FLEA GENETIC DIVERSITY
IN GUNNISON'S PRAIRIE DOG COLONIES
AND ITS IMPLICATIONS FOR FLEA TRANSMITTED DISEASES

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Abstract

Understanding disease-causing organisms from a broader ecological perspective has proven a valuable tool for understanding the causes of disease outbreaks in various organisms. Several insect species act as both parasites and pathogen carriers, making them important players in the spread of diseases in human and wildlife communities. This study aimed to determine what could be used to predict the distribution of flea genetic diversity parasitizing Gunnison's prairie dogs (*Cynomys gunnisoni)* as a foundation for understanding the potential influence and implications this may have for transmission of disease causing microbes such as *Rickettsia, Bartonella,* and *Yersinia pestis*. A much higher level of flea genetic diversity was found in the colonies compared to what has been observed for fleas parasitizing black-tailed prairie dogs (*Cynomys ludovicanus*). Although none of the factors tested (location of colony relative to others, prairie dog genetic diversity, or number of mammals species) were able to predict the genetic diversity of fleas observed across colonies, potential implications for the spread of disease causing microbes are still considered, with recommendations for further research. The present study emphasizes the need to collect further data on mammals that frequently interact with Gunnison's prairie dogs, as well as abiotic factors such as climate and temperature, both of which could be used to further investigate the survival and transmission of pathogens in this system.

Introduction

Historically the studies of diseases and parasitism have been separate from those of ecology and evolution. As knowledge accumulated in each field, the ability to address cross-disciplinary questions became a reality, and the field of disease ecology was born (Gage et al., 1995). In research today there is an emphasis put on understanding disease-causing organisms

from a broader ecological perspective that places these organisms in a network of interactions with other life forms as well as their environment. Understanding how these interactions have changed over time from the perspective of evolutionary biology and population genetics is another step in expanding and unifying these sub-disciplines within biology.

Insect species have played a particularly important role in disease ecology, partly because of the ease in which conducting experiments can be done with them, but largely due to the role of many species of insects as both parasites on other organisms as well as carriers of diseases (Gage et al., 1995).

Fleas

Fleas are a group of wingless insects classified in the order Siphonoptera, of which there are approximately 2,500 species. All Species of fleas are obligatory blood feeders (hematophages) that parasitize on birds and mammals by attaching to the skin (Krasnov, 2008). In addition to their role as parasites, fleas act as carriers of microbes that can cause life-threatening diseases when introduced to their host organisms (Gage et al., 1995; Loftis et al. 2006, Jones et al., 2010). The most well-known microbe carried by fleas is undoubtedly *Yersinia pestis* as it is the causative agent of plague, which resulted in the death of more than one third of the European population in the 14th century (Gage and Kosoy, 2005). Today, plague still infects humans and has caused noticeable declines in different species of prairie dogs over the past several decades (Wilder et al., 2008; Kotliar et al., 1999). Besides *Yersinia pestis*, fleas have been shown to harbor other disease-causing microbes, including the genera, *Rickettsia* (the causative agent of spotted fever and typhus) and *Bartonella* (the causative agent of bartonellosis) (Loftis et al., 2006; Kaewmongkol et al., 2011). In the aim to study this system from a disease

ecology perspective, our attention should largely focus on fleas due to their role as carriers of diseases. Understanding fleas from a perspective of ecology and evolutionary biology should help to elucidate the ecological and evolutionary context from which these disease-causing organisms functions, and may provide insight to their prevention in wildlife and human populations.

Objective

The goal of the present study is to determine variables that can be used to predict the geographic distribution of flea genetic diversity–specifically those that parasitize Gunnison's prairie dogs (*Cynomys gunnisoni)*. This information can be utilized to better understand the transmission of the disease-causing microbes that fleas harbor. Flea genetic diversity may help to understand the spread of disease in prairie dog colonies because there is variation in the ability of different flea species to transmit diseases to their hosts (Burroughs, 1947; Wilder et al., 2008). Different host communities may exhibit different level of disease risk depending on the genetic diversity of the flea communities present in their location. Gunnison's prairie dog colonies consist of several interacting mammals, each of which potentially brings with them their own species of flea (Krasnov, 2008; Davidson et al, 1999). In summary, differences in the genetic diversity of fleas across prairie dog colonies may influence disease transmission and incidence of epidemics in host organisms. Colonies with low levels of flea genetic diversity may pose a greater disease risk to prairie dogs if the fleas present can efficiently transmit a particular pathogen, such as *Yersinia pestis*. Alternatively, each flea species may have a particular pathogen they most efficiently transmit, and prairie dog colonies with higher levels of flea genetic diversity would thus have a larger number of diseases available for prairie dogs to contract.

There are several more hypotheses and questions concerning disease transmission and flea genetic diversity that could be posed, but answering these will first require a foundational knowledge about the genetic diversity of fleas present in Gunnison's prairie dog colonies.

The present study addresses three hypotheses that may predict the geographic distribution of flea genetic diversity, which will be briefly addressed now and considered more fully below:

1. Flea genetic diversity in a given prairie dog colony depends on the ability of fleas from other colonies to migrate there.
2. Maintenance or loss of flea genetic diversity depends on the genetic diversity of their prairie dog hosts.
3. Maintenance or loss of flea genetic diversity in a given prairie dogs colony depends on the number of different mammal species found there.

The three specific aims of this study should contribute to determining what can predict the distribution of flea genetic diversity, which in turn would aid in assessing and preventing diseases in organisms frequently exposed to fleas (including humans). In addition, the findings from this research should contribute to future studies investigating the differential risk of disease transmission across different Gunnison's prairie dog colonies.

Materials and Methods

Collection and storage

The fleas used in this study were collected from 27 sites across Colorado, Utah, New Mexico, and Arizona between 2008 and 2010 (Figure 1, Table 1). The collections were in

concordance with prairie dog capturing efforts in which Tomahawk traps were placed near active prairie dog burrows and baited with cereal grain mixtures after Sackett et al. (2012). Recorded for each flea captured was the date, name of colony, and prairie dog that they came from. Fleas were collected by spraying anesthetized prairie dogs with permethrin (an insecticide), removing them from the fur with tweezers and comb, and placing them in ethanol at -20 °C until DNA extraction.

Table 1
List of Gunnison's prairie dog colonies, their latitude and longitude coordinates, elevation, and month and year for which the flea collections took place.

