

THE INFLUENCE OF HOST ECOLOGY AND LAND COVER CHANGE ON RABIES
VIRUS EPIDEMIOLOGY IN THE FLINT HILLS

by

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Abstract

As human populations increase world-wide, land use and land cover are altered to support the rapid anthropogenic expansion. These landscape alterations influence patterns of zoonotic infectious disease emergence and propagation. It is therefore becoming increasingly important to study emerging and re-emerging diseases to predict and manage for future epidemics. Studies of directly-transmitted infectious diseases should consider three components of disease epidemiology: characteristics of the pathogen, ecology of the host, and habitat configuration of the underlying landscape. I studied the influence of both the host ecology of the striped skunk (*Mephitis mephitis*) and the alteration of the underlying landscape on the epidemiology of rabies virus in the Flint Hills of Kansas. This tall-grass prairie is experiencing woody expansion due to anthropogenic disturbance, altering the landscape on which the rabies virus emerges and spreads. We first studied the behavioral and social ecology of the striped skunk using field and genetic methods. We concluded that 1) striped skunks reached high population densities in anthropogenically disturbed habitats, 2) these individuals were not closely related, and 3) contact rates could be influenced by temperature. Using habitat-specific skunk densities from this initial study, we created spatially-explicit contact networks of skunk populations across the Upper Kansas River Watershed and simulated the emergence and spread of rabies through the system. This modeling approach revealed a threshold of forest habitat beyond which striped skunks became increasingly connected and the rabies virus reached greater extents across the landscape. Based on these findings we recommend fire regimes and land cover alterations to reduce woody encroachment across the Flint Hills and to avoid future disease epidemics in the region.

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CHAPTER 1 - Introduction

The rate at which new diseases are emerging is increasing world-wide due to increased human mobility and rapid landscape alteration (Field 2009). The majority of the diseases emerging or re-emerging share two common characteristics. Almost 75% of emerging infectious diseases are zoonotic (Field 2009) and most are significantly influenced by human expansion and disturbance to the environment (Barrett et al. 1998, Daszak 2001, Patz et al. 2004, Crowl et al. 2008, Grimm et al. 2008). The direct effects of land use change on disease epidemiology are complex, as anthropogenic disturbance alters the ecology and evolution of host species and host-pathogen dynamics (Patz et al. 2004, Wilcox and Gubler 2005). Examples of this phenomenon have been documented for a variety of zoonotic diseases. In the northern United States, anthropogenic factors including forest fragmentation, urbanization, and the subsequent loss of species richness are directly associated with increased risk of Lyme disease (Schmidt and Ostfeld 2001, Patz et al. 2004). West Nile virus exposure in songbirds has been linked to urbanization and related land use alterations in Atlanta, Georgia (Bradley et al. 2008). Additional zoonotic diseases, including Nipah virus (Daszak et al. 2001, Field 2009), Leptospirosis (Vijayachari et al. 2008), and several urban bacterial zoonoses (Comer 2001) have all been associated with developing and expanding urban areas throughout the world. With rapidly changing land use and land cover due to anthropogenic activity, it is critical to study and monitor the subsequent emergence of novel disease to mitigate and prevent human health crises (Grimm et al. 2000, Morens et al. 2004).

The epidemiological properties of a disease are dependent on three components: the pathogen, the host, and the underlying landscape. These three components are interrelated and

should be considered in any comprehensive study of disease emergence and spread. In this thesis, I have studied these three disease components in the rabies virus, which is episodic in the central United States, to determine future risks for humans, companion animals, and livestock. This research not only elucidates future hot spots of disease emergence for the rabies virus, but also provides innovative methods that can be integrated into studies of a broad range of emerging infectious diseases dependent on zoonotic hosts. This introduction will focus on the three epidemiological components associated with rabies and the methods used in this study to consider these disease factors.

The pathogen

Rabies is a zoonotic viral disease that has influenced human populations for at least the last 2,000 years (Kaplan 1985). The virus is a member of the lyssavirus genus of the family Rhabdoviridae (Nel and Markotter 2007). These viruses are ‘bullet shaped’ and are an average 180 nm in length and 75 nm in width (Davies et al. 1963). The RNA genome codes for 5 viral proteins which include nucleoprotein (N), phosphoprotein (P), and RNA polymerase (L) for formation of the condensed nucleocapsid, matrix protein (M) for virus budding, and surface glycoproteins (G) along the host-derived envelope integral in viral pathogenesis (Nel and Markotter 2007). Rabies causes encephalitis in infected individuals and almost always results in death after clinical symptoms have developed (Kaplan 1985). Once infected with the virus, an individual may not display symptoms for several weeks or months, depending on the species. In general, symptoms fall into one of two categories (Kaplan 1985, Brandao 2009). Spill-over into non-host species that are not carnivores typically results in the paralytic form of the disease which results in paralysis prior to death. Host species and carnivore spill-over species most commonly display the furious form of the disease. In the furious form, the infected become

more prone to attack and display abnormal behavior patterns, such as daytime movement in nocturnal animals (Kaplan 1985). In both forms of the disease, the infectious animal excretes the rabies virus in saliva and urine creating a significant risk of spread to other individuals.

Over 70,000 people world-wide die each year due to rabies infection (Dietzschold et al. 2008). In the United States, over \$300 million is spent each year on rabies prevention and control programs, which results in few human fatalities in the country (Center for Disease Control and Prevention 2007). Rabies has been eradicated from the domestic dog population in the United States due to strict vaccination programs (Velasco-Villa et al. 2008); however, the disease is still widely prevalent and circulates through wildlife hosts. There are several terrestrial host species for rabies variants in the United States (Figure 1.1) and variants in bat species are found throughout the country (Blanton et al. 2007).

The host

Striped skunks (*Mephitis mephitis*) are meso-carnivores with a geographic range that covers most of the United States, southern Canada, and northern Mexico (Rosatte and Larivière 2003). As habitat generalists, they take advantage of most habitat types within this range, but typically prefer forest-edge zones (Bixler and Gittleman 2000, Larivière and Messier 2000) and agricultural areas (Larivière and Messier 1999), but is also an urban-adapted species (Gehrt 2005, Weissinger et al. 2009). Body mass in adult skunks ranges from 1.2 to 5.3 kg (Verts 1967). Body mass is lost in the winter, with losses ranging from 31.6-47.7% (Verts 1967) as individuals limit above ground activity during cold weather. Dens are typically located underground during the winter and can shelter multiple females or several females with one male. Communal dens of multiple males are uncommon and communal denning of adults does not typically occur during the summer months (Verts 1967).

Skunks are insectivorous and forage by digging in soil with long, curved front claws (Wade-Smith and Verts 1982). They often carry economic importance due to their consumption of crop-damaging insects (Rosatte and Larivière 2003). They will also feed on berries and nuts as well as small mammals and bird nests (Rosatte and Larivière 2003). As meso-predators, striped skunks play an important role in the ecosystem. Although they do not dominate ecosystem food webs like larger carnivores, meso-carnivores can have significant influences on nutrient flows in a system, drive prey community structure and distribution, and fill distinct niches, such as seed distributors (Roemer et al. 2009). As disease reservoirs, these meso-carnivores can influence and temper the effects of larger carnivores in an ecosystem. With high cross-species infection rates, the skunk reservoir can infect and kill larger carnivores thus altering dominant carnivore densities and population structures (Roemer et al. 2009). The striped skunk is the host species for the rabies virus in California, southern Arizona, and the central United States.

The changing landscape

Human populations are rapidly increasing at a rate of almost 80 million people per year (United States Bureau of the Census 2009). In response to this global increase in human population size, land use and land cover change continues to accommodate the needs of developing and migrating populations (Grimm et al. 2008). These anthropogenic landscape alterations encompass numerous disturbances to natural ecosystem processes. Urbanization, water use and management, and agricultural land uses are all examples landscape alterations at a local scale. These local disturbances influence much broader, global environmental changes including declines in water quality, air pollution, and climate change (Patz et al. 2004). Although these broad scale environmental alterations have strong impacts across the world, local

land use change can have a significant influence on important, more fine-scaled ecological processes, such as species diversity, invasive species, and disease spread (Bradley and Altizer 2006, Crowl et al. 2008). Fine-scale land use alteration is region specific. In considering the effects of anthropogenic disturbance on local ecological processes, one must focus on the alterations occurring at the region of interest.

Over the past 150 years, the natural prairie ecosystem of the Great Plains in the central United States has experienced intense land use alterations. The eastern mesic grasslands have been reduced by 82 to 99% since the initiation of eastern plains homesteads in 1830 (Samson and Knopf 1994). Most of the early reduction in grasslands occurred due to the creation of agricultural fields and intensive grazing practices (Coppedge et al. 2001). Although agricultural expansion has slowed in the last decades, prairie habitat continues to decline due to exurbanization and woody encroachment in the region (Abrams 1986, Samson and Knopf 1994, Briggs et al. 2005). Exurbanization is the movement of urban residents to rural environments. Because of the different land use needs and values of these new rural residents, altered management practices are often enforced in these rural environments (Egan and Luloff 2000). This rural migration transforms the landscape by concentrating resources for local wildlife, and alters basic ecosystem processes, such as fire and grazing patterns. These environmental changes lead to additional land cover alterations, such as woody encroachment. In the Flint Hills of Kansas in the central portion of the Great Plains, there has been a 28% increase in shrub habitat and a 58% increase in forested habitat over the past 60 years (Briggs et al. 2005). This increase may be attributed to two specific causes. First, landscape fragmentation due to agriculture and exurbanization patterns have led to decreased fire frequencies and increased seed source availability (Briggs et al. 2005). Natural prairie fires have been reduced to protect homes

and fields, allowing gallery forest to expand from stream sides and shrubs to increase along slopes. Second, the natural and migratory grazers of the prairie ecosystem, bison, have been virtually eradicated and intensive cattle grazing practices have been introduced. This grazing pattern reduces fuel load for fires, thus stopping fires from reaching intensities strong enough to reduce woody invasion. Even in high and intermediate fire regimes, areas of intense grazing have shown increased woody vegetation in grasslands (Briggs et al. 2002). These land use and land cover alterations may have a significant impact on an endemic zoonotic disease in the area, rabies.

Integrating epidemiological components

The rabies disease study system of the Central Plains is dependent on the integration of the viral properties of rabies, the host ecology of the striped skunk, and the changing habitat of the tall-grass prairie. To consider these disease factors in predicting rabies risk to human health, I first studied the movement and behavioral characteristics of the striped skunk through the combination of field and genetic methods. Skunks were radio-tagged and tracked across anthropogenically disturbed and undisturbed habitats within the Flint Hills region of Kansas. Tissue samples were collected and analyzed from marked individuals as well as additional skunks from the study region. Results from these studies provided information on striped skunk habitat preference, densities, social behaviors, and dispersal characteristics; ecological factors that can have a strong influence on the spread of the rabies virus. These findings are described in the second chapter of this thesis.

Based on habitat-specific density estimates from this study and the literature, striped skunk den locations were integrated into an individual-based contact network across the broader region of the Upper Kansas River Watershed. In this modeling approach I had the

opportunity to integrate properties of the rabies virus and the underlying and altered landscape of the Flint Hills with the characteristics of the disease host. A complex systems network approach was applied to the data, in which real-world systems are modeled in an attempt to avoid unrealistic assumptions (Barrat et al. 2008). With data storage and processing capabilities continually growing, complex interactions are increasingly modeled through the use of network representations in diverse fields such as human social interactions, economic markets, and ecosystem food webs (Barrat et al. 2008, Lewis et al. 2008, Bascompte 2009, Schweitzer et al. 2009). These models are also very useful in ecological forecasting as predicted ecosystem alterations can be used as a basis for network design. This complex network was spatially explicit and was placed on both the current landscape as well as future landscapes predicted based on woody expansion in the region. Using the complex network as a background, an SEIR (susceptible-exposed-infected-removed) approach was used to simulate rabies spread through the network. The disease will spread among individual skunks following a series of infection probabilities based on both spatial distance and specific properties of the rabies virus. This modeling approach incorporated each of the three components of disease spread: 1) host distribution and behavior were integrated via habitat-specific densities and contact rates in the creation of the nodes and links of the network; 2) viral properties were integrated through the probabilities of infection in the SEIR simulations of rabies spread through the network; and 3) the changing landscape was modeled through the spatially explicit placement of the network on varying levels of woody expansion across the Kansas watershed. The results of this modeling approach are described in the third thesis chapter.

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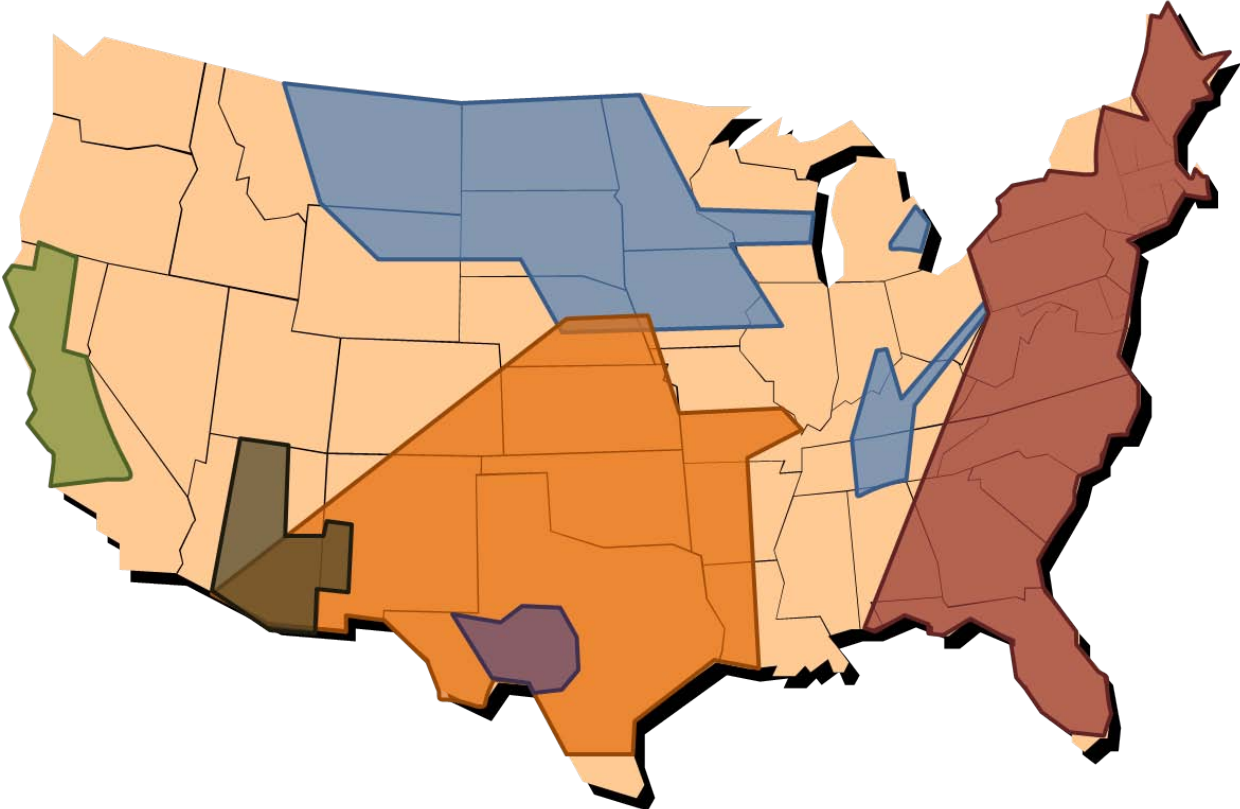
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Figures and Tables

Figure 1.1 Distribution of terrestrial variants of rabies virus in the United States. Blue, orange, and green divisions are variants of rabies in skunks; red represents raccoon rabies; and purple and brown divisions define fox rabies variants. Adapted from Blanton et al. 2007.



CHAPTER 2 - Ecology of the striped skunk (*Mephitis mephitis*) on tall-grass prairie: Implications of anthropogenic habitat alterations on the distribution of a disease host.

