SPATIAL DYNAMICS OF MAMMALS AND THEIR PATHOGENS AND PARASITES

BY

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ABSTRACT

In this dissertation, I explore several aspects of the ecological dynamics of mammals and their pathogens and parasites. I approach this broad topic at various scales, using Ecological Niche Models, field surveys, and theoretical simulations. I focus on two pathogens, hantavirus and plague (*Yersinia pestis*), and a group of parasites, fleas, and address their spatial and ecological relationships. Each of the four chapters presents a set of questions and tests hypotheses regarding the distribution of these taxa. I begin by demonstrating that plague-infected host distributions are not similar to the non-infected host distributions, suggesting that vector ecology may drive the distribution of plague in the western United States. I then show that hantavirus prevalence and flea communities are not mediated by mammalian communities across a contiguous landscape, and flea communities differ with increasing elevation. Finally, I show that re-appearance of hantavirus after a decline in host populations likely is not driven by metapopulation dynamics.

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INTRODUCTION

In this treatment, I present 4 chapters regarding the dynamic relationship between mammals, the environment, and their pathogens and parasites. I focus on the spatial aspects of the distribution and maintenance of the taxa of various scales to present a broad picture of these interactions. Within each chapter, I ask a series of questions to examine particular aspects of the ecological and spatial properties of a system. There are two main pathogens I discuss, hantavirus and plague (*Yersinia pestis*), and a single parasite group, fleas. Hantavirus infects members of the genus *Peromyscus*, but does not cause severe disease or affect mortality in the mice. On the other hand, *Y. pestis* infects virtually all mammals and the hosts develop plague, while fleas are found on all terrestrial mammals and are the vector for plague.

The first chapter asks if mammalian host distributions drive the distribution of plague infections in these hosts. The scale of this chapter is the contiguous United States and parts of southern Canada, as plague is documented throughout much of the western area of the range. The analysis takes advantage of (1) Ecological Niche Modeling to produce estimates of host and infected-host distributions and (2) randomization to address the similarity between these distributions.

The second and third chapters use field surveys to address patterns of mammals and their pathogens and parasites at landscape scales across an elevational gradient. I surveyed the small mammal community in and around Rocky Mountain National Park, in concert with collecting data on hantavirus and plague infection prevalence in these hosts. The surveys included collection of fleas, which were tested for *Y. pestis* DNA and their community was analyzed along the gradient.

In the fourth and final chapter, I present a simulation of *Peromyscus* populations on a single landscape to test a refugia–recolonization hypothesis of hantavirus occurrence. Host populations are known to fluctuate, and low host numbers inevitably lead to a perceived local extinction of the virus, such that dispersal is presumed to be required to re-establish the virus in the local population. I use a spatially explicit, agent-based, cellular automata model to evaluate the likelihood of this scenario under a variety of initial conditions.

CHAPTER 1

Range-wide determinants of plague distribution in North America

Abstract

Plague (*Yersinia pestis*) is established across western North America, and little is known of what determines the broad-scale dimensions of its overall range. I test whether its North American distribution represents a composite of individual host-plague associations (the Host Niche Hypothesis), or whether mammal hosts become infected only at sites overlapping ecological conditions appropriate for plague transmission and maintenance (the Plague Niche Hypothesis). I take advantage of a novel data set of plague records in wild mammals to develop rangewide tests of ecological niche similarity between mammal host niches and plague-infected host niches. Results indicate that plague infections occur under circumstances distinct from the broader ecological distribution of hosts, and that plague-infected niches are similar among hosts; hence, evidence coincides with the predictions of the Plague Niche Hypothesis, and contrasts with those of the Host Niche Hypothesis. This “plague niche” is likely driven by ecological requirements of vector flea species.

Introduction

Spatial scale is now recognized as an element critical to an integrative understanding of ecology (Levin 1992; Wiens 1989). In disease ecology, local-scale studies have dominated (Collinge and Ray 2006; Ostfeld et al. 2008), while synthetic, broader-scale analyses have been less frequent (Biggins and Kosoy 2001; Brooker and Clements 2009; Eisen et al. 2007; Glass et al. 2000). Integrative cross-scale analyses—i.e., detailed understanding of local-level processes placed in the context of regional processes of range limitation and biogeography—are rare, and have been developed for few disease systems.

Plague, caused by the bacterium *Yersinia pestis*, is transmitted among susceptible hosts by the bites of fleas infected previously by feeding on other hosts that were highly bacteremic (Gage and Kosoy 2005; Wimsatt and Biggins 2009). Such transmission is most effective for many flea species within the first few days after taking an infectious host blood meal and at later time points for a smaller number of flea species in which midgut blockage occurs (Barnes 1982; Eisen et al. 2006; Eskey and Haas 1939; Gage and Kosoy 2005; Perry and Fetherston 1997; Wilder et al. 2008; Wimsatt and Biggins 2009). Plague bacteria also may survive outside living hosts on carcasses or in the soil, but only limited evidence exists to suggest that such mechanisms are important for long-term survival of *Y. pestis* in nature (Ayyadurai et al. 2008; Eisen et al. 2008; Gage and Kosoy 2005). Plague likely evolved in Asia (Achtman et al. 1999; Gage and Kosoy 2005; Parkhill et al. 2001), and has since spread broadly by various means (Achtman et al. 1999), including the transport of infected hosts and fleas along overland trade routes or aboard rat-infested ships during the three historically documented pandemics. The third of these pandemics and that most relevant to the present study introduced plague to North American ports in the late nineteenth century; where it afterwards “escaped” into native rodent

populations, eventually spreading east through the Rocky Mountains to the western edge of the Great Plains (Biggins and Kosoy 2001; Gage and Kosoy 2005).

Plague manifests in two distinct types of cycles: epizootic cycles, in which the disease spreads rapidly among highly susceptible rodent species, often resulting in the virtual elimination of local host populations, and enzootic cycles, in which few host individuals die and the disease is maintained in the population over a longer term (Gage and Kosoy 2005; Perry and Fetherston 1997; Wimsatt and Biggins 2009). The epizootic cycle is well-studied in prairie dogs (*Cynomys* spp.) and other social sciurids that live in colonies and exhibit local extinction upon exposure to the bacterium (Barnes 1993; Biggins and Kosoy 2001; Collinge et al. 2005a; Ray and Collinge 2006; Stapp et al. 2004; Stapp et al. 2008; Tripp et al. 2009; Webb et al. 2006). Additional potential rodent hosts are known, and plague was proposed to be enzootic in some, although the role of deer mice (*Peromyscus*) as enzootic hosts was challenged recently (Biggins and Kosoy 2001; Gage et al. 1995; Perry and Fetherston 1997; Salkeld and Stapp 2008). Nevertheless, most mammals are clearly susceptible (Biggins and Kosoy 2001). Another small rodent, the grasshopper mouse, *Onychomys leucogaster*, is relatively resistant to plague in some areas, leading some to propose that it might play a role in long-term maintenance and transmission of plague over its widespread range in North America (Biggins and Kosoy 2001; Stapp 2007; Stapp et al. 2008; Thomas et al. 1988). Wild carnivores also are frequently found to be seropositive, but probably are of little importance as sources of infection for vector fleas and thus play no direct role in the natural host-to-flea-to-host cycle. However, these animals might be important as temporary flea hosts, transporting infected rodent fleas from one site to another. In the face of such complex host relationships, factors that maintain the geographic distribution of plague are not well understood; hence, in this contribution, I propose and test two contrasting hypotheses

regarding the distribution, ecology, and geography of plague at coarse resolutions across western North America.

The “Host Niche Hypothesis” (HNH) postulates that plague distributions are mediated by host distributions, such that the distribution of plague depends on an amalgam of host ranges, and the presence of a particular host species could extend the distributional potential of the pathogen (Figure 1). Alternatively, plague may have its own distinct ecological niche, with infections occurring only in regions where hosts' distributions overlap this ecological and geographic potential (Figure 1). This “Plague Niche Hypothesis” (PNH) suggests that plague distribution is independent of particular host distributions, but rather is mediated by other factors such as vector ecology. Under this view, plague occurs in a particular mammal taxon only if and where its distribution overlaps sites fitting the conditions of the plague niche.

Here, I test HNH and PNH using a framework incorporating ecological niche models (ENMs). HNH predicts that plague infections in hosts will not be distinct ecologically from the overall ecology of the host; PNH, on the other hand, predicts differences between the two. More importantly, HNH predicts distinct ecological profiles for plague-infected distributions of different host species, while PNH would expect those profiles to be similar. Previous efforts have shown that *Y. pestis* occurrence in humans who are accidental hosts of this bacterium is highly predictable using ENMs (Nakazawa et al. 2007; Neerinckx et al. 2008)—in other words, coarse-scale spatial correlates exist that provide a predictive view of human plague geography. Here, I use new ENM-based tools to distinguish between these two views of plague ecology.

Materials and Methods

Input data.—I used digitally captured contents of a massive filing system of zoonotic records of plague-positive samples in North America maintained at the Centers for Disease

Control and Prevention, Ft. Collins, Colorado. Animals were considered plague-positive when samples obtained from them tested positive by one of the following assays: serology, direct immunofluorescence, or bacterial culture (Chu 2000). From the resulting data set, 3777 occurrence points that include host-related ancillary data for a total of 75 host taxa (all mammals) were georeferenced. I focused analyses on the wild mammal taxa showing greatest densities of records: *Canis latrans* (2516 points), ground squirrels (including *Ictonomys* spp., *Callospermophilus* spp., and *Urocitellus* spp.; 150), *Taxidea taxus* (106), tree squirrels and chipmunks (including *Tamias* spp., *Tamiasciurus hudsonicus*, and *Sciurus* spp.; 100), *Cynomys* (including *Cynomys gunnisoni*, *C. leucurus*, *C. ludovicianus*, and *C. parvidens*; 69), *Peromyscus* spp. (69), *Ursus americanus* (43), and *Neotoma* spp. (29; see Figure 2). Geographic coordinates were derived for all of these records following standard point and error radius protocols, (Wieczorek et al. 2004) by means of referring to Terrain Navigator, Topozone (http://www.topozone.com), and Graphical Locator (http://www.esg.montana.edu). Records showing uncertainty radii of >10 km were removed from analyses, as were redundant localities associated with the same host.

Host mammal occurrence data (i.e., general occurrences not associated with plague infections) were collated from records provided online from 29 natural history museums via the MaNIS (http://manisnet.org/) and Arctos (http://arctos.database.museum/) biodiversity data portals (see Figure 2). In addition to the taxa listed above, I gathered *Onychomys leucogaster* occurrence data for inclusion in analyses, in light of its possible role in plague maintenance. Geographic coordinates had been derived previously for these records as part of the MaNIS project, following standard point and error radius protocols; (Wieczorek et al. 2004) again, all records showing uncertainty radii of >10 km were removed from analyses, and all redundant

localities were removed. I pooled occurrence data across host taxa in groups, as described above for plague occurrence data. Host occurrence points were abundant (541–5614 per species), and covered the entire ranges of each host species.

To characterize environmental landscapes across North America, I used climatic layers from the WorldClim (Hijmans et al. 2005) data archive (http://www.worldclim.org/) at a spatial resolution of 5 km, matching the approximate precision of occurrence data. To avoid fitting models in overly dimensional environmental spaces, I assessed patterns of correlation among the 19 bioclimatic variables from WorldClim, and chose 7 that were relatively uncorrelated: annual mean temperature, mean diurnal temperature range, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, precipitation of wettest month, and precipitation of driest month. All of the analyses are based on fitting models in this 7-dimensional space, in a study area extending across southern Canada and the continental United States.

Niche modeling.—ENMs were generated for each host species and for plague-infected individuals of each host species using Maxent (Phillips et al. 2006). This algorithm calculates a probability distribution from the environmental data and occurrence data based on the principle of maximum entropy: i.e., that the best explanation for a phenomenon is that which shows the broadest and most-spread-out probability distribution. Maxent fits this distribution subject to particular constraints, in this case, environmental values associated with known presences (Phillips et al. 2006; Phillips and Dudík 2008).

Because Maxent outputs include significant elements of overfitting to sampling bias (Peterson et al. 2007), it is important to convert continuous raw Maxent outputs (logistic format) to binary formats. This step of thresholding carries a series of assumptions, which I sought to

simplify as much as possible. As a simplest option, I used the minimum suitability value assigned to any training presence record as a threshold for converting logistic (continuous) outputs to binary format (Pearson et al. 2007).

Measuring niche similarity.—Warren et al. (Warren et al. 2008) presented tools for assessing identity and similarity of ecological niches. Given that many of the ranges of the species examined in this study differ in their overall extents, I focused on Warren et al.'s background similarity measures, to avoid the false-positive errors that are common in niche identity tests (A. T. Peterson, unpublished data). I calculated the Hellinger's-based *I* and Schoener's *D* indices (1) for all pairwise combinations of the eight mammal host groups, (2) for all pairwise combinations of plague-infected records of the eight host groups, and (3) comparing overall versus plague-infected records for each host group.

For the background similarity tests, a sampling region must be designated, preferably one that represents the accessible geographic area, in essence the "M" or mobility circle in the BAM diagram presented by Soberón and Peterson (Soberón and Peterson 2005). Accordingly, under a simple assumption of uniform dispersal distance, I generated minimum convex polygons around the set of plague-related points for all taxa, which I buffered by 500 km to represent an area hypothesized as accessible to the species. These polygons were converted to raster-format grids, which were set as areas from which to generate random localities in Warren et al.'s ENMTools (http://enmtools.com/). In ENMTools, for each pairwise comparison, I drew 100 random points from the background 100 times, and generated ENMs based on those points in Maxent; outputs were converted to binary format using the minimum suitability value (see above) as a threshold; and *I* and *D* values determined (Figure 4). In comparing ENMs, I asked the statistical question, "are they similar?" As such, the null hypothesis was that the two species were similar, which I

rejected when observed *I* and *D* values from ENMs based on known occurrence points were in the lowest 5% of the randomized similarity values.

Results

Modern North American plague occurrences are exclusively in the western half of the continent, mostly in the Rocky Mountains (Figure 2). Plague-infected host groups appear—visually, at least—to have different distributional patterns: plague-infected *Canis latrans* and *Taxidea taxus* occur over the broadest areas, whereas other taxa have more restricted areas of occurrence or possible incidental infections. Mammal host taxa are unevenly distributed across the region, with concentrations of plague detections in California, along the eastern edge of the Rocky Mountains, and on the Colorado Plateau.

ENMs based on plague-infected hosts differed markedly from those based on overall host ranges (Figure 2): plague infections do not cover the entirety of any of the host group ranges geographically. Ecologically, for all host taxa, plague-infected hosts were significantly non-similar from overall ENMs (for Hellinger's-based *I* and Schoener's *D*, both $P < 0.05$; Figure 3). Further, plague-infected host ENMs were generally not similar to *any* of the overall host ENMs (for *I* and *D* metrics, both $P < 0.05$ in 55 of 56 comparisons; Figure 4). Only one comparison, *Cynomys* versus plague-infected *Canis latrans*, failed to reject similarity in both metrics ($P > 0.05$). Comparisons of the overall distribution and ecology of *Onychomys leucogaster* to the environmental 'background' of plague-infected hosts were all statistically not similar ($P < 0.05$).

However, ENMs based on plague occurrences in different host taxa tended to be similar to one another, with tests only rarely rejecting the null hypothesis of similarity. Indeed, 70% of such comparisons could not reject ecological similarity between plague-infected host taxon models in at least one of the metrics ($P > 0.05$). Moreover, of the 17 comparisons that rejected

ecological similarity, most (64%) were rejected in only one metric (Figure 3). Most exceptions came from plague-infected *Canis latrans* occurrences, which tended to be more distinct ecologically from the environmental 'background' of records of other plague-infected taxa ($P < 0.05$ unequivocally in 5 of 7 comparisons; Figure 3). Principal components analyses of the environmental data suggest that the plague-infected *C. latrans* ENM represents a broader suite of environmental conditions and that other taxa generally are distributed within a subset of these conditions (Figure 5). Comparisons of other host infections to the environmental background of plague-infected *C. latrans* failed to reject similarity in either metric ($P > 0.05$). The only other non-similar comparisons were plague-infected ground squirrel points compared to plague-infected *Cynomys* and plague-infected *Ursus americanus* backgrounds, and plague-infected tree squirrel points compared to the plague-infected *Cynomys* background.

Discussion

In general, the rangewide analyses showed that plague infections occur in ecologically non-random subsets of the distributions of each host species, but that plague infections in different host taxa occur under similar ecological circumstances. Hence, I found ample support for PNH expectations, but none of the predictions of HNH was fulfilled. In particular, the significant non-similarity between all plague ENMs and associated host ENMs is suggested by the PNH, because host species get infected with plague only where they overlap the plague 'niche.' HNH predictions of non-similarity between plague-infected ENMs were not supported, given that most of these comparisons were similar, which is consistent with PNH predictions.

An additional possibility for consideration is the idea that a single host may drive the geographic distribution of plague, such that its ecological characteristics would be similar to those of plague infections in other taxa. However, of the 64 pairwise comparisons of overall host

ENMs to plague-infected 'backgrounds,' 63 rejected similarity. These results suggest strongly that host distributions differ from plague-infected distributions, and that these taxa do not drive plague distributions at geographic scales. Further, the eight comparisons of the potential enzootic host *Onychomys leucogaster* to plague-infected 'backgrounds' rejected similarity in all cases, suggesting that its distribution does not reflect plague occurrence in other taxa.

