

INTERACTIONS BETWEEN GRASSLAND BIRDS AND THEIR SNAKE PREDATORS:  
THE POTENTIAL FOR CONSERVATION CONFLICTS IN THE TALLGRASS PRAIRIE

by

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B.S., Drake University, 2001  
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AN ABSTRACT OF A DISSERTATION

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## Abstract

The loss, fragmentation, and degradation of grasslands have resulted in widespread declines in grassland birds. Nest predation is the leading cause of avian reproductive failure; therefore minimizing nest predation can lessen the severity of bird declines. Snakes are important predators of bird nests, but little is known about how snakes may enhance predation risk. To address this issue, I studied the habitat use, movement behavior, population genetic structure, and connectivity of snakes in the grasslands of northeastern Kansas. I addressed the connectivity of eastern yellowbelly racer (*Coluber constrictor flaviventris*) populations by using a landscape genetics approach at a broad scale (13,500 km<sup>2</sup>). I also radio-tracked the yellowbelly racer and Great Plains ratsnake (*Pantherophis emoryi*) at Konza Prairie Biological Station to understand their spatial ecology while simultaneously evaluating nest survival in grassland birds. Individual racers had limited dispersal (<3 km), but substantial admixture occurred within 30 km and populations were in migration-drift equilibrium and had high allelic diversity; therefore, racers must be abundant and continuously distributed for gene flow to be fluid throughout the region. Racers may be more likely to encounter bird nests, as they had more frequent movements and traversed greater distances on average than ratsnakes, which exhibited long periods of inactivity between directed movements. As for grassland birds, nest survival rates decreased with increasing shrubs and decreasing vegetation height. Discriminant function analysis revealed that successful nests were likely to occur in tall vegetation but reduced shrub cover, whereas higher shrub cover characterized snake habitats. Because snakes often use shrubs, nests in areas of increased shrubs may be at higher risk of predation by snakes. Targeted removal of shrubs may increase nest success by minimizing the activity of predators attracted to shrubs. Although predator removal is often a strategy for protecting bird populations, it may not be feasible in this instance, especially since snakes are a native component of the grassland community. Efforts to reduce snake predation on grassland bird nests should therefore focus on managing habitat within grasslands (i.e., shrubs) that influence snake activity, as no natural or anthropogenic habitat barriers currently limit snake movement across the landscape.

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## CHAPTER 1- Introduction

Human alteration of the Earth's land surface is estimated to impact one third to one half of the planet (Vitousek et al. 1997). Ten to fifteen percent of the Earth's terrestrial habitat has been completely converted into urban areas or row-crop agriculture resulting in the loss and fragmentation of native ecosystems. Less-intense transformation such as the conversion of native grasslands to rangeland accounts for an additional 6-8% of Earth's surface and results in the degradation of habitat for native species (Vitousek et al. 1997). Worldwide, temperate grasslands have undergone the highest rate of habitat conversion (45.8%) of any biome but have the least amount of land area protected (4.6%; Hoekstra et al. 2005). In North America, the estimated loss of grassland ecosystems has exceeded 80% with < 4% of tallgrass prairie remaining (Samson and Knopf 1994) making it one of the most endangered ecosystems worldwide (White et al. 2000).

The Flint Hills region of Kansas and Oklahoma is the largest, contiguous tallgrass prairie landscape (~2 million ha) remaining in North America (Knapp and Seastedt 1998), and therefore an important conservation area for many native prairie species (Rahmig et al. 2009). Although the Flint Hills region appears to be a well-connected natural landscape, habitat loss and fragmentation may be occurring due to land-management practices. For example, grasslands rapidly become shrublands or savannas when grazing and burning is suppressed (Archer et al. 1995, Briggs et al. 2002), creating unsuitable habitat for grassland-dependent species such as grassland birds (Grant et al. 2004). At the other extreme, habitat degradation occurs through intensive grazing and burning which drastically reduces vegetation structure (With et al. 2008). Although fire and grazing are necessary to maintain tallgrass prairie and avoid succession to forests, the temporal and spatial pattern of current grassland management differs greatly from the

historical regime. Historically, the interval of disturbance in the tallgrass prairie was two to three fires every five years with ungulates that would graze recently burned areas to produce a heterogeneous grassland mosaic (Collins and Gibson 1990). Currently, ~86% of the Flint Hills is privately-owned rangeland with 65% of the managed grassland having season- or year-long stocking and 25% having annual burns with early intensive stocking of cattle (Smith and Owensby 1978, With et al. 2008, With unpubl. data). Elsewhere, such as in areas of exurban development, the lack of grazing and burning promote woody encroachment. Both forms of habitat degradation have been shown to be a major threat to animal populations and overall biodiversity in grasslands (Robbins et al. 2002, Hickman et al. 2004, Wilgers and Horne 2006, With et al. 2008).

A well-documented example of the negative impacts of habitat loss, fragmentation, and degradation are declines in species richness and nesting success for grassland birds (Herkert 1994a, Herkert 1994b), which have suffered the most widespread decline of any North American bird group (Sauer et al. 2004, Brennan and Kuvlesky 2005). Nest predation is the leading cause of avian reproductive failure in many ecosystems including grasslands (Ricklefs 1969, Martin 1993a, Martin 1993b); therefore increasing reproductive success on breeding grounds can lessen the severity of grassland bird declines. In nest predation studies, emphasis has been placed on understanding the predator community in an effort to inform conservation actions attempting to increase the reproductive success of Neotropical migrants (Heske et al. 2001, Klug et al. 2009). For example, it has been hypothesized that variation in snake activity may be responsible for the patterns in nest failure (Zimmerman 1984, Sperry et al. 2008). Therefore, a thorough understanding of the predator's response to habitat loss, fragmentation, and degradation may lead

to a better understanding of the mechanisms by which reproductive success in grassland birds is affected by landscape modification.

The importance of snakes as bird nest predators has received considerable attention (Weatherhead and Blouin-Demers 2004) with the primary focus centered on increasing avian reproductive success by minimizing the abundance or activity of predators in areas important to breeding birds (Heske et al. 2001). Additional information is needed on the ecology of snake species known to depredate bird nests in order to minimize the negative influence of snakes on breeding birds while not negatively impacting the sustainability of snake populations.

Maintaining snake populations is necessary as they are important members of the native tallgrass prairie ecosystem, not only as predators of bird nests but for the ecosystem services they perform as predators of small mammals and invertebrates, which can be considered pests if freed from natural controls (Daily et al. 1997). Understanding snake population structure across the landscape and activity at local scales is essential for predicting relationships between predators and their prey (Stephens et al. 2004). Information on the interactions between birds and snakes can then be used in designing and managing landscapes for both snakes and birds.