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Abstract

Anthropogenic land use and land cover change is a global phenomenon that alters the epidemiological landscape. To understand the altered epizootic potential of rabies in the Flint Hills of Kansas due to human development, we studied the ecology of the host species, the striped skunk (*Mephitis mephitis*), using spatial and genetic methods. A total of 27 striped skunks were radio-marked and tracked in four study areas on the Konza Prairie Biological Station (KPBS) and in Manhattan, Kansas. In addition, we collected 107 tissue and blood samples from striped skunks across the study areas and the broader Flint Hills region. Radio-marked skunks varied habitat preference depending on behavioral activity. Skunks preferred forested habitat for daytime denning, but preferred grassland for nightly foraging movements. We found greater clustering of individuals within anthropogenically disturbed habitats than in undisturbed habitats. Striped skunks in disturbed environments had significantly greater home range overlap than those in undisturbed habitats (ANOVA, $F=4.03$, $p=0.05$). Den site density was significantly greater in disturbed habitats than in undisturbed (ANOVA, $F=18.29$, $p<0.0001$) and all communal dens were located in disturbed habitats. We found no significant correlation between home range overlap and genetic relatedness (Mantel test, $r=0.004$ $p=0.78$) nor between genetic and geographical distances (Mantel test, $r=0.004$ $p=0.156$), and those individuals in communal dens were not more related than the population average. Populations were genetically admixed suggesting high dispersal capacity. In the Flint Hills, host densities are higher and

contact rates are increased in areas of human development and disturbed environments than in undisturbed grassland habitat. The lack of correlation between spatial distribution and genetic relatedness suggests that these populations are highly admixed. The affinity for disturbed habitats, social habits, and dispersal capabilities of this reservoir host contributes to increased disease transmission potential in suburban and exurban areas. By understanding habitat affinities and landscape features which promote host to host contact, disease transmission potential can be reduced by modifying primary skunk habitat and focusing vaccination efforts on these preferred areas.

Introduction

Anthropogenic habitat disturbances, including urbanization, land and water use, pollution, and resulting climatic changes, impact a variety of important ecological processes and dynamics (Grimm et al. 2000, Grimm et al. 2008). Human disturbance has also been implicated for the world-wide increase in the emergence and propagation of zoonotic infectious disease (Daszak et al. 2001). Landscape alterations and increased human mobility have greatly increased rates of disease spread and the spatial distribution of disease outbreaks over recent decades (Patz et al. 2004, Crowl et al. 2008, Grimm et al. 2008). The direct effects of land use change on disease epidemiology are complex and variable, as anthropogenic disturbance influences the ecology and evolution of host species and host-pathogen dynamics (Patz et al. 2004, Wilcox and Gubler 2005). Examples of this phenomenon have been documented for a variety of zoonotic diseases. In the northern United States, anthropogenic factors including forest fragmentation, urbanization, and the subsequent loss of species richness may be directly associated with increased risk of Lyme disease (Schmidt and Ostfeld 2001, Patz et al. 2004). West Nile virus exposure in songbirds has been linked to urbanization and related land use

alterations in Atlanta, Georgia (Bradley et al. 2008). Additional zoonotic diseases, including Nipah virus (Daszak et al. 2001, Field 2009), Leptospirosis (Vijayachari et al. 2008), and several urban bacterial zoonoses (Comer 2001) have all been associated with developing and expanding urban areas throughout the world. To effectively manage and control these zoonotic diseases, we must better understand how the structure of the underlying and changing landscape affects the ecology of the host species responsible for the spread of disease.

Rabies is a globally distributed zoonotic disease with significant impacts to human, companion animal, and livestock health. World-wide more than 50,000 people die from rabies infection each year. In the United States, less than two people die each year due to aggressive control, prevention and treatment programs that cost over \$300M per annum (Center for Disease Control and Prevention 2007). Although domestic dogs have been eliminated as a reservoir for rabies in the U.S. (Belotto et al. 2005, Velasco-Villa et al. 2008), the disease continues to circulate among wildlife reservoirs with spillovers into companion animals, livestock, and humans. The threat of spillover increases as encroachment of urban areas on natural host habitats increases the density and contact of unvaccinated companion animals with urban adapted wildlife (Wunner 2005). This developing human-wildlife interface is of particular concern, as the patterns of disease spread are not thoroughly understood. Thus epidemiological modeling and surveillance of the emerging exurban ecosystem will be valuable for the management and control of rabies (Wunner 2005).

Rabies epidemiology depends on the spread of the virus from infected individuals to susceptible ones. Inoculation of one host from another occurs via the transfer of virions in saliva and animals with a strong bite reflex, like carnivores, are most liable to act as hosts. Once infected, hosts typically display the furious form of the disease involving loss of fear and

heightened aggression, increasing the probability of infecting additional individuals (Kaplan 1985). The most common host of rabies in the United States is the striped skunk (*Mephitis mephitis*, Greenwood et al. 1997, Rosatte and Larivière 2003). Striped skunks range from southern Canada, throughout most of the United States and into the northern portions of Mexico (Wade-Smith and Verts 1982). They occupy a wide range of habitats, but most often occur in forest-edge zones (Bixler and Gittleman 2000, Larivière and Messier 2000) and near agricultural areas (Larivière and Messier 1999). Striped skunks are omnivores with diets consisting largely of insects, which are gathered primarily through digging. The skunk is nocturnal, normally nonaggressive, and does not defend territories (Wade-Smith and Verts 1982). However, when infected with rabies, skunks typically abandon this passive behavior, displaying the furious form of the disease (Aranda and López-de Buen 1999). Because striped skunks are not territorial, they may be more prone to communal denning and other gregarious behaviors, activities that increase contact rates and risk of rabies spread. In addition, skunks are predisposed to human disturbed and exurban habitats (Gehrt 2005, Weissinger et al. 2009) and the current increase in these habitats could increase skunk density and proximity to humans, thus amplifying the risk of human exposure through contact with domestic cats, dogs, and livestock.

In the Great Plains, where the striped skunk is the dominant rabies host, mesic grasslands have decreased by an estimated 82-99% since the initiation of eastern plains homesteads in 1830 (Samson and Knopf 1994). The Flint Hills of Kansas is the largest remaining intact tract of tall-grass prairie, but it is not without human alteration. Exurbanization is occurring across these grasslands as urban residents shift to rural environments. These 'exurbanites' often have different land use needs and values than traditional residents, leading to altered management practices in these rural environments (Egan and Luloff 2000). This rural migration transforms

the landscape by concentrating resources for local wildlife, and alters basic ecosystem processes, such as fire and grazing patterns. In the last 60 years, exurbanization and the resulting fire suppression have resulted in a 28% increase in shrub habitat and a 58% increase in forested habitat in the Flint Hills tall grass prairie ecosystem (Briggs et al. 2005). These habitat alterations have created potential habitat niches for zoonotic disease along with the introduced urban resources.

Because the spread of rabies is heavily dependent on the ecology of the host species (Kaplan 1985) and because host species ecology is greatly influenced by anthropogenic habitat alterations (Bradley and Altizer 2006), it is important to understand how striped skunk populations are distributed across the current landscape and how animals utilize the human-influenced environments. As the landscape and habitat of striped skunks change with anthropogenic expansion, the movement and distribution patterns of the species will change as well. The predicted increase in forested and disturbed habitat across mesic grasslands suggests that suitable habitat for this reservoir host is increasing. In anthropogenically disturbed environments, wildlife often live in close proximity to one another due to concentrated available habitat and suitable resources within these areas. This confined proximity leads to increased host contact rates and greater ease of disease spread (Bradley and Altizer 2006). By understanding host habitat affinity and behavior in this altered ecosystem, we can predict hotspots of rabies spread and disease management can be focused in these areas (Slate et al. 2005).

The central objective of this study was to understand the ecology of the striped skunk in the developing exurban and rural matrix habitat that is increasing throughout the central United States to better explain how zoonotic diseases such as rabies will spread from host to host and ultimately from urban center to urban center. We completed both spatial and genetic analyses of

skunks in human disturbed and undisturbed habitat types in the Flint Hills of northeastern Kansas. We contrasted social patterns, habitat affinities, and population characteristics of the striped skunk in these different habitat types to assist us in developing models of rabies epidemiology.

Methods

Study Area

The Flint Hills extend from northeast Kansas to north-central Oklahoma. The limestone beds underlying most of this region have prevented extensive cultivation, and thus the Flint Hills are considered the largest remaining tract of tall grass prairie. The landscape is dominated by mesic grassland species such as big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), and Indian grass (*Sorghastrum nutans*) and experiences annual and seasonal variation in climate and precipitation (Küchler 1967, Freeman and Hulbert 1985).

We studied the ecology of striped skunks in two habitat types, anthropogenically disturbed and undisturbed, within the northeastern portion of the Kansas Flint Hills (Figure 2.1). Undisturbed habitat was considered to be primarily tall-grass prairie with some cattle and bison grazing bisected by riparian forest. This habitat was encompassed in a single study area, the Konza Prairie Biological Station (KPBS). The station is a 3,487 hectare area of native tall grass prairie and riparian forest located approximately 13 km south of Manhattan (39°05'N and 96°35'W). To study long-term ecological patterns, the KPBS is separated into 60 watersheds which undergo a variety of treatments that alter fire frequencies and large ungulate grazer activity. The southern portion of the station is predominately undisturbed native tall-grass prairie.

Disturbed habitats were comprised of a matrix of exurban development, gallery forest, and agricultural lands and were located in three study areas including the towns of Ashland, Ogden, and the Manhattan suburbs. Skunks were captured and tagged near the small town of Ashland about 3 km north of the KPBS (39°07'N and 96°36'W). The majority of this habitat was large agricultural fields and private residence. Fields were separated by strips of woody vegetation. This area is well connected to the northern portion of the KPBS which has similar habitat composition with buildings surrounded by agriculture and gallery forest. Ashland and northern KPBS were considered a single disturbed study area. Skunks were also captured in Ogden, Kansas about 13 km southwest of Manhattan (39°07'N and 96°42'W). These individuals were trapped in a single barn on a small farm bordering suburban neighborhood development. The final trapping location was an exurban area about 8 km northeast of Manhattan (39°12'N and 96°30'W). This study site consisted of large areas of housing developments surrounding agricultural fields.

Spatial Analysis

Non-lethal, cage traps were set from August 2007 to January 2009 in each study area. These traps were baited with skunk grub lure (Kishel's Quality Animal Scents & Lures, Inc., Saxonburg, Pennsylvania, U.S.A.) and dry cat food, wet canned cat food, or a combination of both grub lure and canned food. The traps were baited at dusk and checked at dawn. Captured skunks were immobilized with an initial intramuscular injection of 0.3 ml of a Ketamine (8.3mg/kg) and Xylazine (1.7mg/kg) mixture. Additional doses were administered as needed to maintain immobilization. Animals were weighed and blood samples were collected intravenously from the jugular vein or ear tissue samples were taken for DNA analysis. Before recovery and release, each skunk was fitted with a radio transmitter mounted on a collar with a

motion sensitive mortality switch (Advanced Telemetry Systems, Inc., Isanti, Minnesota, U.S.A.) and marked with a numbered ear tag. Trapping and animal handling methods followed the procedures suggested by the American Society of Mammalogists (Gannon et al. 2007) and followed Kansas State University IACUC guidelines (Protocol numbers 2291 and 2578).

Radio-tagged skunks were located nightly using triangulation methods. Locations were collected randomly throughout the night from 1800h to 0600h using handheld, 3-element directional Yagi antennae. Locations were estimated using the telemetry program LOAS Version 4.0 (Ecological Software Solutions LLC, Hegymagas, Hungary). Telemetry error was calculated using a double-blind beacon study (White and Garrott 1990) with unused radio-collars. Location data were not used if the observer was > 1 km from the estimated location site due to the relatively hilly terrain of the study areas.

Skunks were also located during the day to determine daytime denning or resting sites. These sites were determined by walking to the exact location of the resting animal. Den sites located through radio-telemetry were monitored with digital trail cameras. Cameras were placed around dens of interest and images were collected over a 48 hour period. The motion-sensitive cameras were triggered as animals entered and left the den. Skunks were identified based on individually unique striping pattern as well as the presence of ear tags placed on radio-marked individuals. Images indicated frequency of communal denning among skunks. These tracking methods were continuous throughout all seasons.

Based on movement locations and home range distributions, striped skunks were divided into two habitat categories: disturbed or undisturbed. Disturbed habitat was described as areas with significant human alterations including building developments, agricultural lands, and major roads. Agricultural lands were used as the defining feature, encompassing homes, yards,

farm buildings, and roads, and 500 m buffers were placed around this habitat to delineate the extent of the disturbed habitat. This buffered area defining the disturbed habitat was composed of 44% agricultural land, 28% woody vegetation, 18% grassland, 6% urban development, and 4% water. Undisturbed habitats were areas outside of this anthropogenic disturbance, dominated by tall-grass prairie vegetation. Portions of this habitat were grazed by the native prairie grazer, bison (*Bison bison*). Skunks with over 50% of their locations within either of the habitat divisions were considered to fall within that category of habitat.

We analyzed habitat use through ArcGIS 9.0. Imagery used for these analyses was generated by the Kansas Applied Remote Sensing (KARS) program from multitemporal Landsat Thematic Mapper (TM) imagery (KARS 2001). The image was created using a two stage hybrid classification method of the TM imagery. This 2001 Kansas GAP land cover database was composed of 30 m raster cells encompassing 43 land cover classes.

Striped skunk locations were categorized by habitat type and season. Seasonal home ranges were estimated using the Hawth's Tools v.3.27 extension for ArcGIS (Beyer 2004) with a 95% fixed-kernel density estimator. Home range (HR) overlap was estimated using the following index (Mitna 1992):

$$\text{Mean overlap} = \frac{\text{HRoverlap} * 100}{\text{HR(A)} * \text{HR(B)}}$$

Habitat preference was determined using a compositional habitat use analysis which compared observed habitat use to habitat availability. Observed habitat use was defined by the locations of radio-marked individuals, while expected, or available, habitat use was based on the percentages of each habitat type within the 100% fixed-kernel estimate of the individual's home range. A multivariate analysis of log-ratios was used to compare habitat use to availability and to rank habitat preference among individuals (Aebischer et al. 1993). As a more detailed

analysis on habitat preference within the larger disturbed habitat matrix, distances between skunk locations and building sites were compared to skunk location distances to random sites throughout the disturbed habitat. General movement patterns and distances among den sites and individuals were measured in the ArcGIS platform.

Estimates of density were calculated for disturbed and undisturbed habitat types. We estimated area based on a 600 m cumulative buffer placed around each trap site. This buffer was determined based on the average nightly movement distance found for skunks in this study. The number of skunks trapped in each habitat type was divided by the total area calculated around the trap sites within each habitat to determine density.

Genetic Analysis

Blood and tissue samples were collected from individuals across all study areas. Approximately 250 μ l of blood was stored in 1 ml lysis buffer. Alternatively, tissue samples were collected from the tip of the ear and stored in 1 ml of 70% ethanol. All samples were stored in a cool, dark location until they could be transferred to storage at 4°C prior to DNA extraction.

We extracted DNA from the blood samples using DNAeasy blood extraction kit (Qiagen, Inc.) following the manufacturer's protocol. Tissue samples were extracted using a phenol-chloroform extraction method. A total of 10-30 μ l of tissue were incubated at 56°C for at least 12 hours in 400 μ l lysis buffer, 20 μ l proteinase K (20mg/ml), 10 μ l DTT (1M), and 2 μ l RNase A (10 mg/ml). The DNA was then extracted with two phenol extractions and one chloroform wash. The DNA was precipitated with 100% ethanol and washed with 100% isopropyl alcohol. Extracted DNA was stored at -20°C. DNA concentrations ranged from 94-3,859 ng/ μ l. All samples were diluted to 25 ng/ μ l for amplification.

We amplified eight microsatellite loci designed for striped skunks: Meph42-73, Meph42-67, Meph22-70, Meph22-19, Meph22-26, Meph42-25, Meph42-15, and Meph22-14 (Dragoo et al. 2009). The universal sequence M-13 was added to the 5' end of the forward primer for each pair and fluorescently labeled M-13 was added to the polymerase chain reaction (PCR). The DNA was amplified using 10 μ l reactions comprised of 1 μ l of the DNA sample, 1x Sigma PCR buffer, 2.7 mM MgCl₂, 0.2 mM dNTPs, 0.1 μ g/ μ l BSA, 0.8 M betaine, 0.2 μ M forward primer with an M-13 complement on the 5' end, 0.5 μ M reverse primer, 0.5 μ M M-13 primer labeled with a fluorescent dye, and 0.5 units *Taq* polymerase. Reactions were amplified using an Eppendorf Mastercycler (Eppendorf, Inc.) using the following protocol: 94°C for 5 min, 30 cycles of 94°C for 30 s, 54°C for 45 s, and 72°C for 45 s, 10 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s, ending with 10 min of extension at 72°C. The annealing temperature was 54°C for all markers. Each run included a negative control to verify a lack of contamination among samples. Genotypes were visualized using a LI-COR Model 4200 IR2 Series DNA Sequencer (LI-COR Inc., Lincoln, NE). Homozygous samples were reamplified 2-3 times to assess allelic dropout.