| colony	latitude	longitude	elev	year	month
RSF	35.09731	-111.6813	2042	2010	June (early-mid)
HUTR	35.7082586	-109.55687	1935	2010	May (late)
BLS	34.15812	-109.30601	2103	2010	May (mid-late)
ELMA	34.9799628	-107.80529	2023	2010	June (late) - July (early)
HOVE	37.3748231	-109.11327	1614	2010	June (mid)
CRL	38.2994034	-109.25173	2078	2010	June (late)
TESW	37.9449716	-107.83391	2664	2009	June (late)
DGO	37.2826459	-107.86814	2010	2009	July (late)
NFF	36.50235	-108.23574	1908	2010	July (early)
SSLM	35.66269	-107.07092	1868	2010	June (early)
SYWS	35.53308	-106.78559	1669	2010	July (late)
DUTS	36.94589	-107.01632	2069	2010	August (early)
WSCM	36.03221	-107.08239	2172	2010	August (early)
BBM	35.97913	-106.87738	2479	2010	July (late) – August (early)
VADO	36.6141	-106.74035	2118	2010	June (late)
FUEN	36.23647	-106.68417	2532	2010	July (late)
HLSP	36.75033	-106.58382	2255	2010	June (late)
CBAR	36.53127	-106.48307	2326	2010	August (early)
VCNP	35.8824736	-106.48795	2624	2010	July (late)
ENSP	36.49235	-105.27218	2524	2010	July (mid)
BLFB	36.32635	-105.28148	2631	2010	July (mid)
TPRR	36.73654	-105.98047	2516	2010	July (mid)
GBTT	38.5436482	-106.88965	2367	2008	May (late)
BVSE	38.7844977	-106.096	2383	2009	July (late)
EMSP	38.942928	-105.51336	2661	2009	September (early)
SAND	35.46654	-106.31748	1713	2010	August (mid)
AGFP	35.6595385	-106.02468	2017	2010	July (mid)
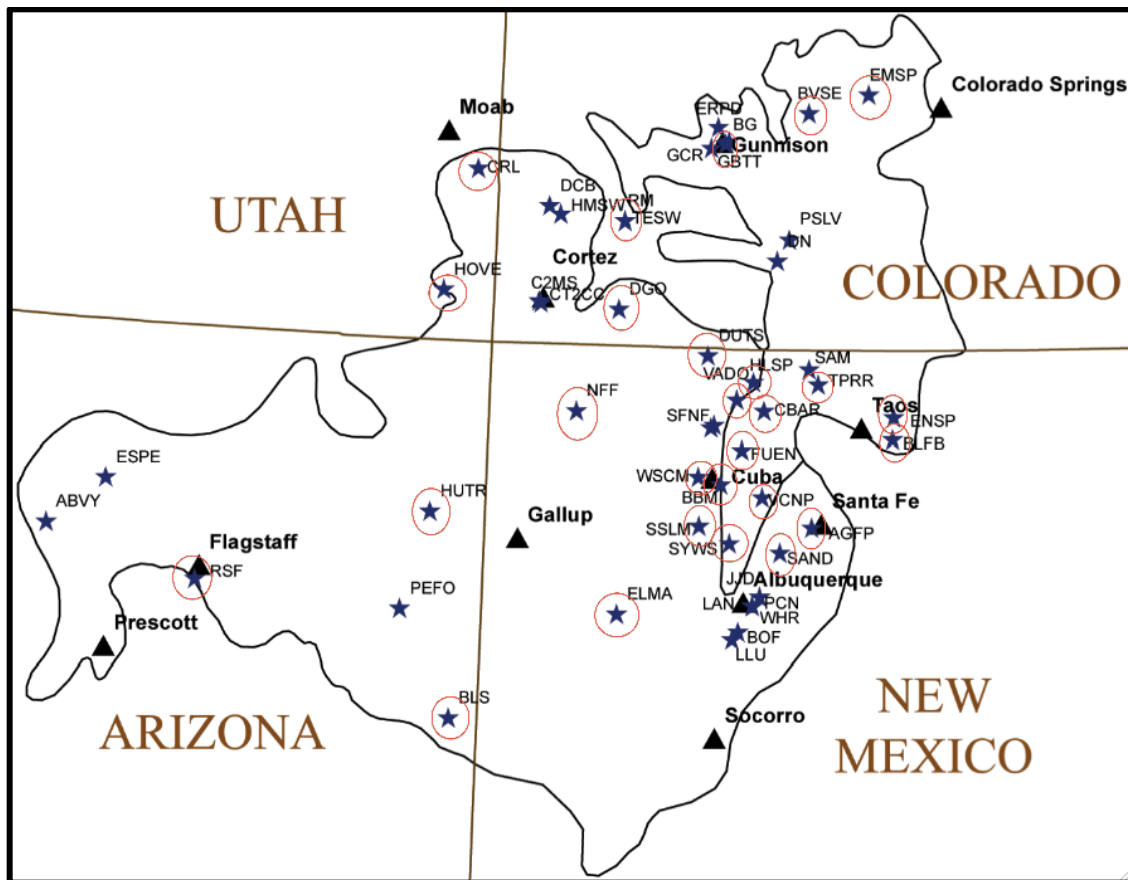
Figure 1 Map of Gunnison's prairie dog colonies. Red circles indicate locations of colonies used for this study.

DNA extraction and molecular protocols[1]

Flea DNA was extracted using the *DNeasy*^{TM} *Purification of Total DNA from Animal Tissues Spin-Column Protocol* (QIAGEN Inc., Venlo, Netherlands), with the modification of decreasing the volume of buffer AE to 150μL in step 7 to increase the concentration of DNA. DNA Primers COIIfLeu (forward) and COIIrLys (reverse) were used in the amplification of the

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[1] The methods used for extracting and sequencing DNA would take too much time to fully describe here. For non-expert readers, it is only important to know that DNA can be attained from an organism's cells, and that we have the technology to read sequences of DNA in order to look for the regions where individuals differ (i.e. mutations). After the differences between individuals are known, there are an immense number of techniques available that allow us to ask interesting and important questions about the genetic characteristics of organisms at the various scales (i.e. individuals, populations, multiple populations, and so on).

mitochondrial gene Cytochrome Oxidase II (Maekawa et al. 1999). All of the genetic analyses in this study were calculated by comparing the cytochrome oxidase II gene (Avise et al., 1987; Liu and Beckenbach, 1992). Polymerase Chain Reaction (PCR) was completed using 1μL DNA, 8μL 1X Master Mix (5 Prime), 1μL of each Primer, and 10μL nuclease free water. PCR was then carried out using the following conditions: 95 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 1 minute, and completing the reaction with 72 °C for 5 minutes post cycles. Crude PCR products were cleaned and sequenced by functional biosciences, Inc. (Madison, WI). The returned sequences were then visually edited for sequencing errors using the program Sequencher, version 4.6 (Gene Codes Corporation, Ann Arbor, Michigan).

Summarizing Flea Genetic Diversity

Before determining what variables predict the distribution of flea genetic diversity, that diversity must be described, summarized, and quantitatively measured. One of the most common and useful ways to summarize genetic diversity is through the use of phylogenetic trees, which infer the relationships between individuals based on the number of shared and unique traits (i.e. mutations in the sequences of their DNA). Phylogenetic trees are similar to family trees, but are used to describe the relationships between groupings at much larger scales (for example, genetic differences between a thousand humans living on different continents).

The genetic region cytochrome oxidase II was used to create the phylogenetic tree (and all the other analyses) in this study because it mutates at a rate that separates insects approximately by species (Liu and Beckenbach, 1992). The phylogenetic tree made for fleas was calculated using the quantitative method of maximum likelihood, which was completed in the program MEGA5 (Tamura et al., 2011).

Identifying fleas by their morphology is very challenging. To infer the species of fleas present in this study, fleas that had already been identified by other researchers were included in the phylogenetic tree by two different means. First, The National Center for Biotechnology Information (NCBI) has a database into which scientists upload the DNA sequences from their studies onto before they are able to publish their findings in a scientific journal (NCBI, 2012). The NCBI database was searched for cytochrome oxidase II sequences that most closely matched those of the fleas in Gunnison's prairie dog colonies. These sequences could then be added to the phylogenetic tree to see how closely related they were to the fleas in the present study. In addition, Dan Tripp from the Colorado Division of Wildlife provided DNA of fleas that had been identified, which were then processed according to the same protocol described above.

In addition to the phylogenetic tree, flea genetic diversity was visualized by mapping the flea DNA haplotypes to the location from which they were sampled. A DNA haplotype is a unique DNA sequence. Multiple individuals can share the same haplotype, and the number and relative proportions of haplotypes found in a colony is indicative of the amount of genetic diversity there. Mapping unique haplotypes was completed in ArcMAP (Esri, California, USA) using the longitude-latitude coordinates of each colony and visualizing the number of haplotypes as pie charts, where each haplotype was assigned a different color, and the number of times a haplotype was sampled was indicated by the pie slice size.

Another important way to summarize genetic diversity is to quantify it. The two metrics used to quantify genetic diversity in this study were theta(S) (also called Watterson's estimator, Watterson, 1975), which was calculated using the program Arlequin (Excoffier and Lischer, 2010). Another measure of genetic diversity used is haplotype diversity (denoted as h) (Nei and

Tajima, 1980), which was calculated by hand[2]. Theta(S) and h were calculated for each colony from which these fleas were collected. The haplotype diversity values used for Gunnison's prairie dogs across colonies were calculated by hand previous to this study (Sackett and Martin, unpublished data). The Gunnison's prairie dog haplotype diversity values are used here with permission from the authors. Once the genetic diversity for each colony was determined, hypotheses as to what variables and characteristics predict the level of flea genetic diversity could then be tested.