The sole occurrence of ecological similarity between a host group and plague-infected individuals of another host group was between overall occurrences of *Cynomys* and plague-infected *Canis latrans*. This similarity presents an intriguing scenario: *Cynomys* exhibits epizootic cycles with extremely high mortality (Ray and Collinge 2006; Stapp et al. 2004; Stapp et al. 2008; Tripp et al. 2009; Webb et al. 2006), and coyotes are known to feed on carcasses and move among *Cynomys* colonies, such that they have been suggested as a dispersal vector for passing plague between prairie-dog towns (Boone et al. 2009). Inferring that *Canis latrans* movements may be driving *Cynomys* outbreaks is intriguing and tempting, but premature, as the bacterium may be maintained by a variety of factors (Webb et al. 2006). Possibly, seropositive *Canis latrans* individuals dispersed into novel areas not representative of plague within the dataset, which would explain the general non-similarity of other plague-infected backgrounds to plague-infected *C. latrans* (Figure 3). More conservatively, if I interpret *C. latrans*-related plague incidence as the ecological extent of plague distribution, as suggested by PCA, then *Cynomys* distribution is simply coincident with plague distribution.

I found widespread similarity among plague-infected mammal host distributions, which suggests strongly that plague occurs consistently within a distinct ecological subset of North America. This study is developed at a scale distinct from that of the individual enzootic and epizootic cycles, which are manifested at local scales. Community interactions and host and

vector population fluctuations no doubt influence host organisms (Brown and Ernest 2002; Brown et al. 1997), and therefore likely mediate plague cycles at local scales (Collinge et al. 2005b; Stapp et al. 2004). Studies to date linking plague occurrences to environmental correlates (Boisier et al. 2002; Cavanaugh and Marshall 1972; Collinge et al. 2005a, 2005b; Enscore et al. 2002; Parmenter et al. 1999; Stapp et al. 2004) focused in large part on host dynamics during epizootics, limiting the possibility of characterization of areas of enzootic plague transmission.

Plague-infected *Canis latrans* points cover the broadest manifestation of the distribution of plague, both geographically and ecologically. The large size and transient behavior of individuals of this species lends well to easy detection, and their ubiquitous presence across many habitats provides an easy point of reference. I do not interpret these results as suggesting *C. latrans* as a driver for plague, but they do suggest that emerging local outbreaks may be signaled by increased rates of seropositivity for *C. latrans*. ENMs of ground squirrel taxa in California were associated ecologically with plague-positive *C. latrans* occurrence sites in a recent study (Holt et al. 2009), providing another example of the general association of plague presence and coyotes.

So what factors drive the "plague niche"? The broad extent of the Third Pandemic suggests that the pathogen itself responds little to the outside environment, and can occur where appropriate hosts and vectors are present, although factors such as temperature and humidity could affect the survival of flea vectors and the development of *Y. pestis* within the flea vector. The analyses presented herein argue against significant effects of particular host lineages. As a consequence, I focus on the distribution and ecology of plague vector species (Siphonaptera) as likely key determinants of plague distributional characteristics. The variety of flea species able to transmit plague (albeit with different efficiencies) is impressive (Gage and Kosoy 2005; Krasnov

et al. 2006a; Perry and Fetherston 1997); a previous study modeled distributions of plague-associated flea species, but did not test for associations with plague occurrence patterns (Adjemian et al. 2006). Clearly, the task of incorporating flea ecology and distributions represents a significant challenge as a next step for understanding the geography and ecology of plague transmission patterns.

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CHAPTER 2

The influence of landscape connectivity on disease prevalence and occurrence

Abstract

Landscape patterns of distributions of organisms have been well-documented, and recent studies have described the landscape effects on disease systems. Sin Nombre virus, the principal cause of hantavirus cardiopulmonary syndrome in the United States, occurs naturally in Deer Mice (*Peromyscus maniculatus*), a species nearly in whose range covers much of North America. Infection of this host leads to a life-long persistent infection without disease, and is passed among individuals through physical contact. This pathogen has been studied in depth at local scales, but the dynamics across relatively contiguous landscapes or environmental gradients have not been documented, previously. Plague, caused by the bacterium *Yersinia pestis*, occurs in western North America in a variety of mammalian taxa, and is spread by flea vectors. Infection is generally fatal, and plague is maintained in epizootic and enzootic cycles. I sampled 7 sites in 2007 and 2008 along an elevational gradient in the Colorado Rocky Mountains to test whether host and pathogen dynamics were detectable at local landscape scales, and if virulence affected these dynamics. My survey showed low levels of hantavirus infection and failed to detect plague in either hosts or vectors. Landscape connectivity metrics and patterns of host richness and diversity did not correlate with hantavirus prevalence. My data suggest this landscape does not impose differences in occurrence or prevalence with regard to hantavirus.

Introduction

The interaction of hosts and infectious agents at local and landscape scales has been examined in a variety of studies (e.g., Collinge et al. 2005a; Abad-Franch et al. 2009) with the patterns seen across landscapes generally being more dynamic than those at local scales (Root et al. 2005). Dispersal mechanisms of hosts and pathogens, and connectivity of the landscape are additional variables and constraints that mediate population interchange (Holdenrieder et al. 2004). The level of disease virulence also can play a significant role in the spread and local stability of pathogen populations (Rushton et al. 2000). More virulent diseases may reduce local host populations and require immigration of disease-free individuals, or their populations must be maintained at a low density if they are to persist, whereas less virulent pathogens may be maintained independent of density and immigration (May and Anderson 1983).

An additional dimension of variability in disease prevalence may be driven by host population structure across landscapes. A large body of literature describes patterns associated with mammal communities and species' distributions (e.g., Arita et al. 2005, McCain 2005). However, few studies have examined pathogen distributions in geographic dimensions and extent in relation to host distributions (but see Mills et al. 1997). Still, as local studies have shown relationships between host diversity and pathogen prevalence (Ostfeld and Keesing 2000; Schmidt and Ostfeld 2001), the interplay between hosts and pathogens in geographic space is critical for exploration (Ostfeld et al. 2005).

In North America, hantavirus (family Bunyaviridae, genus *Hantavirus*) infections occur most often in mice of the genus *Peromyscus* (Rodentia: Cricetidae), with specific viral species associated with specific host taxa (Yates et al. 2002). The wide-ranging Deer Mouse, *P. maniculatus*, occurs across much of North America and is associated with Sin Nombre virus

(SNV) (Childs et al. 1994; Biggs et al. 2000; Boone et al. 2002; Kuenzi et al. 2007; Adler et al. 2008). Deer Mice exhibit considerable temporal variation in abundance and hantavirus prevalence (Calisher et al. 2005, Pearce-Duvet et al. 2006, Adler et al. 2008), with additional variation in infection rates perhaps related to the presence of other species that interact with the host species (Peixoto and Abramson 2006; Clay et al. 2009b; Dizney and Ruedas 2009; Suźan et al. 2009). Fluctuations in Deer Mouse populations are generally associated with environmental conditions (Calisher et al. 2005), particularly relating to food source variability (Wolff 1996).

Horizontal transmission from infected to susceptible Deer Mice is thought to occur via direct contact, possibly during copulation or fights (Yates et al. 2002; Clay et al. 2009a). Therefore, prevalence of the virus should relate closely to host population density (Biggs et al. 2000). While this idea is clear in theory (Adler et al. 2008), the pattern is not obvious *in situ* and empirical tests have been inconclusive (Boone et al. 2002; Madhav et al. 2007). The virus persists for the lifespan of an individual (Meyer and Schmaljohn 2000, but see Kuenzi et al. 2005), and may produce higher mortality rates in some host species (Kallio et al. 2007). Hantavirus pathology can be significantly more severe for human infection, where inhalation of virus in aerosol form can be fatal (Duchin et al. 1994).

Plague, caused by the bacterium *Yersinia pestis*, is generally transmitted by fleas and can occur in a variety of mammal species (Gage and Kosoy 2005; Krasnov et al. 2006a; Wimsatt and Biggins 2009). Although originally from Asia, it now has a distinct distribution in North America, seemingly limited to the western portion of the continent (Gage and Kosoy 2005; Nakazawa et al. 2007; Maher et al. *in review*). Plague cycles in two distinct forms: (1) epizootic, characterized by widespread mortality, and (2) enzootic, a less well-defined cycle that probably involves numerous host species (Gage and Kosoy 2005; Wimsatt and Biggins 2009). In general,

and regardless of cycle, infection is usually lethal, and spread is dependent upon vectors and possibly the presence of certain mammalian hosts, such as the Grasshopper Mouse, *Onychomys leucogaster*.

These two diseases co-occur at many sites in western North America, providing unique opportunities to examine how mammal communities affect the occurrence of pathogens (and vice versa) across real landscapes. Here, I examine occurrence and prevalence of Sin Nombre virus and *Y. pestis* over geographic space in association with landscape metrics and host population characteristics. These pathogens have different levels of virulence (*Y. pestis* is high, Sin Nombre virus is low), and therefore provide the opportunity to compare effects of virulence in a landscape *in situ*. I explore distributional patterns of mammals and pathogens across a 1,000 m elevational gradient that is part of the dramatic transition seen from the Great Plains up to the high Rocky Mountains. This area is a region from which both pathogens are known and the mammal hosts have been documented (Telleen 1978; Bergstrom 1992; Fitzgerald et al. 1994; Armstrong 2007).

Methods

Study area

Arapaho–Roosevelt National Forest (ARNF) and Rocky Mountain National Park (RMNP) are located in north-central Colorado (Figure 6). RMNP (~107,556 ha) is a generally continuous protected area, including mountain meadows, tundra and forests within its landscape; it receives ~3,000,000 visitors annually. ARNF encompasses ~607,050 ha and includes adjacent areas east, west, and south of RMNP. It contains large tracks of forest and meadows interspersed with anthropogenic disturbance.

A set of 7 sites, ranging in elevation from 2,181–3,064 m, was sampled in 2007 and 2008 between the end of May and the beginning of August in both years. One site (Hollowell Park) was mostly within a meadow, but with forested edges; the remaining sites were entirely forested. At low elevations, Ponderosa Pine (*Pinus ponderosa*) is the dominant tree, while higher elevations hold Lodge-pole Pine (*P. contorta*) and Limber Pine (*P. flexilis*). Near water, spruces (*Picea engelmannii* and *P. pungens*) and firs (*Abies bifolia* and *Pseudotsuga menziesii*) are common and dominant at some sites.

Sampling

The sampling design for capture of small mammals consisted of a total of 60 traps of different sizes and different styles: 30 LFATDG Sherman Live Traps (7.62 × 8.89 × 22.86 cm), 10 XLF15 Sherman Live Traps (10.16 × 1.43 × 38.1 cm; H.B. Sherman Traps, Tallahassee, FL), 10 rat-sized snap traps, and 10 pitfall traps (~10 cm diameter) at each site. Multiple trap types were used to attempt to maximize the diversity of mammals captured, particularly larger mammals such as woodrats (genus *Neotoma*) that can be too large to fit in smaller live traps. The arrangement of traps was 10 replicates of 1 LFATDG, 1 XLF15, 1 LFATDG, 1 snap trap, 1 LFATDG, and 1 pitfall. Traps were set 8–10 m apart along linear transects, such that multiple vegetation classes could be sampled at a single site, but variation in elevation within any single site was minimal. Traps were checked in the morning and evening for four consecutive days, then removed and disinfected prior to placement at the next locations to avoid transfer of pathogens. Mammals captured in live traps were marked on their pelage to avoid double-counting of captures, but individuals were not marked uniquely.

Individuals captured in snap traps were placed in plastic bags and transported to a secure site for processing. Animals were removed from the bag (which was resealed immediately), and

brushed for parasites for 2 min. Parasites thus dislodged were placed in saline solution for storage until identification and testing for plague. Time spent searching for dislodged parasites was not included in the 2 min period. After brushing, I examined the sealed bag for additional parasites. Fleas were identified to species, and tested for *Yersinia pestis* DNA at CDC Fort Collins. Blood samples were then extracted from potential hosts for use in hantavirus and plague testing. Plague tests on mammal blood were completed only for the samples from 2008.

To test for hantavirus infection, I drew at least 10 μL of blood from voucher specimens of *Peromyscus*, and placed each sample in a unique, sterile vial. Blood samples were tested for antibodies to hantavirus nucleocapsid antigen with a rapid field test (RFT, Schountz et al. 2007) by Tony Schountz and his lab at University of Northern Colorado. The samples were diluted 1:100 with phosphate-buffered saline (PBS) and added to polyvinyl chloride plates coated in recombinant SNV nucleocapsid. After a 30 min incubation and 3 additional washes of PBS/0.5% Tween-20, staphylococcal protein-A/streptococcal protein-G horseradish peroxidase conjugate was added for 30 min. Plates were then washed with PBS-Tween-20 and activated ABTS substrate was incubated for 15 min at ambient temperature. Wells were scored based on absorbance at 405 nm, where values greater than 0.100 were considered positive for hantavirus antibodies. Because historical sampling of potential hosts in the region failed to detect the virus in non-*Peromyscus* species (C. Calisher, pers. comm.), other mammal species were not tested for hantavirus antibodies.

PCR was used to determine whether fleas contained *Y. pestis* DNA, and mammal blood was tested for antibodies using a serological assay. After identification, fleas were grouped by species and location, ground with glass balls, and submitted for PCR testing. I followed procedures detailed in Engelthaler et al. (1999), specifically using primers Yp1 (59-

ATCTTACTTTCCGTGAGAA-39) and Yp2 (59-CTTGGATGTTGAGCTTCCTA-39) that were originally described in (Hinnebusch and Schwan (1993). Thermal cycling procedures followed Hinnebusch and Schwan (1993), and included positive and negative controls. Electrophoresis of PCR products were visualized under ultraviolet light on 2% agarose gels stained with ethidium bromide.

Procedures detailed in Wolff and Hudson (1974) were followed to test host samples for plague antibodies. Blood drawn from host voucher specimens was placed onto Nobuto blood filter strips (Toyo Seisakusho Kaisha, Ltd., Tokyo, Japan), then dried and submitted for the serological test. Samples were soaked overnight at 4º C in 0.4 ml of borate buffer at a pH of 8.0, followed by inactivation by incubation at 56° C for 30 min. The extract was absorbed with washed sheep erythrocytes for 20 min at room temperature. Samples were then centrifuged, and the supernatant was exposed to the 1A envelope protein of *Y. pestis* to test for reaction.

Analysis

Data from 2007 and 2008 were pooled at each location as there was no statistical difference among sites between years. Mammal species richness (SR) was calculated in two ways: first, as a simple count of the number of species captured in traps and second, using Chao estimates of likely full species richness as calculated in EstimateS ver. 8.0 (Colwell 2006). I calculated both Chao 1 and Chao 2 scores using default settings, but only report Chao 1, as the estimators were essentially equal. I excluded Red Squirrels (*Tamiasciurus hudsonicus*) from these calculations because detection in traps was low, although they were observed or heard calling at all 7 sites. Host community structure was quantified using Shannon's Diversity Index and Simpson's Index. I calculated Bray-Curtis distance and a Sørensen-based metric to quantify

differences between host communities. These community metrics were calculated using EstimateS ver. 8.0 using default settings.

I used Fisher's exact test to determine the probability that sites had similar hantavirus prevalence. I examined relationships between disease prevalence and host characteristics or location in several ways. To address the ability of host and community variables to predict disease occurrence (defined as a positive test result for an individual host or at least one positive host in the community), I used logistic regression to test for relationships with host sex, host size (mass of individual), host abundance (defined as the number of unique hosts at a location), and elevation (defined as the elevation at the beginning of each transect), and linear regression to examine the relationships between and among elevation, host community metrics, and disease prevalence. I used Minitab ver. 15 (http:///www.minitab.com) for all regressions and Fisher's exact tests.

Landscape patterns were evaluated using Mantel tests comparing the Fisher's exact probability to differences in elevation, community metrics, and host abundance. The Mantel uses permutation of rows and columns to generate a null distribution to compare the observed correlation between two matrices. A significant result suggests the observed patterns are significantly non-random. I used R ver. 2.10.1 (R Development Core Team 2009) and the package vegan (Oksanen et al. 2010) for Mantel tests.

Permits for this work were provided by the Colorado Division of Wildlife and RMNP; research protocols were approved by the University of Kansas Institutional Animal Care and Use Committee and RMNP.

Results

I regularly captured 8 species of rodents over the elevational gradient, in addition to incidental captures of 2 species—Red Squirrels and Snow-shoe Hares (*Lepus americanus*; Table 1). In 2008, I detected Bushy-tailed Woodrats (*Neotoma cinerea*) and Colorado Chipmunks (*Tamias quadrivittatus*), which were not detected during 2007. Presumably, these observations are related to fewer captures of *Peromyscus maniculatus* in 2008, which resulted in greater trap availability to capture other taxa. When years were pooled, *P. maniculatus* was ubiquitous, and the dominant species both locally and overall.

Observed local rodent species richness, SR_{Actual}, varied, from a minimum of 2 to a maximum of 6, with a median of 4 (Table 2). Chao estimates of species richness, SR_{Chao}, were similar but slightly higher: minimum of 2, maximum of 8, and median of 5. Local habitats varied, but dominant vegetation or quality did not mediate SR or abundance.

Neither SR_{Actual} nor SR_{Chao} showed a strong statistical relationship to elevation (P = 0.203, R^2 = 30.0% and P = 0.086, R^2 = 47.7%, respectively), although a trend toward higher SR values at higher elevations is evident (Figure 7). Mammal diversity was related to elevation for both Simpson and Shannon indices (P = 0.020, R^2 = 69.2% and P = 0.015, R^2 = 72.4%, respectively; Figure 7). Mantel tests comparing correlations between mammal communities to differences in distance and elevation between sample sites were not significant ($P > 0.05$ for both Sorenson and Bray-Curtis distances in all cases).