We used GENEPOP v.3.3 (Raymond and Rousset 1995) to test Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium. To assess relatedness among individuals, the program Relatedness 5.0 (Queller and Goodnight 1989) was used to estimate Grafen's relationship coefficient (r) between 2 individuals. First-order relatives were defined by r -values > 0.375 and encompassed full siblings and parent to offspring pairs. Second-order relatives were defined by r -values ranging from 0.125 to 0.375 and included half sibling pairs. We compared relatedness among individuals with potentially close contact, such as those sharing dens or with overlapping home ranges, to the average relatedness of individuals across the Flint Hills. Mean

relatedness (R) was estimated and compared for communally denning individuals and those not in shared dens with 95% confidence intervals calculated around each estimate using a jackknife procedure in Relatedness 5.0. Program Genelex (Peakall and Smouse 2006) was used to look for correlations between home range overlap and genetic relatedness as well as correlations between genetic and geographic distance using Mantel permutation tests.

We used Program STRUCTURE v.2.0 (Pritchard et al. 2000, Pritchard and Wen 2002) to assign each multilocus genotype to a genetic cluster. A population admixture model was used due to high expected gene flow. We conducted a spatial autocorrelation analysis (Smouse and Peakall 1999) in Genalex by comparing the genetic correlation coefficient (r) to geographic distance within each of a series of geographic distance classes.

Results

Data Collection

Trapping was conducted across the four study areas from 22 August 2007 to 15 January 2009, with the majority of trapping during the fall and opportunistic trapping during the remaining seasons. Eight striped skunks were captured and radio-tagged on the southern, undisturbed portion of the KPBS, one skunk was captured and radio-tagged in the suburbs of Manhattan, and 18 were radio-tagged in Ashland and on the disturbed northern portion of the KPBS. Of the 27 radio-marked individuals, 17 were male and 10 were female and all were adults, >1 year old. In comparisons of habitat use among disturbed and undisturbed habitats, 19 skunks were located in disturbed habitat and 8 were located in undisturbed habitats based on movement and home range patterns. In addition to the radio-tagged individuals, we collected tissue samples from 16 individuals from a den site of skunks in Ogden, 34 additional samples from Ashland and northern KPBS, and 57 samples of road-killed individuals from across the

Flint Hills. Striped skunk weights were collected before and after the winter months (December through March). Weights in skunks after winter averaged 1.93 ± 0.089 kg (mean \pm S.E.) and were significantly less than weight before winter (2.7 ± 0.69 kg; paired t-test, $t=3.63$, $p=0.0023$).

Telemetry efforts were conducted from 11 October 2007 through 01 April 2009. We identified > 900 locations and 59 den sites. We calculated a standard deviation of $\pm 6.8^\circ$ from the true telemetry bearing and a mean error polygon of 0.0003 ± 0.0002 km² based on telemetry error testing. We considered these error estimates reasonable for our study and habitat analyses. The estimated error polygon was within the 30 m pixel size of the habitat composition GAP land cover images used in the study.

Spatial Analysis

Average movement between daytime den locations and nightly foraging locations was 604 ± 81 m. The maximum distance an individual skunk moved between daytime den and nighttime foraging locations over the course of the study was 3.6 km. These movements were shorter during the winter of 2007 (average high temperature: 6.6°C) with an average of 197 ± 44 m between den and foraging sites. Summer (average high temperature: 23.6°C) movements were an average of 596 ± 102 m. During the warmer winter of 2008 (average high temperature: 7.9°C), movements from dens to foraging sites were at their highest with an average of 948 ± 207 m between locations. The average high and low temperatures were significantly lower when skunks remained in their dens ($13.3 \pm 0.13^\circ\text{C}$, $-1.2 \pm 0.12^\circ\text{C}$, respectively) compared to nights when skunk chose to leave their dens and forage ($18.5 \pm 0.03^\circ\text{C}$, $4.8 \pm 0.17^\circ\text{C}$, linear regression, $F_{1,825}=35.37$, $p<0.0001$, $r^2=0.04$, $F_{1,825}=46.34$, $p<0.0001$, $r^2=0.05$, respectively, Fig. 2.2).

Of the radio-tagged skunks, 13 in the disturbed habitats (4 females and 9 males) and 5 in the undisturbed (all males) had sufficient data to analyze home range characteristics. The average

home range size for all individuals was 5.2 km^2 . Male home ranges were significantly larger ($5.8 \pm 0.48 \text{ km}^2$) than females ($3.1 \pm 0.58 \text{ km}^2$; ANOVA, $F_{1,16}=8.29$, $p=0.01$). Home range areas were not significantly different among seasons (ANOVA, $F_{2,20}=0.51$, $p=0.606$). Home ranges in disturbed habitats were smaller ($4.8 \pm 0.54 \text{ km}^2$) than those in undisturbed habitats ($6.6 \pm 0.77 \text{ km}^2$), but this difference was also not significant (ANOVA, $F_{1,16}=2.60$, $p=0.1263$); however, skunks with home ranges within disturbed habitat had significantly greater overlap ($41.34 \pm 3\%$) than those in undisturbed habitat ($29.00 \pm 5\%$; ANOVA, $F_{1,51}=4.03$, $p=0.05$). Based on trapping results, the calculated density of striped skunks in the disturbed habitat type ($2.8 \text{ skunks}/\text{km}^2$) was higher than that calculated for the undisturbed habitat ($0.6 \text{ skunks}/\text{km}^2$).

Den sites varied in structure and habitat type (Figure 2.3). In both disturbed and undisturbed habitats, 34% were dug into the ground, 22% were in rocky outcrops, 15% were located in holes beneath or within the base of large trees, and 15% were found in human structures such as barns and junk piles. There were no significant differences in den type between disturbed and undisturbed habitats. The average distance among den sites was $1.8 \pm 0.005 \text{ km}^2$. Den sites in disturbed habitats were an average of $1.68 \pm 0.002 \text{ km}^2$ apart from one another and were significantly closer than those in undisturbed habitat with an average distance of $2.19 \pm 0.01 \text{ km}^2$ (ANOVA, $F_{1,841}=18.29$, $p<0.0001$). Communal denning occurred only during winter months from 18 to 29 February 2008 (average low -6.0°C) and from 02 November 2008 to 06 March 2009 (average low -5.8°C). Out of 28 winter dens, 5 were confirmed to be shared by three or more individuals. These communal dens ranged in size from 3 to 16 individuals. All communal dens were located within disturbed habitats. Of the communal dens, 2 were located in barns, 2 were located in forested habitat with entrances dug into the ground, and 1 den was located in tree habitat under a fallen tree.

We found that habitat use was non-random and that woody habitat ranked highest in the analysis, slightly preferred over grassland and significantly preferred over agricultural lands (Wilks' $\lambda=0.495$, $F_{2,16}=8.15$, $p=0.0036$; Table 2.1a, Figure 2.4). However, variation in habitat preference occurred between nighttime foraging locations and den sites. Grassland habitat ranked the highest in comparisons of foraging locations to available habitat, although this rank was not significant (Wilks' $\lambda=0.898$, $F_{2,16}=0.90$, $p=0.4246$; Table 2.1b). Woody vegetative habitat ranked significantly higher than the other habitat types in comparisons of den site locations to available habitat (Wilks' $\lambda=0.291$, $F_{2,15}=18.29$, $p<0.0001$; Table 2.1c). We found no differences in habitat preference rankings among skunks in disturbed and undisturbed habitat divisions. Within the disturbed habitat, skunks were significantly closer to building sites (241.7 ± 6.2 m) than to the random sites throughout the disturbed study area (321.4 ± 7.5 m; ANOVA, $F_{1,1164}=66.35$, $p<0.0001$).

Genetic Analysis

We analyzed 107 genetic samples. We found high levels of heterozygosity in the eight microsatellite markers, ranging from 0.888-0.913, and allelic diversity varied from 11-18 alleles per locus (Table 2.2). We found a single microsatellite, 42-15, to be slightly out of HWE. One loci pair was found to be in linkage disequilibrium at the 0.05 level, 42-67 and 42-25.

Out of the 11,131 individual pairs analyzed in program Relatedness, we found 15 pairs of first-order relatives and 831 pairs of second-order relatives. The average distance among first-order pairs was 21.3 ± 4.7 km and was 20.8 ± 0.08 km for second-order relatives. We found no significant correlation between home range overlap and the pairwise relatedness values of striped skunks (Mantel test, $r=0.004$ $p=0.78$), nor between genetic and geographical distance (Mantel

test, $r=0.004$ $p=0.156$). Individuals denning communally were not more closely related ($R= -0.004 \pm 0.006$, C.I. 0.0138) than those not sharing den sites ($R= -0.0091 \pm 0.002$, C.I. 0.0051).

No unique populations were delineated through analysis in program STRUCTURE; all individuals were classified into a single, geographically-expansive population. We found no significant structural differences in populations delineated based on disturbed and undisturbed habitat types (AMOVA). We found a significant correlation between genetic and geographic distance in the 0-100 m distance class ($p=0.006$, Figure 2.5). This distance class included individuals within communal dens.

Discussion

Based on the spatial and genetic results of this study, we have distinguished three broad conclusions on striped skunk host ecology and subsequent impacts on rabies epidemiology in the Flint Hills. In our study sites, there was a greater density of skunks near areas of anthropogenic disturbance. Contrary to our expectations, skunks in these dense populations were not closely related based on genetic population analyses. Striped skunks displayed high levels of genetic admixture and mobility. Additionally, temperature had a significant impact on the potential for contact among these highly mobile and densely populated disease hosts. These conclusions are interrelated and have a strong influence on rabies epidemiology in the Central Plains. As the current prairie landscape changes with greater human disturbance and increased woody expansion, habitat composition and resources will better support these dense host populations and the potential for serious rabies epidemics will increase near human and domestic animal populations.

High skunk densities in disturbed landscapes

Striped skunks reached higher densities in the disturbed habitat matrix of our study areas than in the relatively undisturbed grasslands. In areas of anthropogenic disturbance, we found high skunk densities of skunks and of den sites, a greater degree of home range overlap, and more instances of communal denning. These findings were the result of comparisons made between large habitat matrices, consisting of a variety of habitat types, including open agricultural fields and forest. To validate that these high densities were related to direct human presence, additional analyses were conducted within the disturbed habitat matrix and revealed that skunks were significantly more likely to remain near human structures and building sites than would be expected if individuals displayed no preference for these structures.

This dramatic clustering of individuals is likely driven by increased resource availability in human disturbed landscapes. These resources may include increased foraging opportunities and denning habitat. In our study sites, nightly foraging movements were located in all habitat types including grassland and agricultural land. However, skunks preferred to den in wooded habitats over grassland or agricultural lands and thus appear to rely on a matrix of wooded and open habitats. These results are consistent with skunk habitat use of field-tree edges in areas such as the wooded valleys of the Great Smoky National Park in eastern Tennessee (Bixler and Gittleman 2000) and the cropland and woodland habitat matrix of south-central Saskatchewan (Larivière and Messier 2000). In the disturbed habitats of the Flint Hills, the surplus of agricultural fields, in addition to existing grassland, provides excellent habitat for digging and foraging and these fields are surrounded by wooded habitat for den settlement. The availability of wooded habitat and human structures also provide protection for individuals as they move to forage. This configuration of habitat types makes it highly suitable for skunks and their non-territorial nature allows for high densities within this disturbed matrix.

These patterns of habitat use and spatial distribution on the forest/field matrix have important implications for disease emergence and spread. The high densities of striped skunks and increased incidence of communal denning in areas of human disturbance increase intraspecific contact rates and disease transmission among skunks which will likely be amplified with continuing land use and land cover changes. As human populations continue to expand in the southern Great Plains, woody encroachment will increase as a result of fire suppression and exurbanization (Briggs et al. 2002a, Briggs et al. 2005). This land cover change will not only involve wooded riparian habitat, but also wooded vegetation along the perimeter of agricultural fields and near human developments (Knopf and Samson 1995, Briggs et al. 2002b). The creation and expansion of highly suitable skunk habitat is projected to facilitate transmission corridors for disease spread not only in and around urban centers but facilitate the spread of disease among urban centers as exurbanization connects these areas.

Our data suggests that exurbanization increases resource availability and that those resources occur in close proximity to human dwellings. This proximity of suitable habitat to human habitation increases the risk of disease spread to companion and livestock animals and therefore increase the risk of human exposure. Indeed, in Kansas, domestic cats are the species most frequently exposed to rabid skunks and to contract rabies (Kansas State University Veterinary Diagnostic Laboratory). As humans move to rural areas, the increase of domestic animals in the rural landscape may amplify the risk of human exposure to zoonotic diseases.

The evolving matrix of human development and natural host habitat is a newly developing ecotone, where two adjacent ecological systems meet and ecological processes are amplified at the boundary (Despommier et al. 2007). Ecotones are often reported as areas for emerging infectious disease in wildlife, especially as anthropogenic habitat encroaches on that of

disease hosts. This dynamic process appears to be occurring in the developing exurban ecotone in the Flint Hills of Kansas. Disease management efforts may therefore benefit from habitat management efforts and thoughtful zoning ordinances.

Genetic admixture and mobility in striped skunks

Striped skunks displayed high levels of genetic admixture and mobility within the Flint Hills of Kansas. Genetic analyses revealed that individuals with high levels of home range overlap were not more closely related than those outside of these groupings and skunks in communal dens were not more closely related than those outside of the dens. We found no correlations between geographical and genetic distances across the study area and very low relatedness among animals in our study areas. Closely related (first or second order) animals were distant from one another. Highly related pairs were > 20 km from one another, suggesting large dispersal distances and no philopatry either among males or females.

This lack of population structure on the landscape may be attributed to two biological characteristics of the striped skunk. First, the skunk is not a territorial animal. The home ranges of several unrelated individuals can overlap significantly (Wade-Smith and Verts 1982). Second, skunk populations typically display high dispersal rates in young males (Wade-Smith and Verts 1982). These dispersal rates lead to high admixture among populations and in areas of high quality resources large numbers of individuals will congregate. Increase population admixture and tolerance of high densities and close proximities further amplifies the risk of disease transmission. Other studies in carnivore and meso-carnivore species have found similar increases in social behavior and contact with increased resource availability (Prange et al. 2004, Riley 2006, Davison et al. 2008). This social behavior is amplified by the existing

characteristic of non-territoriality in skunks. Dispersing males will be attracted to these habitats and meet no resistance in foraging and mating by resident, non-territorial individuals.

These host characteristics add to the disease risks created by high density in and habitat preference for areas with high levels of human disturbance. The unrelated clusters of individuals indicate greater levels of contact among skunks beyond that expected solely by familial groups and mating events. An increase in contact rates among the rabies host species provides greater potential for disease spread in human developed habitats. The high mobility in striped skunks also allows for rabies to rapidly amplify among host populations. For example, the rabies virus could lie dormant in an individual for several months to a year, allowing a young male infected with rabies to travel from his natal den to a new, densely populated area before symptoms of rabies affect his behavior.

Implications of climate change on host contact rates

Seasonality and temperature elicited dramatic changes in striped skunk behavior and physiology. Skunks remained in their dens during the coldest part of winter and ventured outside only after the temperature rose. Likewise, communal denning among individuals occurred only during the colder temperatures of winter months in our study. Skunks suffered significant weight loss through the winter. Future climate change may alter seasonal patterns of behavior which might both enhance and mediate the degree of rabies in striped skunk populations.

In eastern Kansas, models of future climate scenarios predict warmer winters with higher nightly temperatures over the next decades (Brunsell et al. 2008). We envision two possible scenarios: 1) when skunks are out of the dens and foraging, the chance for contact with other individuals is greatest, especially in areas of high skunk densities. Thus, the risk of rabies transmission may be amplified by warmer night-time temperatures, particularly in winter when

striped skunks may be more likely to move during night hours instead of remaining in dens. The increased movement could lead not only to higher intra-specific contact rates, but also increased rates of skunk-companion animal contact in disturbed habitats. 2) On the other hand, transmission rates may be lessened if warmer winters result in a decline in communal denning rates and decrease direct contact among multiple individuals. Additionally, transmission may be reduced if skunks remain healthier over a warmer winter. Cold winter months often lead to decreased nutrition and weight loss in wildlife compromising immune system health (Altizer et al. 2006). Warmer winters could reduce the prevalence of a weakened immune state, thus reducing disease transmission risk.