The overall goal of this dissertation was to understand nest predation in grassland birds by evaluating the predator ecology of two snake species known to depredate bird nests in the tallgrass prairie. The dissertation focuses on the landscape ecology of a predator-prey interaction, and how predation risk may be mediated through predator response to landscape heterogeneity at broad (i.e., northeast Kansas) and fine (i.e., Konza Prairie Biological Station, KPBS) spatial scales. The structure and connectivity of snake populations at broad landscape and regional scales will allow us to better understand if snakes populations are continuous and panmictic or if any habitat features restrict the movement of snakes to create discrete populations. Few studies

have addressed snake population responses to habitat fragmentation at landscape scales relative to the number of bird studies that have addressed the impact of fragmentation on avian nesting success (Stephens et al. 2004, Weatherhead and Blouin-Demers 2004). For example, many nest predation studies have considered the density and configuration of habitat patches in the landscape to explain nest predation rates (Robinson et al. 1995, Bergin et al. 2000), but few studies have addressed the response of snake populations to landscape variables (Cagle 2008). This is especially significant given that much emphasis has been placed on understanding the influence of landscape factors at broad scales on the abundance and behavior of nest predators and thus predation rates on birds (Chalfoun et al. 2002). In addition, little is known about habitat overlap between snakes and birds at the local scale (i.e., within grasslands). Understanding habitat at the local scale will be valuable in establishing management practices that minimize nest predation by removing habitat features that attract predators (e.g. shrubby structures that act as perch for foraging or thermoregulation) as opposed to predator removal strategies (Côté and Sutherland 1997).

The dissertation is organized into this introductory chapter, three data chapters, a synthesis chapter covering conclusions, and one appendix. The three data chapters focused on snakes with the premise that in order to understand nest predation in birds we must understand the predator (Chalfoun et al. 2002). I have chosen to study the eastern yellowbelly racer (*Coluber constrictor*) and Great Plains ratsnake (*Pantherophis emoryi*) due to their documentation as predators of bird nests on the KPBS (Sandercock et al. 2008; per.obs.) and in other grassland regions (Thompson et al. 1999, Stake 2001, Weatherhead and Blouin-Demers 2004, Klug 2005). In addition, the eastern yellowbelly racer and the Great Plains Ratsnake were two of the most abundant snakes on the KPBS (Klug et al. In revision), and therefore I was able to acquire

sufficient numbers for study. In chapter five, I focus on the major conclusions of my work and how the results are applicable to managing grasslands for declining songbird populations while simultaneously acknowledging the importance of multi-species management to maintain sustainable populations of native snakes.

Specifically, in chapter two, I analyzed the landscape genetics of the eastern yellowbelly racer to elucidate the genetic patterns and structure of snake populations in the tallgrass prairie. The second chapter defines species-specific landscape connectivity across the broader landscape of northeastern Kansas (i.e., 13,500-km<sup>2</sup> region) by using geospatial mapping of land cover (Kansas Gap Analysis Project; Egbert et al. 2001) in conjunction with genetic information. I defined landscape connectivity and identified how snake populations scale and respond to heterogeneity across the landscape as assayed by gene flow (i.e., dispersal followed by successful reproduction).

In chapter three, I evaluated space use, movement behavior, and ecology of the eastern yellowbelly racer and Great Plains ratsnake by radio-tracking snakes at a local scale on the KPBS. By working on the KPBS, I was able to address the spatial ecology of two snake species in a relatively contiguous grassland habitat while qualitatively examining the influence of grazing and burning on their ecology. A study of the spatial ecology of snakes and their responses to habitat management will contribute to a better understanding of their potential impact on prey species of conservation concern in grassland systems (e.g., grassland birds). In addition, this chapter focused on snakes as potential species of concern given that snakes as a whole have shown regional and global declines (Gibbons et al. 2000, Cagle 2008) and the status of snakes throughout the Flint Hills is uncertain.

In chapter four, I compared habitat use of two snake species and five species of nesting grassland birds within the experimental landscape of KPBS, which is subjected to different fire and grazing treatments. Although overwhelming evidence has shown snakes to be important predators of bird nests in many habitat types (Weatherhead and Blouin-Demers 2004, Klug 2005), few studies have looked at habitat use of snakes in relation to nest-predation patterns (Sperry et al. 2009). For example, almost nothing is known about how current land-management practices (e.g., grazing and burning) affect the habitat use of snakes during the avian breeding season. Land management may be enhancing predation risk through habitat modification that creates “hotspots of predation risk” that pose a threat to nesting songbirds (i.e. songbirds may be forced to nest in areas that attract high snake activity). Therefore, the aim of chapter four was to achieve an understanding of predation risk for grassland birds through an analysis of how snakes respond to habitat shaped by different management practices. Insights into snake habitat use can inform conservation efforts for grassland birds by gauging the plausibility of managing habitat features intensively used by predators.

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## **CHAPTER 2- Population genetic structure and landscape connectivity of the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) in the tallgrass prairie of northeastern Kansas**

### **Abstract**

In North America, the estimated loss of grassland ecosystems has exceeded 80% with <4% of tallgrass prairie remaining. The Flint Hills region of Kansas and Oklahoma is the largest, contiguous tallgrass prairie landscape remaining in North America and is considered an important conservation area for many native prairie species and therefore an ideal landscape to study the population genetic structure of a still-common snake. Understanding the population genetic structure of animals in contiguous habitats sets a baseline for evaluating changes in genetic structure when the habitat is fragmented. We adopted a landscape genetics approach to identify how landscape structure (i.e., habitat composition and resistance) affected the dispersal range, population genetic structure and landscape connectivity of the eastern yellowbelly racer (*Coluber constrictor flaviventris*) across a 13,500-km<sup>2</sup> tallgrass prairie landscape in northeastern Kansas, USA. The yellowbelly racer population had high heterozygosity, high number of alleles per locus, and was shown to be maintaining migration-drift equilibrium, all indicating that no subpopulations were isolated from each other and genetic drift was continually offset by migration of new individuals. Autocorrelation between genetic and geographic distance revealed that yellowbelly racers exhibit restricted dispersal within 3 km, but substantial admixture occurred within 30 km, indicating that racers must be abundant and continuously distributed for gene flow to be fluid throughout the region. Significant isolation-by-distance eventually occurred at broad regional scales (> 100 km), but because no subpopulations were completely isolated, we were unable to define discrete subpopulations using Bayesian clustering analyses based on allele

frequencies. The resistance distance, which considers the permeability of different habitats, did not explain a significant amount of the variation in genetic distance beyond Euclidean distance alone suggesting that yellowbelly racers are not heavily influenced by landscape composition. In the tallgrass prairie of northeastern Kansas, eastern yellowbelly racers appear to be an abundant and continuously distributed snake that perceives the landscape as well connected, with no barriers large enough to alter dispersal and gene flow.

## **Introduction**

Dispersal is defined as the movement of an individual from its natal site to the site where it produces its own offspring (Howard 1960), or as the movement from one breeding site to another (Greenwood and Harvey 1982). Although dispersal and gene flow are often correlated, they are not synonymous (Bohonak 1999). For dispersal to result in gene flow, individuals must successfully reproduce after dispersal (Greenwood 1980). Thus, the dispersal ability of a species has the potential to influence evolutionary processes such as genetic drift, local adaptation, and speciation by promoting or limiting gene flow (Dieckmann et al. 1999). Dispersal is vital in maintaining viable populations as it decreases inbreeding (Perrin and Goudet 2001) and increases recolonization (Hanski and Gilpin 1998). Dispersal success is influenced by landscape structure (i.e., amount, size, and distribution of suitable habitat) and the movement behavior of the organism attempting to disperse (With and King 1999). Landscape modifications, such as habitat fragmentation and degradation, have the potential to influence population genetic structure especially when loss and fragmentation of suitable habitat has created expanses of hostile matrix in which the organism cannot disperse (King and With 2002). Therefore dispersal is one of the most important life-history traits for understanding the historical, present, and future distribution

of genetic variation and how population structure may be impacted by landscape change (Vandergast et al. 2007; Zellmer and Knowles 2009).