In some regard, sample size was limited for pathogen detection, and positive detections were even more limited. After removing smaller individuals (less than 15 g) to avoid maternal antibody positives, of 78 *P. maniculatus* tested for hantavirus, 10 were positive for antibodies. Of

all tests of fleas and mammal blood for plague, however, none were positive; therefore, the remainder of analyses focus on hantavirus prevalence and occurrence.

In *P. maniculatus*, positive tests were skewed towards males (Fisher's exact test, $P = 0.045$). Neither host size nor elevation was a significant predictor of hantavirus occurrence ($P = 0.865$ and 0.475). The logistic regression of hantavirus occurrence by mass and sex showed a significant relationship ($P = 0.049$), but only sex had a significant contribution to the model (sex, $P = 0.053$; mass, $P = 0.306$, respectively). Plots of the data showed that larger males were disproportionately infected. Julian day was also a poor predictor of a positive test ($P = 0.624$).

Linear regression suggested no relationship between hantavirus prevalence and elevation or host abundance ($P > 0.05$ in both cases). Examination of bivariate plots of these variables did not suggest discernable trends or patterns. Comparisons of prevalence among sites using Fisher's exact test showed sites were not different ($P > 0.05$ in all tests). Mantel tests comparing the probability of prevalence difference to landscape features (elevation differences and distance) and host community structure were not significant ($P > 0.05$ in all tests).

Discussion

My survey of mammals in the Colorado Rocky Mountains showed no statistically detectable pattern of species richness with respect to elevation, although I found diversity indices to be higher at higher elevations. Richness was relatively stable at elevations above 2,200 m, and several species at these elevations are expected to occur at lower, unsampled elevations (i.e., see Bergstrom and Hoffmann 1991; Fitzgerald et al. 1994). The study region has undergone considerable change in land use and development over the last century (Buchholtz 1983), and this impact might have local influences on diversity. Impacts of the increasing populations of the Mountain Pine Beetle (*Dendroctonus ponderosae*) in this region will no doubt cause changes in

species occurrence (e.g., Holdenrieder et al. 2004). Although others suggest that environmental qualities such as historical disturbance (Rowe 2005), climate (McCain 2007), or physical boundaries (Colwell and Lees 2000; McCain 2003) can drive patterns of species occurrence, I did not test these hypotheses.

With regard to hantavirus, I found similar levels of low prevalence across sites between years, and limited evidence that connectivity correlates with disease incidence. While landscape composition may be important (Langlois et al. 2001; Lehmer et al. 2008; Torres-Pérez et al. in press), my sites are located within a relatively continuous habitat matrix. My results do provide additional evidence of a sex bias in hantavirus occurrence (Borucki et al. 2000), with males more frequently infected than females. Recent evidence suggests that even a few large individuals are sufficient to sustain the overall prevalence of the disease (Clay et al. 2009a).

Hence, the pattern of mouse dispersal, particularly of males (both infected and non-infected) is probably key in understanding the local distribution and maintenance of hantavirus. While abundance of potential hosts no doubt affects local prevalence (Madhav et al. 2007; Adler et al. 2008), the vagility of infected individuals will dilute patterns on a landscape through spread (Douglass et al. 2006; Lonner et al. 2008). For a species such as *P. maniculatus,* which has a wide distribution and is able to move relatively large distances given its small size, certain perceived barriers (e.g., mountains or rivers) may be of little consequence.

Perhaps the most surprising aspect of my survey is the failure to detect evidence of *Yersinia pestis* in the system, thus limiting my ability to compare the effect of transmission and virulence on landscape patterns. My failure to detect plague does not necessarily indicate complete absence of the pathogen from my sites, but rather does elaborate upon the difficulty of detection of this bacterium (MacKenzie et al. 2006). Plague occurred in a population of

Wyoming Ground Squirrels (*Urocitellus elegans*) in this region in the 1970s (J. Visty and J. Connor, pers. comm.), but plague has not been documented in the study area since. Transmission of plague from infected to susceptible host relies on vectors (i.e., fleas), the effectiveness of which varies and requires opportunity to transfer to a new host (Eskey and Haas 1939; Gage and Kosoy 2005). Epizootics, by definition, are unstable with regards to local maintenance, as the disease sweeps through and wipes out hosts, then disappears, only to re-emerge later. The enzootic phase has been difficult to identify, and may be critical in maintaining *Y. pestis* in the local environment (Wimsatt and Biggins 2009). The result is a situation in which any living individual that might be sampled is unlikely to be infected by this pathogen.

A positive test for plague requires one of several possible circumstances. For instance, tests for antibodies, such as those completed on the mammal blood samples, require infection and immune response within the time frame of sampling and prior to mortality (Shepherd et al. 1986). Additionally, detecting plague in fleas requires them to have fed from an infected host, with pathogen transfer to the gut of the flea (Eskey and Haas 1939; Perry and Fetherston 1997; Gage and Kosoy 2005; Wimsatt and Biggins 2009). The likelihood of capturing a flea on a susceptible individual that has previously bitten an infected individual during an enzootic cycle is expected to be quite low, given the expected low levels of bacteria ingested (Perry and Fetherston 1997). Furthermore, transmission of the bacteria to a flea from an infected host is inefficient (Eskey and Haas 1939). I do not want to discourage further work on potential systems that may exhibit enzootic plague, but rather wish to highlight the difficulty in detection, particularly in light of my results.

With my analysis, I acknowledge the possibility of Type II error, in that landscape effects may exist that I failed to detect. I had generally small sample sizes at each locality (for some

locations, fewer than 10 samples) and short trapping durations (4 days at each site per year). These factors may limit my ability to make inferences and estimate parameters associated with prevalence and occurrence of disease. Abundance of *P. maniculatus* can change dramatically from year to year, and the raw data suggest that abundance was low across sites in 2008, as compared to 2007 trapping (data not shown). Fluctuations in populations have been suggested as correlates to changing prevalence of the pathogen, particularly as it relates to human disease risk (Glass et al. 2000; Madhav et al. 2007). Continued testing and additional localities are required to determine an exact empirical relationship between sites and landscape structure. While seasonal variation in seroprevalence has been observed (Pearce-Duvet et al. 2006), I found no detectable effect over the summer months in the survey.

In conclusion, I expected that if strong landscape patterns are driving disease prevalence and occurrence, then differences among sites would correspond to connectivity metrics. However, if local processes dominate, host dynamics will be important and local sites should be distinct from each other. For hantavirus, I found that neither local nor landscape dynamics was associated with prevalence or occurrence, suggesting that my surveys may derive from a single entity (or population) despite the fact that I sampled a nearly 900 m elevational range.

The results are consistent with the hypothesis of rarity of a more virulent pathogen, and a greater occurrence of a less virulent pathogen. Thus, hantavirus has evolved to persist in a single host without causing significant mortality, whereas *Y. pestis* has evolved a more dynamic life-cycle, relying on independent hosts and vectors, and leads to high, if not complete, mortality. Perhaps the influence of landscape connectivity and host dynamics may be most important in transmission for highly virulent pathogens, but of limited influence for less virulent pathogens. For instance, during an enzootic cycle, an infected host must be able to move into a region with

susceptible individuals for the disease to spread, such that a fragmented landscape would impede the spread.

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CHAPTER 3

Landscape patterns of flea diversity along an elevational gradient

Abstract

Analyses of host-parasite relationships have generally focused either on broad scale patterns of coincidence of host and parasite distributions, or on more local patterns such as variation in parasite load and the diversity of parasites on a single host. The patterns of host–parasite relationships at landscape scales, however, are poorly known, even where hosts are well studied. In an effort to fill this gap, I conducted a survey of hosts and fleas over a 1000 m elevational gradient in northern Colorado, at sites in and around Rocky Mountain National Park. I found that host species richness (the number of species) was related positively to flea species richness, but flea community structure (incorporating richness, evenness, and species identity) was independent of the host community. Flea species richness was positively associated with elevation, and the changes in community structure were positively associated with elevation. While some flea species exhibited host specificity, most flea species were found on multiple host species. The data suggest that patterns of host–parasite relationships are scale-dependent, and that host richness impacts flea species richness. However, local environments likely play a significant role in determining the make-up of parasite communities.

Introduction

Scale is an important context for understanding ecological patterns (Levin 1992). Evolutionary patterns at broad scales are inherent to understanding the mechanisms of isolation and speciation and how they vary in space and time (Rosenzweig 1995), and macroecological patterns at continental scales can suggest consistent mechanisms that produce diversity (Brown 1995; Hubbell 2001). Local scales, however, can provide the context for interactions and population shifts that lead to evolutionary changes in species (e.g., Badyaev et al. 2008; Grant and Grant 2002; Lively 2009). Between these 2 extremes is the landscape perspective, where spatial arrangement of local sites and movement among areas by individuals can be examined, tested, and quantified (Turner 1989; Wiens et al. 1993).

In parasites, broad-scale analyses have shown interesting patterns in diversity within and between hosts (Poulin 1995) and differences in relationships of host and parasite taxa in biogeographic regions (Krasnov et al. 2007). Recent studies also elucidate host characteristics that are drivers in parasite evolution and distribution. Combes (2001) presented 15 hypothesized mechanisms that may mediate parasite distributions, both in evolutionary and ecological contexts. Among these, body mass and geographic range are considered important factors, but tests of these characters do not designate the geographic clustering of parasites, nor do they generally consider parapatric distributions of hosts or parasites (Krasnov et al. 2004a; Krasnov et al. 2004b). Environmental data were used successfully to predict geographic distributions of some parasites and pathogens, independent of explicit host data, suggesting climatic influences on distributions of parasites and pathogens (Adjemian et al. 2006; Krasnov et al. 2005b; Nakazawa et al. 2007; Peterson et al. 2002).

At the most local scale, comparisons of parasite communities focus on infracommunity structure, providing insight into the role of parasite competition for hosts and other species interactions (Combes 2001; Krasnov et al. 2006b). This approach has been expanded to study patterns of occurrence and abundance of parasites on a focal taxon to show the relationship between abundance and occurrence (Krasnov et al. 2006d). Treating the hosts as biogeographic islands and applying the theory of nested subsets (Patterson and Atmar 1986) demonstrated that parasite species richness on a given host varies as a function of the overall community of parasites (González and Oliva 2009; Krasnov et al. 2005a; Timi and Poulin 2008). Therefore, the component community, the community of parasites on a single host, is a subset of local parasite community and a landscape analysis of parasite richness must incorporate various component communities to estimate total local parasite richness.

Asking questions within a landscape context provides the opportunity to examine local determinants in a spatial context (Turner 1989), which should provide insight into the interactions among and between variables. Specifically for parasites, this approach provides the opportunity to evaluate environmental influences on occurrence and examine how host community patterns influence parasite communities (Ostfeld et al. 2005). For instance, the "dilution" effect suggests that increased host species richness negatively effects pathogen occurrence owing to the increase in non-target hosts in the population (Ostfeld and Keesing 2000; Schmidt and Ostfeld 2001). Independent of the host community, changes in habitat have facilitated changes in flea communities (Brouat et al. 2007; Krasnov et al. 2006c).

Fleas (Siphonaptera) are generally free-living from their mammalian or avian hosts, but also are intricately tied to the host throughout their life cycles. All species of fleas are obligate blood-feeding parasites; adults feed on host blood and larval stages feed on organic matter,

particularly fecal matter from adult fleas. Fleas generally inhabit the nest or burrow of their focal host, but many species also feed opportunistically on other hosts. Medvedev and Krasnov (2006) provide an excellent synopsis of the general biology and ecology of fleas.

Krasnov et al. (2007) presented evidence that flea species richness is independent of host richness in the Nearctic, but not in the Palearctic. Still, within the Nearctic, Bossard (2006) showed that specific aspects of host biology and ecological traits explain community structure of fleas in the Great Basin of Utah, which suggests that patterns of parasite communities may be scale-dependent. It is possible to reduce the scale of analysis further, and thus increase the depth of understanding by testing for patterns along an environmental gradient in lieu of a survey over an entire continent or biogeographic region. Previous work on elevational gradients has demonstrated that flea richness in highlands is higher (Ponce and Llorente 1993), and that fleas often have more limited elevational ranges than their hosts (Eads and Campos 1983; Wenzel and Tipton 1966), a pattern seen in a few other parasites.

Here, I present an analysis of landscape patterns of mammal flea communities to test whether host community and spatial structure influence flea richness and community structure, based on a survey of small mammals and their fleas over a 1000-m elevational gradient in Colorado (Fig. 1). Early work in this region by Eads and Campos (1983) focused on fleas from a single host species in a specific alpine zone; I extend this survey to a more general inventory of the fleas of the small mammal community, across a broad elevational range. Given patterns seen at broad scales, I expect that host community and flea community patterns should be independent and that local site dynamics play an important role. I ask the following questions: 1) Do patterns of flea species richness associate with those of host richness?, 2) Are flea communities driven by

host communities?, 3) Is variation in flea richness and community structure related to distance and/or elevation?, and 4) Are fleas host specific?

Methods

Study area and data collection

Details of the sampling methods are found in Maher et al. (*in review*); here, I present only the fundamental aspects of data collection. Rocky Mountain National Park (RMNP) is in north-central Colorado, USA, encompassing ~107,556 ha, and various forest types and meadows. Arapaho–Roosevelt National Forest (ARNF) is considerably larger (~607,050 ha) and extends down slope towards short-grass prairie. In 2007 and 2008, I sampled 7 sites that ranged in elevation from 2181–3064 m (Figure 6). All sites but 1 were forested; the dominant species of tree varying among elevations and local environments. The Hollowell Park site contained a large area of meadow, but was bookended by areas of tree cover. Pine species varied depending upon elevation, changing from Ponderosa (*Pinus ponderosa*) at the low elevation, to Lodge-pole (*P. contorta*), and then to Limber (*P. flexilis*). Throughout the transect, spruce (*Picea engelmannii* and *P. pungens*) and fir species (*Abies bifolia* and *Pseudotsuga menziesii*) occurred, particularly in wetter areas.

At sites, each transect consisted of 60 traps, 30 LFATDG Sherman Live Traps (7.62 × 8.89 × 22.86 cm), 10 XLF15 Sherman Live Traps (10.16 × 1.43 × 38.1 cm; H. B. Sherman, Tallahassee, FL), 10 rat-sized snap traps, and 10 pitfall traps (~10 cm diameter). Potential hosts were placed in a unique plastic bag prior to transport to a secure site for parasite survey and collection. Each host was removed from the bag, which was then resealed, and the mammal was brushed for a total of 2 min, accounting for time spent collecting parasites. All parasite samples were placed in a saline solution and stored in a freezer until identification.

I used Hubbard (1947) and Furman and Catts (1982) to identify the fleas. As part of my attempt to detect plague in this region (see Maher et al. *in review*), fleas were destroyed during PCR analysis. However, I maintained a flea voucher collection, securing a male and female, when available, of each species. Mammals were identified to species using Armstrong (1987) and Fitzgerald et al. (1994) as references. All voucher specimens are deposited in the University of Kansas Biodiversity Institute, Lawrence, Kansas.

Analysis

I used regressions, permutation tests, and rarefaction analyses to assess the importance of host community and geographic and elevational proximity in determining the flea community of this region. I pooled host and flea data for each locality between years and counted numbers of species of hosts (SR_{host}) and fleas (SR_{flea}) for each locality. To account for the possibility that some taxa went unsampled, I also calculated estimates of species richness of hosts and fleas, specifically mean Chao 1 and Chao 2 values, using EstimateS ver. 8.0 (Colwell 2006). Chao values and confidence limits (Chao 1984, 1987) are calculated through counts of singletons (single observations of a species) and doubletons (at least 2 observations of a species). For both hosts and fleas, these 2 estimators yielded identical values or broadly overlapping confidence intervals. Therefore, I report the Chao 1 estimates only for both groups ($Chao_{host}$ and $Chao_{flea}$), respectively. I regressed SR_{flea} and $Chao_{flea}$ on numbers of host samples across localities to determine if differential sampling of hosts among sites influenced these observations.

Host community richness varied among sites (Maher et al. *in review*) and I chose to rarify host data to reduce effects of differential sampling. This step was achieved by selecting at random a subset of hosts, and determining the sampled flea and host richness. I used a VBA script written by SPM (available upon request). Rarefied estimates were developed by sampling

2 populations—hosts containing fleas and all hosts sampled, regardless of flea occurrence. To determine if localities differed in flea species richness, I examined overlap within ± 2 SD around the rarefied means and compared subsets of 5, 10, 15, and 20 hosts. If these values overlapped between sites, I concluded no difference in flea richness between them.

I used EstimateS ver. 8.0 (Colwell 2006) to calculate mean Simpson reciprocal and mean Shannon indices for each locality to assess simultaneously evenness and richness of the sampled flea species. To describe differences in communities, I used default settings in EstimateS ver. 8.0 (Colwell 2006) to generate Bray–Curtis similarity and Chao-based Sørensen values between each pair of locations for hosts and fleas and subtracted these values from unity to generate dissimilarity estimates. I recorded elevation as the value at the beginning of each trap transect, and calculated the difference between sites to characterize elevational difference between sites. Geographic distance was calculated for the beginning of each transect to the beginning of each other transect using ArcGIS (Environmental Systems Research Institute, Inc., Redlands, Calif.).

In Minitab ver. 15 (http:///www.minitab.com), I completed a series of simple linear regressions to examine relationships between host and flea species richness and community structure. Previously, I have shown that mammal richness, both actual and Chao1 estimates, is not related to elevation and that community structure was not related to differences in elevation or distance (Maher et al. *in review*). Thus, I analyzed flea richness data for similar patterns.

