>90%, see Chapter 2), we based equation 2 on a relative humidity of 85%.

Because the field sites in the northern plains (MT, ND, and SD) have a cooler, wetter climate than those in the southern plains (CO and NM), we estimated development rates for each region separately. In the northern plains, the average June mean monthly high temperature is 28°C, with a predicted burrow temperature of 14.6°C and a development rate of 0.021. Thus, it takes 47.1 days for a newly laid egg to complete development. In the southern plains, the average June mean monthly high temperature is 34°C, with a predicted burrow temperature of 19.5°C. At this temperature, the estimated

development rate is 0.033, and adults emerge 30.1 days after eggs are laid.

Under the CMIP3 A2 scenario, the average temperature in the Great Plains is expected to rise by 4.4°C by 2100 (Kunkel et al. 2013a, 2013b). If current mean monthly high temperatures rise by 4°C, burrow temperatures in the northern plains are predicted to increase to 17.9°C in June. This will increase the development rate to 0.029, and adults will emerge 34.2 days after eggs are laid; 13 days (28%) faster than current rates. In the southern plains, burrow temperatures will increase to 22.7°C in June, and the development rate will increase to 0.041. Adult fleas will emerge 24.2 days after eggs are laid; 6 days (20%) faster than current rates.

Discussion

Since fleas are ectothermic, changes in their environment can significantly impact their development. In both the northern and southern plains, prairie dog flea development rates are projected to increase with warming temperatures. *Oropsylla hirsuta* are currently most abundant for about 150 days from June to October (Salkeld and Stapp 2008), and current mean monthly high temperatures in the northern plains are lower than the minimum threshold necessary (6°C) for flea development to occur for 7 months, thus limiting the season of active flea recruitment. As temperatures increase, the number of days required to complete development will decrease, leading the number of generations produced over 150 days to increase from 3.2 to 4.4 in the northern plains, and 5.0 to 6.2 in the southern plains by 2100. These correspond to a potential flea population increase of 138% and 124% in the northern and southern plains, respectively.

One of the consequences of warming climates is a decrease in the number of cold

days, leading to shorter winters. In temperate regions, warming throughout the 20th century has been correlated with earlier spring emergence for many insect species. If cold temperatures start later and end earlier, fleas could have an even longer period of successful development than they do now. In addition, the fecundity of several flea species increases with temperature (Krasnov 2005), leading to higher rates of recruitment. Together, faster development rates over longer growing seasons coupled with higher recruitment rates could increase flea abundance. However, further research is necessary to determine how *O. hirsuta* and *O. tuberculata* respond to warmer and longer periods of favorable temperatures.

Flea bites are responsible for >70% of plague transmission during epizootic plague (Richgels et al. 2016). If fleas are present for longer periods of time at higher abundance, it is possible that outbreaks could last longer and be more severe than what we see currently. This pattern is similar to the impacts of climate change on avian malaria in the Hawaiian Islands. Currently, high altitude forests provide a refuge for highly susceptible birds, as cool winter temperature and rainfall limit the ability of the mosquito vector to persist year-round and the malaria parasite to replicate in the vector (Ahumada et al. 2004). As the climate warms, however, this refuge is expected to shrink, as increasing temperatures allow for a more abundant year-round mosquito population, facilitating higher transmission of malaria for a longer period of the year (Liao et al. 2017). Shorter, milder winters have also been implicated in epizootic outbreaks of winter ticks (*Dermacentor albipictus*) causing mortality among moose. Warmer temperatures during fall questing decrease tick mortality and increase the likelihood of successfully finding a host (Dunfey-Ball 2009, Holmes et al. 2018). Similarly, survival of *Ixodes*

ricinus, a vector of Lyme disease and tick-borne encephalitis, has become active year-round in Scotland as have warmer winter temperatures (Furness and Furness 2018); increasing the chances of an infected tick transmitting disease to a host.

Conclusion

Flea development is sensitive to environmental conditions. Higher relative humidity and temperature lead to higher development rates. The climate in the Great Plains is projected to warm by 4.4°C by the end of the 21st Century, unless significant programs are implemented to limit the production of greenhouse gasses. As the climate warms, prairie dog flea development rates are expected to increase, allowing fleas to produce more generations in a single growing season and leading to a population increase. As winters become shorter, there will be a longer period of favorable temperatures for fleas to survive and reproduce. Because flea bites are the primary mechanism of plague transmission, an increase in flea abundance and a longer annual period with warm temperatures could lead to more and longer plague outbreaks in the future.

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