Although dispersal ability is an important aspect of an organism's biology, the life stage in which dispersal often occurs (i.e. juvenile) is the most difficult to observe in field studies (Koenig et al. 1996). Dispersal is often underestimated due to logistical difficulties such as the need to mark a large number of individuals in order to witness a dispersal event and the limited size of study areas relative to the dispersal range. Studies on dispersal often use satellite radio-tracking equipment, which require large batteries and are biased toward animals > 300 g and capable of carrying heavy radio-transmitters (Wikelski et al. 2007). The alternative to intensive observational studies of movement or dispersal is the use of genetic data, which permit the detection of dispersal and population genetic structure across broad landscape extents. In addition, genetic metrics are able to effectively measure low dispersal rates and only include meaningful dispersal events (i.e., those that result in breeding or permanent emigration), both of which are difficult to attain in field studies (Cushman et al. 2006).

The population genetic studies often use allele frequencies (e.g. Wright's F-statistics; Wright 1943) to quantifying genetic differentiation among isolated and internally panmictic subpopulations to identify gene flow and migration. A drawback to this approach is the need to group organisms as discrete subpopulations, which is not realistic for many terrestrial organisms that have continuously distributed populations or populations that are patchily distributed but have low density of individuals between patches. Gene flow within a continuous population occurs according to the isolation-by-distance theory (Wright 1943) with an inverse relationship between genetic relatedness and geographic distance. The ability to measure genetic distance between individuals (Rousset 2000), along with spatial analyses borrowed from the discipline of

landscape ecology, allows us to detect the scale of genetic discontinuities as well as the correlation between those genetic discontinuities and landscape features (i.e., landscape genetics; Manel et al. 2003). For example, spatial autocorrelation can be used to measure the spatial scale at which pairwise relatedness between individuals is significantly positive or negative, thus revealing the dispersal range of the species (Epperson and Li 1996; Sokol and Oden 1978). Techniques in landscape genetics also include defining landscape connectivity for an organism by elucidating the degree to which the landscape facilitates or impedes dispersal and therefore gene flow to shape genetic patterns (Holderegger and Wagner 2006). For example, many studies have used geospatial software (e.g., Geographic Information Systems) to quantify resistance-to-movement values for different habitats in an effort to establish the effective distance between individuals, as opposed to straight-line distances, for use in isolation-by-distance analyses (Clark et al. 2008; Coulon et al. 2004). A landscape genetics approach to identifying species-specific landscape connectivity is necessary to understand how populations are genetically structured on the landscape and how landscape connectivity influences dispersal (i.e., gene flow) in order to better inform conservation efforts as habitats become fragmented (Diniz-Filho and Telles 2002).

Dispersal in snakes is particularly difficult to observe due to their cryptic habits and lack of easily identifiable nesting sites in which to mark and follow dispersing juveniles. In addition, visual surveys and mark-recapture methods are often ineffective for following snakes, estimating population densities, or delineating population structure because snakes are inconspicuous and have extended periods of inactivity (Parker and Plummer 2001). Although the study of snakes has been facilitated by the invention of small-bodied radio-transmitters, our ability to link movement to dispersal events, which ultimately influence population structure, is still limited.

Genetic technologies are one way to bridge this gap in our current understanding of individual dispersal and the population genetic structure of snakes.

Although little is known about how genetic structure of snake populations is shaped by dispersal ability and species' responses to landscape structure (Gibbs and Weatherhead 2001), the few studies conducted on snakes have shown that they possess limited dispersal abilities, resulting in subpopulation structure occurring at < 6 km (Clark et al. 2008; Gibbs et al. 1997; Lougheed et al. 1999). The majority of snake studies concerning population genetics use traditional methods of evaluating snake populations as discrete subpopulations marked by hibernacula use (Clark et al. 2008; Manier and Arnold 2005). The genetic structure of a snake population not limited by fidelity to hibernacula or other patchily distributed resources such as water bodies (e.g. *Thamnophis* spp.) and large rock outcrops (e.g. *Crotalus horridus*) have rarely been studied (Keogh et al. 2007). Therefore, expanding the scope of studies to include snakes of varying ecological needs (i.e.. habitat generalist in a relatively contiguous landscape) will give us an understanding of the variety of constraints influencing population genetic structure in snakes.

The population genetic structure of snakes in the tallgrass prairie and how they perceive the connectivity of habitat are not known, but are important given that many regions of the historical tallgrass prairie have suffered drastic declines in snake populations possibly due to loss and fragmentation of grassland habitat by agriculture and urbanization (Cagle 2008). The Flint Hills region of Kansas and Oklahoma represents the largest remaining tract of tallgrass prairie (Knapp and Seastedt 1998), and appears to be a relatively contiguous landscape, in that more than half (52%) of the region is grassland. However, landscape connectivity is species-specific and may not correspond to our perception; therefore each habitat in the landscape (i.e., grasslands, forests, urban areas, water bodies, and wetlands) is associated with a different degree

of permeability for a given species (With et al. 1997). The Flint Hills region is thus an ideal landscape in which to study the population genetic structure of a still-common snake in a relatively intact grassland habitat. Understanding the population genetic structure of animals in contiguous habitats sets a baseline for evaluating changes in genetic structure when the habitat is fragmented (Banks et al. 2005; Dayanandan et al. 1999).

We used geospatial modeling and population genetics to elucidate the landscape genetics of a common snake in the tallgrass prairie of northeast Kansas. The goal of this study was to use spatial and genetic information to understand the population genetic structure of the eastern yellowbelly racer (*Coluber constrictor flaviventris*) and how habitat heterogeneity across the landscape influenced gene flow. The three main objectives of the study were to 1) identify population subdivisions (or lack thereof) using Bayesian clustering procedures to assign individual snakes to discrete subpopulations; 2) use spatial autocorrelation to analyze fine-scale genetic structuring within snake populations and identify the scale at which individuals within a population are genetically similar, admixed, and differentiated; and 3) define landscape connectivity of yellowbelly racers in the tallgrass prairie by analyzing if the resistance distance, which considers the permeability of habitats, better explains genetic distance between individuals than straight-line distance. By evaluating landscape connectivity, we will be able to determine if landscape structure is limiting dispersal (i.e., gene flow) and impacting genetic structure.

## **Methods**

We collected genetic material from yellowbelly racers in northeastern Kansas encompassing a 75 x 180 km extent (13,500 km<sup>2</sup>). We collected samples from individuals (n = 57 individuals) from Riley, Geary, Pottawatomie, and Marshall Counties in the Flint Hills ecoregion, which included the Fort Riley Military Reservation (FRMR; n = 12) and Konza

Prairie Biological Station (KPBS; n = 32). The Flint Hills ecoregion is the largest tallgrass prairie remaining in North America and contains limestone and shale hills with steep narrow valleys. We also obtained samples of individuals (n = 23) from Douglas, Jefferson, and Leavenworth Counties in the Central Irregular Plains ecoregion, which included the University of Kansas Field Station and Ecological Reserves (KSR; n = 21). The Central Irregular Plains ecoregion also contains native tallgrass prairie, but a transition zone representing a grassland/forest mosaic. Tissue samples were obtained from snakes between May and October in 2007 and 2008. We collected tissue from road-killed specimens (i.e. muscle or liver) encountered opportunistically and from live specimens (i.e. scale or tail clip) encountered during snake surveys. Snake surveys included setting out coverboards (60 x 180 cm plywood sheets) as passive traps (KPBS and KSR) as well as turning over rocks (KPBS, KSR, and FRMR; Parmelee and Fitch 1995).