Patterns of community structure were compared via multiple Mantel tests, which compare the observed element-by-element correlation between 2 data matrices to a null distribution generated by permutation. I compared differences in flea community structure to elevation differences, geographic distance, and differences in host community structure. When both geographic distance and elevational difference matrices were significantly related to the

community matrix, partial Mantel tests were used to test for correlation between the community matrix and 1 of the landscape matrices adjusting for correlations with the second landscape matrix. I used R ver. 2.10.1 (R Development Core Team 2009) and the package vegan (Oksanen et al. 2010) for Mantel tests. I used a series of χ^2 tests to test whether flea species were uniformly distributed among host species in my sample, essentially testing for host-specificity.

Permits for this work were provided by the Colorado Division of Wildlife and RMNP; research protocols were approved by the University of Kansas Institutional Animal Care and Use Committee and RMNP. All procedures involving handling wild mammals followed ASM Guidelines (Gannon et al. 2007).

Results

Across the elevational transects, I captured 9 species of mammals and identified 11 species of fleas (Table 3). I sampled 220 potential hosts for fleas: 114 Deer Mice (*Peromyscus maniculatus*), 33 Least Chipmunks (*Tamias minimus*), 25 Southern Red-back Voles (*Myodes gapperi*), 23 Uinta Chipmunks (*T. umbrinus*), 16 Golden-mantled squirrels (*Callospermophilus lateralis*), 3 Snowshoe Hares (*Lepus americanus*), 3 Wyoming Ground Squirrels (*Urocitellus elegans*), 1 Bushy-tailed Woodrat (*Neotoma cinerea*), 1 Colorado Chipmunk (*T. quadrivittatus*), and 1 Red Squirrel (*Tamiasciurus hudsonicus*). Details of mammal occurrence are found in Maher et al. (*in review*). I included 10 of the flea species in the analyses, excluding *Hoplopsyllus glacialis*, which was found only on a single *L. americanus*, a species not included in the analysis of mammal occurrence owing to low capture rates.

The flea *Aethica wagneri* was the most common species, both in number and distribution, and was collected at all sites. *Eumolpianus eumolpi* and *Peromyscopsylla hesperomys* were common also and widely distributed, being found at 5 and 4 of 7 sites, respectively. The

remaining flea species associated with rodents were relatively uncommon but, with the exception of *Epitedia wenmanni*, occurred at more than 1 locality. There was considerable variation in abundance of fleas at sites, with a minimum of 4 and a maximum of 74 individuals (Table 1).

SR_{flea} varied with respect to locality (Table 4), and $Chao_{flea}$ values also varied considerably with confidence intervals (± 2 SD) quite broad for localities with high SR_{flea}. The correlation between SR_{flea} and $Chao_{flea}$ values was high ($r = 0.960$, $P = 0.001$), but Chao estimates also suggest that I may have under-sampled some of the communities, particularly Wind River, Lower Boulder Brook, and Upper Boulder Brook (Table 4). Still, regressions of SR_{flea} and $Chao_{flea}$ on the number of hosts sampled failed to show a relationship ($P = 0.472$, $R^2 = 10.8\%$ for SR_{flea}; $P = 0.583$, $R^2 = 6.4\%$ for $Chao_{flea}$). Additionally, I found no relationship between the number of fleas in a sample and the number of hosts sampled at that site ($P = 0.920$, $R^2 = 0.2\%$).

When I rarefied host data, 6 sites were essentially statistically indistinguishable from each other with regard to SR_{flea} (Figure 8). The Roosevelt National Forest site, which had 1 flea species, deviated from the other groups, although this may be due to a *depauperate* host community (Maher et al. *in review*). When the number of hosts approached 20 in the rarefied sample, estimates of SR_{host} were asymptotic (Figure 8). The plot of rarefied SR_{host} and estimated SR_{flea} shows that other localities had a larger SR_{host} with fewer hosts (Figure 9), which may have driven the increase in SR_{flea}.

Multiple linear regression of SR_{flea} on elevation and SR_{host} showed a strong relationship ($P = 0.003$, $R^2 = 94.1$). While the SR_{host} variable was significant in the model ($P = 0.008$), inclusion of elevation was only marginally significant ($P = 0.062$). A similar model replacing observed SR with Chao values also was marginally significant at the 0.05 level ($P = 0.070$, $R^2 =$

73.6), but still explained much of the variation. In this case, neither elevation (P = 0.425) nor $Chao_{host}$ (P = 0.161) were significant predictors in the non-significant model.

Additional regressions showed that Shannon indices were positively related to elevation (P = 0.031, R^2 = 63.8), but Simpson indices were not (P = 0.196, R^2 = 30.8). The Simpson result may be due to a single large value (5.0) with a large residual in the model (2.174) at Hollowell Park (Table 4). Removal of this point resulted in a significant model (P = 0.041, R^2 = 69.0). Regardless, both Simpson and Shannon indices exhibited a trend of increased value with elevation. Correlations between Simpson and Shannon indices for host and flea were also equivocal (r = 0.306, P = 0.504; r = 0.838, P = 0.019, respectively), but Spearman rank correlations for the same comparisons were not significant (rho = 0.250, P = 0.595; rho = 0.464, P = 0.302, respectively).

Mantel tests comparing differences in community structure were generally non-significant. When I used the Bray–Curtis dissimilarity metric, all tests were non-significant (P > 0.05 for all tests); Chao-based Sørensen values were associated significantly with geographic distance (Mantel r = 0.777, P = 0.004) and changes in elevation (Mantel r = 0.531, P = 0.030), but they were not associated with host community structure (Mantel r = 0.167, P = 0.238). The partial Mantel test that maintained the relationship between Chao-based Sørensen values and geographic distances was significant (Mantel r = 0.678, P = 0.031), but the test was not significant when maintaining the relationship between Chao-based Sørensen values and elevation (Mantel r = 0.147, P = 0.220).

Certain flea species were obviously more abundant than others (Table 3), and abundance of host species also varied (Maher et al. *in review*). Therefore, I examined host specificity at all sites for the 3 most abundant fleas, *Eumolpianus eumolpi*, *P. hesperomys*, and *A. wagneri* within

the 4 most abundant hosts, *Peromyscus maniculatus*, *Myodes gapperi*, *Tamias minimus*, and *T. umbrinus*. Each of the flea species was unevenly distributed across hosts (*A. wagneri*: $\chi^2 = 16.47$, $P < 0.05$; *Peromyscopsylla hesperomys*: $\chi^2 = 10.70$, $P < 0.05$; and *E. eumolpi* $\chi^2 = 56.57$, $P < 0.05$). Two flea species were over-represented on a single host taxon (*E. eumolpi* on *Tamias* and *A. wagneri* on *Peromyscus maniculatus*), and *Peromyscopsylla hesperomys* was under-represented on *Tamias*, over-represented on *Myodes gapperi*, but proportionally represented on *Peromyscus maniculatus*. Three flea species were documented by multiple individuals but detected on only a single host taxon: *Megabothris abantis* only on *Myodes gapperi*, and *Malareus telchinum* and *Opisodasys keeni* only on *Peromyscus maniculatus*.

After completing the outlined analyses I tested the influence of additional hosts on the richness of fleas found on *P. maniculatus*. As such, I calculated the flea richness from only *P. maniculatus* at each site and regressed this value against the number of additional host species at each site. The regression was significant and positive ($P = 0.003$, $R^2 = 85.9\%$; Figure 10), and the flea richness from *P. maniculatus* was highly correlated with the overall SR_{flea} ($\rho = 0.789$, $P = 0.035$). I then tested whether inclusion of *P. maniculatus* as a host created a bias in my first regressions by regressing flea species richness of non-*P. maniculatus* hosts on host species richness, excluding *P. maniculatus*, and found a significant, positive relationship ($P = 0.038$, $R^2 = 61.0\%$).

Discussion

The present survey and analysis is complementary to and expands upon the earlier work of Eads and Campos (1983), who sampled in this region in the mid and late 1970s, but focused their work at higher elevations and in different habitats. Regarding the flea species associated with *Peromyscus maniculatus*, my results are consistent with their findings only in the common

occurrence of *Aethica wagneri*. I found *Peromyscopsylla hesperomys* more frequently than they reported below tree line and this species was common in the tundra during their survey. They found a larger proportion of *Opisodasys keeni*, which was rare in my survey, and overall their survey resulted in greater richness. Perhaps this difference is reflected in the larger Chao estimations of the flea richness as compared to the observed richness values.

Within my sample, estimates of host richness were not exceptionally variable (Maher et al. *in review*), but estimates of flea community richness were. This result derives from the number of singletons of flea species at some sites (Table 4), whereas few mammal species were represented by singletons (Maher et al. *in review*). For example, I observed 6 flea species at the Lower Boulder Brook site, 2 of which were singletons. Therefore the Chao estimate for this site was 8.0, with an upper limit of 28.13. Perhaps this is a matter of statistical inference, but realistically the difference in observed and estimated richness may reflect the difficulty in detection of rare species.

Regressions of SR_{flea} and $Chao_{flea}$ on the number of hosts sampled show that the observation of richness is not dependent upon the number of hosts I sampled. The regression of the number of fleas at each site on hosts sampled indicated no relationship as well. These important observations suggest that variation in sampling at each site did not contribute to a major bias in the later analyses, and, perhaps, that the flea community was more depauperate during my survey than during that of Eads and Campos (1983). During the 6 years of the Eads and Campos (1983) survey, they examined 2090 *Peromyscus maniculatus* in all life-zones, whereas over the 2 years in my survey, I examined 114. With such a large sample size, the expectation for the number of observations of rare flea species increases. I used Chao estimators that approach the true number of groups such that I was likely to converge on a reasonable

estimate of species richness without a large sample (Peterson and Slade 1998). By simply comparing species lists and prevalence, it is apparent that there is a difference between the surveys in flea species composition.

It is reasonable to consider that the infracommunity of a given host may contribute heavily to the local estimate of SR_{flea}, particularly if the flea species is represented by a singleton. While the regression analyses suggest that sampling did not affect SR_{flea}, these parametric methods do not account for the variability from the host perspective, and I reach the same conclusion. If host sample sizes are low, then estimates of SR_{flea} must be treated with caution, and I suggest that rarefaction of host data at richer sites be used to compare to depauperate sites. I suggest that at least 15 hosts are required for estimate of SR_{flea}, as the rarefaction plots of SR_{flea} estimate ± 2 SD include the observed value of SR_{flea} at such a sample.

When I rarefied host data, a complex relationship between flea richness and host richness emerged (Figures 8 and 9). Flea richness and host richness at the Roosevelt National Forest site was significantly lower than those of the other sites. The remaining 6 sites exhibited a consistent trend of increasing SR_{flea} with increasing SR_{host}, and that as the number of rarefied hosts increased, the estimate of SR_{flea} increased. I note that the plots show extensive overlap between sites and between rarefied host values (either rarefied hosts or rarefied SR_{host}; Figures 8 and 9) based on the estimate of SR_{flea} ± 2SD, suggesting that the pattern may be independent of location. Thus, the apparent increase in SR_{flea} with rarefied hosts is not due to increased sample size, but the interaction between the estimate of SR_{host} and sample size (Figure 8).

The structure of the flea community changed with elevation, with explicitly higher values of Shannon and Simpson indices at higher elevations. While flea Simpson values are independent of host values for each metric, the flea Shannon values are clearly related to host

values. The host Simpson value for Hollowell Park was smaller than the value for the flea community due to a much more even flea community. I note that number of fleas captured, which could be correlated to their overall abundance, are greater at higher elevations, and this correlation will affect the value of each index.

The results of the Mantel tests are not interpreted easily, as the 2 community distance metrics lead to different conclusions. The Bray–Curtis distance, a metric common in community ecology, failed to show similarity in any comparison. However, when I used the Chao-based Sørensen values in the same analysis, both geographic distance and elevation seemed related to flea community structure. Partial Mantel tests show that, while controlling for geographic distance between sites, the elevational structure is statistically correlated to the diversity matrix, but the converse is not significant. Therefore, flea communities were structured with changes in elevation and not associated with geographic distance.

The Chao-based Sørensen value uses an estimate of unseen species that may be shared (Chao et al. 2005), while the Bray–Curtis value does not. While I feel my survey was complete and thorough for the component of the mammal fauna surveyed, the possibility of additional unsampled mammalian taxa at each location exists. As such, it is reasonable to consider that the Chao-based Sørensen value is more representative than the actual observed differences. Other studies used the Chao-based Sørensen value derived from raw abundances (e.g., Maas et al. 2009), the differences in the values in my sample were equivocal. The Mantel tests incorporating geographic distance and elevational difference were sensitive to the choice of distance metric; the Chao-based Sørensen resulted in significant values, but the Bray–Curtis did not. Nevertheless, differences in flea community structure were independent of host community regardless of metric.

The distance metrics of flea and host communities used in the Mantel test represent differences in community structure and do not quantify the local diversity or richness. It is possible to have similar values for Simpson and Shannon indices, despite differences in community structure. I observed differences in SR_{flea} and SR_{host} in my transect, which can influence the value of either index. Therefore, I are inclined to support the results of the Mantel tests over the correlation between diversity indices of hosts and fleas, and to assert that changes in the flea community structure were independent from changes in host community structure.

I am still left with different results regarding the similarity in flea community structure and distance metrics. While it seems logical that nearby locations are more similar than sites that are distant (Tobler 1970), the host community is likely a confounding factor. Specifically, while the host community may not completely determine a flea community, it is likely to mediate it to some extent, as flea species occur where host taxa also occur. The analysis of host specificity shows that at least two species of fleas (*Aethica wagneri* and *Eumolpianus eumolpi*) were over-abundant on single host species and relatively sparse on the other well-sampled hosts. These fleas did occur on other hosts, however, suggesting opportunistic feeding (Table 3). From my data, some species (*Megabothris abantis*, *Malareus telchinum,* and *Opisodasys keeni*) seem to be host specific, but sample sizes limit my ability to make inference. Hubbard (1947) and Holland (1949) reported that *M. telchinum* is present on many hosts of various lineages, and *O. keeni* prefers *Peromyscus*, but they disagreed on host-specificity of *Megabothris abantis;* Hubbard (1947) reported a variety of hosts and Holland (1949) reported only *Peromyscus*. My data show *M. abantis* from only *Myodes gapperi* and Eads and Campos (1983) reported it from *Peromyscus* also, so it is likely that it is not host specific. Thus, to suggest that flea species act completely independent of their hosts would be unfounded from a biological standpoint. Environmental

influences are clearly important in the large-scale distribution of species, and fleas are no doubt subject to climatic constraints as well as well as seasonal and yearly abundances of hosts. It is with this in mind that I suggest that flea communities are influenced by small-scale environmental and habitat effects in addition to slight changes in host community.

Conclusions

I found a diverse community of fleas and hosts along the 1000-m elevational transect in the Colorado Rockies. In general, the results show that, at this landscape scale, flea species richness is significantly and positively related to host richness. This observation runs counter to general patterns found in the Nearctic and matches the pattern in the Palearctic as proposed by Krasnov et al. (2007). The community structure of fleas was not dependent on host community and was driven by changes in elevation, which would correlate to changes in environment. This observation is counter to Bossard (2006) who suggested that communities and richness were driven by host characteristics. Perhaps the landscape scale provides a contextual view of variability in host and parasite occurrence that is blurred at broader scales.

When parasites are host specific, patterns of parasite richness will be tied heavily to the availability of hosts. Occurrence of generalist parasites should be independent of host species richness, so long as changes in species richness are associated with maintenance of overall host abundance. Alternatively, changes in host species richness will be reflected in parasite diversity and richness when parasites are host specific. The flea community is dynamic in space and potentially dependent on available host species. Further work regarding the relationship of flea and host communities on a landscape scale should be explored, particularly where environmental and habitat influences can be quantified.

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CHAPTER 4

Evaluating the refugium–recolonization hypothesis for hantavirus

Abstract

Metapopulation dynamics are inherent to maintenance and dispersal of pathogens in a natural landscape. The North American hantaviruses have low virulence, while maintaining low prevalence in their primary host, *Peromyscus* species. When host populations decline, the virus seemingly becomes locally extinct, probably due to a reduction in host contact rates reducing transmission. When host populations recover, viral prevalence generally returns to pre-decline levels. It has been hypothesized that refugia for the virus are required to re-infect neighboring populations. I tested this hypothesis using spatially explicit simulations of hantavirus transmission based on cellular automata in an agent-based context. I used published demographic parameters and varied a suite of behavioral and pathogen-related parameters to determine the minimum time required for infection to occur across an entire 400-cell grid. The results of the simulation suggest that the amount of time required to spread the infection is at least 9 months, although the parameters for this iteration are not necessary biologically reasonable. Under dispersal conditions that are similar to empirical observations, the virus fails to spread to all cells, even when the simulation begins with infected individuals in 88% of the cells. Thus, it is likely that the refugium–colonization hypothesis alone cannot account for the re-occurrence of the virus once it disappears on a landscape.

Introduction

Spatial dimension of disease ecology is a growing field with important implications for conservation biology and public health (Gog et al. 2002; Hess 1996; Hess 1994; Omenn 2010). Monitoring the spread of new pathogens can be complex when identification of the primary host is unclear or when the pathogen or host is very mobile (Morens et al. 2004; Riley et al. 2003). Dynamics even of known pathogens can be difficult to predict in natural systems, especially when multiple hosts and vectors are involved (Gage and Kosoy 2005) or transmission and virulence vary (Antolin 2008). Identifying the patterns and processes by which diseases spread or persist in environments can shed light onto the mechanisms of maintenance (Altizer et al. 2006).

Classical disease experiments show that equilibrium states are difficult to maintain in lab conditions. In many respects, metapopulations (Levins 1969) are inherent to pathogen systems, particularly if virulence is high, such that dispersal and connectivity are required for the pathogen to find additional hosts (Stapp et al. 2004; Thrall and Burdon 1997). After host populations have recovered within a location, metapopulation dynamics are required for re-establishment of a pathogen (Grenfell and Harwood 1997; Keeling 2000). When virulence is low, metapopulation dynamics may have a more limited role in structuring prevalence and occurrence because host populations dynamics will be independent of the pathogen prevalence (Thrall and Burdon 1997).