Analyses

The distribution of flea genetic diversity observed in this study could be explained by limitations in the distances that fleas are able to disperse. If fleas are only able to disperse over a limited distance, the genetic diversity of fleas in a given colony will only be affected by the genetic diversity of fleas from colonies within that dispersal distance, suggesting the location of a colony in relation to other colonies will have an effect on the level of flea genetic diversity found there.[3] A ubiquitous method used in population genetics to infer the dispersal patterns of an organism is known as the Isolation by Distance Test, which compares the geographic and genetic differences among populations (Wright, 1943; Rousset, 1996). If isolation by distance is occurring, populations that are farther apart will exchange fewer individuals than populations that are closer together. Under isolation by distance, populations that become far enough apart

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[2] Understanding the exact mathematical calculations for these two measures of genetic diversity are not necessary for understanding their use in this study. Simply note that these two metrics are used to quantify the amount of flea genetic diversity present in each of the prairie dog colonies sampled.

[3] Recall this is not a discussion about genetic changes in individual fleas, but rather about changes in the genetic make-up of the flea population due to the immigration of genetically unique individuals.

will no longer exchange any individuals and will show the highest levels of genetic differentiation. The measure of genetic differentiation used when testing for isolation by distance is called FST, which describes the amount of genetic variation between any two populations compared to the total variation both within and between them; FST is an indicator of how similar or different populations are genetically. Applied to the study at hand, the isolation by distance test can be used to infer to what degree fleas are limited in their ability to disperse across geographic space, and will help to explain how the genetic diversity of fleas in a given colony may increase or decrease based on the colony's geographic locations relative to the position of other colonies.

The FST values used to compare genetic differentiation among colonies were calculated using the program Arlequin (Excoffier and Lischer, 2010). The geographic distances among all colonies were calculated in the program ArcMAP (Esri, California, USA) using the longitude-latitude coordinates for each colony. Once the FST and geographic distances were acquired for all colony comparisons, isolation by distance was tested for using a mantel test, implemented in the R-package Vegan (R Core Team, 2012; Oksanen, 2012).

Besides the effect that the location of colonies may have on flea genetic diversity, it is also important to consider what is present at each specific colony that could allow for the persistence or loss of their genetic diversity. One possible predictor for the persistence of high genetic diversity of fleas in a colony is the level of genetic diversity of their prairie dog hosts. Research has shown that there is variation in the level of host specificity among different flea species (Poulin et al. 2006, Krasnov 2008), suggesting that some fleas have adapted to optimally feed on one species, but have lost the ability to feed well on multiple hosts. Expanding on this idea, it may be possible that there is enough genetic diversity within Gunnison's prairie dogs that

fleas have adapted to feeding optimally on one genetic type within the species, and in doing so have lost the ability to feed on other genetic types. If this were the case, the genetic diversity of fleas would depend on the diversity of their hosts. Colonies with high prairie dog genetic diversity would allow for high levels of genetic diversity in fleas, while colonies with low prairie dog diversity would result in a loss of flea genetic diversity. It is important here to recognize that there are multiple potential explanations for why the distribution of prairie dog genetic diversity could predict that of fleas, and it would require further research to determine the true cause. The case for host specificity driving flea genetic diversity as described is just one possible explanation that justifies testing this hypothesis. However, for the purpose of this research, it isn't necessary to know exactly *why* the distribution of flea genetic diversity is or is not explained by prairie dog genetic diversity, only whether or not it *does* explain it.

To test this hypothesis, haplotype diversity of fleas was compared to that of Gunnison's prairie dogs using Pearson's Product Moment Correlation Coefficient implemented in the program R (R Core Team (2012).

Diversity of Rodents

It is possible that the genetic diversity of prairie dogs has no effect on that of fleas. However, the concept of host specificity described earlier may still be able to assist in predicting the distribution of flea genetic diversity. Rather than adapting to specific genetic sub-types of Gunnison's prairie dogs, different flea species may be adapting to optimally feed on different species of mammals. This would result in colonies having an increase in flea genetic diversity as the number of mammal species found in that colony increase. Again, there are likely several

reasons besides host specificity that could explain this pattern; the goal at this stage is to see if the pattern does or does not exist.

To test if the number of mammal species predicts the amount of flea genetic diversity in a colony, freely available shape files providing the current distribution of North American mammals were downloaded from the International Union for Conservation of Nature and Natural Resources (IUCN, 2012), and were visualized using ArcMAP (Esri, California, USA). The known location of Gunnison's prairie dog colonies have a different degree of overlap with each mammal species, creating variation in the number of mammals possible in any given colony (figure 2). The mammal species considered were a smaller subset of the total number of mammals in the IUCN data set. Mammals included in the data were chosen by the following criteria: their distribution had to be available from the IUCN, they had to have a range overlap with at least one of the colonies where flea data was available, and their habitat preferences had to be similar to that of prairie dogs to ensure interaction between the species could occur (for example, the big horn sheep, *Ovis canadensis* fit the first two criterion, but they prefer very different habitats from prairie dogs, making it extremely unlikely that they play any role in changing the amount of flea genetic diversity).

Once the overlap between the mammal ranges and the Gunnison's prairie dog colonies were determined, both measures, theta(S) and h, of flea genetic diversity for each colony were compared to number of overlapping mammal distributions. A Model I Linear Regression was implemented in the program R (R Core Team (2012) in order to test for a predictable linear association between both theta(S) and h against the number of species present.
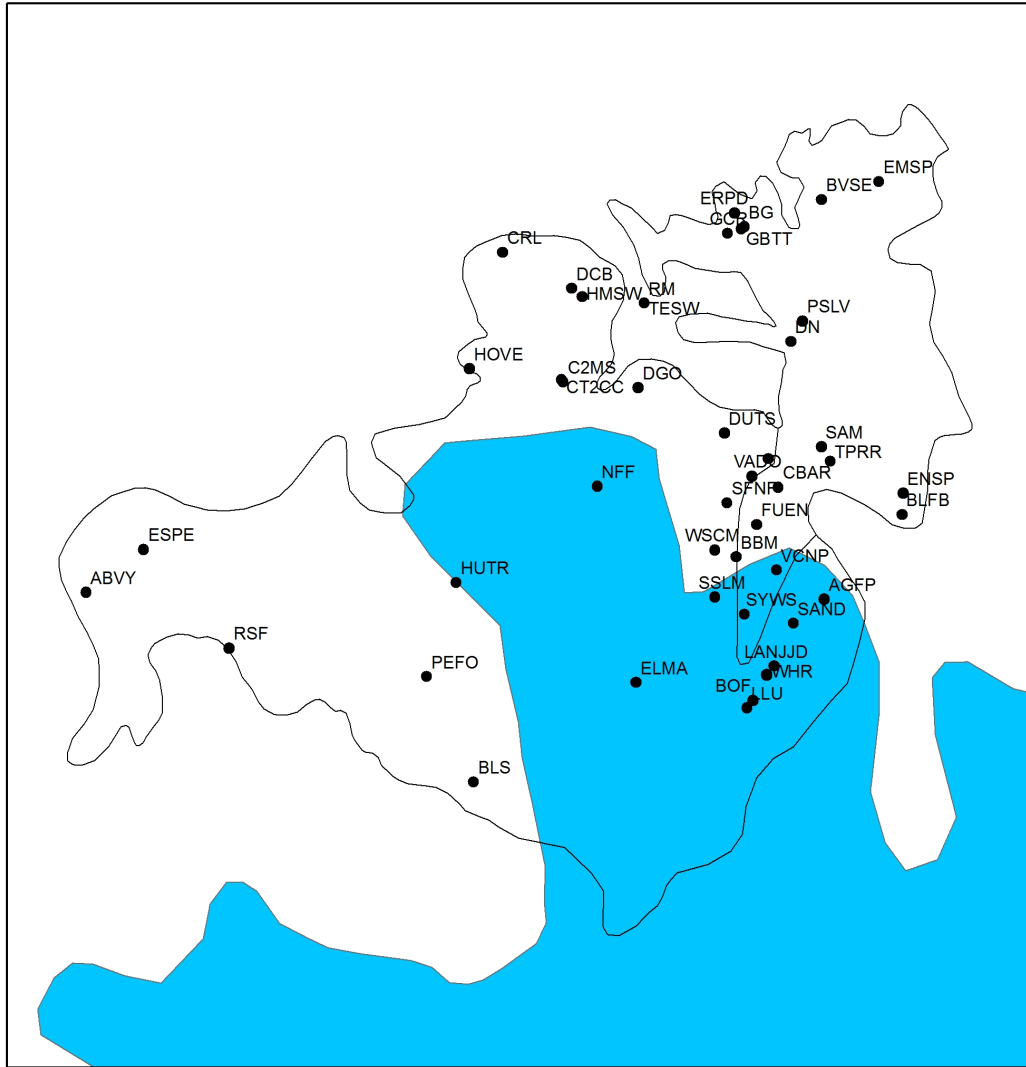
Figure 2. Overlapping geographic distributions of Gunnison's prairie dog colonies (black dots) and *Dipodomys spectabilis,* the banner-tailed kangaroo rat (shaded in blue).