Eastern yellowbelly racers have a broad geographic and ecological distribution that encompasses a variety of habitat types including forests, native grasslands, old fields, wetlands, and urban centers, and are found from Montana to western North Dakota and from eastern Iowa to southern Texas (Conant and Collins 1998). Although the subspecies known as eastern yellowbelly racer is considered a habitat generalist, in the Great Plains region they are mainly found in tallgrass prairie and pastureland (Fitch 1999). Dispersal in yellowbelly racers occurs in two ways: 1) during the juvenile stage, when young establish summer home ranges and hibernacula, and 2) when adults change hibernacula or summer home ranges, which can occur throughout their lifetime (Fitch 1963). The average distance from summer home ranges to hibernacula was 400 m for snakes in the tallgrass prairie of northeastern Kansas, but can range from 0-1,225 m (Fitch 1963). The average size of summer home ranges for males was 21.6 ha (SE = 6.4) and for females was 6.4 ha (SE = 1.5) on KPBS (Klug et al., In review). In



yellowbelly racers, fidelity to a particular hibernaculum is not strong, and individuals may shift hibernacula or summer home ranges during their lifetime (Fitch 1963). In Kansas, yellowbelly racers breed from May to June after they have dispersed away from the hibernaculum (Fitch 1963; Fitch 1999; Parker and Brown 1973). Male racers are capable of breeding at 20 months and females likely begin breeding at three years of age after a larger body size has been attained (Fitch 1999). The female racer will lay eggs in the vicinity of a hibernaculum that is different from her own in late June (Fitch 1963). Hatching occurs August to September (Fitch 1999). Evidence indicates that racers are promiscuous and multiple males may mate with one female (Fitch 1963). From long-term trapping data on the KSR, racers occurred at 4-6 snakes per ha with a sex ratio of 49% males (i.e., with samples biased against first-year young; Fitch 1999).

We used microsatellite markers to elucidate genetic structure of the eastern yellowbelly racer. Microsatellite markers are selectively neutral, have high mutation rates ( $10^{-6} - 10^{-2}$  mutations per locus per generation), and exhibit high allelic diversity, which is necessary for genetic studies interested in contemporary, intraspecific population genetic structure as opposed to the geological timescales of phylogeography (Schlötterer 2000). We employed 12 microsatellite markers specifically developed for eastern yellowbelly racers (Molecular Ecology Resources Primer Development Consortium et al. 2009; Molecular Ecology Resources database accessions 37758–37769; GenBank Accession GQ371177-88). A full description of the development of these loci is available on the Molecular Ecology Resources database (<http://tomato.biol.trinity.edu/>, accessions 37758–37769 and Appendix 1). DNA was extracted from scales, tails, or the liver using Dneasy™ Tissue Kit (Qiagen). Products were amplified using polymerase chain reactions (PCR), visualized using an ABI PRISM™ 3730 DNA Sequencer with ROX labeled size standard, and analyzed using GeneMarker® (Softgenetics). We

used MICROCHECKER v. 2.2.0 (Van Oosterhout et al. 2004) to statistically test for genotyping errors and the presence of null alleles using the Brookfield-1 equation as the null estimator.

### ***Population Genetic Characteristics***

We used GENEPOP (<http://wbiomed.curtin.edu.au/genepop>) to calculate diversity statistics and to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (Raymond and Rousset 1995). Linkage disequilibrium between pairs of loci was tested with a Fisher's exact test of contingency tables of allele frequencies for all pairs of loci (Markov chain of 1000 dememorizations, 100 batches, and 100 iterations per batch). We tested for heterozygote deficiency using Hardy-Weinberg exact tests for each locus (Markov chain: 1000 dememorizations, 600 batches, 1000 iterations per batch) within the whole sample. We also analyzed Riley/Geary County and Douglas/Jefferson County populations separately because sampling occurred in two different ecoregions. If these are actually two subpopulations that are in Hardy-Weinberg equilibrium, then a reduction in heterozygosity may occur in the total sample (i.e., Wahlund effect). We qualitatively checked for a potential Wahlund effect by comparing the heterozygote deficit in both the entire sample and the two subpopulations.

We used the Wilcoxon sign-rank test within BOTTLENECK 1.2.02 (Piry et al. 1999) to analyze if the number of loci with heterozygote excess was significantly higher than what would be expected by chance (50%) at mutation-drift equilibrium. As suggested for microsatellite data, we employed the two-phase mutation model (TPM) with a variance of 12 and 95% of the stepwise mutation model (SMM) in the TPM (Piry et al. 1999). Any recent reductions in the effective population size will cause the allele number to be reduced faster than the genetic diversity (heterozygosity), which causes increased heterozygote excess when using HWE compared to heterozygosity calculated from the number of alleles (Luikart et al. 1998). We did

not expect yellowbelly racers to have experienced a reduction in effective size as yellowbelly racers are habitat generalists and appear to be a common snake in the Flint Hills region over the past 80 years (Busby and Parmelee 1996). We nevertheless tested for a possible bottleneck as we do not fully understand the status of yellowbelly racer populations in the tallgrass prairie and are unsure of how land or wildlife management may have impacted racer populations in this region.

### ***Bayesian Clustering Assignment Test***

Discrete population boundaries could not be distinguished a priori for yellowbelly racers in Kansas because the snakes did not occur in distinctly patchy habitats where populations could be defined (Fig. 2.1). Although we hypothesized that the distribution of yellowbelly racers may be continuous because its grassland habitat is relatively intact across the region and racers are habitat generalists, tissue samples from racers were obtained from two distinct ecoregions (Flint Hills and Central Irregular Plains), which may give rise to subpopulation structure. We used STRUCTURE version 2.3.1 (Pritchard et al. 2000) to deduce if population genetic structure (i.e., subpopulations) existed within the study area or if gene flow was continuous. Bayesian clustering methods such as STRUCTURE do not make a priori assumptions of population assignment based on geographical information. Instead, unbiased patterns of population genetic structure can be realized for a species that does not occur in discrete population patches but is one population exhibiting genetic structure. We used the program STRUCTURE to estimate the probability of a set of allelic frequencies given  $K$  populations  $\ln\text{Pr}(X/K)$ . The program calculates the posterior probability for each value of  $K$  and produces estimates of log-likelihood, which are used to discern the optimal number of population clusters in the population. In STRUCTURE, we ran 500,000 steps after a burn-in of 100,000 under the admixture model with correlated gene frequencies. We ran five separate iterations for each value of  $K$  and used the average value of  $\ln$

$\Pr(X|K)$  and the variation to select the probable number of population clusters ( $K$ ). The genetic clustering approach used in STRUCTURE was appropriate because the population boundaries are uncertain and there was the possibility that not all sources were sampled. If the results had indicated genetic population structure, we would have proceeded with traditional estimates of allele frequencies or  $F_{ST}$  statistics between populations to describe the genetic structure of yellowbelly racer populations across the landscape. Since the results indicated that the snakes were likely one large, panmictic population (see Results), we used the individual-based approach to understand the scale of population genetic structure as explained in the next section.