Conclusions

The use of both spatial and genetic analyses can greatly improve the accuracy of studies on the spatial distributions and behavior of wildlife species. The combination of both analyses often leads to different conclusions than would be established if only one technique was used (Aubry et al. 2004, Fedy et al. 2008). Telemetry studies provide information on home range size and composition, daily movement patterns, and individual contact rates, while genetic analyses compliment these traditional studies with insight into dispersal patterns and fine-scale population structure. We implemented both techniques to consider the impact of striped skunk ecology on the epidemiology of rabies at the evolving urban/rural interface. The conclusions from these analyses have implications for rabies management and control.

The habitat preferences and denning behaviors of the striped skunk in this study provide a scenario for rabies spread in the region that must be carefully considered in management and control of rabies spread. We see the potential for increased contact rates and high levels of population mixing in areas close to human development due to the skunk's affinity for these

habitats, its high dispersal capacity, and gregarious behavior. As human development and fire suppression increase in the Flint Hills, woody encroachment will increase and more companion animals will inhabit the exurban ecotone (Briggs et al. 2005). Thus, we envision increased disease transmission with the predicted land use and land cover changes for the Flint Hills of Kansas.

A greater understanding of host habitat use patterns can also be advantageous for disease control efforts. Given the affinity of striped skunks for encroaching woodlands, landowner incentives to increase prescribed fire regimes within the Flint Hills should be implemented to reduce the current expansion of wooded vegetation into the prairie ecosystems. Land cover management changes now could reduce future rabies epidemics ignited by the expanding forested habitat. In addition, vaccination and culling efforts can be directed at forest and field habitat boundaries for more efficient control of disease spread. Currently, oral rabies vaccination (ORV) programs are used in North America through the use of bait, targeting specific host species (Slate et al. 2005). Striped skunk ORV is being improved for higher consumption by the animal and the trap-vaccinate-release method offers another, more direct effort for vaccination (Rosatte et al. 1992). Whatever method is proven most effective for striped skunk rabies eradication, vaccination efforts should be concentrated on hot spots of disease spread, such as the forest/field matrix.

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Figures and Tables

Figure 2.1 Map of four study areas near Manhattan, Kansas. Study areas are labeled as follows: 1) Konza Prairie Biological Station (KPBS) southern tall-grass prairie (undisturbed), 2) town of Ashland and northern KPBS (disturbed), 3) suburbs of Manhattan (disturbed), and 4) town of Ogden (disturbed).

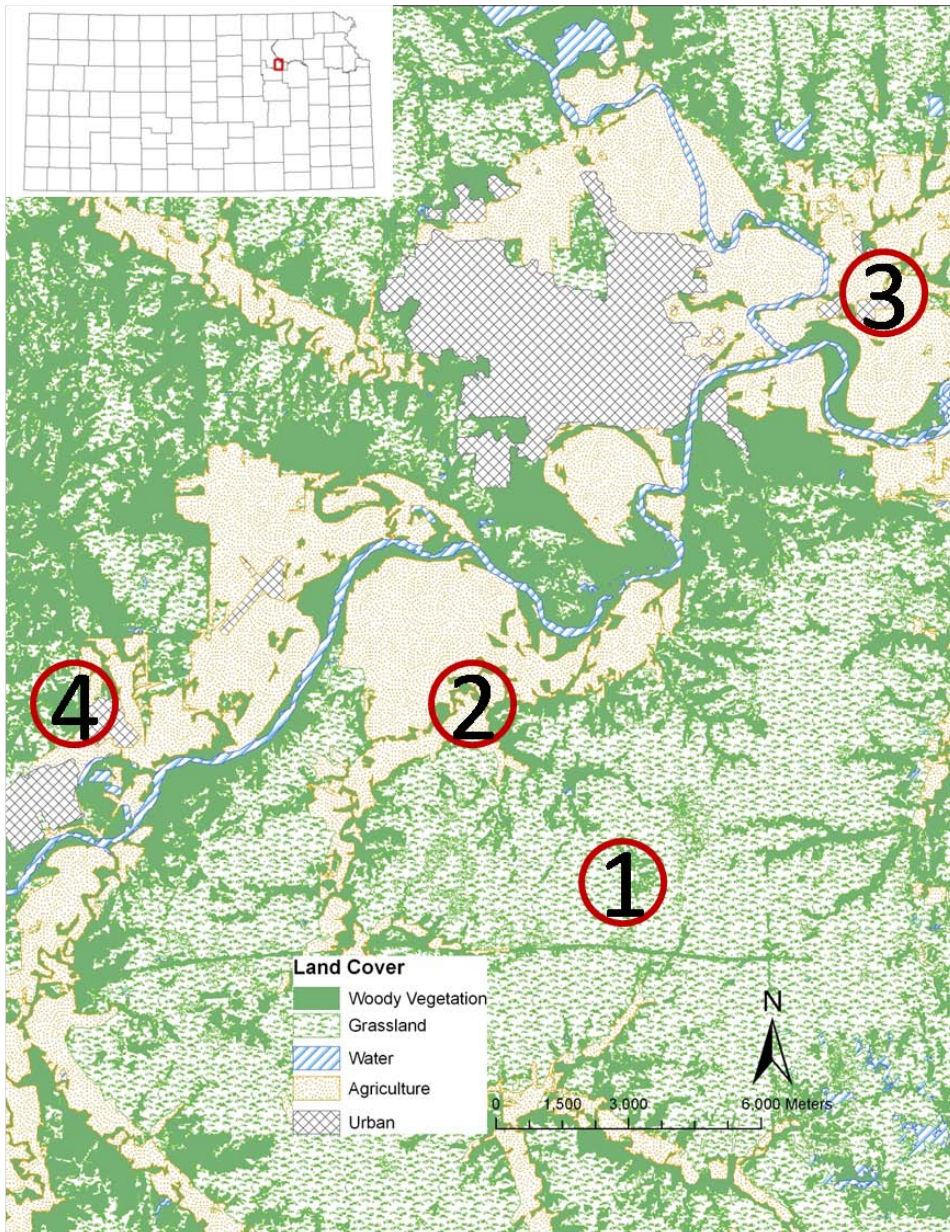


Figure 2.2 High and low temperatures for the extent of the study period with percentages of skunks in and out of den sites. High, or daytime temperatures, are shown in red (solid), while low, or nighttime temperatures, are displayed in blue (dotted). Black vertical bars on the upper portion of the figure represent percentages of located skunks moving at night, while vertical bars on the lower portion represent percentages of skunks in den sites at night. Average daytime temperatures were significantly lower for den locations than movement locations (ANOVA, $F=35.37$, $p<0.0001$). Nightly temperatures were also significantly lower for den locations (ANOVA, $F=46.24$, $p<0.0001$). Horizontal bars at the base of the graph represent lengths of time when communal dens were observed.

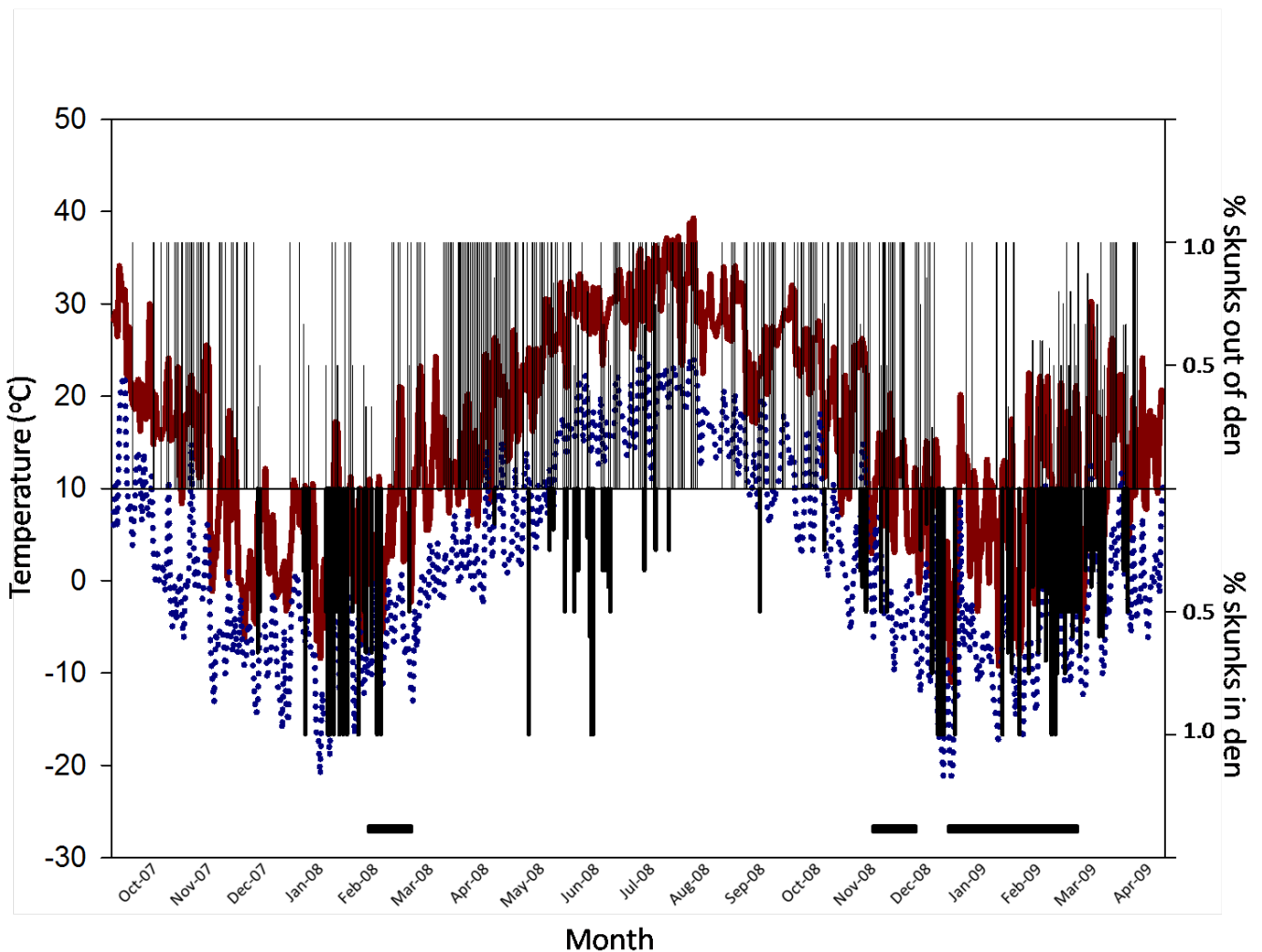


Figure 2.3 Den site descriptions for 59 located dens for radio-tagged striped skunks. The categories in the legend describe the actual opening of the den site. The ‘hole’ label refers to an entrance dug into the soil, ‘rock’ describes a den within a rock outcrop, ‘tree’ describes an entrance burrowed into the base of a living tree or within a dead tree, and ‘unknown’ indicates that the exact entrance was not located due to increased cover.

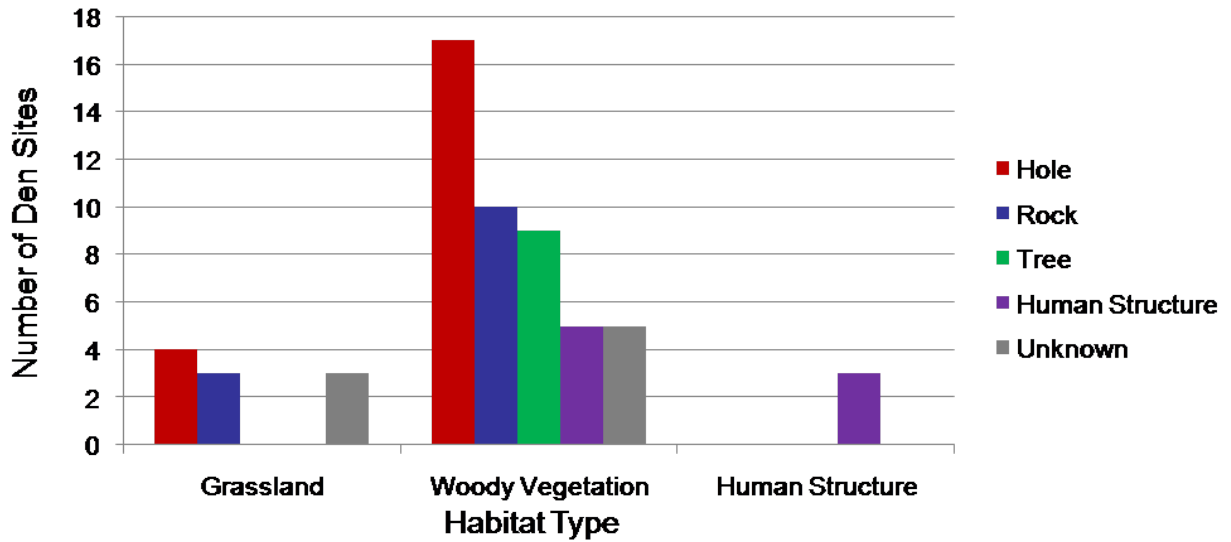


Figure 2.4 Observed versus expected percentages of striped skunk locations in three habitats.

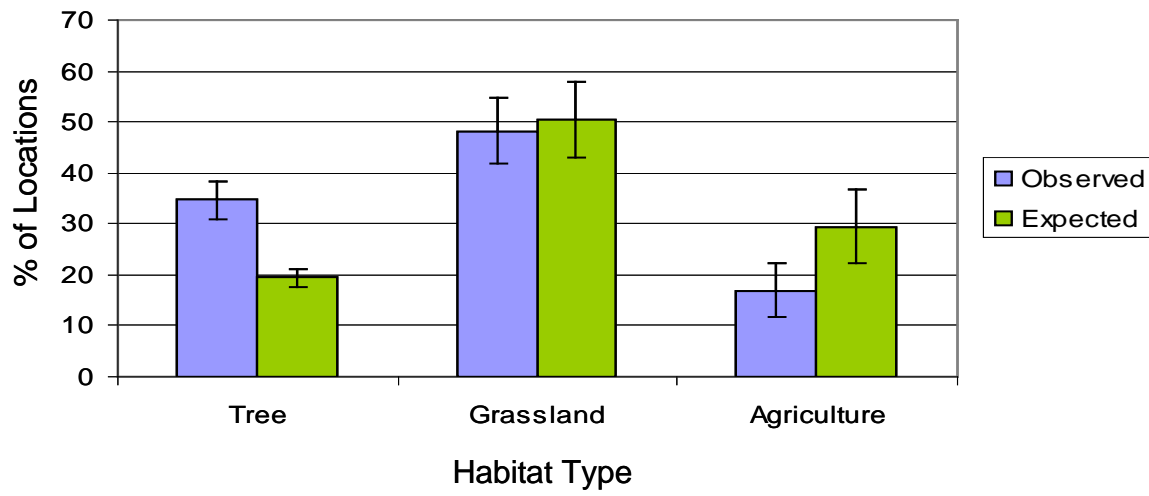


Figure 2.5 Correlogram plot of the genetic correlation coefficient (r) as a function of distance for striped skunk samples with known locations ($n=85$). The 95% confidence intervals are shown as dashed lines and the bootstrapped 95% confidence error bars are shown with the r values. All individuals within the '0' distance class are members of a communal den.

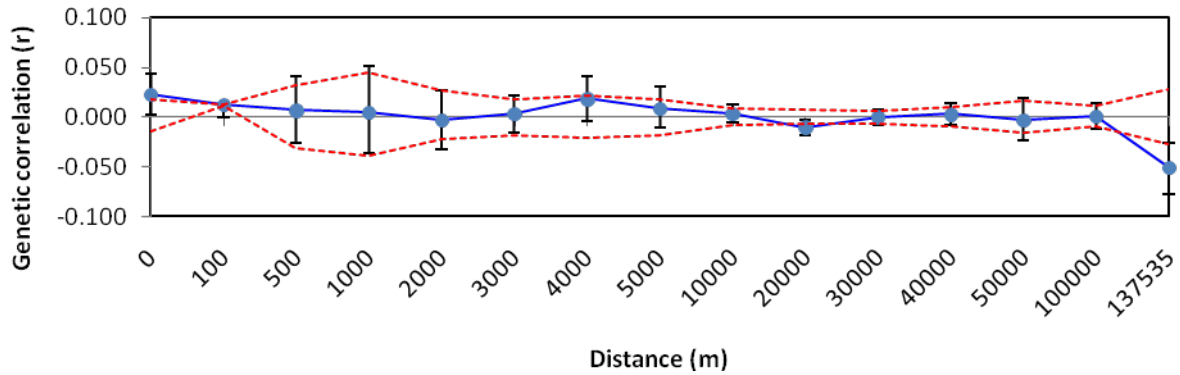


Table 2.1 Ranking matrices for striped skunk habitat preference comparing location data to home range habitat composition based on a) all location data, b) movement locations only, c) den site locations only. The sign of each t-value is indicated by + or -. A triple sign indicates a significant deviation from random at $p < 0.05$.

a)

Habitat Type	Habitat Type			Rank
	Woody cover	Grassland	Agriculture	
Woody cover		+	+++	2
Grassland	-		+++	1
Agriculture	---	---		0

b)

Habitat Type	Habitat Type			Rank
	Woody cover	Grassland	Agriculture	
Woody cover		-	+	1
Grassland	+		+	2
Agriculture	-	-		0

c)

Habitat Type	Habitat Type			Rank
	Woody cover	Grassland	Agriculture	
Woody cover		+++	+++	2
Grassland	---		+	1
Agriculture	---	-		0

Table 2.2 Summary statistics of eight microsatellite markers for striped skunks and Hardy-Weinberg equilibrium values.