Results

A total of 251 fleas from 27 different Gunnison's prairie dog colonies were collected. The average number of usable nucleotides amplified per individual at the cytochrome oxidase II region was 736 base pairs (+/- 58 standard deviations). 101 unique haplotypes were discovered, with an average haplotype diversity of 0.637 across all colonies, ranging from 0 to 0.867. The average theta(S) diversity across all colonies was 17.83 sites per individual. The maximum likelihood tree (figure 3) had a log likelihood of -5698.83.
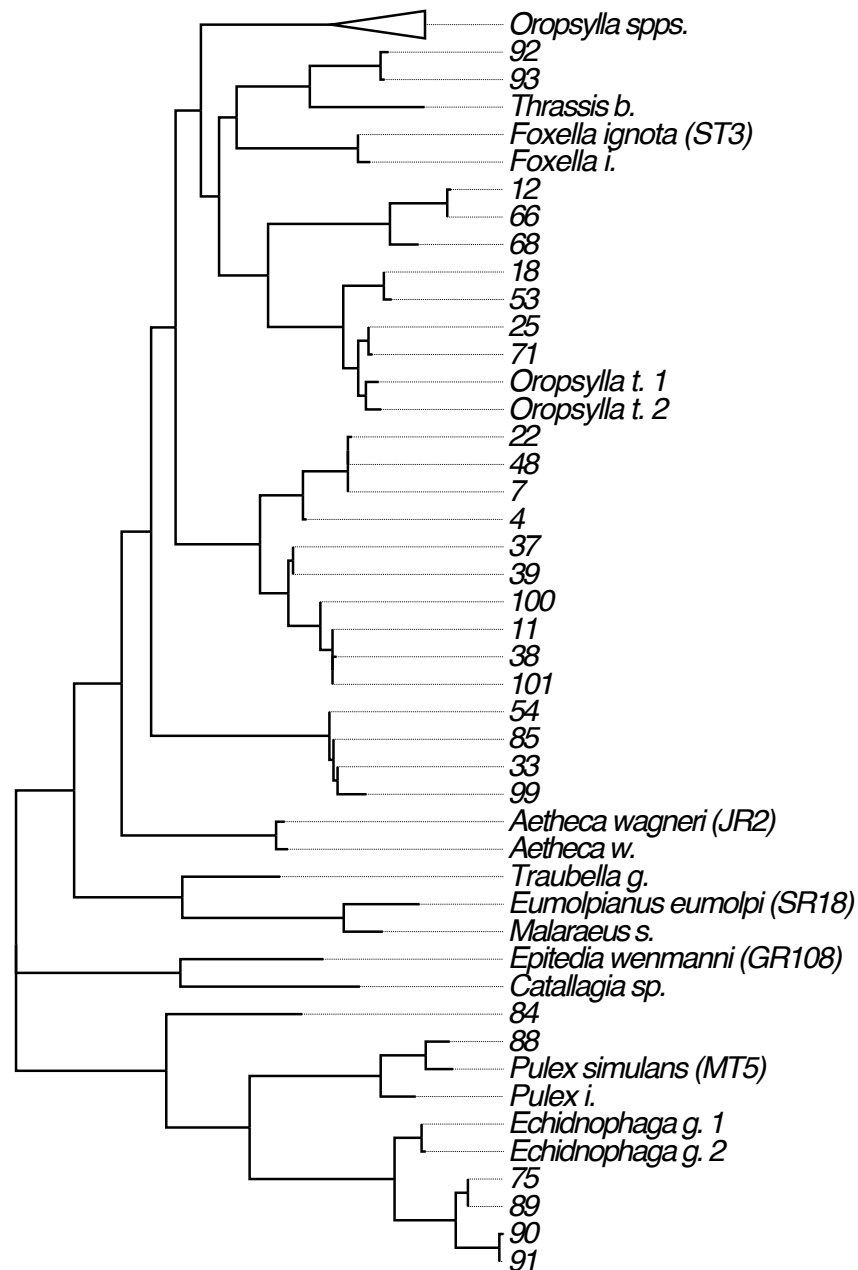
Figure 3. Flea phylogenetic tree produced using maximum likelihood. The length of horizontal branches indicates the number of DNA nucleotide differences that have occurred since the last union between two branches. The numbers are used as names to indicate unique haplotypes sampled from Gunnison's prairie dogs. The other IDs are the species names of identified fleas that were not collected in this study. Dan Tripp provided the flea species that are followed by two letters and a number in parenthesis. The abbreviated species names are fleas taken from the NCBI database. Note that the triangle shaped branch at the top-most part of the tree consists of 76 more haplotypes that have far shorter branch lengths compared to the rest of the tree, and were collapsed for visual clarity. This part of the tree consists of only one identified species, *Oropsylla hirsuta*, (also known as the common prairie dog flea), and is thought to be the primary vector of *Yersinia pestis* in prairie dog colonies (Jones and Britten, 2010).
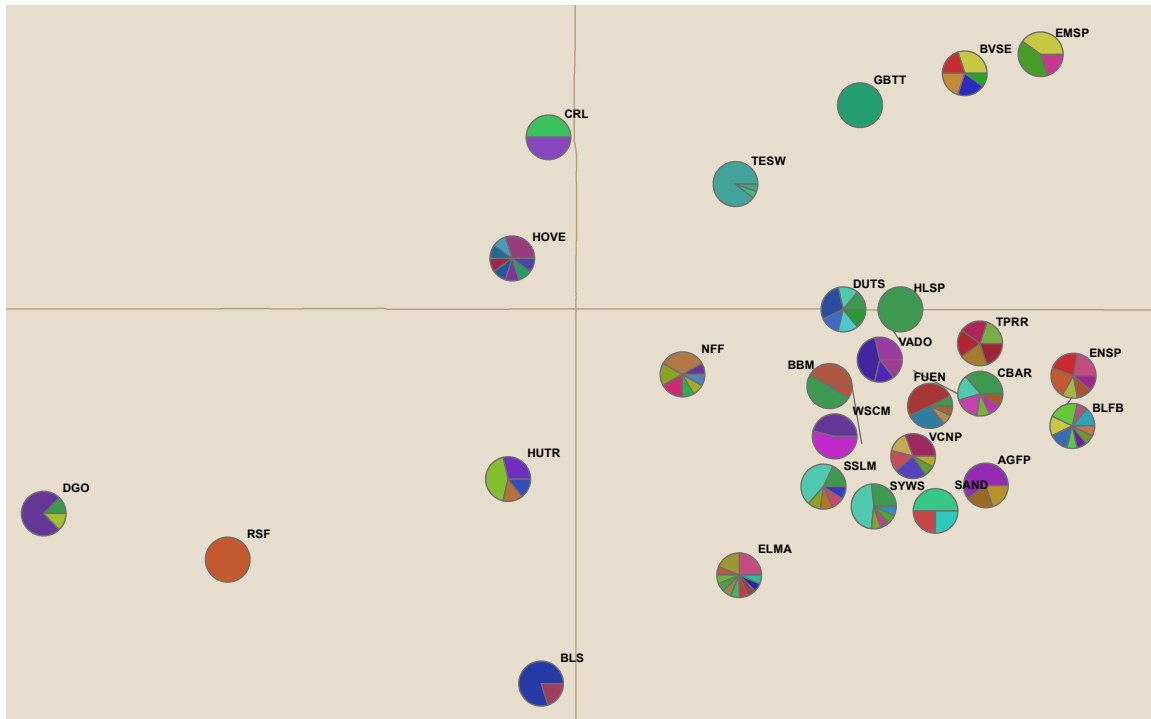
Figure 4. The number and proportions of unique flea haplotypes across colonies. Each color represents a unique flea haplotype (i.e. a sequence of DNA unique from others by at least one base-pair difference), which serves as a means to visualize how diverse a given colony is. This also depicts the level of flea relatedness between colonies based on how many haplotypes they share (for example SAND, SYWS, and SSLM all share the teal, dark green, and orange haplotypes, although the abundance of each of the haplotypes differ among the three colonies). Figure constructed using ArcMAP (Esri, California, USA). Note: colors chosen at random, similar colors *do not* depict more closely related haplotypes.