Members of the virus genus *Hantavirus* (family Bunyaviridae) occur in a variety of small mammals, particularly rodents and shrews, across several continents. In North America, associations between hantaviruses and specific primary hosts, members of the genus *Peromyscus* (Rodentia: Cricetidae) have been studied over the last decade, particularly after a series of fatal

infections in humans (Abramson et al. 2003; Calisher et al. 2007; Dragoo et al. 2006; Glass et al. 2007; Mills et al. 1998; Yates et al. 2002). Surveys indicated that a single specific virus lineage is associated with each species of *Peromyscus* that is a known primary host (Yates et al. 2002). Infection was assumed to have little effect on hosts (Abbott et al. 1999; O'Connor et al. 1997), although some evidence exists for increased mortality rate compared to uninfected individuals (Douglass et al. 2001; Kuenzi et al. 2005). Transmission of the virus is horizontal, presumably occurring most frequently in fighting bouts and copulation, but also may occur through sharing nests (Clay et al. 2009a; Glass et al. 1988; Yates et al. 2002). Shedding of the virus varies during infection, which doubtlessly plays a role in the probability of transmission (Kuenzi et al. 2005; Safronetz et al. 2008).

Patterns of hantavirus occurrence and prevalence, defined as the proportion of individuals infected, were documented in a number of recent studies. Generally, prevalence is related to host population cycles and patterns in demography (Calisher et al. 2007; Kuenzi et al. 2007; Luis et al. 2010; Madhav et al. 2007), but some evidence indicates that presence of other mammal taxa influences the frequency of infection (Clay et al. 2009b; Dizney and Ruedas 2009). Modeling efforts have shown that a small subset of individuals ultimately may be responsible for infection, and these individuals are characterized as larger in size and more aggressive in behavior (Clay et al. 2009a).

Analyses of landscape patterns of the infection have begun to illuminate the relationship between viruses, hosts, and spatial dimensions. When the landscape is fragmented, viral prevalence is reduced in isolated habitats (Langlois et al. 2001), perhaps owing to associated low host population densities or lack of contact with new sources. Contiguous landscapes can show little consistent variation in prevalence even across relatively large areas when one considers

mouse dispersal capabilities (Maher et al. *in review*). In field surveys over multiple years, variation in prevalence from year to year can be considerable, such that, when host populations are low, the virus may go undetected, only to reappear when host populations rise (Boone et al. 2002; Calisher et al. 2005; Kuenzi et al. 2007). Clustering of individuals may also be an important factor regarding local prevalences (Abbott et al. 1999; Root et al. 2005). It has been hypothesized that the virus becomes locally extinct, requiring recolonization from local refugia in neighboring host populations to re-establish virus populations at a site (Boone et al. 2002).

This refugium–recolonization hypothesis relies on host metapopulation structure and rather ample connectivity of the landscape. Herein, I test the feasibility of this hypothesis using spatially explicit simulations of hantavirus transmission based on cellular automata in an agent-based context. These simulations incorporate realistic demographic data while simultaneously allowing for variation in disease transmission and host dispersal. I tested how long it would take for disease occurrence and prevalence to establish throughout a landscape when pathogens in specific areas have become locally extinct, and how the dispersal characteristics can mediate the time to complete spread across a landscape. I should find support for the local refugium–recolonization hypothesis if areas lacking the disease can be repopulated within a short time frame under biologically reasonable dispersal parameters.

Methods

Simulation

I developed a Visual Basic for Applications (VBA) script that establishes a dimensionless 20 × 20 grid, representing a continuous landscape, and that tracks the spread of a pathogen in mouse populations across the landscape through multiple generations on a month-by-month basis. The characteristics of the simulation follow, as closely as was possible, known

characteristics of hantavirus infections in rodents, particularly Sin Nombre Virus in *Peromyscus maniculatus.* The simulation was structured as follows.

Initially, 10,000 individuals were generated at an even sex ratio, assigned ages of 1–6 months at random, and placed in a cell. Initial "seed" sites for infection were spread across the landscape in various ordered configurations designed to minimize clustering. Between replicates, the initial pattern was held constant and only refugia were populated with the infected individuals. Dispersal dynamics were mediated by two parameters: (1) crowding effects, which was a proxy for the effects of resource availability and density on rodent populations, and (2) dispersal probability. Monthly demographic parameters were taken from Reed and Slade (2007), and all replicates were initiated in June.

The simulation progressed as follows:

1. Mice interacted with all other mice in their grid cell.
    a. Within each grid cell, residents were paired sequentially with one another.
    b. When the pairing was between a male and female, they would copulate and the female was considered pregnant (see below).
    c. If either of the pair was infected with the virus, transmission of the virus would occur if a value from a uniform random draw was smaller than the transmission probability.
2. The number of infected individuals, the total number of individuals, and the number of cells holding infected individuals were tallied.
3. New mice were added through a reproductive cycle.
    a. If the population of the cell was <60, then each female mouse had a chance to give birth. If the population was ≥ 60, then no mouse would give birth in that cell.

   b. A mouse would give birth if the probability of pregnancy was greater than a value from a uniform random draw.
   c. The number of pups to the new mother was determined from a draw of a uniform random variable
      i. A value <0.25 was assigned 4 pups.
      ii. A value ≥0.25 but <0.75 was assigned 5 pups.
      iii. A value >0.75 was assigned 6 pups.
   d. All pups were introduced into the population in the same cell as their mother and were infection-free.
   e. Pups became adults and were treated as such in the month following the birth month and were treated as such in the following month.
4. Mice died and were removed from the population:
   a. If the mouse was 12 months of age, or
   b. If the mortality probability exceeded a uniform random number; infected and uninfected mice were assigned equal mortality probabilities.
5. Mice would disperse from their cells to a neighboring cell if both of two conditions were met:
   a. If the mouse was the only resident in the cell or if more residents were present in the cell than the "crowding effect" variable (see below).
   b. If the dispersal probability exceeded a uniform random number.
6. The script returned to step 1, and looped for 54 months.

The simulation was run in Excel 2007 and is available from the author upon request.

Analysis

I replicated each iteration 50 times from the same initial conditions. Eight different refugia arrangements were explored, with 352, 304, 254, 200, 144, 64, 40, and 20 refugia, varying prevalence in initial conditions as discussed earlier. Two levels of prevalence for refugia were explored: 0.15 and 0.35, which represent a commonly viewed regular prevalence and a commonly found maximum prevalence, respectively (e.g., Abbott et al. 1999, Douglass et al. 2001, Kuenzi et al. 2007). On average, 25 individuals were in each cell, such that 3–4 and 8–9 infected mice were in each refugium under the two prevalence conditions, respectively. The crowding effect was fixed between replicates at 25 or 40, and dispersal probability varied from 0.125 to 1.0, depending on the iteration, but was fixed between replicates. The crowding effect parameters represent the average cell population from the initial conditions and an estimate of the number of mice required to maintain the virus in a population (A. D. Luis, pers. comm.). I used two transmission probabilities: 0.025, which is higher than expected, and 0.015, which is approximately the value that has been estimated from data (A. Luis, pers. comm.). As a consequence, I explored 512 different initial conditions.

My analysis focused on the number of months necessary for the entire grid to be populated with the virus at any prevalence. For each month during each simulation, I calculated the number of cells with the infected mice. To summarize overall tendencies in the results, I used a factorial ANOVA to test the effects of the following predictors on the time to complete occurrence: number of initial refugia, refugium infection prevalence, dispersal rate, and the cluster effect. I included interaction terms for each possible combination of predictors and Pareto plots to understand the effects of the parameters. For the ANOVA, I used MINITAB ver. 15.1 (http://www.minitab.com).

Results

The transmission probability of 0.015 failed to spread the virus to the entire grid under all sets of parameter values that were explored. Furthermore, this parameter value led to the loss of the virus on the grid when the number of refugia were <144, and the virus became very rare (<5 cells) or disappeared in the vast majority of simulations with higher numbers of refugia. Of the replicates where >5 cells were infected after 54 months, the total number of cells infected was invariably less than the starting number of refugia. Using a transmission probability of 0.025, the majority of iterations resulted in at least one infected individual in a cell for one month, but prevalence in all grid cells never attained a minimum of 0.15 within 54 months. Different iterations resulted in different times to complete occurrence, and in some cases produced variable numbers of replications reaching complete occurrence (Table 5). The larger crowding effect led to a longer time to complete occurrence, as did a lower prevalence in the refugia (in all but three iterations).

Increasing the number of refugia shortened the time needed for all cells to have non-zero prevalences of the virus. The fastest time to complete coverage was 9.9 months ± 1.1 (1 SD), under the parameters of 352 refugia, refugia prevalence of 0.35, crowding factor of 25, and dispersal of 0.625. Exemplar plots of average occurrence over time and histograms show the pattern of quick increase in distribution before reaching, or nearly reaching complete occurrence (Figures 11 and 12).

The factorial analysis of the simulation output was significant for main effects ($P < 0.001$), and interaction of 2-way and 3-way terms also were significant ($P < 0.001$), though the 4-way interaction term was not ($P = 0.161$). The Pareto plot of the interaction terms shows that the dominant effects were the number of refugia, followed by the crowding effect, and then dispersal

probability (Figure 13). The remaining terms that were significant were dispersal probability × crowding effect, refugium prevalence, dispersal probability × number of refugia × crowding effect, number of refugia × crowding effect, number of refugia × refugia prevalence, dispersal probability × number of refugia × refugia prevalence, and dispersal probability × refugia prevalence. Thus, the average time to complete occurrence was dependent on the parameter settings such that each setting was relatively unique.

Discussion

Spread and prevalence in grid cells

Perhaps the most striking trend in the simulations was that a transmission probability of 0.015 failed to spread the virus to every cell in the grid, and generally led to extinction of the virus across the grid in those simulations. Also surprising was that the prevalence in all grid cells never exceeded 0.15 regardless of the starting conditions, even though one set began with 88% of the cells at 0.35 prevalence. Within the context of the refugium–recolonization hypothesis, the expectation would be that local prevalence would return to "normal" levels once infected individuals dispersed into the region (Boone et al. 2002). The simulation results do not support this expectation, however, as prevalence in all cells did not reach the 0.15 threshold, the minimum refugium prevalence. As described earlier, the 0.15 prevalence threshold is an approximate median value from natural populations and would represent a return to normal infection status in the populations.

Demographic parameter realism

Parameter values in this simulation are a mixture of realistic values derived from on-ground studies (e.g., Abbott et al. 1999, Douglass et al. 2001, Kuenzi et al. 2007), and some values that are more extreme. For instance, the demographic patterns are taken from

measurements of a real population in Kansas (Reed and Slade 2007). The number of pups produced incorporates the number of progeny and the probability of survival to adulthood in a single parameter, and does not reflect seasonal variation in offspring survival (Luis et al. 2010). Adding these independent sources of variation may add further reality to the simulation in terms of density dependent and seasonal shifts in prevalence that are not as dramatic as in the present version.

Dispersal probabilities

Dispersal is limited to occurring between neighboring cells, and this simulation does not allow for long-distance movements that can be seen in nature. *Peromyscus* individuals have been documented as moving far distances (e.g., >500 m, Bowman et al. 2001; Bowman et al. 1999; Maier 2002), and this may be seasonally specific (Rehmeier et al. 2004). In this simulation, the value of the dispersal probability between iterations changed dramatically and increased the flow of individuals at higher values. Thus, I invoked more local movement than that found in field surveys (e.g., Rehmeier et al. 2004, Reed and Slade 2007, Luis et al. 2010), while also restricting long-distance dispersal. Further, when the dispersal probability approached 1.0, every individual in a cell meeting the crowding requirements would move to a new cell. It might be unrealistic to force an entire local population to emigrate, but such considerations represent a greater mixing of the population and facilitate the transmission of the virus across cells.

Allowing for dispersal beyond nearest neighbor cells could lead to a shorter time to complete viral coverage, as infected individuals from distant cells would be able to reach cells without infection sooner. For example, infected individuals from non-edge refugium cells can reach 24 cells if dispersal is extended to include second neighbors, compared to the simulated

limit of the 8 nearest neighbors. These longer distance events often are viewed as rare (Bowman et al. 2001, Maier 2002, but see Rehmeier et al. 2004), and would have to be simulated accordingly.

Single species and uniform environments

This simulation does not consider multi-species interactions and their consequences on disease prevalence. An increase in mammal diversity may lead to between-species interactions, and may reduce transmission rates (Dizney and Ruedas 2009; Suźan et al. 2009). If such were the case, then refugia containing infected individuals would be concentrated in the regions that are least species-rich. Assuming that species-rich and species-depauperate sites were adjacent, the environment or habitat also would have to change quickly to account for an abrupt turn-over in species composition.

Peromyscus populations fluctuate markedly with changes in their environment (e.g., Luis et al. 2010; Reed and Slade 2007; Wolff 1996), which may lead to the reduced populations associated with absence of hantavirus detection. Refugia would have to have populations that are more stable (i.e., resistant to environmental fluctuations) or occur in a region not affected by the conditions causing the reduction in host populations. The present simulation works under the latter assumption that the refugia populations were able to maintain population sizes independent of an environmental fluctuation, while absence cells represent sites with a recovering population. Under the refugium hypothesis, populations could act alternatively as sources and sinks depending on the local environmental conditions. The scale of such environmental effects would no doubt have to be quite small or landscape dependent, as neighboring populations must experience independent (or relatively so) conditions.

Including additional species or environmental heterogeneity or both conceivably could increase the time for the virus to occur in every cell. These parameters would lead to a decrease in *Peromyscus* individuals, though through different mechanisms, and reduce the opportunities for transmission of the virus. For instance, additional species would result in a reduction of resources through competition (Abramsky et al. 1979; Dueser and Hallett 1980; Redfield et al. 1977), while harsher environments simply would have fewer resources (Chesson et al. 2004; Chesson and Huntly 1997; Yarnell et al. 2007). Therefore maintenance of the virus in such cells would be more difficult and additional dispersal would be required for the host to populate the cell if local extinction were to occur.

"Refugia" vs. local absence

The number of cells selected as viral refugia was quite broad, from 5% to 88% of the available cells. The lower values are more indicative of the term refugium in that only a few sites have infected individuals and the majority of the cells are not host to infected individuals. Whereas higher values represented local extinction of the virus in a small number of cells and maintenance of infected individuals in the majority of cells. Thus, my simulations present a spectrum of potential circumstances for absence of hantavirus across a landscape.

Interpretation of Results

The most important parameter with regard to time to complete virus coverage was the number of refugia, followed by the crowding effect. The importance of these parameters is obvious: (1) The more cells infected, the fewer cells required to spread the infection to the entire grid, and (2) the lower the value of the crowding effect, the more often dispersal will happen. Dispersal was important, particularly in context with the crowding effect. Again, this result is logical, in that spread of the virus will be mediated by the number of infected individuals that

move to infection-free cells. Infection prevalence within viral refugia had a significant effect also, but it was not as large as the previous effects. One could assume that higher prevalence in the refugia would greatly decrease the time to complete occurrence, but perhaps the numbers of individuals infected in the initial conditions were not high enough to do so, given the influence of the other parameters.

The factorial analysis of the simulation results demonstrates that the impact of interactions between the initial parameters. The 2-way and 3-way interaction terms were significant, but the 4-way was not. Half of the 3-way interactions had significantly strong effects, and 4 of the 6 2-way interactions had significantly strong effects (Fig. 3). The interaction between refugium prevalence and the crowding effect was not significant, nor were the two 3-way interactions incorporating both of these variables. Thus, little association exists with these two parameters regarding the time to complete occurrence. Interestingly, interaction between the dispersal probability and the number of refugia was not significant; adding either of the remaining two parameters to this combination did result in a significant effect. Perhaps it is the interaction between the third variable and one or both of dispersal probability and number of refugia that results in the significant effect.

The fastest average time for complete re-colonization was ~10 months, although the parameters associated with this result include a very high dispersal probability and low tolerance of crowding, leading to a lot of movement between cells. More realistic parameter values regarding dispersal are lower probabilities with greater tolerance of crowding that are in accord with recent estimates for *Peromyscus* (A. D. Luis, pers. comm.) Under these conditions, only a small percentage of replicates showed complete spread of the virus within the 54-month limit. Even when >88% of the grid was infected initially, the grid did not generally reach complete

virus coverage with low dispersal probability and high tolerance of crowding. At minimum, a dispersal probability of 0.25 was required to reach complete occurrence, and this occurred commonly when initial conditions also included fewer refugia. This probability is equivalent to one quarter of the mice moving to a new grid cell each month, which would exceed known dispersal rates, and thus is not realistic biologically.

Broader Impacts & Future Directions

This simulation shows that the refugium–recolonization hypothesis for hantavirus is viable only so long as the expected time to re-colonize is >12 months. Alternatively, these results may suggest that the refugium hypothesis is untenable because the amount of dispersal required to populate all cells with the virus is high, especially when infection is expected to return within a year. In addition, with a broad-scale decline in populations of *Peromyscus* resulting in occurrence of the virus in only a few locations, it will take 3–4 years under the smallest dispersal values before the infection occurs throughout a region. While expectation of infected mice in every cell may be viewed as too broad, it avoids the possibility that a single site remains absent throughout the simulation.