Locus	n	Alleles	T_a(°C)	He	Ho	Fis	HW (p-value)	HW (SE)
Meph42-73	107	15	54	0.898	0.916	-0.021	0.230	0.026
Meph42-67	107	18	54	0.908	0.953	-0.050	0.157	0.024
Meph22-70	106	13	54	0.899	0.943	-0.049	0.295	0.021
Meph22-19	101	11	54	0.889	0.871	0.020	0.160	0.014
Meph22-26	105	16	54	0.913	0.876	0.041	0.185	0.022
Meph42-25	104	14	54	0.891	0.808	0.094	0.580	0.026
Meph42-15	103	18	54	0.898	0.816	0.092	0.001*	0.001
Meph22-14	105	17	54	0.912	0.914	-0.002	0.571	0.030

CHAPTER 3 - Modeling disease spread on spatially-explicit contact networks: The influence of woody encroachment on rabies epidemiology in the Flint Hills

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Abstract

The emergence and propagation of infectious zoonotic disease is significantly influenced by anthropogenic alteration of land use and land cover. Mathematical models are important tools to predict and control future epidemics, but to realistically simulate disease spread, models should be spatially explicit and consider both host and pathogen ecology. The development of complex systems networking provides an excellent platform for exploring epidemiological modeling on heterogeneous landscapes. In the central United States, natural prairies ecosystems are currently experiencing a rapid expansion of natural and introduced woody vegetation as a result of shifts in anthropogenic disturbance, intensive grazing practices, and altered fire frequencies. Rabies is a disease of concern in the region as it remains enzootic in wildlife populations and presents a persistent threat to human health that may be influenced by the changing landscape. We modeled the spread of the rabies virus across the Upper Kansas River Watershed in eastern Kansas using spatially explicit contact networks based on habitat-specific distributions of the disease host species in this region, the striped skunk (*Mephitis mephitis*). To model future land cover change, forested habitat was increased across the watershed at forecasted levels of expansion 27, 54, and 85 years from now. This simulated woody expansion resulted in networks with higher connectivity than those representing the current habitat configuration. Higher average node degree and site betweenness allowed the disease to spread

broader distances and to sustain itself for significantly longer periods of time across the landscape. In scenarios of increased woody encroachment, habitats that acted as barriers to spread on current habitat configurations became ineffective at containing the disease. With only a 25% increase in woody vegetation, rabies spread dramatically increased. Disease management strategies should focus on reduction of existing woody vegetation and control of ongoing woody encroachment to avoid future epidemics. Controlling woody expansion now may be more cost-effective and efficient than after the threshold of woody cover is reached and the landscape is substantially more connected.

Introduction

As anthropogenic development rapidly increases and continues to alter land use and land cover patterns in the United States, it becomes increasingly important to understand how these landscape changes will influence ecological systems and processes (Grimm et al. 2000, Crowl et al. 2008, Grimm et al. 2008). The emergence of infectious zoonotic disease and its epidemiological processes are often linked to human habitat alteration (Patz et al. 2004, Crowl et al. 2008), yet understanding the influence of habitat alteration on disease spread is complex. Habitat alteration can affect the ecology and evolution of host and pathogen as well as host-pathogen dynamics (Wilcox and Gubler 2005). Land use change has been implicated as a driver of zoonotic disease emergence around the world, including Nipah virus in China (Daszak et al. 2001, Field 2009), Lyme disease in the northern United States (Schmidt and Ostfeld 2001, Patz et al. 2004), Leptospirosis in India (Vijayachari et al. 2008), and West Nile virus in Atlanta, Georgia (Bradley et al. 2008). As established diseases expand to new environments and newly emerging diseases begin to affect human populations as a result of anthropogenic disturbance, it

is crucial to model and understand how these ecosystem changes affect epidemiological processes and to predict both current and future hotspots and distributions of disease spread.

Mathematical models of disease spread are important tools in understanding the epidemiology of emerging and re-emerging infectious disease (Hethcote 2000). For directly transmitted diseases, three components of epidemiology must be considered to begin to understand disease dynamics. First, properties of the disease pathogen should be studied and integrated into models of the disease spread (Fowler 2000, Huang and Li 2009). These properties include parameters such as the infective capability of the pathogen and disease latency. Variability in these parameters can significantly influence the emergence and sustainability of the disease in the environment (Fowler 2000, Wisely et al. in review). Second, the ecology of the host species responsible for the spread of the disease can shape and alter patterns of disease expansion and maintenance across the landscape (MacDonald and Voight 1985). Host ecology includes habitat preferences, population densities, and probabilistic contact rates among individuals. Closely linked to the ecology of the host is the third component of disease epidemiology, the underlying landscape (Parham and Ferguson 2006). The habitat of the host continually changes, especially with rapid human development. As the underlying landscape and habitat configuration of host populations changes with anthropogenic expansion, the movement and distribution patterns of the species change as well. Thus, spatial heterogeneity of the landscape which influences the movement and organization of individuals is pivotal to understanding how this habitat alteration influences pathogen-host processes (Deal et al. 2000, Real and Biek 2007). Currently few epizootiological models incorporate all epidemiological components including realistic ecological properties of host dynamics, variation in pathogenesis, and consideration of the changing habitat matrix. The emerging of the field of

complex systems modeling provides an excellent format in which to consider all components of disease epidemiology.

Complex networks encompass a broad range of systems including areas as diverse as human social interactions, economic markets, and ecosystem food webs (Lewis et al. 2008, Bascompte 2009, Schweitzer et al. 2009). With increased data storage and processing capabilities, we are able to study increasingly complex interactions through the use of network representations (Barrat et al. 2008, Barabasi 2009). The network approach to the study of complex systems attempts to describe and model real-world systems without many of the restraints and assumptions imposed on analytical models (Barrat et al. 2008). For modeling disease spread, host and disease parameters can be directly incorporated into the network along with the underlying, heterogeneous landscape on which the disease propagates. The compartmentalized approach to epidemiology (e.g. SEIR or susceptible-exposed-infected-removed) is a common method for exploring how host disease states and the transition from one stage to the next affect disease emergence (Lavine et al. 2008). Incorporating this disease model to a network of individual-based nodes placed across a realistic landscape allows for a spatially explicit examination of the interaction among pathogen, host, and environment.

We applied this complex network approach in considering the rabies virus in the central United States. This directly transmitted zoonotic disease continues to threaten livestock, domestic animals, and human populations in the United States despite >\$300 million spent each year on rabies prevention and control (Center for Disease Control and Prevention 2007). In the Central Plains, the striped skunk (*Mephitis mephitis*) is the reservoir host for the rabies virus. A recent study of skunk habitat affinity and distribution in tall grass prairie (Chapter 2, Bowe 2009) revealed a preference among individuals for woodland forest; within this habitat, home ranges

overlapped to a greater extent and den densities were higher than in the surrounding grassland habitat. In the last 60 years, this tall-grass prairie region has experienced a 28% increase in shrub habitat and a 58% increase in forested habitat (Knight et al. 1994, Briggs et al. 2005). This increase, due to fire suppression near areas of exurbanization in the region, creates potential habitat niches and transmission corridors for zoonotic diseases by connecting rural environments to urban centers as well as linking urban centers, which increases the risk of human exposure. Therefore to understand the effect of the land cover change on epidemiological processes, predictive modeling of rabies spread in the region must be spatially explicit to consider the future expansion of woody vegetation in the Flint Hills.

This study aimed to model and predict rabies spread across the Upper Kansas River Watershed of the Central Plains based on three future scenarios of forested habitat distribution. Rabies virus was propagated across spatially-explicit contact networks through the use of a compartmentalized epidemiological model. Characteristics of viral spread were compared among models to determine the influence of woody encroachment on disease emergence and maintenance. Management strategies and applications in future studies of emerging infectious diseases are discussed.

Methods

Network Construction

Spatially explicit contact networks were created across the Upper Kansas River Watershed. This watershed is located in north central Kansas at the northern end of the Flint Hills. The Kansas River passes through the center of the watershed, separating the watershed horizontally with distinct habitat divisions north and south of the river. To the north of the Kansas River, forested habitat dominates the landscape with substantial urban development near

the river. The northern tip of the watershed and south of the Kansas River is dominated by agricultural and grassland habitats with considerably less forest. These differences in forest distribution are likely due to fire suppression near northern urban areas, and due to annual prescribed burning of grasslands and maintenance of agricultural fields in the southern rural areas. These patterns of habitat configuration shaped the distribution of skunk populations within the disease networks.

Four spatially heterogeneous contact networks were created based on habitat-specific density estimates of striped skunks from the Flint Hills (Chapter 2, Bowe 2009) and published studies in similar habitats. Nodes representing striped skunk dens were placed randomly across the Upper Kansas River Watershed based on springtime habitat-specific density estimates. We used a density of 2.5 skunk dens per km² for the forested habitat type based on spring telemetry data in the region. Skunks were less common in open habitat; we used a density of 0.06 skunk dens per km² for both the grassland and agricultural habitats. These estimates were similar to the wide range of published striped skunk density estimates in similar habitats (0.7 to 18.5 km², Wade-Smith and Verts 1982). Based on data from Gehrt (2004) and our own field work, skunk densities from urban areas were estimated to be 2.1 skunk dens per km². These estimates were applied to habitat specific node densities for all networks.

Variation among networks was based on underlying habitat composition. To simulate predicted woody encroachment across the landscape over the next 27, 54, and 85 years, wooded habitat was increased by 25%, 50%, and 78%, respectively, in the Upper Kansas River watershed. These simulated habitat increases were based on average forest succession over the past 60 years (Knight et al. 1994, Briggs et al. 2005). These increases were produced by creating buffers around existing forested habitat using ArcGIS 9.0. We constrained the expansion of

forest habitat only into grassland habitat; agricultural, urban, and water cover types were not affected by the increase in forest. Imagery used for these analyses was generated by the Kansas Applied Remote Sensing (KARS) program from multitemporal Landsat Thematic Mapper (TM) imagery (KARS 2001). The image was created using a two stage hybrid classification method of the TM imagery. This 2001 Kansas GAP land cover database was composed of 30 m raster cells encompassing 43 land cover classes. These land cover classes were generalized into five habitat types for analysis: forest, grassland, agriculture, urban, and water. The total area of the Upper Kansas River Watershed was about 1,385 km². Forest habitat comprised approximately 300 km² of the current landscape (Network 1), grassland habitat was ~704 km², agricultural lands were ~335 km², urban areas were ~38 km², and water comprised ~9 km² of the total area. Based on the estimated densities, a total of 892 skunk den locations were randomly placed across the study area with 751 locations in forest, 42 in grassland, 20 in agricultural areas, and 79 in urban habitat. A 25% increase in forest habitat (Network 2) resulted in 376 km² of forest and 628 km² of grassland, a 50% increase (Network 3) resulted in 450 km² of forest and 554 km² of grassland, and a 78% increase (Network 4) resulted in 534 km² of forest and 470 km² of grassland (Figure 3.1). Striped skunk den densities remained constant in all networks, but the number of dens increased with increasing forested habitat.

To determine pairwise contact rates based on proximity among individuals on the network, probability of contact was estimated using 100,000 Monte Carlo simulations. Probability of contact was determined by average fixed kernel estimates of spring home ranges. For simulations, home ranges were defined as circles surrounding a centroid den site, in which each concentric isopleth represented a probability of occupancy. An individual had a 25% probability of being found within a 384 m radius of its den, a 50% probability of being found

within 591 m of the den, a 70% probability of being within 770 m of the den, a 95% probability of being within 1,188 m of the den and a 100% probability of being within 1,656 m of its den site. Using these occupancy probabilities and Monte Carlo simulations, we created a distribution of pairwise probabilistic contact rates based on the distance between each two centroid den site locations. Contact was considered to occur if striped skunks were within 50 m of one another due to the social nature of these meso-carnivores (Verts 1967, Chapter 2, Bowe 2009).

Simulations of disease spread

We simulated spread of rabies through the network using a susceptible-exposed-infected/mobile-infected/stationary-removed (SEIIR) model. At the initiation of each simulation a single node was infected while all other nodes were categorized as susceptible. If a susceptible animal, j , came into contact with an infected animal, i , based on the probabilistic contact rates, w_{ij} , this individual converted to the exposed category at time t , with probability, $p_{i,t}$, based on a constant infection capability, β_f , of 0.85, as calculated in the following formula:

$$p_{i,t} = 1 - (1 - p_{i,t-1}) \prod_j (1 - \omega_{ij} \beta_f p_{j,t-1})$$

The amount of time the disease was latent in each node in the exposed category was based on a uniform distribution with a minimum of 14 days and a maximum of 172 days. These estimates represented the range of incubation times of ex situ transmission studies of wild type rabies in skunks (Parker and Wilsnack 1966). When in the exposed category, infected individuals incubated but were not capable of transmitting the disease. From the exposed category, all individuals moved into the infected category and actively transmitted the disease to other susceptible animals with which they came into contact for 7 days. Of this infection period, 5 days were spent in an active state, in which the infected skunk moved normally around its

home range, and 2 days were spent in a stationary state, in which the skunk did not move from the den site. Following the 7 days of infectivity, infected skunks were removed from the population. These period lengths were based on ex situ and in situ studies of rabid striped skunks (Parker and Wilsnack 1966, Greenwood et al. 1997).

Each simulation began in one of 5 locations in the Upper Kansas River Watershed: 4 locations were in the northern urban/forest matrix of Junction City, Kansas, Manhattan, Kansas, the Fort Riley Army Post, or the small town of Riley, KS. The town of Riley bordered the agricultural northern tip of the watershed. The final entry location was in the southern part of the watershed in a matrix of row crop agriculture and grassland. We had four spatially explicit contact networks representing the succession of increasing forest habitat and five epidemic initiation locations. Disease spread was simulated through each of the 20 models a total of 10,000 times. These simulations were conducted in C++ programming software and visualized using KiNG version 1.22 imaging software (<http://kinemage.biochem.duke.edu>).

Network Analyses

Basic network parameters were estimated including average node degree (k), network diameter, and betweenness centrality. The degree (k) of a node is defined by the number of links that are connected to that node (Barrat et al. 2008). This measure provides a description of network connectivity based on the individual components. The diameter of a network is the maximum shortest path length among nodes, with the shortest path length calculated as the number of links forming the shortest path between nodes. These measures describe the size and connectivity of the network. Betweenness centrality is the number of shortest paths between two nodes that pass through a node of interest (Freeman 1977, Newman 2001). Therefore, nodes with high site betweenness act as bridges, connecting one part of a network to another (Barrat et

al. 2008, Lewis et al. 2008). We also measured the density of the network, which described network sparseness. We calculated this measure as follows:

$$D = E / [N(N-1) / 2]$$

where E is the number of edges or links and N is the number of nodes in the network. If $D \ll 1$ than the network is said to be sparse, indicating that most of the values of probability of contact, w_{ij} , of the network are defined by edges of value 0. These parameters were compared among habitat types and among the four networks.

Connected components were delineated within the networks. A connected component describes a division of the network that contains paths that connect any two nodes within that division. Two components are disconnected if it is not possible to create a path that connects any of the nodes between the components (Barrat et al. 2008). This provides a simple description of network connectivity and is not based on the weights of contact, but simply on the edges among nodes. To determine epidemic hotspots based on habitat composition, a cluster analysis was conducted to identify areas of high connectivity on the landscape. An edge clustering coefficient was estimated by calculating the number of triangles an edge belonged to divided by the number it could belong to based on the node degree of adjacent nodes (Radicchi et al. 2004).