There was a significant positive correlation between flea genetic differentiation (FST) and geographic distance among the 27 colonies sampled (mantel test, r = 0.307, p = 0.003, See figure 5). Data for prairie dogs were not available at every location for fleas, so only 22 of the 27 colonies sampled could be used to test the effect of prairie dog genetic diversity on that of fleas. Of the 22 colonies sampled, there was no evidence that flea haplotype diversity is correlated with that of Gunnison's prairie dogs (r=0.193 p=0.390, figure 6). There was no evidence that either theta(S) or h measures of flea genetic diversity are linearly or predictably associated with the number of mammals (theta(S): b=0.180, p=0.188, haplotype diversity: b=1.16 , p=0.160 , see figures 7 and 8).
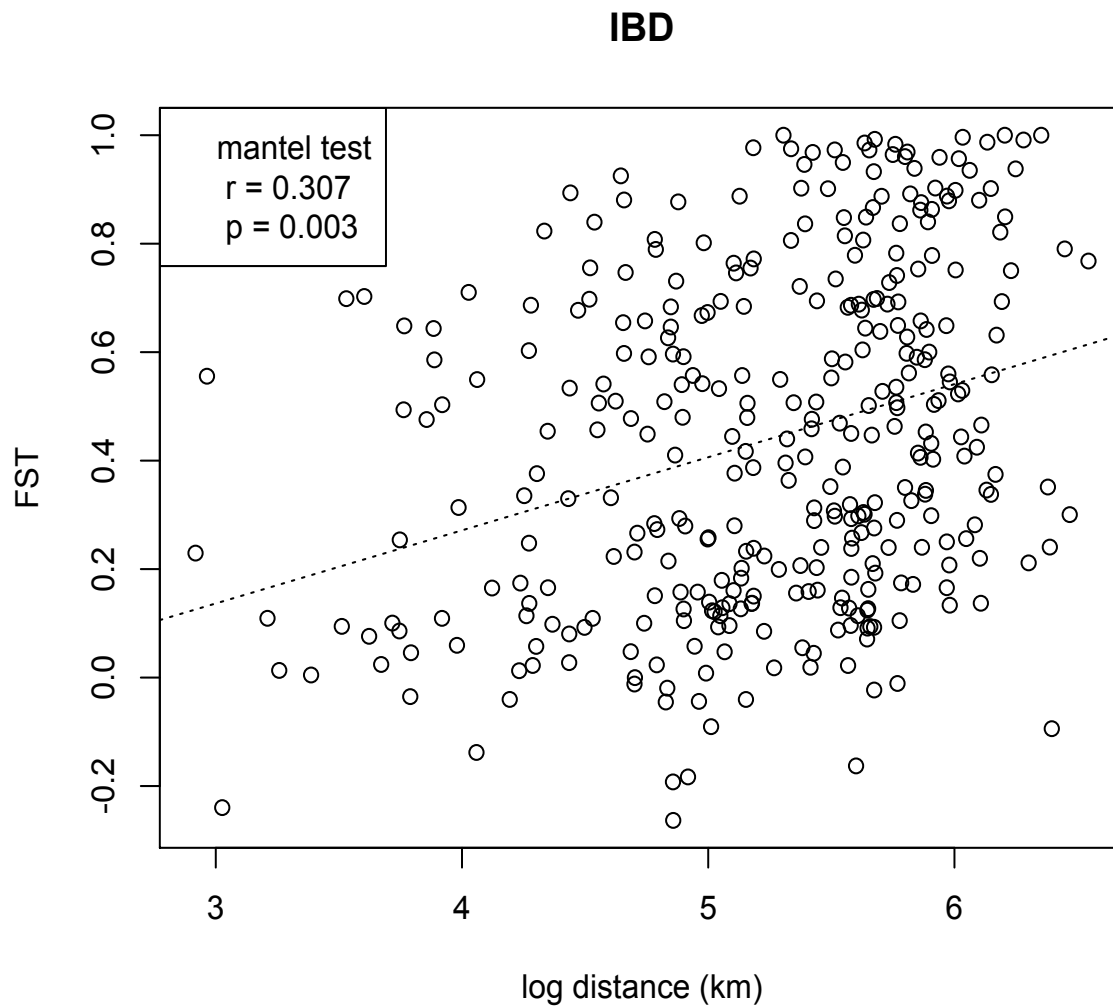
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Figure 5. Correlation between geographic distance (natural log of kilometers) and genetic differentiation among fleas (FST) used for inferring if fleas are dispersal limited across colonies due to geographic distance.
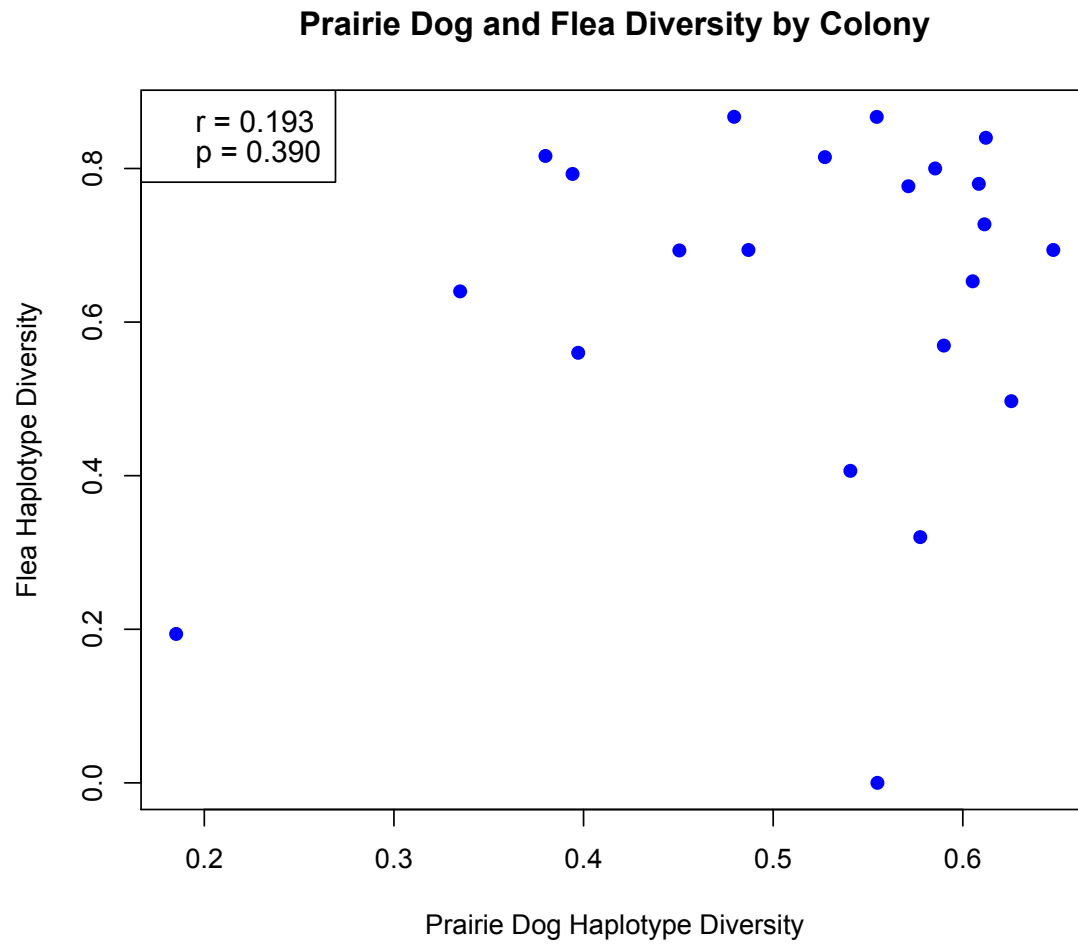
Figure 6. Relationship between genetic diversity of Gunnison's prairie dogs and fleas captured from the same colony.
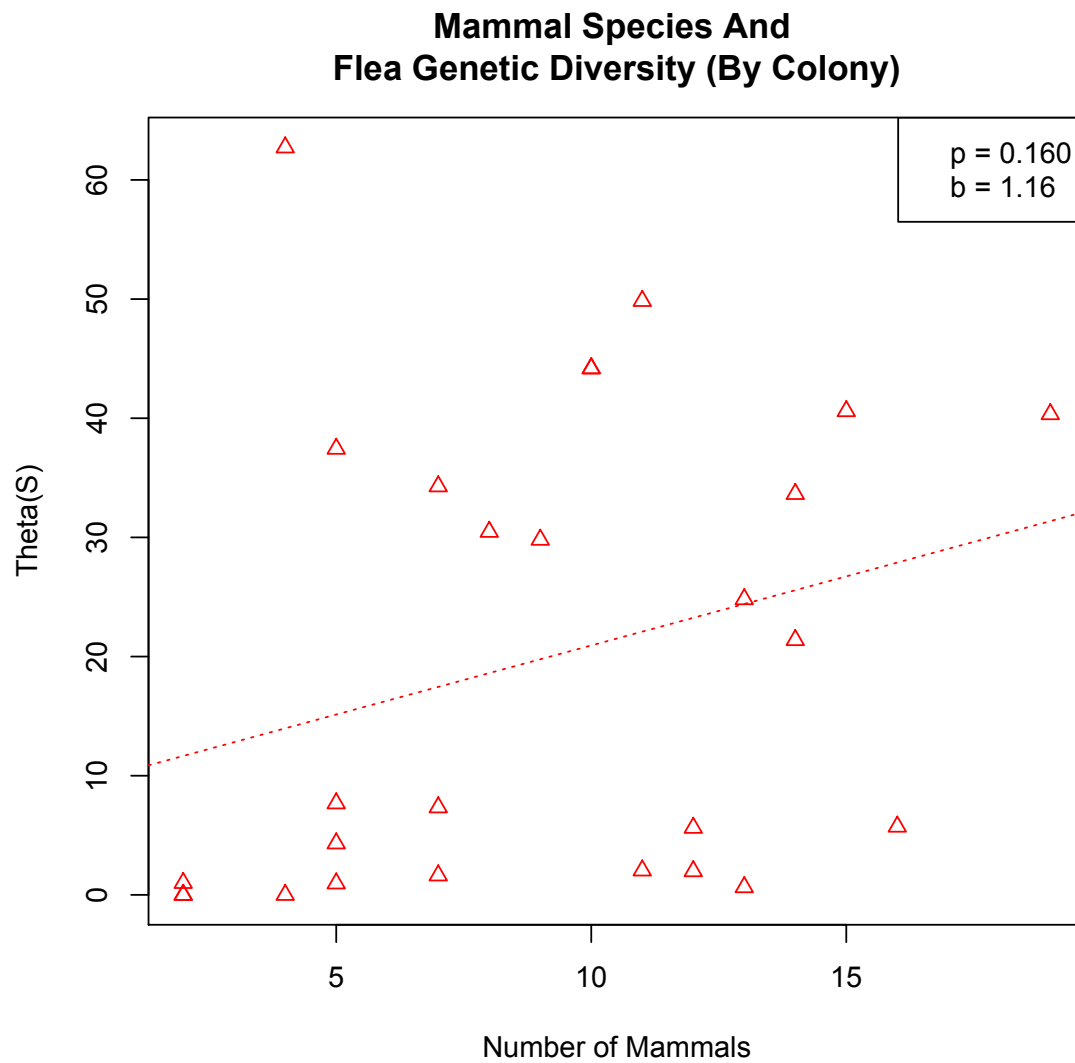
Mammal Species And
Flea Genetic Diversity (By Colony)