### ***Spatial Autocorrelation***

Spatial autocorrelation was used to analyze fine-scale patterns of genetic structure, which is valuable in investigating within-population processes when population boundaries cannot be defined (Double et al. 2005; Peakall et al. 2003). We used genotypic data from all 81 snakes and assessed the spatial signal produced by 12 codominant microsatellite loci using a multivariate approach, as opposed to one allele at a time (Smouse and Peakall 1999). Pair-wise genetic distance was calculated for each locus and summed across all loci for total genetic distance (Smouse and Peakall 1999). Geographic distance (km) was the Euclidean distance between the latitude and longitude of each snake on a xy-coordinate system. A correlation coefficient ( $r$ ) for each distance class was calculated with a pair-wise matrix of genetic distance and a pair-wise matrix of geographic distance in GENALEX 6 (Peakall and Smouse 2006).

We created a genetic autocorrelogram by plotting  $r$  as a function of geographic distance to elucidate the scale at which genetic differentiation occurs in eastern yellowbelly racers of the tallgrass prairie. If positive genetic structure is found, the point at which the correlogram crosses the x-axis indicates the approximate scale where genetic differentiation begins or the extent of

the genetic neighborhood (Sokal and Wartenberg 1983). The ability to correctly estimate the extent of genetic structure relies on the extent of the actual genetic structure, the distance class size, and the sample size within each distance class. We chose the size of distance classes so that each had approximately equal sample sizes and varied the number of distance classes, which effectively changed the size of distance classes. In reporting results from multiple correlograms, we are assured that distance class intervals were not greater than the scale of genetic structure or smaller than necessary so as to compromise statistical power (Peakall et al. 2003).

We tested statistical significance at each distance class using 1000 random permutations in which individual genotypes are shuffled between the geographic locations to achieve a recalculated estimate of  $r$ . The upper and lower bounds of the 95% confidence intervals were defined by the 25<sup>th</sup> and 95<sup>th</sup> values of the estimated  $r$ , sorted in ascending order. These random permutations generated an estimate of  $r$  around the null hypothesis (i.e., no genetic structure). Where  $r$  was greater than the 95% confidence interval, significant positive autocorrelation occurred, which is expected if dispersal is restricted (Peakall et al. 2003). We also estimated  $r$  for each distance class using 1000 bootstraps and used the sorted and ranked  $r$ -values to define the 95% confidence interval. We confirmed significance if the actual  $r$ -value met two criteria: 1)  $r$  exceeded the 95% confidence interval around the null hypothesis (i.e., permutation test), and 2) the 95% confidence interval around  $r$  did not overlap zero (i.e., bootstrapping).

We designed the autocorrelograms with distance classes of equal samples (i.e., all distance classes have similar sample size) in an effort to reduce noisy confidence limits due to uneven sample sizes. We present multiple correlograms to assure that chosen distance classes do not influence the scale of genetic structuring but reflect the true genetic structure. For example, correlogram A gives the first three distance classes as 2, 3, and 7 km; correlogram B gives 1, 3,

and 7 km; and correlogram C gives 1, 2, and 3 km. Comparisons among these three correlograms enabled us to pinpoint the scale at which positive genetic structure occurred below 7 km.

### ***Landscape Connectivity***

Population genetic structure was correlated to landscape heterogeneity using isolation-by-distance (i.e., genetic distance vs. geographic distance between individuals) to define the connectivity of the landscape and identify any important corridors or barriers to yellowbelly racer movement. We calculated genetic distances between individuals ( $a_r$ ) as described by Rousset (2000) in GENEPOP (<http://wbiomed.curtin.edu.au/genepop>). High values of  $a_r$  indicate high dissimilarity between two individuals. Previously, for the spatial autocorrelation analyses we used linear genetic distance (Smouse and Peakall 1999) as opposed to  $a_r$ . Therefore we employed a Mantel test to compare the two genetic distance measures; a significant correlation ( $r = 0.999$ ,  $p < 0.001$ ) indicated that both measures would give similar results.

We used CIRCUITSCAPE, a computational tool employing circuit theory to model landscape connectivity (McRae and Shah 2009). CIRCUITSCAPE uses resistance distances between pairs of nodes (i.e. individual racers) on a raster landscape. The resistance distance is the distance between two snakes and takes into account all possible pathways (i.e., uses circuit theory to create a graph-theoretic distance metric). For example, multiple routes and wider habitat swaths allow greater dispersal between nodes (i.e., analogous to multiple and wider conductors connecting electrical nodes allowing greater electrical current; McRae and Beier 2007). This method of quantifying geographic distance may therefore be better than least-cost path models, which only allow for one path through the landscape (Ray 2005). In addition, resistance distances in CIRCUITSCAPE may better evaluate genetic isolation-by-distance than

Euclidean distance because the metric is based on assumptions regarding the permeability of landscape features during dispersal, which ultimately impacts gene flow (Verbeylen et al. 2003).

We used the Kansas GAP Analysis (Egbert et al. 2001), which included 43 land cover classes at a spatial accuracy of 15 m to determine habitat composition. We condensed the 43 land-cover categories into seven categories: gallery forests, native prairie (never cultivated), wetlands/marshes, grasslands (CRP fields and other agricultural fields restored to grassland), cultivated fields, water bodies (rivers, lakes, and reservoirs), and urban centers. To assess how yellowbelly racers might respond to landscape heterogeneity, we constructed resistance values for each habitat type by creating multiple working hypotheses of the influence of land cover on snake dispersal. Friction grids were constructed by coding each pixel based on the habitat's resistance to dispersal (i.e., 1 = most permeable, 100 = least permeable, barrier = not permeable). We exported eight, hypothetical friction grids (cell size = 150 m<sup>2</sup>) from ArcMap 9.0 that varied in the resistance values for each habitat type (Table 2.2). Although we hypothesize that movement in yellowbelly racers would be tied to grasslands, it is not known what type of habitat juvenile racers prefer to move through during dispersal. Therefore, six of the eight friction grids had one habitat type as more permeable than all others because we did not want to limit dispersal to one habitat type a priori (native prairie, grasslands, gallery forest, cultivated, urban, and water; Table 2.2). The other two friction grids comprised a gradient of resistance values depending on the presumed degree of human alteration of terrestrial habitats. The 'Gradient' hypothesis coded all natural land cover as least resistant, cultivated fields at a mid-resistance, and urban centers as high resistance (water bodies were also high resistance due to racers being terrestrial organisms although they are capable of swimming). The 'Barrier' hypothesis was similar to 'Gradient' but had urban centers and water bodies as a complete barrier to yellowbelly racer movement (Table

2.2). We ran each friction grid separately in CIRCUITSCAPE to calculate resistance distances between individual racers for a total of eight resistance distances.