Transmission clusters were defined by removing edges with clustering coefficients $< 7.4 \times 10^{-4}$, a value which maximized clustering of nodes.

These clusters were analyzed in ArcGIS 9.0 using the Kansas GAP land cover data to determine the underlying habitat composition. We also analyzed the simulated habitat use of the rabies virus in the same platform. The rabies spread analysis was accomplished by comparing the observed habitat use of the rabies virus among all networks. Viral habitat use was delineated by creating minimum convex polygons around the extent of nodes removed by the rabies virus

across the landscape in each simulation. In addition to habitat compositional analyses, we employed several landscape metrics that measure habitat configuration and fragmentation of viral habitat use using the program FRAGSTATS (McGarigal and Marks 1995). We described various elements of the landscape using the parameters of patch and edge density, contagion, and Shannon's diversity and evenness indices. All metrics were calculated based on habitat raster images with 90 m pixels. Patch density is defined as the total number of habitat patches on the landscape divided by the total area of the landscape. Edge density is defined as the total length of all habitat pixel edges between land cover types on the landscape divided by the landscape area. The contagion metric describes whether a landscape is clumped or fragmented and is calculated using the following formula:

$$C = \frac{1 + \sum_{i=1}^s \sum_{j=1}^s (P_{ij}) \ln (P_{ij})}{2 \ln (s)} * 100$$

where P_{ij} is the probability that two randomly chosen adjacent pixels belong to cover type classifications i or j , respectively, and s is the number of cover types on the landscape. This index ranges from zero to one, with zero describing a completely fragmented landscape and one describing a landscape with cover types completely clumped and separate from all other habitat cover types.

Shannon's diversity and evenness indices provide measures of landscape heterogeneity. The diversity index (H') is based on the proportional abundance of each habitat cover type on the landscape and is calculated using the following formula:

$$H' = - \sum_{i=1}^s P_i \ln (P_i)$$

where P_i is the proportion of cover type i on the landscape, and s is the total number of cover types. Shannon's evenness is a related measure based on the comparison of the diversity index and the maximum heterogeneity of the landscape, $\ln(s)$. The evenness index is calculated as follows:

$$E = \frac{H'}{\ln(s)}$$

These metrics were compared among networks for the habitat underlying both the calculated transmission clusters and the simulated maximum extent of rabies spread.

Results

Network statistics

In the original habitat configuration, representing contemporary land cover, the northern tip and the southern half of the watershed were dominated by agriculture and grassland habitats. In contrast, habitat north of the Kansas River was predominantly forest with substantial urbanization near the river. This northern forested area expanded with woody encroachment, with forested habitat increasing by ~7% in habitat proportion on the landscape with each 25% increase in the area of forested habitat. However, the southern agriculture and grassland matrix remained fairly constant, due to the lack of existing forested habitat in the region. Here forested habitat increased by only ~4% in habitat proportion with each 25% forest habitat increase (Figure 3.2). Areas of disease spread and connected clusters were related to these patterns of habitat configuration.

The number of nodes and edges within each network increased with increasing forested habitat area (Table 3.1). Network 1, derived from current land cover, contained 2 isolated nodes, but the remaining networks had no singleton nodes that did not share at least one link. Density

remained fairly constant but showed slight declines; Network 1 maintained the highest density and density decreased in the subsequent network with increasing forest habitat (Table 3.1).

These density measures indicated that as forest habitat increased the networks became slightly sparser, with less links between nodes compared to the number of nodes within the network.

However, this difference in density was slight.

The average node degree was significantly different among all networks with an increase in node degree with increasing forested habitat (ANOVA, $F=86.01$, $p<0.0001$, Network 1 was $k=32$, Network 2 was $k=37$, Network 3 was $k=41$, and Network 4 was $k=45$). Node degree distributions varied among habitat types in all networks, with highest average node degree in forested and urban habitats. Node degree was influenced by node location on the watershed as forested habitat increased. In Network 1, nodes north and south of the watershed had similar degree distributions (Figure 3.3, 'a' and 'b'). However, the simulated increase in forested habitat substantially increased the node degree in the northern portion of the watershed, but did not have as strong of an effect in the south (Figure 3.3, 'c' and 'd'). This difference in node connectivity was influenced by the initial habitat conditions of the northern and southern portions of the watershed, with significantly less forest south of the Kansas River.

The number of connected components decreased as forest area increased across the networks as nodes joined the giant connected component of the northern portion of the watershed. Network 4 was fully connected signifying that any two nodes within the network were connected by some combination of links (Table 3.2). The average site betweenness increased with increasing forest habitat in the networks, with the highest levels in Network 4. This indicated that more skunks were acting as bridges across the landscape with increasing forest. The diameter of connected nodes across the landscape also increased with expanding

forested habitat, indicating that network breadth had increased. The cluster analysis with a cluster coefficient of $> 7.4 \times 10^{-4}$ revealed high variation in cluster number and size with increasing forest (Table 3.2). Surprisingly, the increase in forest habitat did not result in greater clustering. We found no expansion of the large cluster initiated in the first network. In fact, the largest cluster declined in size with increasing forest. In addition, the clusters delineated in networks with increase forest did not predict the patterns of disease spread displayed in the epidemic simulations.

Simulations of disease spread

The rabies virus did not spread completely through the networks in any of the model runs. Because of the distribution of skunk dens across the landscape and the contact probability among nodes, only Network 4 was fully connected. However, all connected components within the networks, including Network 4, contained several links with very low probabilities of contact, limiting the spread of the disease.

Patterns of disease spread differed greatly between Network 1 and the remaining networks. Rabies spread was limited in Network 1, reaching a maximum area of spread of about 350 km^2 , while the virus spread to $1,000$ to $1,200 \text{ km}^2$ in the remaining networks (Figure 3.4). In Network 1, the disease reached significantly different spatial areas of spread depending on the initiation location. In the remaining networks, the disease consistently reached a similar area of spread for every initiation point, except initiation in the southern portion of the watershed. In the southern portion of the watershed, where there was little forested habitat, the disease did not spread more than 14 km^2 in any of the networks. In Network 2, the disease did not spread further than the initially infected skunk.

Habitat analyses

The habitat composition underlying transmission clusters varied among networks (Figure 3.5). Forest dominated the areas inside of the clusters in all networks, but the matrix of other habitat types surrounding forest varied among the networks. Landscape metrics also varied in areas of the clusters in ways that did not correlate with increased forest. These findings are due to the inconsistencies in the clusters themselves as the size and numbers of clusters were not influenced by the increasing forested habitat.

The habitat composition of the minimum convex polygons that encompassed simulated viral spread varied throughout the networks (Figure 3.6). The percent forest cover through which the virus moved decreased initially with Network 2 and then increased through the remaining networks. The reverse pattern was evident for grassland, with an increase of grassland habitat from Network 1 to Network 2 and then a decrease in the remaining networks. The percent of agricultural lands covered by the virus increased with increasing forested habitat.

Landscape metrics varied among simulated models (Table 3.3). Both patch and edge densities decreased with increasing forested habitat, signifying that the virus spread in areas of lower habitat heterogeneity and that habitat heterogeneity decreased with forest succession. Contagion increased with increasing forest, indicating greater connectivity of the landscape through time which facilitated viral spread. Landscape diversity and evenness decreased as forest increased, indicating a greater dominance of a single habitat type in the area through which the disease spread. This pattern was altered when rabies infection was initiated in the town of Riley, the contagion was much higher underlying the area of viral spread in Network 1 and diversity and evenness was much lower than the other initiation locations. This pattern is due to the differences in habitat surrounding the small rural town of Riley. As forest increased around the town, the virus was able to use the matrix of forest and agriculture more effectively.

Discussion

We simulated the spread of the rabies virus through spatially explicit networks of contact among individual striped skunks and found that as forest habitat increased, connectivity among skunks, and therefore potential disease transmission, increased. The heightened connectivity in the simulated skunk populations was demonstrated by an increase in the number of connections among individuals, an increase in average number of individuals to which a skunk was connected (average node degree), and an increase in the number of skunks acting as bridges, connecting groups of individuals (betweenness centrality). This increased connectivity influenced patterns of rabies spread in three ways. First, the area the rabies virus covered dramatically increased with increasing forest habitat. In addition to this increased area of disease spread, we found that the location of disease initiation in large urban centers with areas of forest surrounding them did not influence the spatial spread of the virus with increased forest succession. Second, agricultural habitats no longer acted as buffers to rabies spread with only a 25% increase in forested habitat. In the initial network based on current land cover, agriculture and grassland habitats limited rabies spread across the watershed. With high levels of connectivity, existing habitat barriers on the landscape were unimportant in shaping patterns of disease spread. Finally, the cluster analyses were not accurate predictors of the extent of disease spread as skunk density increased. This finding could have significant implications for interpreting cluster analyses in future disease studies.

Increased network connectivity

As would be expected with an increase in the population size of a reservoir host across a landscape, the contact network connectivity increased with the expansion of densely populated forested habitat across the watershed. This increased connectivity was evident by the greater

number of connections among individual skunks and the decreased number of connected components as the individuals became more connected. Network 4, with a 78% increase in forested habitat, was fully connected such that every striped skunk was linked by some combination of connections. The average node degree also increased with increased expansion of forest. Because the degree of a given node provides a direct quantification of the connectivity of that individual to others in the network (Barrat et al. 2008), the high average node degree we observed signifies substantial connectivity within the network. In addition, average site betweenness increased with increasing forested habitat, with a dramatic increase in Network 4. These results indicate increasing connectivity, increased contact rates and ultimately increased transmission rates among disease hosts in areas with woody encroachment.

Increased proximity and contact are often found in areas of preferred habitat for a disease host species (Bradley and Altizer 2006). Besides increasing disease risk due to higher contact rates, wildlife may also be more susceptible to infection in these environments due to decreased physical condition and infection-fighting defenses (Bradley and Altizer 2006). Perhaps more critical to rabies management efforts is the increase in average site betweenness with increased woody encroachment. These highly connected and bridging nodes are often targeted in disease control and management efforts (Ueno and Masuda 2008, Youm et al. 2009). Yet an increase in the number of nodes with high site betweenness decreases the ability to target hot spots for disease spread. Therefore there are a greater number of skunks acting as ‘bridges’ connecting segments of the network that must be targeted to break up the connectivity of the network (Figure 3.7). Under present habitat conditions (Network 1), the number of nodes with high betweenness, i.e. the number of skunks with high connectivity, was low enough to target for disease control. With the increasing forested habitat, however, the number of highly connected

skunks became very high. Thus once betweenness becomes high, it may be a more productive control measure to reduce the amount of forest, rather than to target individual disease hosts for disease control. The increased site betweenness also demonstrates the risk woody vegetation poses as corridors for rabies spread. As woody encroachment continues not only in isolated riparian habitats but also along the perimeters of agricultural field and urban areas (Knopf and Samson 1995, Briggs et al. 2002b), skunk populations may be able to bridge viral spread from rural habitats to more densely populated urban centers in these highly connected corridors.

Increased forest propagates disease emergence

As we simulated woody encroachment across the watershed, the extent of the area over which rabies spread increased. In Network 1, based on current land cover, the rabies virus reached a maximum area of 311 km². However, with an increase of only 25% forest, this area increased by 345% (1,072 km²). In the remaining networks, there were not any significant, additional increases, with only a 40% additional increase (~1,200 km²) of rabies spread for 50% and 78% increases in forested habitat. Once the amount of forest increased, the urban area from where it originated did not influence how the disease spread. In Network 1, the four northern locations of disease emergence had the broadest variance in area of viral spread from 57 km² to 311 km². In Network 2, these areas affected by the virus only ranged from 915 km² to 1,072 km². The variance in area of disease propagation decreased further, with only a 16 km² difference in area between northern initiation locations in the final networks. Thus although area affected by the virus increased, variance in disease spread among initiation points decreased as forested habitat increased. The expansion in the area of viral spread as forest increased was evident only in the northern portion of the watershed where urban centers were located and did not increase in the southern portion of the watershed. At the southern initiation points for

Networks 1 and 3, the disease spread only 10 km², there was no spread in Network 2, and a maximum spread of 14 km² in Network 4. This suppression of disease emergence was due to the lack of forested habitat and domination of agricultural habitat in the southern watershed.

These findings reveal two important conclusions on the effects of woody expansion on rabies spread. First, there appears to be a threshold effect in the connectivity of the disease. With only a 25% increase in forested habitat (76 km² added to a 1,385 km² watershed), the area of rabies spread increased by 71%. With additional increases at 25% increments, the area of spread only increased by 3-8%. Thresholds have important implications for habitat management strategies (Field 2009). If woody encroachment is not slowed or reduced before passing this threshold, it may become much more difficult to control epizootic outbreaks. Because anthropogenic change is characterized by rapid expansion, these ecological thresholds are increasingly crossed and are often attributed to the current emergence of several zoonotic diseases.

The second conclusion derived from these analyses considers the fact that the location of disease initiation did not influence rabies spread with increased forested habitat in the northern forested portion of the watershed. In Network 1, disease spread was fairly limited when initiated in the towns of Riley and Junction City which were both surrounded by agriculture or grassland, compared to extensive spread when initiated in the more forested urban areas of Fort Riley and Manhattan. However, with only a 25% increase in forest, initiation in any of the northern locations resulted in large areas of spread due to the increased connectivity of the network. Because initiation location became irrelevant to patterns of disease spread with increased forested habitat, rabies emergence and propagation may be more difficult to control once woody encroachment passes the threshold. On a more connected landscape, hotspots for disease

emergence become increasingly difficult to pinpoint, making direct vaccination or control programs ineffectual.

These patterns of disease spread found in the northern portion of the watershed were not seen in initiation in the southern portion of the watershed which was limited in all models. This is due to differences in the habitat composition in the south, where the forested habitat did not reach the extent of the other areas. Network connectivity did not increase enough in the southern portion of the watershed to sustain greater disease spread. With the lack of substantial forested habitat and the subsequent dampened landscape connectivity, viral expansion was significantly slowed.

Decreased effectiveness of habitat barriers

As forested habitat increased in our networks, the barrier effects of agricultural and grasslands became ineffectual against rabies spread. When comparing the areas of disease spread to the underlying habitat, we found that in the original network, the largest area of spread closely outlined the configuration of forest habitat. The disease did not cross large areas of agricultural or grassland habitats. As forested habitat increased in the networks, rabies expansion no longer followed the forested habitat area. In Network 1, based on current habitat configurations, the percentage of agricultural habitat within the area of rabies spread was low. The amount of agricultural habitat within the rabies spread area was greater in the remaining networks as the virus increasingly crossed these barriers. Once forest expanded beyond the epidemic threshold, the effects of barriers to connectivity, such agricultural areas and grasslands, were overshadowed by the strong connectivity among forested habitat patches. This increase in disease transmission was driven by the increased connectivity of the landscape and decreased

habitat diversity. As forest encroaches on grassland habitat, skunks will have increased contact among forest patches separated by grassland and agricultural habitats.

Woody expansion is occurring rapidly in the Flint Hills of Kansas as prescribed burning decreases in exurban and suburban environments (Knopf and Samson 1995, Briggs et al. 2002b, and Briggs et al. 2005). Higher contact among host individuals is predicted to occur with the expansion of this preferred habitat and is amplified by the reduction of grassland and agricultural barriers. This loss of existing transmission barriers occurs as a threshold effect and exemplifies the concept of ecological and epidemiological thresholds. Similar occurrences have been noted in the northeastern United States where reforestation in the region has led to increased prevalence of Lyme disease (Barbour 1998, Wilcox and Gubler 2005). In the Flint Hills, prescribed burning regimes could be implemented to keep wooded habitat below the threshold. Managing for disease outbreaks by managing habitat rather than controlling host populations will likely be more effective both in cost and in its ability to suppress epizootic outbreaks.

Unpredictable cluster formation

In the original network, viral spread closely followed the clusters produced by the cluster analysis. This provided a reliable method of predicting rabies spread. However, in the subsequent networks, transmission clusters were variable in size and number and were not dependent on the increasing forested habitat, nor were the clusters related to the patterns of rabies spread. The clusters no longer accurately predicted the viral spread across the altered landscapes.

The decrease in the predictive ability of the cluster analysis offers a warning that network analyses must be carefully considered in terms of biological importance. The unreliability of the cluster analysis may be attributed to alterations in the cluster coefficient delineation. Increases in

node density may influence accurate measures of this coefficient. Definition of a cluster may change with changing landscape habitat configuration (Radicchi 2004). Accurate delineation of these communities should be considered in future analyses.