Figure 7. Plot of dependency of flea theta(S) on the number of mammal species present for each colony.
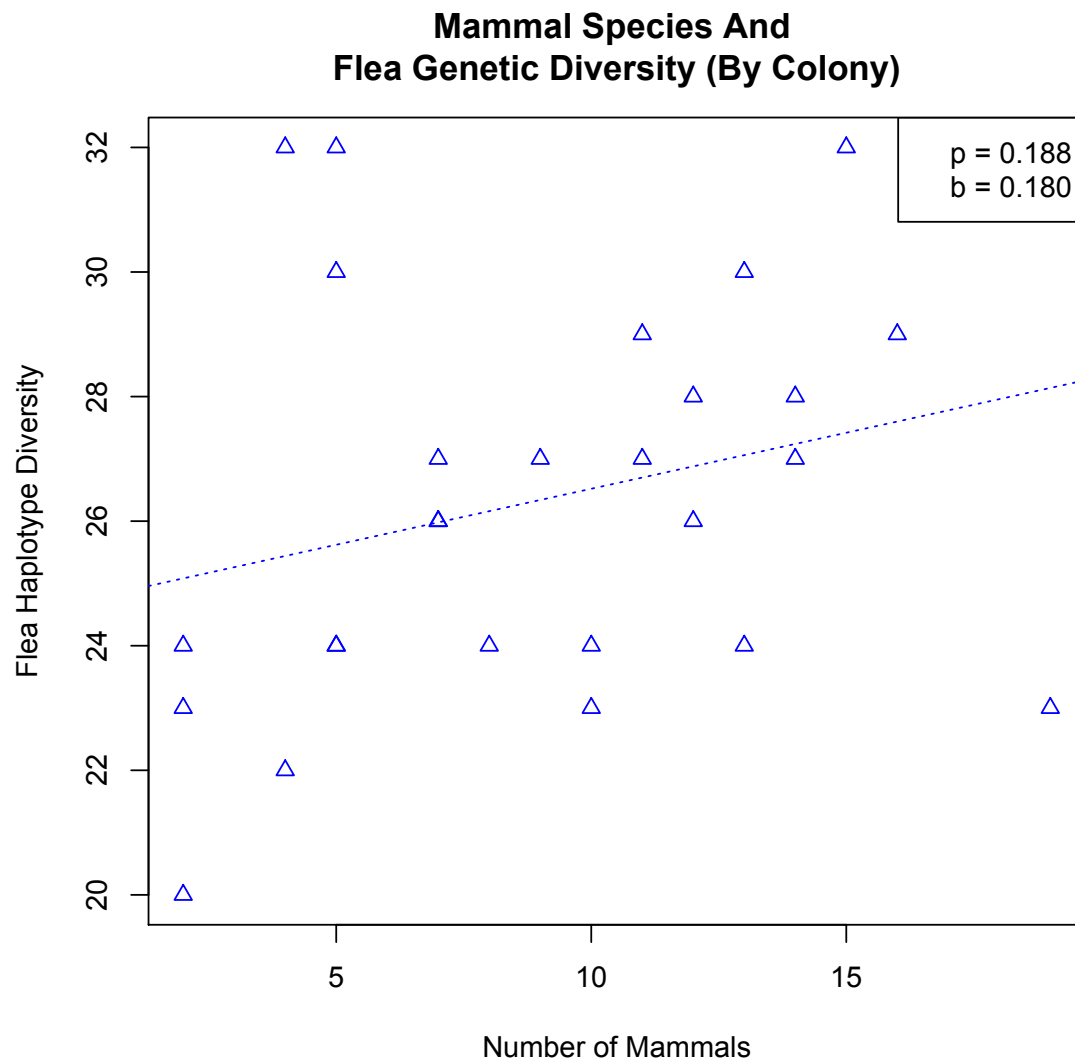
Figure 8. Plot showing the dependency of flea haplotype diversity on the number of mammal species present for each colony.