Realistically, it seems more probable that the virus may go undetected on local landscapes, rather than disappear entirely and then reappear as a result of re-colonization. Transmission of the virus requires interaction between infected and potential hosts, such that low populations levels may prove difficult for spread. Local foci of infected individuals were noticed by Abbott et al. (1999), wherein the virus stayed in the overall population, but only in specific locations mediated by habitat and nests. When environmental conditions improved, hosts began moving through the landscape and the virus spread to the remaining study area. Employment of

grids for trapping may fail to sample the entire habitat space and potentially could miss local foci when host populations are low.

Future simulations should attempt to vary the population sizes between cells or the crowding effect. The crowding effect is analogous to resource availability in this simulation, as more tolerance is proportional to available resources. Seeding refugia with larger population sizes than infection-free cells and assuming a higher prevalence than in this simulation will lead to a higher number of dispersing individuals that are infected, thus spreading the virus in absent cells. By means of addition of species that could disrupt host interactions or host densities through competition, one could estimate a parameter for the dilution effect in this system.

The refugium–recolonization hypothesis for hantavirus infection as suggested by Boone et al. (2002) is logically and intellectually appealing. When host populations decline, prevalence declines as well until the virus seemingly vanishes from the landscape, only to return once host populations recover. This simulation however found little support for the hypothesis as a mechanism for re-establishing the virus into a landscape or region. Only when I invoked extreme dispersal and began with many refugia did the virus re-populate all cells under a year. Under more biologically reasonable conditions, the virus rarely spread to all cells, and when few cells were initiated as refugia, the time required for the virus to re-populate all cells was much longer than seen in field surveys. The conclusion made from this simulation is that the refugium–recolonization hypothesis cannot be the lone driver for appearance of infection after local extinction. Instead, other mechanisms, that may not be mutually exclusive from dispersal, are required to explain infection at this scale.

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Conclusion

This dissertation presents four chapters that evaluate the patterns and processes regarding the distribution and maintenance of pathogens and parasites in mammal communities. While each represents a single manuscript, taken together they build an inclusive picture about the structure and occurrence of disease at various scales. In particular, this treatment shows pathogens and parasites, that while inherently tied to their hosts, have different ecological requirements than the hosts and occurrences are mediated by these needs. Additionally, I call attention to discrepancies in some of the current hypotheses regarding pathogen occurrence in landscapes.

In Chapter 1, I demonstrate that plague-infected hosts occur within a subset of the host distributions, and that plague-infected host distributions generally are similar. In Chapter 2, I show the hantavirus prevalence is independent of landscape connectivity and mammal community structure. In Chapter 3, I show that flea communities are structured independently from the host communities, and that flea species richness and community structured are related to changes in elevation. In Chapter 4, I tested the refugium–colonization hypothesis using a simulation and found that this idea is tenuous as viral populations are not restored under biologically reasonable parameters within naturally observed time frames.

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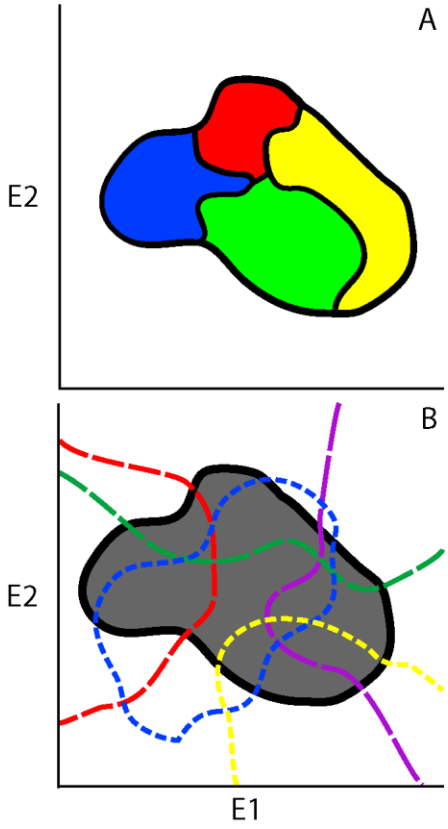

Figure 1. A conceptual diagram of relationships between plague occurrences, host occurrences, and environmental variation under the Host Niche Hypothesis (A) and the Plague Niche Hypothesis (B). The HNH states that the occurrence of plague (thick black line) is a result of a combination of individual host ranges (colored polygons). The PNH, on the other hand, states that plague has its own ecological niche (gray polygon), and that occurrence of plague in mammal hosts occurs only where host ranges (colored outlines) coincide with the plague niche.

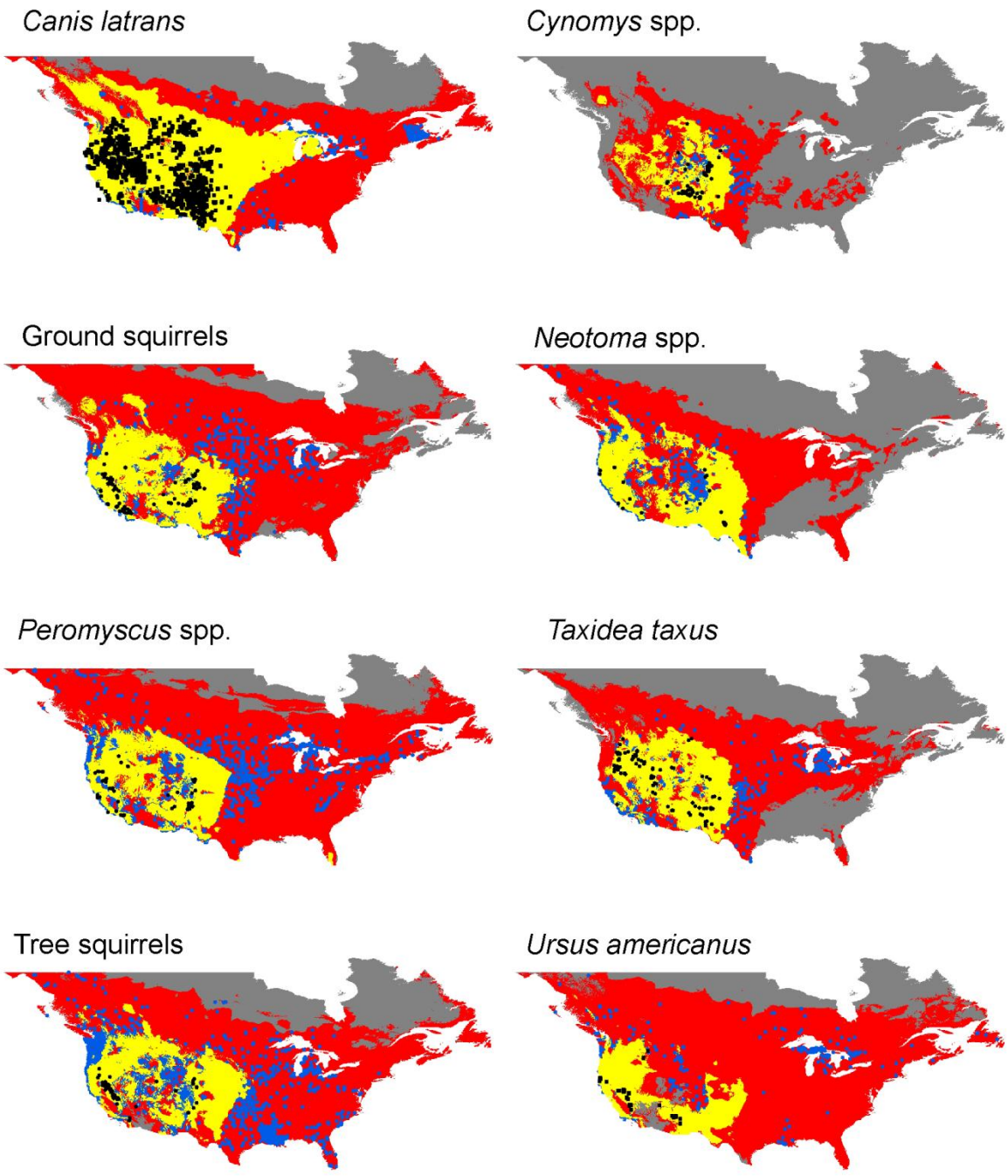

Figure 2. Comparison of overall host niche models and plague-infected-host niche models. In each map, the host's overall modeled potential distribution is shown in red, the plague-infected portion of the host's distribution in yellow, host occurrence points as blue dots, and plague-infected host occurrence points as black dots (for simplicity, host occurrence points are not shown in plague-infected areas).

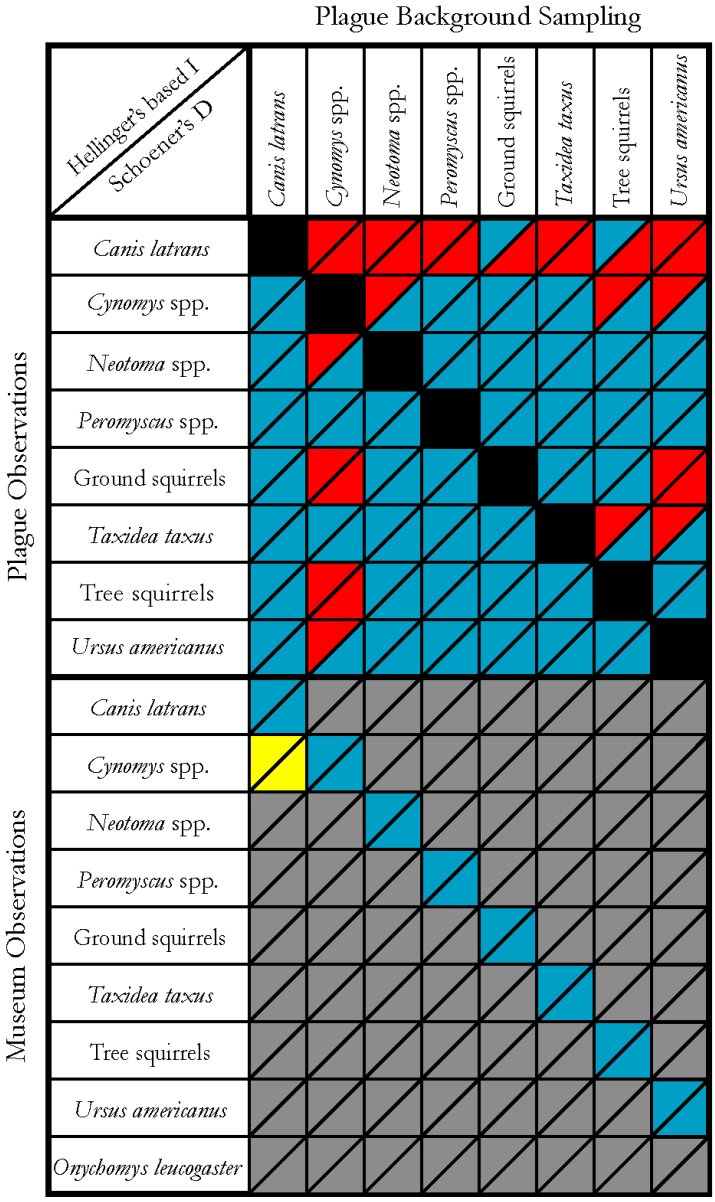

Figure 3. Results of background similarity tests. Rows represent point occurrence data, and columns relate plague-infected-taxon comparisons (i.e., background areas in the similarity tests); the upper portion of each cell represents the similarity test results based on Hellinger's *I*, and the lower portion represents the similarity test results based on Schoener's *D*. Blue cells support the predictions of the PNH, while red cells fail to support the predictions of the PNH. Additional comparisons of plague-infected hosts to other host taxa are designated in grey (statistically not-similar $P < 0.05$) or yellow ($P > 0.05$).

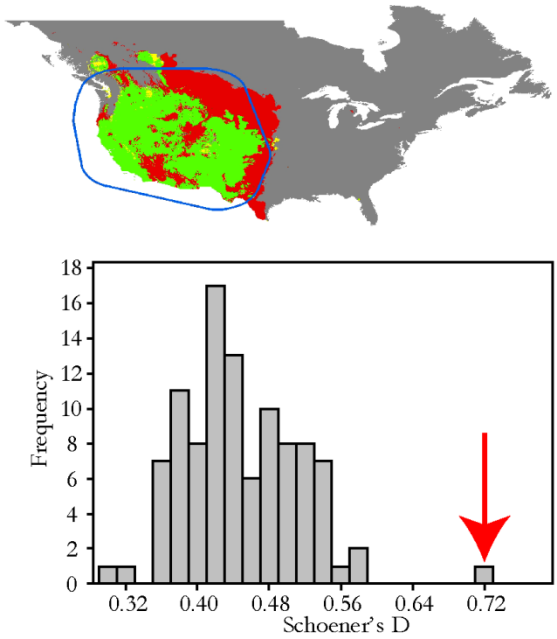

Figure 4. An example of the background similarity test in ENM Tools. The map shows two niche-model-based predictions (1) plague-infected-ground squirrels and (2) and a random draw of points from the M of plague-infected *Taxidea taxus*, shown as the blue polygon. Red cells represent predictions only from the random draw, yellow cells represent predictions only from the plague-infected-ground squirrels, and green cells represent overlap between the two. The histogram shows the entire range of Schoener's *D* values from the 100 random-draw models; the observed Schoener's *D* is shown as the red arrow. In this example, similarity between the two plague-infected host taxa is greater than that expected given the environments surrounding the distributional area of *Taxidea*.

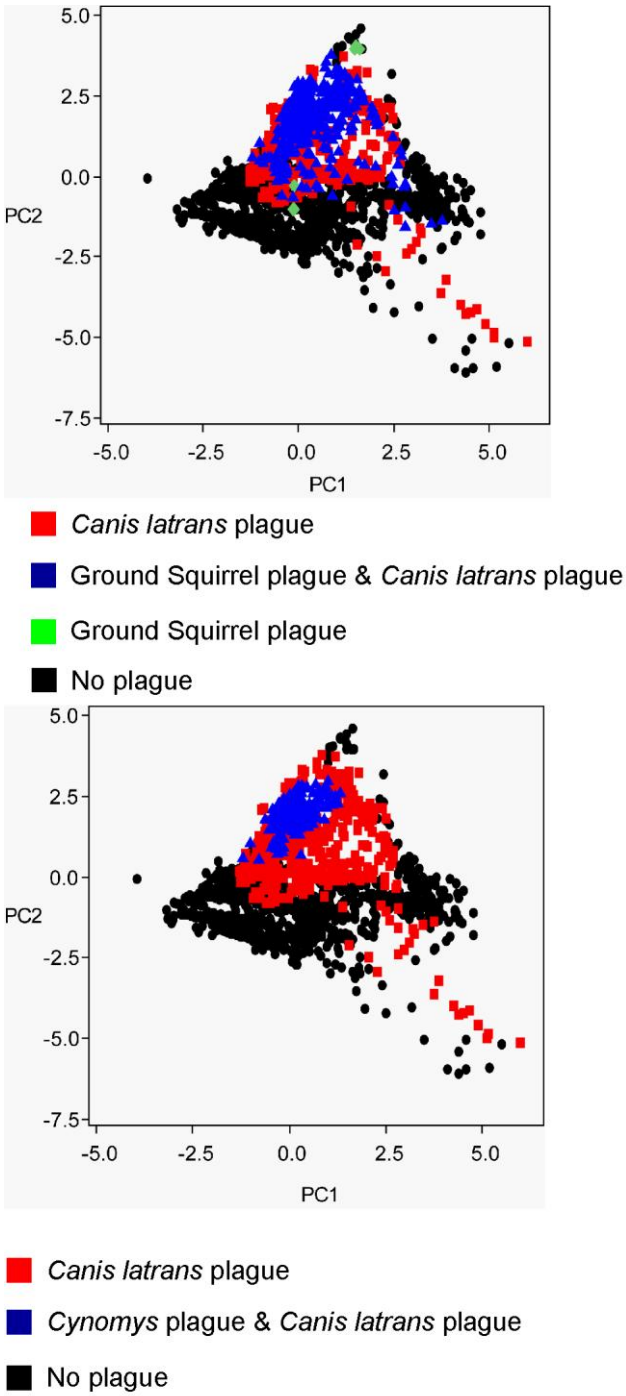

Figure 5. Principal components analysis of the environmental data used to generate ecological niche models from plague-infected records. Groups are labeled to show how they differ in ecological space, and particularly to illustrate that the ecological niche model of *Canis latrans* plague-infected data subsumes most, if not all, of the area of environmental space associated with other plague-infected taxa.

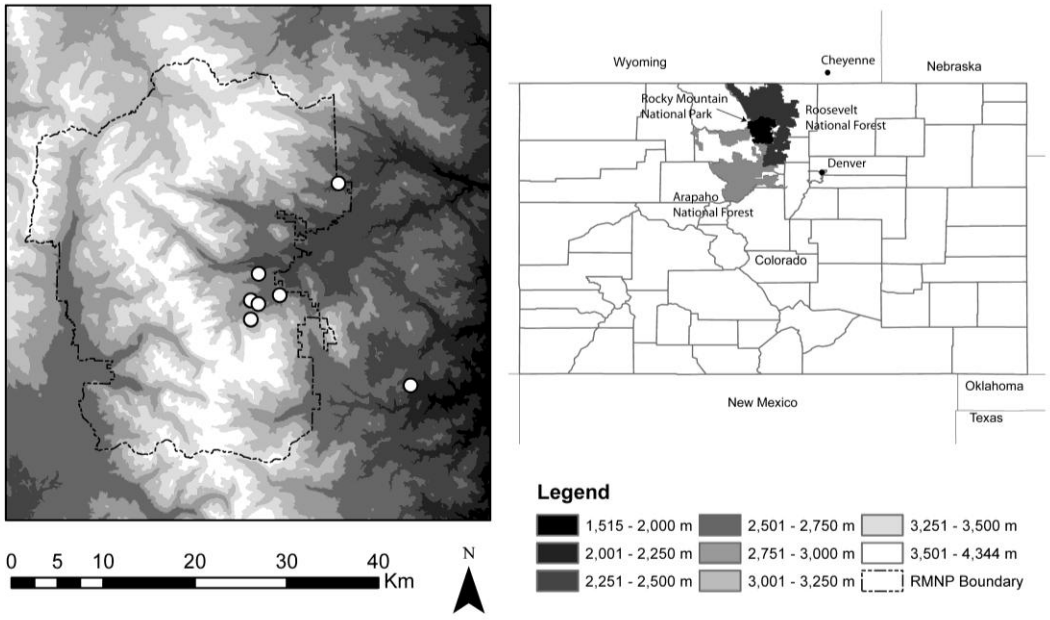

Figure 6. Map of the study region, showing the Rocky Mountain National Park boundary and the 7 sampling locations distributed within distinct elevation bands.