We used Mantel tests (Mantel 1967) to compare the correlation between the pair-wise genetic and pair-wise geographical matrices to test for the impact of landscape heterogeneity on gene flow (Smouse et al. 1986). We analyzed isolation-by-distance in two analyses including the entire study region and for the Flint Hills ecoregion alone. We did not analyze the Central Irregular Plains ecoregion due to small sample size ( $n = 23$ ). We visualized the relationship between the genetic and geographic distances using linear regression and a lowess regression (sampling proportion = 0.6 and one polynomial) in SIGMAPLOT version 11.0 to smooth the noisy data and reveal underlying patterns. The Mantel tests were performed in library VEGAN version 1.6-7 (Dixon 2003; Oksanen et al. 2007) of R (R Development Core Team 2005). The nine models tested included the Euclidean distance and each of eight-resistance maps. After assessing significance of the Mantel test for all nine models, we then ran a partial mantel test (function = 'partial.mantel'), which compared the genetic and resistance matrices while controlling for Euclidean distance. Although we acknowledge the debate over whether the partial Mantel test produces biased estimates of  $P$  values (Castellano and Balletto 2002; Raufaste and Rousset 2001; Rousset 2002), we considered significant partial Mantel tests to indicate if resistance maps explained variation in genetic distance beyond Euclidean distance alone. The partial Mantel test is similar to multiple regression, but in testing significance incorporates non-independence of points by creating a null distribution through the random redistribution of points (Mantel 1967). If the resistance distance was a better estimator than Euclidean distance, then we could conclude that landscape structure may have been affecting gene flow, such that the overall landscape was functionally fragmented and habitats were differentially permeable to yellowbelly racers.



## Results

### *Population Genetic Characteristics*

We sampled 81 yellowbelly racers across the study area with the most intensive sampling occurring within the Flint Hills ecoregion (1,000 km<sup>2</sup>; Figure 2.1). All 81 samples were genotyped at the 12 microsatellites with no redundant genotypes (i.e., no pairs had completely matching genotypes). Allelic variation ranged from 8 to 51 alleles per locus (mean = 20.8, SD = 11.2). Expected and observed heterozygosities ranged from 0.29 to 0.97 and 0.31 to 0.88, respectively, for the entire sample (Table 2.1). No pairs of loci exhibited linkage disequilibrium. We found consistent evidence of significant deviation from HWE for one microsatellite (i.e., GQ371178; Table 2.1). We found evidence for null alleles at two microsatellites (i.e., GQ371178 and GQ371183;  $nf > 0.10$ ). Yellowbelly racers in eastern Kansas have not experienced population numbers low enough to have caused a founder's effect because there were not significantly more heterozygotes than expected based on mutation-drift equilibrium using either the sign test ( $p = 0.32$ ) or the Wilcoxon test ( $p = 0.30$ ; two-tailed) within BOTTLENECK. The expected number of loci displaying heterozygote excess (7.27) was not substantially different from the observed number of loci displaying heterozygote excess (6) or heterozygote deficiency (6).

### *Bayesian Clustering Assignment Tests*

For the analysis genetic structure using a Bayesian clustering assignment test, there was evidence for population subdivision at  $K = 1$ ,  $K = 2$ , and  $K = 3$  (Fig. 2.2A). Often when a program such as STRUCTURE finds clustering solutions with similar probabilities at multiple  $K$  values, the  $K$  with the highest  $\ln\text{Pr}(X|K)$  and lowest variation is chosen, which would be  $K = 1$  (mean = -4781.4, SE = 0.36) (Pritchard et al. 2000; Pritchard et al. 2009). The next optimal solution would have been  $K = 2$ , which has a mean  $\ln\text{Pr}(X|K) = -4763.5$  (SE = 1.26). When

evaluating the proportional membership of each racer in the two clusters with the plot of ancestry estimates ( $K = 2$ ), we observed a separation between those snakes found in the Flint Hills (i.e., KPBS, FRMR, and surrounding counties) and those found in the Central Irregular Plains (i.e., KSR; Fig. 2.2B). Although the number of subpopulations could be  $K = 2$ , sufficient gene flow must be occurring between these locations as we were not able to define discrete subpopulations because  $K = 1$  was just as likely. Therefore, we continued with individual-based methods to understand the scale at which racers begin to exhibit genetic differentiation.

### *Spatial Autocorrelation*

For correlogram A, the  $r$ -values are positive and significant at 3 km with an  $x$ -intercept of 29 km (Fig. 2.3A). For correlogram B, the  $r$ -values are positive and significant at 1 km with an  $x$ -intercept at 16 and 29 km (Fig. 2.3B). For correlogram C, the  $r$ -values are positive and significant at 1 and 3 km with an  $x$ -intercept at 2, 16, and 29 km (Fig. 2.3C). Through the use of multiple correlograms, we deduced that snakes within 3 km of each other showed significant genetic similarity (i.e., positive genetic structure). Of the three correlograms, none show  $r$  as significantly negative. The correlograms maintain positive genetic structure with few 95% confidence intervals and few  $r$ -values dropping below  $r = 0$  until a distance class between 70 and 117 km, where snakes separated by these distances show genetic dissimilarity (i.e.,  $r$  became consistently negative). The distance of 70 to 145 km was the distance between the eastern (Central Irregular Plains) and western (Flint Hills) regions, indicating the dissimilarities may be due to differences in ecoregions. The inability to pinpoint the exact scale at which the population becomes admixed ( $r = 0$ ) or genetically differentiated ( $r < 0$ ) may be due to the discrete sampling distribution in which pairwise comparisons between 40 and 100 km are underrepresented.

### ***Landscape Connectivity***

Mantel tests indicated that isolation-by-distance partially explained the observed genetic structure of yellowbelly racers when considering all samples ( $n = 81$ ,  $r = 0.075$ ,  $P = 0.006$ ; Table 2.3A), but not when considering only those samples in the Flint Hills ecoregion ( $n = 57$ ,  $r = 0.042$ ,  $P = 0.286$ ; Table 2.3B). The genetic distance was also partially explained by distance in space for the eight hypothetical resistance distances when considering the entire data set (Table 2.3A) but not the restricted data set (Table 2.3B). None of the eight resistance maps explained further variation in genetic distance after controlling for Euclidean distances (Table 2.3). The isolation-by-distance model was significant, but only when considering the broadest landscape scale (75 x 180 km) and not the Flint Hills ecoregion alone (25 x 40 km; Table 2.3 and Fig. 2.4). Although the low  $r$ -values from the Mantel tests do not appear to explain a large amount of the variation, this is a common observation of individual-based models (Rousset 2000).

### **Discussion**

We adopted a landscape genetics approach to explore how habitat heterogeneity at a regional scale influenced gene flow, population genetic structure, and landscape connectivity for yellowbelly racers in the largest tallgrass prairie landscape remaining in North America. Through spatial autocorrelation, we found that yellowbelly racers appear to be restricted in dispersal at fine spatial scales ( $< 3$  km), but substantial genetic exchange occurred across broad scales ( $\sim 30$  km), which was corroborated by a lack of a significant isolation-by-distance (Mantel test) in the Flint Hills ecoregion (25 x 40 km). A large number of individuals in a near-continuous distribution must exist for a population with dispersal-limited individuals to be admixed over broad spatial scales; our results suggest this is the case for yellowbelly racers in this grassland region. For example, the population showed no signs of a genetic bottleneck and was

maintaining migration-drift equilibrium, indicating that no subpopulations were isolated from each other and genetic drift was continually offset by migration of new individuals. A heterozygote deficit was only significant for one of the 12 loci (GQ37117), which was most likely the result of a null allele. The observed high heterozygosity (mean = 0.73, SE = 0.05) suggests that the loss of heterozygosity that often occurs with genetic drift in small populations was not evident. The population also exhibited a high number of alleles per locus (mean = 20.9, SE = 3.2) suggesting a large number of individuals contributing to the gene pool. This genetic evidence in conjunction with the snake survey data from this study (i.e., racers were 47% of total snake captures, Klug et al. In revision) indicates that the yellowbelly racer is abundant and has a fairly continuous distribution across this predominantly grassland region. At present, the tallgrass prairie of northeastern Kansas seems capable of maintaining a genetically viable population of yellowbelly racers and could serve as a baseline for evaluating population genetic structure of snakes in other areas of the tallgrass prairie where grassland habitat is extremely fragmented.