Discussion

The flea phylogeny produced using alignments of the cytochrome oxidase II gene resulted in a far greater level of flea genetic diversity than anticipated compared to the genetic diversity of fleas found in the colonies of black-tailed prairie dogs (Jones et al., 2010; Jones and Britten, 2010). The tree consists of multiple haplotype groups that are both independent from other groups, and independent from the flea species that have been identified in other studies. Phylogenetic work done by Whiting et al. (2008) was conducted using multiple genetic regions, including cytochrome oxidase II, in order to determine the relationships for a vast number of flea species; several of the identified flea species in the phylogenetic tree from this study originally came from the work by Whiting et al. The number of flea haplotypes that group together independently of the identified species, combined with the fact that extensive work has been done on fleas using cytochrome oxidase II, signifies the possibility that previously undescribed species of fleas are occurring in Gunnison's prairie dog colonies.

Unfortunately, no information was recorded about the physical characteristics of the fleas captured for this study, so it is difficult to know if the genetic diversity of these fleas is reflected in the diversity of their morphology. Though still somewhat controversial, it is generally thought that morphological traits (physical and observable) underestimate the true rate of evolutionary changes (Bromham et al., 2002). Applied here, it is unlikely then that the vast genetic diversity of fleas in Gunnison's prairie dog colonies would be equivalently matched by morphological changes. Following studies interested in comparing genetic and morphological changes are advised to carefully examine the fleas before crushing them into pieces for DNA extraction.

The phylogenetic tree effectively describes the large amount of genetic diversity of the fleas in Gunnison's prairie dog colonies. Having described the genetic diversity of these fleas,

the next component of this study was to explain the distribution of that diversity. The map of

unique flea haplotypes across colonies summarizes the immensity and span of genetic diversity

occurring in Gunnison's prairie dog colonies (figure 4). The most important information gained

from this map is the large amount of variation in the number of haplotypes that can be found in

any given colony, and the lack of any observable pattern in the levels of genetic diversity across

colonies, even for those located right next to each other. Though visually mapping the unique

haplotypes effectively describes the distribution of genetic diversity of fleas in Gunnison's

prairie dog colonies, it does not quantitatively evaluate what patterns may be underlying the

distribution.

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Finding a statistically significant result of the comparison between geographic distance

and FST in the flea populations suggests colonies that are closer together are more likely to be

composed of a genetically similar community of fleas than those that are far apart (figure 5).

This finding should be reflected in the haplotype diversity map (figure 4), where colonies nearest

to each other should share many of the same haplotypes. However, this pattern does not clearly

emerge from the map of colony haplotypes. Similarly the isolation by distance plot is highly

scattered, indicating an abundance of noteworthy exceptions in the trend of increasing

differentiation (FST) with increasing geographic distance. In the flea communities studied, the

scatter of points illuminate that there are several populations of fleas that do not follow the

idealized expectations of isolation by distance. To be more specific, each data point on the top-

most left section of the graph represent a comparison between two colonies that are a relatively

short distance apart geographically, but are comprised of very different populations of fleas

genetically. Likewise, points on the bottom right of the graph are comparisons between colonies that are relatively far apart but consist of genetically similar flea populations. An idealized example of isolation by distance would result in a scatter of points that increased in FST as geographic distance increased (that is, diagonally moving upward from left to right) and would additionally show a tight clustering of points, unlike the scatter shown in figure 5.

To summarize, the distance between colonies has little explanatory power for how genetically similar or different the flea communities located there will be. This assertion is supported both by the scatter of points in the isolation by distance, as well as the haplotype map. Together these figures show the possibility of finding genetically dissimilar flea populations in neighboring colonies, and highly similar genetic communities of fleas in populations that are far apart from each other. This result is comparable to that found by Jones and Britten (2010) in their assessment of fleas parasitizing black-tailed prairie dogs (*Cynomys ludovicianus*) in Montana, which found no evidence for isolation by distance between the colonies. Both of these studies suggest that the relative location of a prairie dog colony has little to no effect on the diversity of fleas that can be located there. These findings emphasize the importance of considering alternative possibilities as to what can predict the distribution of flea genetic diversity found in Gunnison's prairie dog colonies.

Although testing for isolation by distance is an effective method for inferring large scale dispersal patterns, knowing more about the ability and frequency of flea migration would be a valuable place for further investigation. One way to gain higher confidence in migration patterns of fleas would be to completely exterminate their standing populations in a given colony, and then resample that population after enough time has passed to allow fleas from other regions to recolonize. Assessing the genetic diversity and abundance of fleas that recolonized could provide

information about the rate of migration, as well as possible locations from which fleas are migrating. Knowing more about flea migration across colonies would greatly enhance our ability to make the best management decisions in times of disease outbreak. Seery et al. (2003) showed that dusting black-tailed prairie dog colonies with the insecticide deltamethrin noticeably reduced the number of fleas that could be found in burrows and living on prairie dogs. In addition, the authors suggest deltamethrin may act to protect prairie dogs from plague outbreaks, based on the knowledge that colonies which went undusted in their study were decimated during a plague outbreak, while the deltamethrin dusted colonies nearby remained without casualties. Having the ability to control flea populations within prairie dog colonies–combined with understanding of how flea migration occurs among colonies could together increase the ease and efficiency of suppressing plague and other flea transmitted diseases.

Diversity of Gunnison's Prairie Dogs

Given the data, there is no evidence that prairie dog genetic diversity has an effect on that of fleas. This finding may suggest there is not enough variation in Gunnison's prairie dog's defense mechanisms against fleas to drive them towards host specificity. As Krasnov (2008) discusses, hosts have three main lines of defense against their parasites: avoidance (preventing contact), repelling (grooming), and immune response. Even though the fleas sampled are very diverse genetically, likely constituting multiple species, they may be equipped with similar mechanisms to evade their host's defenses, suggesting selective pressures other than feeding have driven genetic divergence among these fleas.