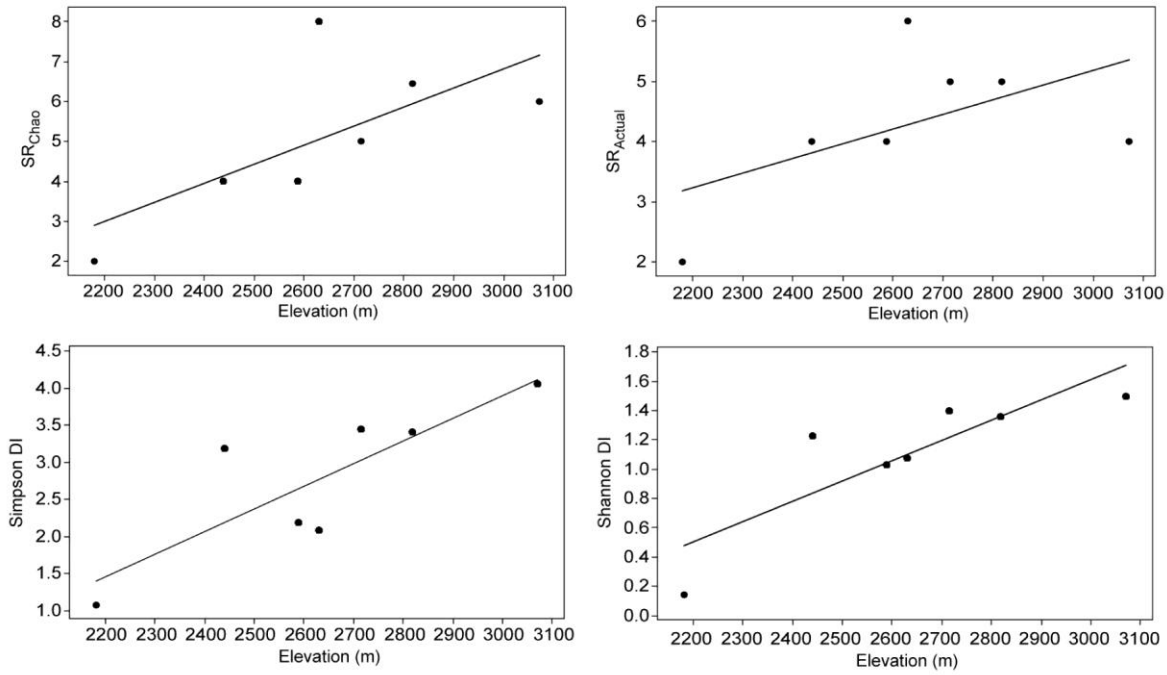

Figure 7. Regressions of host richness and diversity measures on elevation.

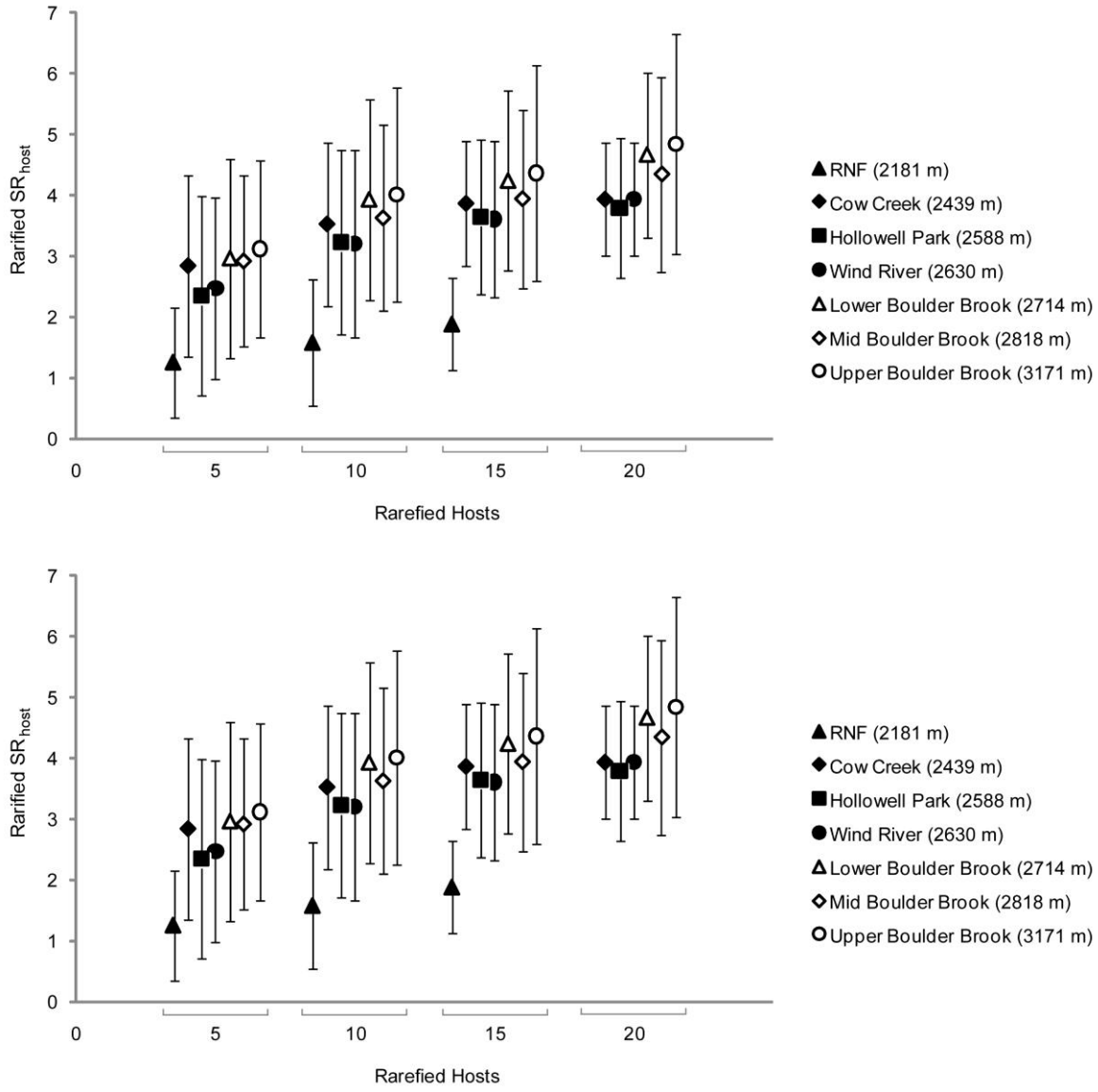

Figure 8. Plots of rarefied hosts against estimates of SR_{flea} (± 2 SD) and SR_{host} (± 2 SD) show that the estimates increases with the number of random hosts selected.

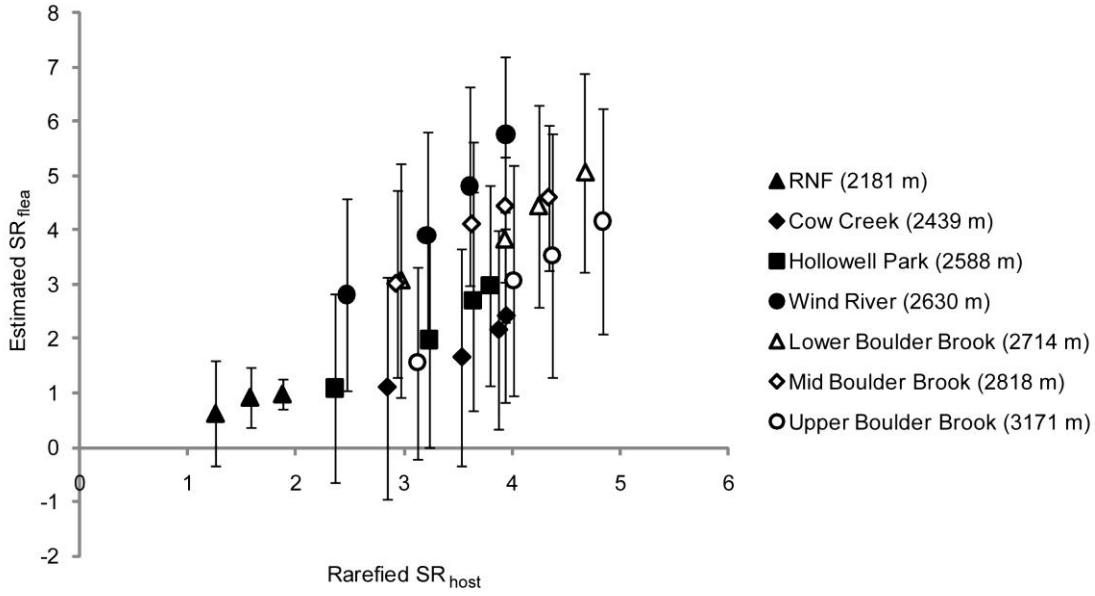

Figure 9. The plots of rarefied SR_{host} and associated $SR_{flea} \pm 2$ SD show that as the estimate of SR_{host} increases so does SR_{flea}, although error bars suggest significant overlap and limited differentiation between sites.

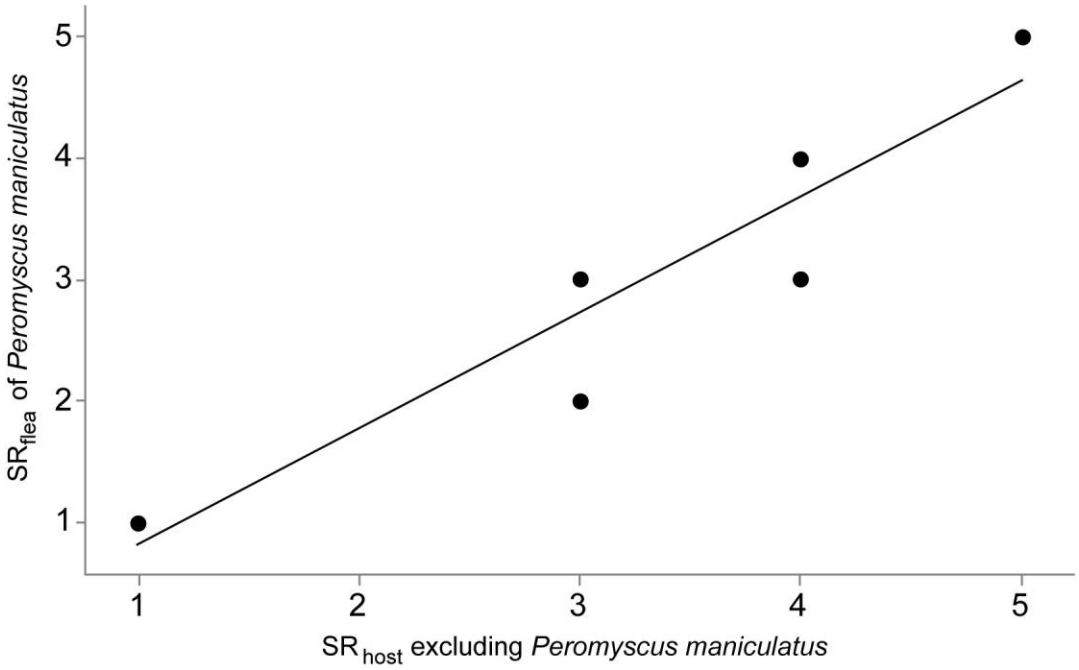

Figure 10. The regression of SR_{flea} of *P. maniculatus* on the remaining host richness was signifcant (P = 0.003, R^2 = 85.9%) and suggests that presence of other hosts predicts the number of flea species on Deer Mice.

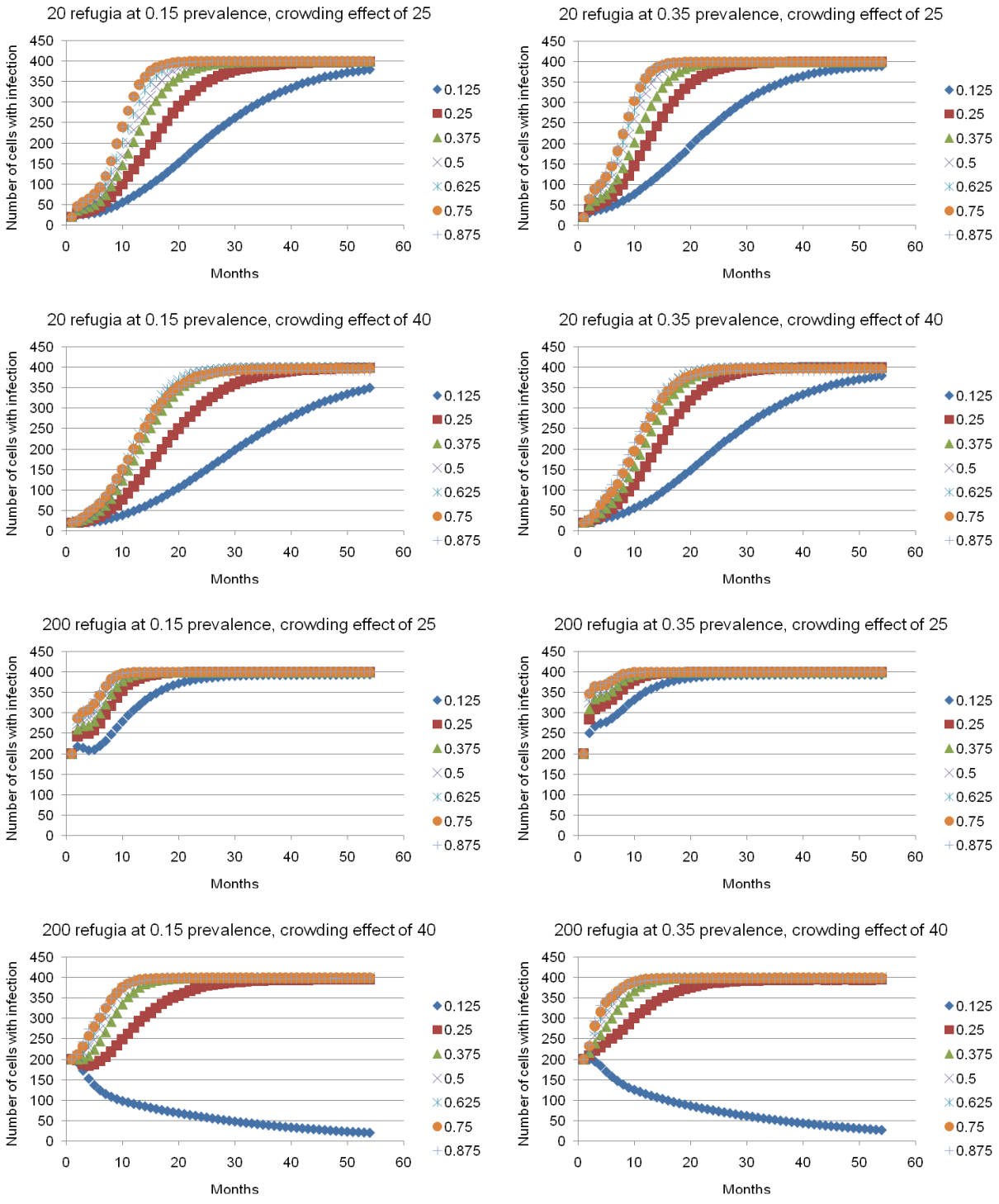

Figure 11. Exemplar plots of the average number of cells with the virus over time, from 20 and 200 refugia, both values of refugia prevalence and crowding effects. Transmission probabilities from 0.125 to 0.875 are shown.