We found that gene flow occurred throughout the study area, as we were unable to confidently define discrete subpopulations in STRUCTURE (i.e., K could have been 1-3 subpopulations). We chose a conservative  $K = 1$ , following the rules of parsimony, despite the plot of ancestry estimates depicting a separation of racers from the Central Irregular Plains ecoregion (i.e., KSR and surrounding) and those from the Flint Hills ecoregion (i.e., KPBS, FRMR, and surrounding) when  $K = 2$ . Further support for the existence of two subpopulations ( $K = 2$ ) comes from the Mantel tests for isolation-by-distance in which we found a significant positive relationship between genetic distance and geographic distance when using the entire study area, but not when the analysis was limited to the Flint Hills ecoregion. The fact that isolation-by-distance was not significant within the Flint Hills ecoregion suggests a large

admixed population of racers within the 25 x 40 km (i.e., 1,000 km<sup>2</sup>) extent of that study area. The results from the genetic autocorrelation analyses also show genetic dissimilarity occurring between individuals from different sampling locales (i.e., Flint Hills and Central Irregular Plains), as  $r$  became negative between 30-115 km. Although we were unable to pinpoint the exact distance at which the genetic neighborhood becomes differentiated due to our sampling scheme (i.e., distances between 40 and 100 km are underrepresented), we can say that differentiation occurs at a scale greater than 30 km but less than 115 km (i.e., the distance between the Flint Hills and the Central Irregular Plains sampling locales). As the Bayesian clustering analysis uses the frequency of alleles (F-statistics), a clear barrier to dispersal is necessary between subpopulations to create isolated populations for genetic drift to occur and allele frequency to change (and to be detected in STRUCTURE). Although the racers in the Flint Hills and Central Irregular Plains are genetically differentiated (i.e., significant isolation-by-distance and genetic neighborhoods in the autocorrelation analyses) enough gene flow occurs for migration to offset genetic drift across broad regional scales.

The inability to assign discrete subpopulations using Bayesian clustering methods for a species that shows limited dispersal is somewhat surprising, especially given the size of the study area (13,500 km<sup>2</sup>). In previous studies on snakes, genetic differentiation was found between populations of massasauga rattlesnakes (*Sistrurus c. catenatus*) found within 2 km (Gibbs et al. 1997), timber rattlesnakes within 2-8 km (Clark et al. 2008), and black ratsnakes (*Elaphe obsoleta*) within 6 km (Lougheed et al. 1999), all of which were attributed to limited dispersal ability. A shared characteristic of these snakes is that they exhibit high philopatry to communal hibernacula or are habitat specialists. For example, population subdivisions were also evident in water snakes (*Nerodia sipedon*) and garter snakes (*Thamnophis* spp.), which are strongly

associated with water sources that tend to be patchily distributed on the landscape (Prosser et al. 1999, Manier and Arnold 2005). A lack of genetic differentiation between subpopulations was recently found in the small-eyed snake (*Rhinoplocephalus nigrescens*), a habitat generalist. Thus, discrete subpopulations may only be found in snake species that are habitat specialists, exhibit strong philopatry, or are confined to patchily distributed habitat. Given that the racer is a habitat generalist, does not exhibit strong philopatry to summer home ranges or hibernacula, and can inhabit multiple summer home range or hibernacula in their lifetime (Fitch 1963), perhaps it is not surprising that discrete subpopulations were not evident, despite its limited dispersal range.

Given that yellowbelly racers are habitat generalists, the population genetic structure was not heavily influenced by landscape composition. Through a combination of isolation-by-distance theory and geospatial mapping, we were able to identify the dispersal range and landscape connectivity of yellowbelly racers. We found no natural (i.e., forest or bodies of water) or anthropogenic habitat barriers (i.e., row-crop agriculture and urban centers) that currently limit snake dispersal (i.e., gene flow). When considering the entire study area, all Mantel tests were significant for isolation-by-distance. We recognize that the correlation coefficients were generally low, but we expected low r-values because there is a great deal of inherent variability in our data given that our analyses were conducted at the individual level (Rousset 2000, Coulon et al. 2004). Studies that have used inter-individual distances as opposed to inter-population distances have all consistently reported low r-values that were often statistically significant (Sumner et al. 2001, Leblois et al. 2004). Although the resistance distances obtained from maps describing the landscape as a gradient of permeability (i.e., barrier and gradient maps; Table 2.2) had correlation coefficients that were slightly stronger than Euclidean distance, the partial Mantel tests were not significant. Therefore, it can be inferred that the landscape is functionally

connected, and dispersal (gene flow) is presumably not limited by landscape structure, in terms of either the current amount or distribution of habitat in the landscape.

The greatest challenge in landscape genetics is matching the temporal scale of the observed genetic patterns to the temporal scale of landscape modification (Balkenhol et al. 2009). Although, we are unable to identify the exact temporal scale at which the observed genetic patterns occurred, general land cover within the Flint Hills has changed relatively little during the past 50 years. For example, barriers such as urban centers (e.g., Manhattan, Kansas) have been well established for the past 50 years. The conversion of grasslands to row crop agriculture was mostly completed by the 1940's in the Midwestern United States (Waisanen and Bliss 2002), and large bodies of water such as the Tuttle Creek and Milford Lake Reservoirs were established and filled in the early 1960's. In general, the land cover types included in the models of potential landscape connectivity have changed little over the past 50 years, and we feel our results are robust to changes in land cover. A time frame of 50 years is also reasonable for the observed genetic patterns to emerge given that racers experience a generation time of about three years (i.e., males breed at 20 months and females at three years; Fitch 1963).

Although the general land cover categories (e.g., prairie) have likely changed little over the past 50 years, land-management practices within grasslands have experienced more recent changes. Currently, half of the Flint Hills is grassland and much of that is managed for cattle production (86%; With, unpubl. data). Traditional rangeland management such as season- or year-long grazing has been in practice since the late 1800's and still occupies 65% of the Flint Hills landscape, but more recent changes in land management have occurred since the 1980's (With et al. 2008). For example, the practice of annual burning coupled with early-intensive stocking (i.e., cattle stocked at twice the rate for half the season) now makes up 25% of managed

grasslands in the region and drastically reduces vegetation structure (With et al. 2008). In general, up to two-thirds of the Flint Hills grassland is burned annually every spring (With et al. 2008). This intensive use of fire and grazing has homogenized grassland habitats within the Flint Hills, producing reduced vegetation structure that may prove detrimental to many native species.