Krasnov (2008) additionally discusses the fact that adult flea's survival depends entirely on gaining access to a host's blood, imposing strong selection on their ability to do so. In contrast, a prairie dog's survival is far less dependent on flea avoidance, which imposes weaker

selection on their defensive traits. Based on Krasnov's assertions, the differential selection pressures on fleas and prairie dogs may explain the ability of genetically diverse fleas' continued abilities to parasitize Gunnison's prairie dogs; flea evolution is constrained by their ability to gain access to a host's blood. In contrast, while Gunnison's prairie dogs may benefit by defending themselves against fleas, their survival does not depend on fleas the same way fleas' survival depends on them. [4]

Limitations of the findings for this test are worth consideration. The means of testing if Gunnison's prairie dog genetic diversity influences flea genetic diversity was done by comparing single values of genetic diversity for fleas and prairie dogs from the same colony. This is problematic because it does not assess the genetic diversity of fleas parasitizing particular prairie dogs, or the potential variation of prairie dog genetic diversity within a colony. An alternative way to test this hypothesis would be to ask what amount of flea genetic diversity is partitioned among individual prairie dogs within colonies, and if genetic differences exist between prairie dogs. In doing so, it would also be necessary to determine if all prairie dogs harbored the same number of fleas, or if there was significant variation in the number of fleas found on each prairie dog, all the while keeping in mind the potential effect of genetic differences individual prairie dogs may have on fleas. These tests would more effectively evaluate the influence of Gunnison's prairie dog genetic diversity on the diversity of fleas parasitizing them.

Diversity of Mammal Community

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[4] This assertion may be weakened when considering the added selective pressure for prairie dogs to avoid fleas due to the risk of disease. However, there is evidence that prairie dogs have only recently been introduced to these microbes (Jones et al., 2010; Girard et al., 2004), so natural selection may not have had the time nor the genetic variation needed to induce noticeable changes in heritable defense strategies against fleas as disease transmitters.

No relationship was found between flea genetic diversity of a colony and the number of overlapping mammal species distributions. The same arguments about host specificity discussed concerning prairie dogs may still apply here; the groups of mammal species considered in the data may be too similar in their defense adaptations against fleas to drive signatures of host specificity. If true, this would indicate fleas in Gunnison's prairie dog colonies have evolved to be host generalists. Poulin et al. (2006) studied trends in flea host specificity over a wide geographic range in an attempt to determine if fleas have become more or less host specific over their evolutionary history. While the results of the authors' investigation were admitted to be equivocal, they found some evidence for an overall decrease in host specificity signified by younger flea lineages having a larger number of potential hosts. Suggesting the same for fleas in this study would be unfounded because they were collected straight from the sleeping bodies of Gunnison's prairie dogs without any evidence or reason to believe they had prior interaction or contact with any other mammal species. Determining if fleas in Gunnison's prairie dog colonies are able to effectively parasitize multiple mammal species, fleas would need to be collected while feeding from these mammals, and genetically compared to those caught feeding on Gunnison's prairie dogs. If the fleas collected in this data set were identified as genetically similar (using FST, or perhaps their relative location in a phylogeny) to those collected feeding on different mammal species, this would indicate them being host generalists.

Future Studies

An active area of research not considered here is the influence of a location's biodiversity and the potential effect this has directly on pathogen transmission. The disease ecology principle known as the dilution effect is described as the decrease in infection risk of a

primary host as a result of increasing the species diversity in the same location (Ostfeld and Keesing, 2000). A laboratory study by Eisen et al. (2008) showed that *Yersinia pestis* is able to survive within a flea vector for different durations depending on if the source of a fleas' blood meal came from a rat, rabbit, or mouse, where rat blood allowed *Y. pestis* to persist for the longest duration. Ostfeld and Keesing (2000) showed a decrease in percentage of rodents infected with *Bartonella* decreased as their community biodiversity increased. Together these findings provide evidence that the number of suitable hosts for fleas and insect vectors may not equate to suitable environs for the pathogenic microbes they harbor. As it was mentioned earlier, the species of flea may also have an effect on the ability of a pathogen to be transmitted to a host. Taken together, these phenomena could lead to several more studies concerning the diversity and number of mammal species that can be found in a colony, and how this might affect pathogen transmission directly rather than secondarily though the flea vector.

The differing climatic conditions across Gunnison's prairie dog colonies have been completely ignored in this study, but previous research has shown climate and temperature to have clear effects on both fleas and their microbial pathogens. Flea diversity has been shown to increase with warm moist climatic conditions, as it positively affects their ability to reproduce, their rate of metamorphosis and their survival at immature stages. In addition, the persistence of plague has been shown to be limited to climatic conditions of arid to semi-arid, where humid tropical conditions and extreme desserts show very few cases of plague in any organisms (Ari et al., 2011). Although the Gunnison's prairie dog colonies occur over a wide range of climatic conditions, knowing if there is enough variation among the colonies to influence the diversity of fleas and the persistence of *Y. pestis* within them is not known, indicating another potentially fruitful area of future research.

Conclusion

The goal of the study presented was to determine what variables predict the geographic

distribution of genetic diversity of fleas parasitizing Gunnison's prairie dogs. This information is

valuable because of the differential abilities of flea species to transmit disease-causing microbes

to their host organisms. Although none of the factors tested provided viable explanations for the

observed distribution of flea genetic diversity, the study's results still provide implications for

the spread of disease-causing microbes that fleas harbor. First, the large amount of overall flea

genetic diversity observed in Gunnison's prairie dogs was far greater than expected given the

results from studies in other species of prairie dogs. The genetic differences found between fleas

in this study and those previous studies suggests the possibility that multiple undescribed species

of fleas are parasitizing Gunnison's prairie dogs. Additionally, the genetic diversity of fleas was

randomly scattered across colonies without signatures of consistent flea migration patterns.

Combined with knowledge that flea species have different levels of efficiency in transmitting

pathogenic microbes, different colonies of Gunnison's prairie dogs are likely to have differing

risk levels of flea transmitted disease outbreaks, and that the future location of outbreaks will be

very challenging to predict. Whether high flea genetic diversity increases or decreases the risk of

disease spread is unknown, but this is a valuable place to continue research. Second, there is no

evidence that the distribution of prairie dog genetic diversity influences the distribution of flea

genetic diversity. This likely indicates all species of fleas collected are similarly able to evade

Gunnison's prairie dog defenses. This finding suggests that all individual Gunnison's prairie

dogs will have a similar susceptibility of disease regardless of which flea species most efficiently

transmits a given pathogen. Third, there is no evidence that the number of mammal species

available as hosts influence the distribution of flea genetic diversity. Further analyses are needed to better understand the impact of the mammal community, as they are likely to play an important role in disease transmission in Gunnison's prairie dog colonies.

Further investigations on the distribution of flea genetic diversity in Gunnison's prairie dog colonies are recommended to incorporate climatic and environmental data, as these have been shown to impact both fleas and the causative microbial agents they harbor.

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Appendix 1. IUCN Mammal species' distributions used in study

Dipodomys ordii
Dipodomys spectabilis
Neotoma albigula
Neotoma micropus
Onychomys arenicola
Onychomys leucogaster
Perognathus flavescens
Peromyscus leucopus
Peromyscus truei
Reithrodontomys megalotis
Reithrodontomys montanus
Ammospermophilus interpres
Ammospermophilus leucurus
Chaetodipus hispidus
Chaetodipus intermedius
Cynomys leucurus
Cynomys ludovicianus
Dipodomys merriami
Lepus americanus
Lepus californicus
Lepus townsendii
Macrotus californicus
Microtus mexicanus
Microtus montanus
Microtus ochrogaster
Microtus pennsylvanicus
Neotoma cinerea
Neotoma leucodon

Neotoma mexicana
Neotoma stephensi
Notiosorex crawfordi
Peromyscus crinitus
Perognathus fasciatus
Peromyscus nasutus
Sorex cinereus
Sorex monticolus
Sorex palustris
Spermophilus lateralis
Spermophilus spilosoma
Spermophilus tridecemlineatus
Spermophilus variegatus
Sylvilagus audubonii
Sylvilagus floridanus
Sylvilagus nuttallii
Tamias cinereicollis
Tamias dorsalis
Tamiasciurus hudsonicus
Tamias minimus
Tamias quadrivittatus
Tamias rufus
Thomomys bottae
Thomomys talpoides
Vulpes macrotis
Vulpes velox
Vulpes vulpes