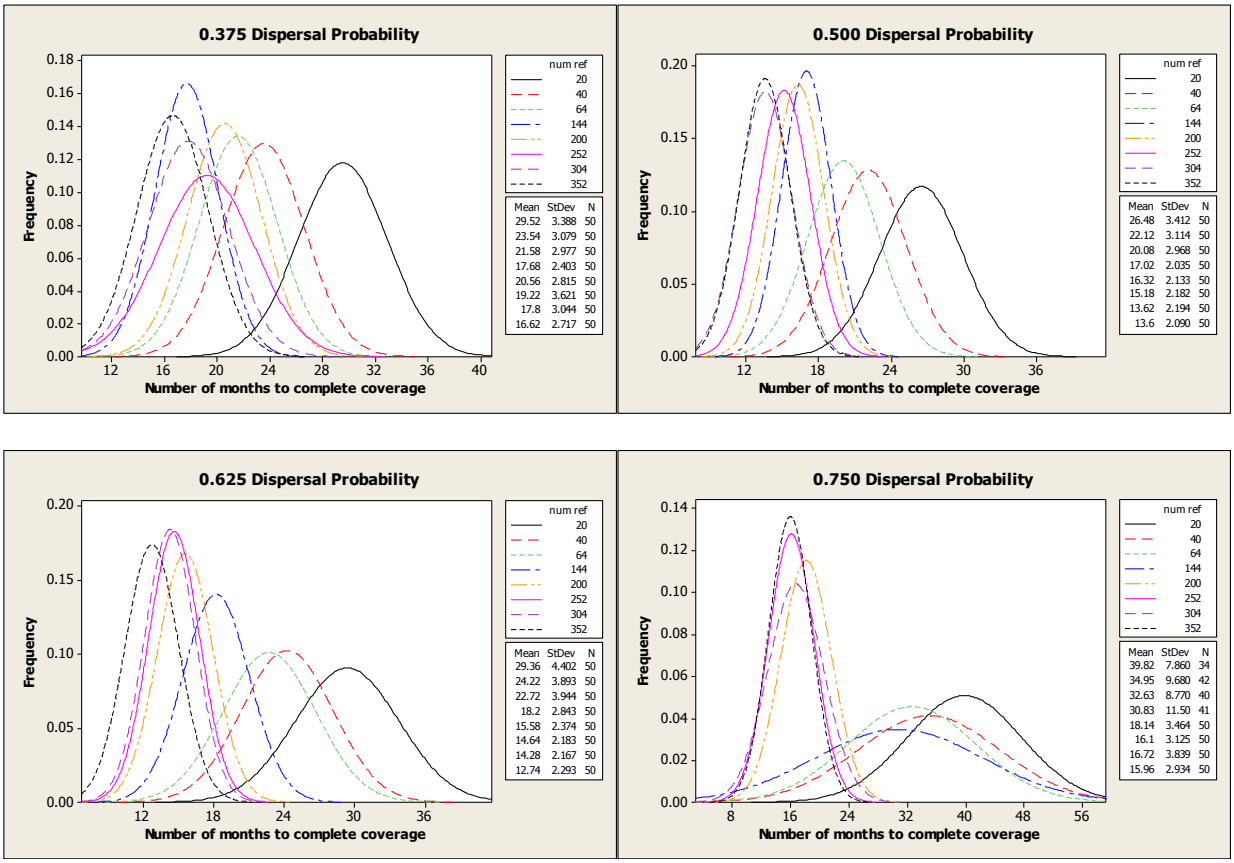

Figure 12. Exemplar histograms of the number months it took for the replications to reach complete occurrence over the grid.

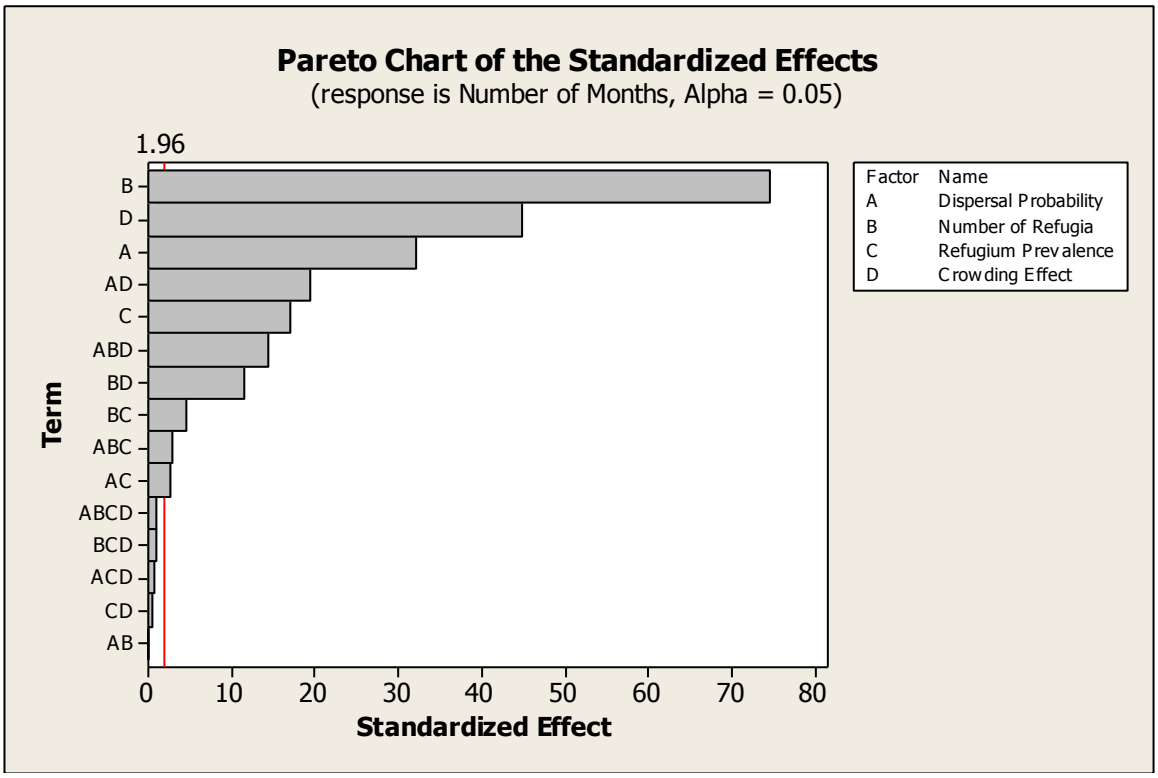

Figure 13. The Pareto plot of the factorial analysis of the simulation.

Table 1. Occurrence and number of unique individuals captured of small mammals captured or detected along the transect.

Host species	Roosevelt National Forest	Cow Creek	Hollowell Park	Wind River	Lower Boulder Brook	Mid Boulder Brook	Upper Boulder Brook
Callospermophilus lateralis	0	8	15	3	0	2	1
Myodes gapperi	0	0	0	1	6	15	18
Neotoma cinerea	0	0	0	3	6	1	0
Peromyscus maniculatus	30	21	68	30	26	22	20
Tamias minimus	0	12	10	1	6	0	13
Tamias quadrivittatus	1	0	0	0	0	0	0
Tamias umbrinus	0	4	0	5	10	9	0
Tamiasciurus hudsonicus	0	0	0	1	0	1	1
Urocitellus elegans	0	0	13	0	0	0	0

Table 2. Locality information and local variables from sampling of an elevational transect of 2,181–3071 m in Rocky Mountain National Park and Arapahoe–Roosevelt National Forest.

Site	*Elevation (m)*	SR_{flea}	$Chao_{flea}$	$Chao_{flea}$ *95% CI Lower Bound*	$Chao_{flea}$ *95% CI Upper Bound*	*Flea Shannon's DI*	*Flea Simpson's Reciprocal DI*
Roosevelt National Forest	2181	1	1.0	1.00	1.29	0.00	1.00
Cow Creek	2439	3	3.5	3.03	11.44	0.72	1.78
Hollowell Park	2588	4	4.0	4.00	5.30	1.34	5.00
Wind River	2630	6	8.0	6.18	28.13	1.22	2.82
Lower Boulder Brook	2714	6	8.0	6.18	28.13	1.23	3.00
Middle Boulder Brook	2818	5	5.0	5.00	5.00	1.41	4.02
Upper Boulder Brook	3171	5	7.0	5.18	27.13	1.21	3.01

Table 3. Occurrence and abundance of flea species at locations along the elevational transect.

Host	*Flea*	*Roosevelt National Forest (2181 m)*	*Cow Creek (2439 m)*	*Hollowell Park (2588 m)*	*Wind River (2630 m)*	*Lower Boulder Brook (2714 m)*	*Mid Boulder Brook (2818 m)*	*Upper Boulder Brook (3171 m)*
Lepus americanus	*Hoplosyllus glacialis*	0	0	0	0	0	0	1
Myodes gapperi	*Aethica wagneri*	0	0	0	0	1	0	5
	Catallagia decipiens	0	0	0	0	0	0	1
	Eumolpianus eumolpi	0	0	0	0	0	0	1
	Megabothris abantis	0	0	0	0	0	16	1
	Orchepeas sexdentatus	0	0	0	0	1	0	0
	Oropsylla idahoensis	0	0	0	0	0	0	1
	Peromyscopsylla hesperomys	0	0	0	0	4	21	0
Neotoma cinerea	*Aethica wagneri*	0	0	0	0	1	0	0
	Eumolpianus eumolpi	0	0	0	0	1	0	0
	Orchepeas sexdentatus	0	0	0	0	1	0	0
Peromyscus maniculatus	*Aethica wagneri*	0	9	3	20	26	12	3
	Catallagia decipens	0	0	0	1	1	0	1
	Epitedia wenmanni	0	0	0	1	0	0	0
	Eumolpianus eumolpi	0	0	1	0	0	0	0
	Malaerus telchinum	4	0	4	2	0	1	0
	Opisodasys keeni	0	2	0	0	1	0	0
	Peromyscopsylla hesperomys	0	1	0	14	7	4	0
Tamias minimus	*Eumolpianus eumolpi*	0	0	1	3	5	0	10
Tamias umbrinus	*Aethica wagneri*	0	0	0	1	0	1	0
	Eumolpianus eumolpi	0	0	0	0	25	16	0
Urocittelus elegans	*Oropsylla idahoensis*	0	0	2	0	0	0	0
	Grand total	4	12	11	42	74	71	24

Table 4. Locality elevation and associated flea community and richness metrics

Site	*Elevation (m)*	SR_{flea}	$Chao_{flea}$	*$Chao_{flea}$ 95% CI Lower Bound*	*$Chao_{flea}$ 95% CI Upper Bound*	*Flea Shannon's DI*	*Flea Simpson's Reciprocal DI*
Roosevelt National Forest	2181	1	1.0	1.00	1.29	0.00	1.00
Cow Creek	2439	3	3.5	3.03	11.44	0.72	1.78
Hollowell Park	2588	4	4.0	4.00	5.30	1.34	5.00
Wind River	2630	6	8.0	6.18	28.13	1.22	2.82
Lower Boulder Brook	2714	6	8.0	6.18	28.13	1.23	3.00
Middle Boulder Brook	2818	5	5.0	5.00	5.00	1.41	4.02
Upper Boulder Brook	3171	5	7.0	5.18	27.13	1.21	3.01

Table 5. Average time in months (± SD) and number of replicates to complete occurrence of the virus (maximum of 54 months)

Number of refugia	*Refugia prevalence*		*Crowding factor*		*Dispersal Probability*																	
	0.15	0.35	25	40	0.125		0.250		0.375		0.500		0.625		0.750		0.875		1.000			
20	×		×		*		39.217 ± 6.359	46	29.440 ± 4.769	50	25.780 ± 3.303	50	22.660 ± 2.973	50	20.680 ± 2.622	50	20.800 ± 3.523	50	21.300 ± 2.823	50		
		×	×		*		35.500 ± 4.718	50	24.780 ± 2.690	50	21.260 ± 2.783	50	19.420 ± 1.939	50	18.720 ± 2.658	50	17.900 ± 2.121	50	18.420 ± 1.642	50		
	×			×	*		43.435 ± 5.679	46	31.740 ± 4.139	50	30.220 ± 3.945	50	31.120 ± 5.317	50	40.90 ± 7.19	50	*		*			
		×		×	*		38.380 ± 5.142	50	29.520 ± 3.388	50	26.480 ± 3.412	50	29.360 ± 4.402	50	39.82 ± 7.86	34	*		*			
40	×		×		*		32.460 ± 5.104	50	23.340 ± 3.243	50	20.040 ± 2.099	50	18.720 ± 1.750	50	18.200 ± 2.231	50	17.960 ± 2.030	50	18.120 ± 1.955	50		
		×	×		46	1	26.540 ± 3.530	50	20.160 ± 2.271	50	18.340 ± 1.791	50	16.300 ± 1.799	50	15.700 ± 2.03	50	15.560 ± 1.680	50	16.240 ± 1.721	50		
	×			×	*		35.100 ± 4.808	50	27.340 ± 3.712	50	25.440 ± 3.296	50	26.340 ± 3.723	50	35.83 ± 8.68	50	49.00 ± 2.83	2	*			
		×		×	*		30.720 ± 5.395	50	23.540 ± 3.079	50	22.120 ± 3.114	50	24.220 ± 3.893	50	34.95 ± 9.68	42	41	1	*			
64	×		×		48.00 ± 2.83	2	29.180 ± 4.628	50	21.420 ± 2.627	50	18.680 ± 2.075	50	16.940 ± 1.984	50	16.080 ± 2.137	50	15.880 ± 1.586	50	16.740 ± 1.639	50		
		×	×		44.50 ± 12.02	2	24.720 ± 2.886	50	18.220 ± 1.844	50	16.520 ± 1.887	50	15.160 ± 1.683	50	14.340 ± 1.599	50	14.000 ± 1.616	50	15.680 ± 1.845	50		
	×			×	*		31.300 ± 5.040	50	24.520 ± 3.333	50	21.720 ± 2.195	50	24.080 ± 3.275	50	35.64 ± 8.93	50	*		*			
		×		×	47.67 ± 4.04	3	26.720 ± 3.76	50	21.580 ± 2.977	50	20.080 ± 2.968	50	22.720 ± 3.944	50	32.63 ± 8.77	40	*		*			
144	×		×		44.60 ± 4.62	5	23.240 ± 3.101	50	17.180 ± 2.310	50	15.560 ± 1.950	50	13.820 ± 1.453	50	13.640 ± 1.509	50	13.860 ± 1.512	50	15.040 ± 1.895	50		
		×	×		44.75 ± 6.70	4	20.920 ± 2.947	50	15.440 ± 2.072	50	13.560 ± 1.312	50	12.560 ± 1.296	50	11.820 ± 1.304	50	11.940 ± 1.621	50	13.420 ± 2.031	50		
	×			×	51	1	25.080 ± 3.556	50	18.780 ± 1.730	50	18.280 ± 2.339	50	21.340 ± 4.452	50	33.59 ± 10.60	50	*		*			
		×		×	49.00 ± 2.55	5	22.760 ± 3.761	50	17.680 ± 2.403	50	17.020 ± 2.035	50	18.200 ± 2.843	50	30.83 ± 11.50	41	*		*			
200	×		×		42	1	21.740 ± 3.256	50	16.640 ± 1.903	50	14.380 ± 1.354	50	13.140 ± 1.414	50	12.720 ± 1.415	50	12.400 ± 1.355	50	13.920 ± 2.078	50		
		×	×		37	1	19.640 ± 3.056	50	14.700 ± 2.225	50	12.560 ± 1.343	50	11.460 ± 1.034	50	11.000 ± 1.245	50	11.360 ± 1.711	50	13.300 ± 1.607	50		
	×			×	*		45.50 ± 2.52	4	22.980 ± 2.896	50	18.060 ± 2.298	50	17.700 ± 2.873	50	19.380 ± 3.356	50	32.67 ± 10.13	46	41	1		
		×		×	*	*	47.00 ± 9.90	2	20.560 ± 2.815	50	16.320 ± 2.133	50	15.580 ± 2.374	50	18.140 ± 3.464	50	29.77 ± 10.78	39	29.00 ± 13.53	3		
252	×		×		41.50 ± 4.95	2	20.880 ± 3.895	50	15.520 ± 2.112	50	13.300 ± 1.542	50	12.580 ± 1.279	50	11.940 ± 1.316	50	12.000 ± 1.471	50	13.660 ± 1.768	50		
		×	×		45.83 ± 5.64	6	17.920 ± 3.225	50	13.980 ± 1.635	50	12.020 ± 1.505	50	11.040 ± 1.228	50	10.480 ± 1.403	50	10.800 ± 1.340	50	12.340 ± 1.624	50		
	×			×	*		45.00 ± 5.66	2	21.720 ± 3.470	50	16.900 ± 2.435	50	16.280 ± 2.167	50	18.380 ± 3.096	50	32.79 ± 8.82	43	*			
		×		×	*		45.67 ± 4.84	6	19.220 ± 3.621	50	15.180 ± 2.182	50	14.640 ± 2.183	50	16.100 ± 3.125	50	27.17 ± 9.67	46	46	1		
304	×		×		45.00 ± 9.90	2	19.660 ± 3.679	50	14.440 ± 1.580	50	12.900 ± 1.344	50	12.120 ± 1.507	50	11.560 ± 1.264	50	11.380 ± 1.323	50	13.480 ± 1.821	50		
		×	×		47.00 ± 6.76	8	17.620 ± 2.975	50	13.160 ± 2.122	50	11.800 ± 1.616	50	10.360 ± 1.453	50	10.220 ± 1.389	50	10.360 ± 1.382	50	12.060 ± 1.659	50		
	×			×	*		39.50 ± 5.51	4	20.380 ± 3.050	50	15.740 ± 2.008	50	15.400 ± 2.204	50	17.220 ± 2.852	50	28.85 ± 9.68	41	*			
		×		×	*		40.00 ± 10.55	7	17.800 ± 3.044	50	13.620 ± 2.194	50	14.280 ± 2.167	50	16.720 ± 3.839	50	31.20 ± 10.65	46	*			
352	×		×		39.75 ± 7.63	4	18.700 ± 3.221	50	14.580 ± 1.949	50	12.020 ± 1.253	50	11.380 ± 1.105	50	11.420 ± 1.430	50	10.980 ± 1.286	50	13.220 ± 1.718	50		
		×	×		50	1	16.700 ± 3.666	50	12.500 ± 1.982	50	11.100 ± 1.432	50	9.920 ± 1.085	50	9.960 ± 1.564	50	10.060 ± 1.376	50	11.780 ± 1.776	50		
	×			×	*		40.50 ± 7.56	6	19.380 ± 2.594	50	15.640 ± 2.238	50	14.520 ± 1.764	50	17.460 ± 4.186	50	28.18 ± 9.62	45	30	1		
		×		×	*		36.57 ± 8.04	7	16.620 ± 2.717	50	13.600 ± 2.090	50	12.740 ± 2.293	50	15.960 ± 2.934	50	31.19 ± 10.72	48	46	1		