Conclusions

Ecological systems are composed of intricate interactions at small scales and from these microscopic interaction, large-scale, macroscopic patterns emerge. Consideration of these interactions is critical to realistic modeling of ecosystem function. Over the last several years, data availability and storage capability have increased allowing for the study of these large complex networks (Barrat et al. 2008). In modeling the rabies disease system, we used an individual-based complex network approach to attempt to realistically simulate disease spread across spatial-explicit backgrounds. By placing nodes based on habitat-specific densities across a landscape and by simulating disease spread based on empirically derived transmission values, we have attempted to accurately predict patterns of rabies spread across future landscapes given predicted land cover changes.

From this method of modeling, we found that with only a 25% increase in woody vegetation resulted in a greatly increased extent of rabies spread. This resulting area of rabies spread was not limited by agricultural barriers or habitat fragmentation, and displayed high connectivity among clustered nodes. These results indicate that there is a threshold level of forest expansion, beyond which rabies spread may impact substantially larger areas. Methods of wildlife control and vaccination may be effective at the current level of forest encroachment, but continued fire suppression will make this method control difficult and ineffectual. Preemptive rabies management, to avoid future epidemics, would involve annual prescribed burning and vegetation removal to reduce the amount of woody vegetation on open grassland.

As land use and land cover continues to change with rapidly expanding areas of exurbanization, it is crucial to public health to be able to predict future areas of disease spread. The complex modeling system presented in this study provides a simple method of delineating future areas of disease spread on realistic landscapes. By overlaying network models of disease spread on projected landscape scenarios, one can estimate future hot spots of disease transmission and respond with appropriate management actions. Novel infectious diseases often emerge in zones of habitat alteration, and the development of complex system and network modeling approaches are useful methods to model and predict disease spread across potential land cover distributions.

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Figures and Tables

Figure 3.1 Four network node distributions based on increases in forest habitat across the Upper Kansas River Watershed. The original network (a) is the current habitat composition of the watershed, while Network 2 (b) represents a 25% increase in forest, Network 3 (c) is a 50% increase in forest, and Network 4 (d) has a 78% increase in forest. Nodes were placed in each network based on the same habitat specific densities of 2.5 skunks/km² in forest, 2.1 skunks/km² in urban habitat, and 0.06 skunks/km² in grassland and agricultural lands.

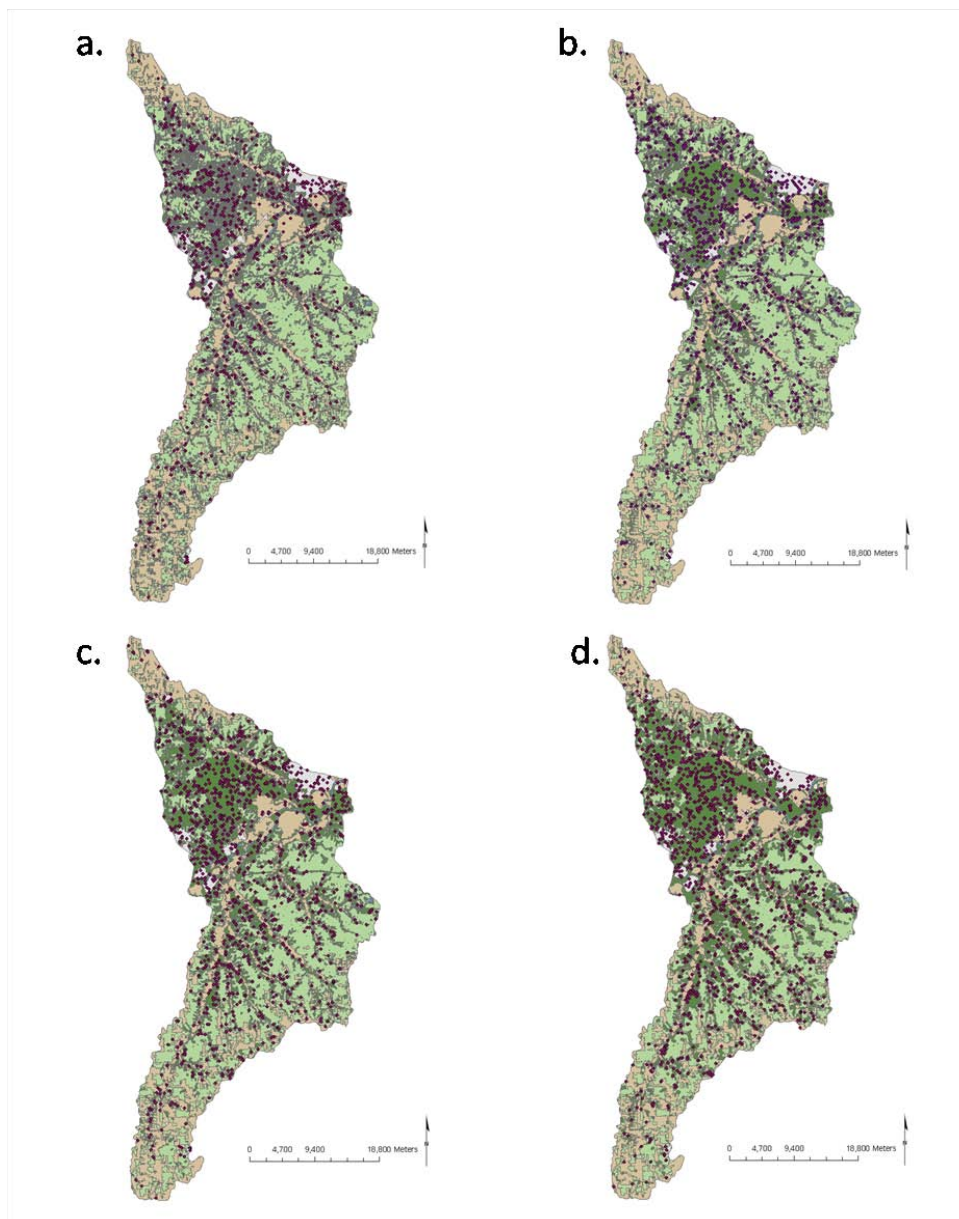


Figure 3.2 Forest habitat increase in proportion to total habitat area in each of the four watersheds divided by the Kansas River. Forest habitat north of the Kansas River is indicated in blue and south is indicated in red. The watersheds were delineated based on simulated increases in forested habitat area, with Watershed 1 representing the original habitat configuration, Watershed 2 representing a 25% increase in forest habitat, Watershed 3 representing a 50% increase, and Watershed 4 representing a 78% increase. Forest proportion in the south increased much less with each 25% increase in forest area than in the north.

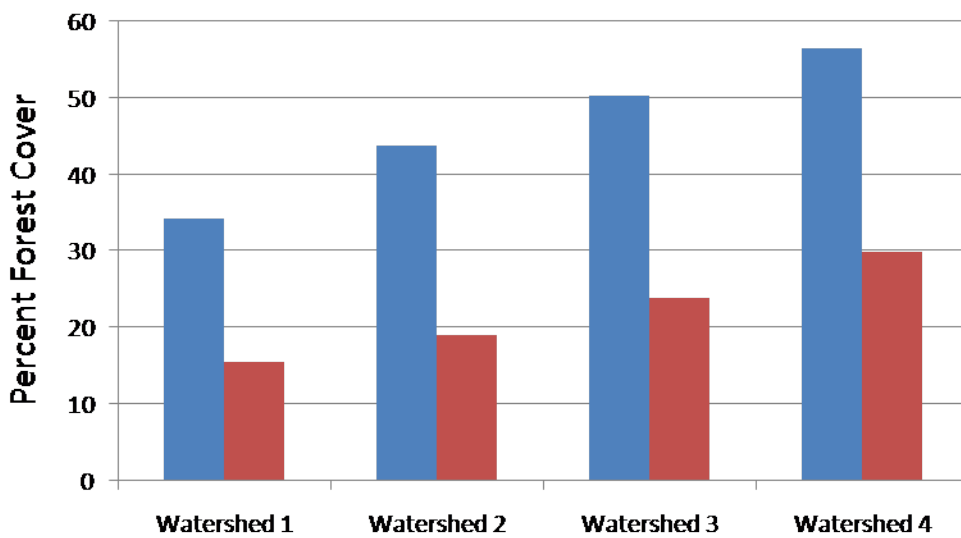


Figure 3.3 Node degree by habitat for Network 1 nodes south of the river (a) and north (b) and Network 4 nodes south of the river (c) and north (d). Dark green lines represent forest habitat, light green represent grasslands, red represent agricultural lands, and blue represent urban habitat. Networks 1 and 4 represent the contact networks of striped skunks before and after a 78% increase in forested habitat. The north of the watershed is dominated by forested habitat and the 78% increase greatly increased the connectivity of the network. The southern portion of the watershed is predominantly grasslands and agriculture and the increase in forested habitat did not have as significant effect on connectivity.

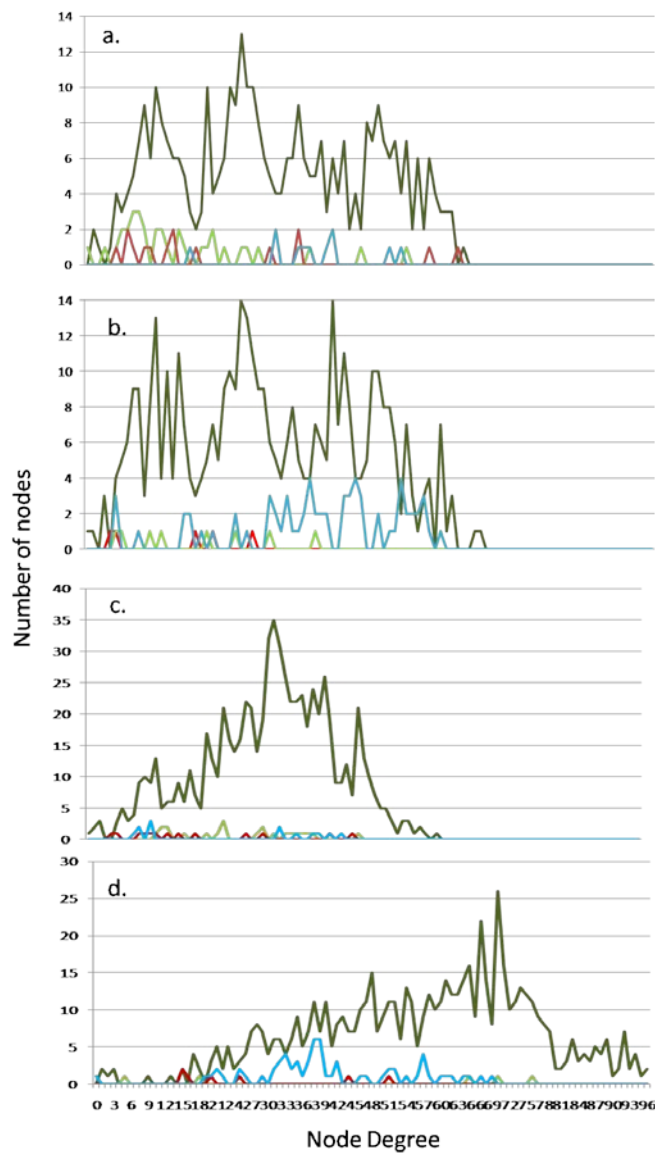


Figure 3.4 Disease spread by viral initiation location for each of the four networks (a.-d.). Red squares represent initiation in Junction City, green triangles are Manhattan, purple crosses are Riley, blue stars are Fort Riley, and orange circles are initiation in the south of the watershed.

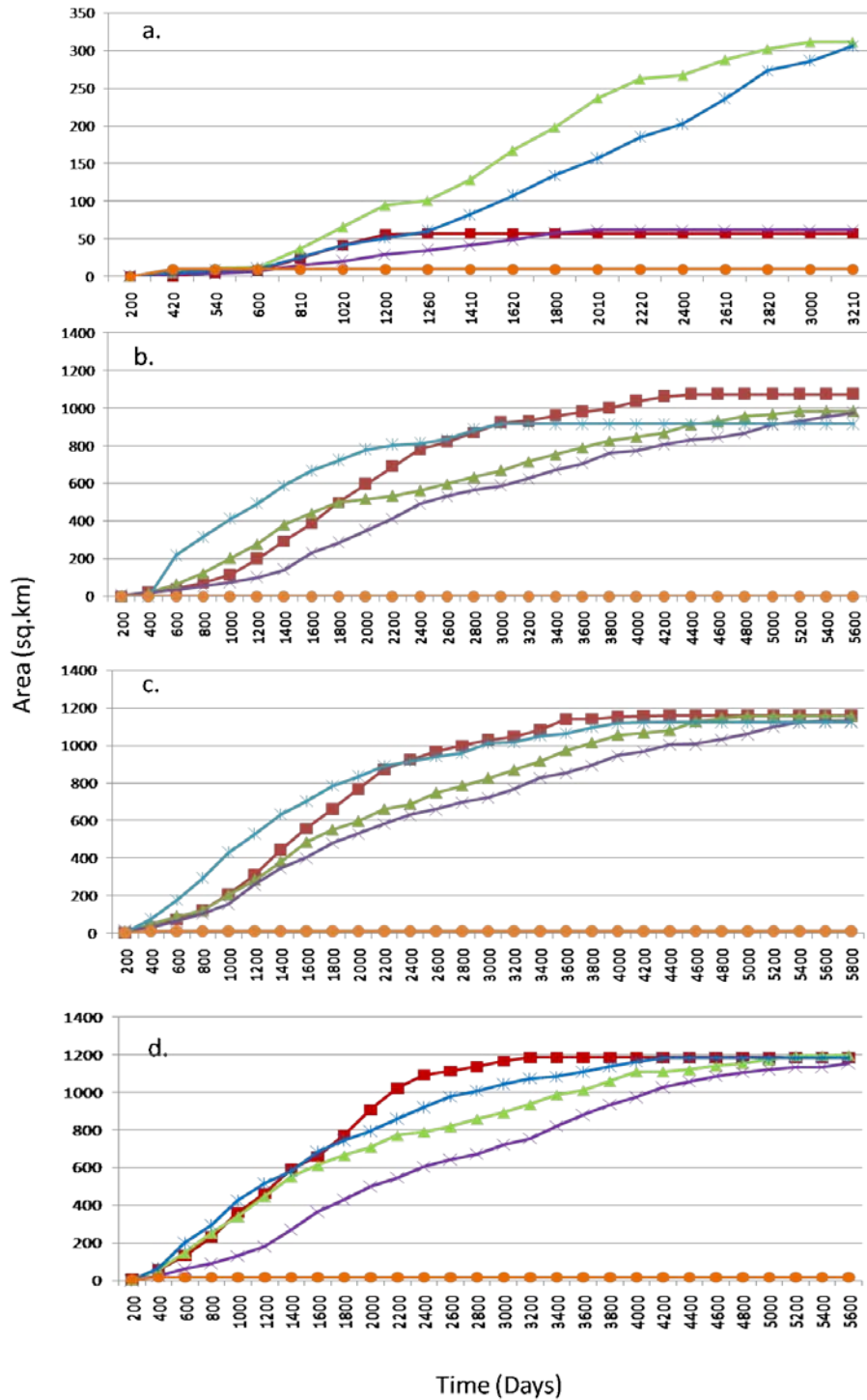


Figure 3.5 Comparisons of habitat composition for underlying landscape within network clusters (blue bars) and landscape outside of the clusters (red bars) for each of the four networks (a-d). These clusters were delineated to determine areas of high connectivity and to identify epidemic hotspots. An edge clustering coefficient was estimated by calculating the number of triangles an edge belonged to divided by the number it could belong to based on the node degree of adjacent nodes. Clusters were defined by removing edges with clustering coefficients $< 7.4 \times 10^{-4}$, a value which maximized clustering of nodes.

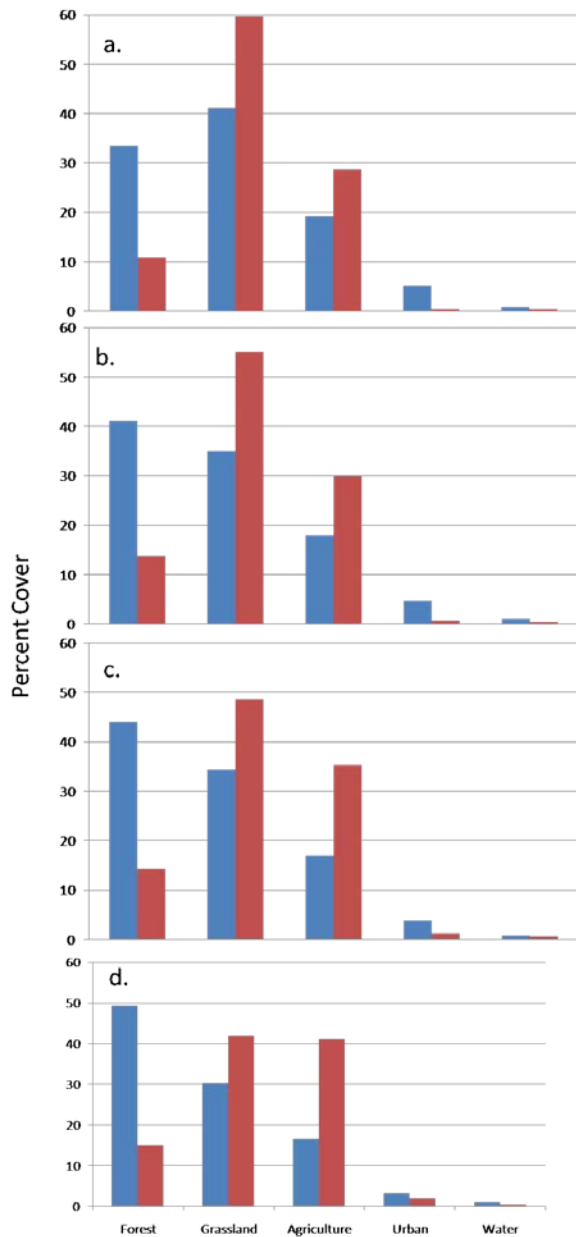


Figure 3.6 Habitat composition of the area of rabies spread through each network.
Network 1 is blue, Network 2 red, Network 3 green, and Network 4 purple. Each graph represents an initiation location: a) Junction City, b) Manhattan, c) Fort Riley, d) Town of Riley, and e) South.

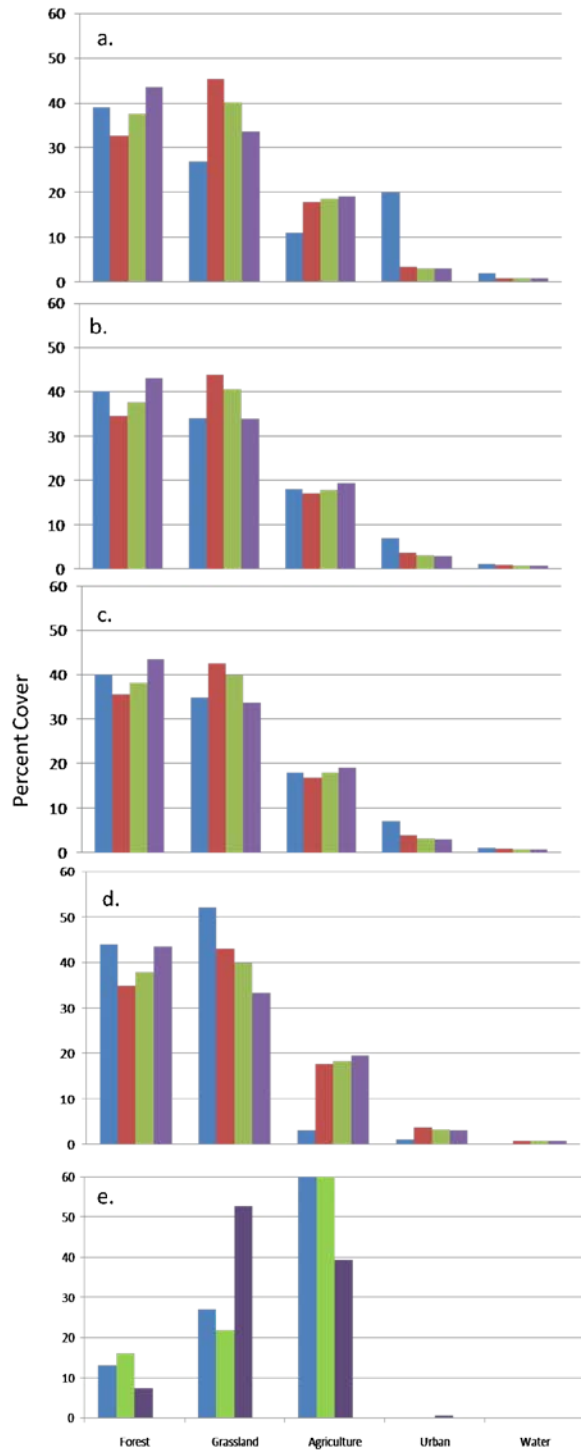


Figure 3.7 Example of an increase in the number of nodes with high site betweenness. Node 1 has high site betweenness and removing this node would separate the network. However, the addition of nodes 2 and 3 increases the average site betweenness and separation of the network requires the removal of 3 nodes.

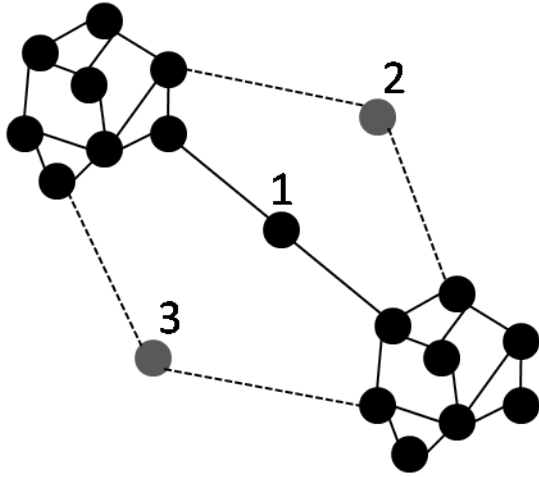


Table 3.1 General network descriptors for the four spatially explicit contact networks based on increases in tree area. Network 1 is based on the current habitat configuration of the Upper Kansas River Watershed, while Networks 2-4 are based on increased forested habitat as indicated. Nodes representing individual skunk dens were placed across these habitat configurations based on habitat-specific density estimates. Edges, or links, between 2 nodes were determined based on pairwise proximity probabilities. Density is a measure of network sparseness calculated based on the number of edges versus the number of nodes within each network. Densities $\ll 1$ indicate a sparse networks with most nodes not connected to one another.

	Forest Increase	Number of Nodes	Number of Edges	Density
Network 1	Original	892	14,273	0.03592
Network 2	25%	1,076	19,678	0.03402
Network 3	50%	1,257	25,770	0.03265
Network 4	78%	1,463	32,755	0.03063

Table 3.2 Analyses of connectivity for the four spatially explicit contact networks based on increases in tree area across the Upper Kansas River Watershed. Network 1 is based on the current watershed habitat configuration, Network 2 is a 25% increase in forest habitat, Network 3 is a 50% increase, and Network 4 is a 78% increase. A connected component describes a division of the network that contains paths that connect any two nodes within that division. Two components are disconnected if it is not possible to create a path that connects any of the nodes between the components. Site betweenness is the number of shortest paths between two nodes that pass through a node of interest. The diameter of a network is the maximum shortest path length among nodes, with the shortest path length calculated as the number of links forming the shortest path between nodes. Clusters were defined by removing edges with clustering coefficients $< 7.4 \times 10^{-4}$, a value which maximized clustering of nodes.

	Connected Components	Average Site Betweenness	Diameter	Isolates	Clusters	% Nodes in Largest Cluster
Network 1	5	6494 \pm 279	25	16	47	51
Network 2	4	7637 \pm 247	25	16	53	46
Network 3	3	8944 \pm 270	28	9	41	25
Network 4	1	110534 \pm 10047	30	11	40	66

Table 3.3 Landscape metrics comparing habitat configuration of observed viral habitat use against expected habitat use for the four spatially explicit contact networks based on predicted forest increase across the Upper Kansas River Watershed at each rabies initiation location for disease spread simulations.

Patch Density					
<i>Network</i>	<i>Junction City</i>	<i>Manhattan</i>	<i>North</i>	<i>Riley</i>	<i>South</i>
1	5.7255	5.274	7.065	5.5861	7.2378
2	3.8711	3.8839	3.8821	4.067	-
3	2.753	2.6984	2.7116	2.688	5.6537
4	2.1747	2.1708	2.1699	2.1722	3.0364

Edge Density					
<i>Network</i>	<i>Junction City</i>	<i>Manhattan</i>	<i>North</i>	<i>Riley</i>	<i>South</i>
1	52.2857	52.73	60.8123	53.598	53.5083
2	42.8798	43.0915	43.2132	43.587	-
3	39.1766	38.8466	38.6903	38.9632	54.2141
4	34.3232	34.3045	34.0869	34.309	34.9047

Contagion					
<i>Network</i>	<i>Junction City</i>	<i>Manhattan</i>	<i>North</i>	<i>Riley</i>	<i>South</i>
1	36.2187	40.0345	54.2358	40.1194	29.1826
2	43.9991	43.8907	43.6829	43.4777	-
3	44.4949	44.7265	44.5944	44.6778	26.5611
4	45.9883	45.9416	45.9798	46.0078	47.5774

Shannon Diversity Index					
<i>Network</i>	<i>Junction City</i>	<i>Manhattan</i>	<i>North</i>	<i>Riley</i>	<i>South</i>
1	1.3514	1.2779	0.8342	1.2713	0.8783
2	1.1847	1.1909	1.1953	1.1997	-
3	1.1933	1.1886	1.194	1.1901	0.9191
4	1.1878	1.1891	1.1909	1.1875	0.9286

Shannon Evenness Index					
<i>Network</i>	<i>Junction City</i>	<i>Manhattan</i>	<i>North</i>	<i>Riley</i>	<i>South</i>
1	0.8397	0.794	0.5183	0.7899	0.7995
2	0.7361	0.7399	0.7427	0.7454	-
3	0.7414	0.7385	0.7419	0.7394	0.8366
4	0.738	0.7388	0.7399	0.7378	0.6699

CHAPTER 4 - Conclusions

Zoonotic infectious disease epidemiology is dependent on three important factors: properties of the pathogen, ecology of the disease host species, and the habitat configuration of the underlying landscape. Multiple approaches should be integrated to study, manage, and control emerging and re-emerging infectious disease across rapidly changing landscapes. In our work on rabies virus in striped skunk populations in the Flint Hills of Kansas, we combined field data, genetic analyses, and mathematical modeling to characterize the epidemiology of an important zoonotic disease. I believe integration, along with cross-discipline collaborations, have greatly enhanced the completeness and breadth of our findings. Telemetry data collected on and around the Konza Prairie Biological Station provided fine-scaled information on individual habitat use and spatial distributions in anthropogenically disturbed and undisturbed environments. Contributing to the findings from these field data, genetic analyses revealed a lack of population structure delineations and high admixture in striped skunks in the telemetry study and individuals across the Kansas Flint Hills. Field and genetic analyses provided a basis for mathematical modeling of rabies spread across a broader spatial scale, the Upper Kansas River Watershed. Working with researchers in computer and electrical engineering, we were able to predict disease spread based on future land cover configurations based on habitat-specific densities of striped skunk populations from the telemetry study. The combination of these three methods has led to several important conclusions on striped skunk ecology and predicted patterns of rabies spread across an altered landscape.

Through the integration of field and genetic methods in Chapter 2, we concluded that 1) striped skunks reached high population densities in anthropogenically disturbed habitats, 2) these individuals were not closely related, and 3) contact rates could be influenced by temperature.

Striped skunks were found at higher densities in the disturbed habitat matrix of our study areas than in the relatively undisturbed grasslands demonstrated by high skunk and den densities, a greater degree of home range overlap, and more instances of communal denning. These densities indicate the potential for increased levels of contact among individuals of this disease host species. Adding to these elevated contact probabilities is the fact that striped skunks displayed high levels of genetic admixture and mobility within our study areas. Genetic analyses revealed that individuals with high levels of home range overlap were not more closely related than those outside of these groupings and skunks in communal dens were not more closely related than those outside of the dens. Distances among first and second order related pairs were high, with average distances greater than 20 km, indicating large dispersal ranges. These results can have implications for human health and risk of disease across the Flint Hills. The dense populations of skunks are clustering in habitats near human development and these individuals prefer to den within forested habitat. Contact rates are therefore significantly heightened at the interface of human and domestic animal populations. Rabies risk will increase with increasing preferred skunk habitat. Adding to this scenario, ease of movement among skunk populations as demonstrated by the genetic analysis will allow for the disease to spread among regional urban centers. Rabies can lie dormant in individuals for extended lengths of time, allowing for a dispersing male to carry the disease from his communal den in one disturbed area to another disturbed habitat area in search of a mate. The infected skunk will meet little resistance from the non-territorial residents.

Anthropogenic land use change also has broader reaching consequences. As highly populated urban centers expand around the world, the resulting pollution and climate change affect landscapes on a global scale. In eastern Kansas, models of future climate scenarios predict

warmer winters with higher nightly temperatures over the next decades (Brunsell et al. 2008). Climate change may interact with rabies spread in these highly clustered and genetically unrelated populations in complicated ways, exacerbating issues caused by habitat change in the Flint Hills. Skunks move from their dens during warmer winter nights, therefore increasing the potential for disease spread. Greater winter movement will increase contact rates among skunks as well as contact with domesticated animals and other spill-over wildlife species. However, climate change may also help reduce rabies risk. Communal denning among individuals occurred only during the winter months in our study areas and skunk weights declined in individuals over the cold winter months of our study. The increased contact among individuals induced by communal denning would decline in a warmer winter scenario. Weight loss often indicates a compromised immune system in wildlife species (Altizer et al. 2006). Warmer winters would help maintain more resilient immunity in striped skunks, potentially reducing susceptibility to disease.

Based on the spatial analysis of the telemetry data, we found that striped skunks preferred to den within forested habitat. This habitat preference may lead to high densities of the disease host within increasing woody cover across the Flint Hills. To analyze how the predicted increases in woody encroachment on open grassland will influence rabies spread, we simulated an increase in forested habitat through a contact network system of modeling, as described in Chapter 3. These models revealed that rabies spread does increase in area as forested habitat increases due to both heightened landscape connectivity and reduced efficiency of agricultural and grassland habitat buffers. In addition, there was a threshold to this increase and rabies spread reached its maximum rate of spread after only a 25% increase in woody cover. This finding emphasizes the need to manage woody encroachment now to avoid serious disease

epidemics in the future. If woody encroachment continues at its current rate of spread, the threshold for these large scale epidemics will be reached in less than 30 years.

A greater understanding of host habitat use patterns can also be advantageous for disease control efforts. Given the affinity of striped skunks for encroaching woodlands, landowner incentives to increase prescribed fire regimes within the Flint Hills should be implemented to reduce the current expansion of wooded vegetation into the prairie ecosystems. Land cover management changes now could reduce future rabies epidemics ignited by the expanding forested habitat. In addition, vaccination and culling efforts can be directed at forest and field habitat boundaries for more efficient control of disease spread. Currently, oral rabies vaccination (ORV) programs are used in North America through the use of bait, targeting specific host species (Slate et al. 2005). Striped skunk ORV is being improved for higher consumption by the animal and the trap-vaccinate-release method offers another, more direct effort for vaccination (Rosatte et al. 1992). Whatever method is proven most effective for striped skunk rabies eradication, vaccination efforts should be concentrated on hot spots of disease spread, such as the forest/field matrix.

The synthesis of a variety of research strategies to model patterns of an infectious disease is essential for disease spread predictions and the coordination of management efforts to prevent future epidemics and human casualties. Emerging infectious diseases are becoming increasingly widespread with increased human mobility and landscape alteration (Field 2009) and are typically zoonotic and influenced by anthropogenic disturbance (Patz et al. 2004, Crowl et al. 2008, Grimm et al. 2008). Therefore, accurate descriptions of these diseases must not only consider the pathogen, but also its animal host and the changing landscape on which it spreads. The increased emergence of zoonotic diseases have caused well-publicized scares in the United

States and many European and Asian countries with outbreaks worldwide, including West Nile virus, severe acute respiratory syndrome (SARS), and most recently, H1N1 influenza virus. The development of a system to rapidly and accurately study, predict, and control future outbreaks is critical to human health worldwide (Brug et al. 2009) and should involve the integration of all components of disease epidemiology including disease pathogenesis, host ecology, and the underlying landscape. The methods and research strategies presented in the thesis may be useful and applicable in future studies of rapidly emerging infectious disease.

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