A reduction in vegetative cover could potentially expose dispersing yellowbelly racers to temperature extremes and higher predation risk (Wilgers and Horne 2007) during emergence from hibernacula after spring burns (Fitch 1963). In addition, yellowbelly racers are shown to have an affinity for increased vegetation structure (Klug et al., In revision), and thus may avoid grasslands managed with annual burns and early-intensive stocking of cattle. Although we can hypothesize that this land-management practice may negatively impact yellowbelly racer populations, even this type of intensive management may not act as a complete barrier to snake dispersal. These intensively managed grasslands are left to recover after cattle are removed in mid-season (mid-July; Smith and Owensby 1978). Previous studies on yellowbelly racers in the Flint Hills have shown that although racers avoid recently burned areas they are able to recolonize these areas later in the growing season (Cavitt 2000). In addition, female racers lay eggs in early August and hatching occurs in September (Fitch 1963), after the peak of the growing season when sufficient vegetation structure is present to facilitate juvenile dispersal to a hibernaculum. Although we ascertain that land management would not be a complete barrier to dispersal, land management is certainly capable of negatively influencing snake populations. Had we the resolution to include grassland management as a variable in our land-cover map, we may have been better able to deduce the impact of land management on gene flow in yellowbelly racers by evaluating population genetic structure under the current distribution of land



management and comparing it to historical land cover within the past 100 years to distinguish which best explains the observed genetic patterns (e.g., Zellmer and Knowles 2009).

At the other extreme, a lack of active land management (especially fire) may actually facilitate dispersal and gene flow for yellowbelly racers. Increased exurban development in this region curtails burning and grazing, which promotes shrub encroachment and the succession of grasslands to forests. Although racers have restricted dispersal, they nevertheless appear to have a near continuous population distribution across the region possibly due to the amount of native grassland in the landscape (e.g., ~52% native grassland in the Flint Hills ecoregion; With, unpubl. data). In addition, snakes are able to use other habitats such as forests to disperse and may actually prefer a mosaic of habitat types, which offer a variety of environments for thermoregulation (Wilgers and Horne 2006). Therefore, the increased woody encroachment in the Flint Hills (Briggs et al. 2005) and the mosaic of forest and grasslands in the Central Irregular Plains may benefit yellowbelly racer populations. In fact, the historical suppression of fire resulted in drastic expansion of gallery forests from 1859-1939 (Abrams 1986) and continues today (Briggs et al. 2005). As reliable survey data on snakes does not predate 1859, it is difficult to surmise the population size of yellowbelly racers before the increase in woody vegetation. Although increased woody encroachment provides heterogeneity for thermoregulation or protection from predators, this type of landscape change will only benefit snakes until some threshold at which grasslands have converted to forests and grassland species, such as the yellowbelly racer, are excluded (Fitch 2006). Although it appears from our landscape genetic analyses that no habitat currently poses a barrier to dispersal (i.e., gene flow) for racers, this does not mean that they would be able to withstand increased levels of habitat fragmentation. Dispersal success is affected by landscape pattern when the scale of movement for an organism

is less than the scale of fragmentation (With and King 1999). Currently, gallery forests do not restrict dispersal in yellowbelly racers, but if the intensity and scale of forest cover increases beyond the scale of a racer's ability to disperse, large expansive forests could turn into a barrier to gene flow. The same could be said for other land cover types that are not preferred habitat of racers (i.e., row crop agriculture and urban centers). Future research should evaluate the influence of shifts in grazing regimes, increased woody cover, exurban expansion, and increased fragmentation on gene flow to deduce how land modification may alter the connectivity and population genetic structure of native species like the yellowbelly racer.

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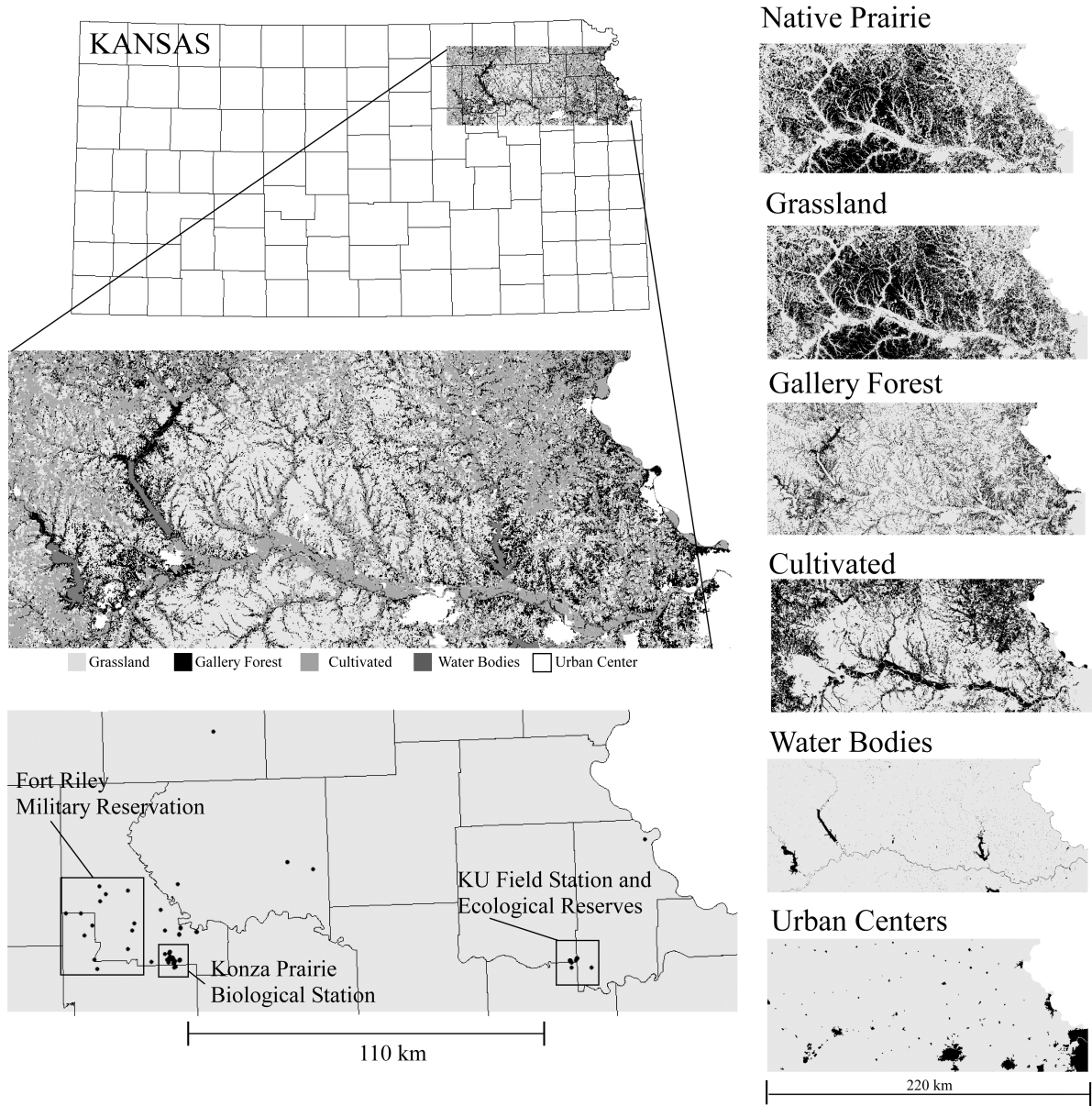
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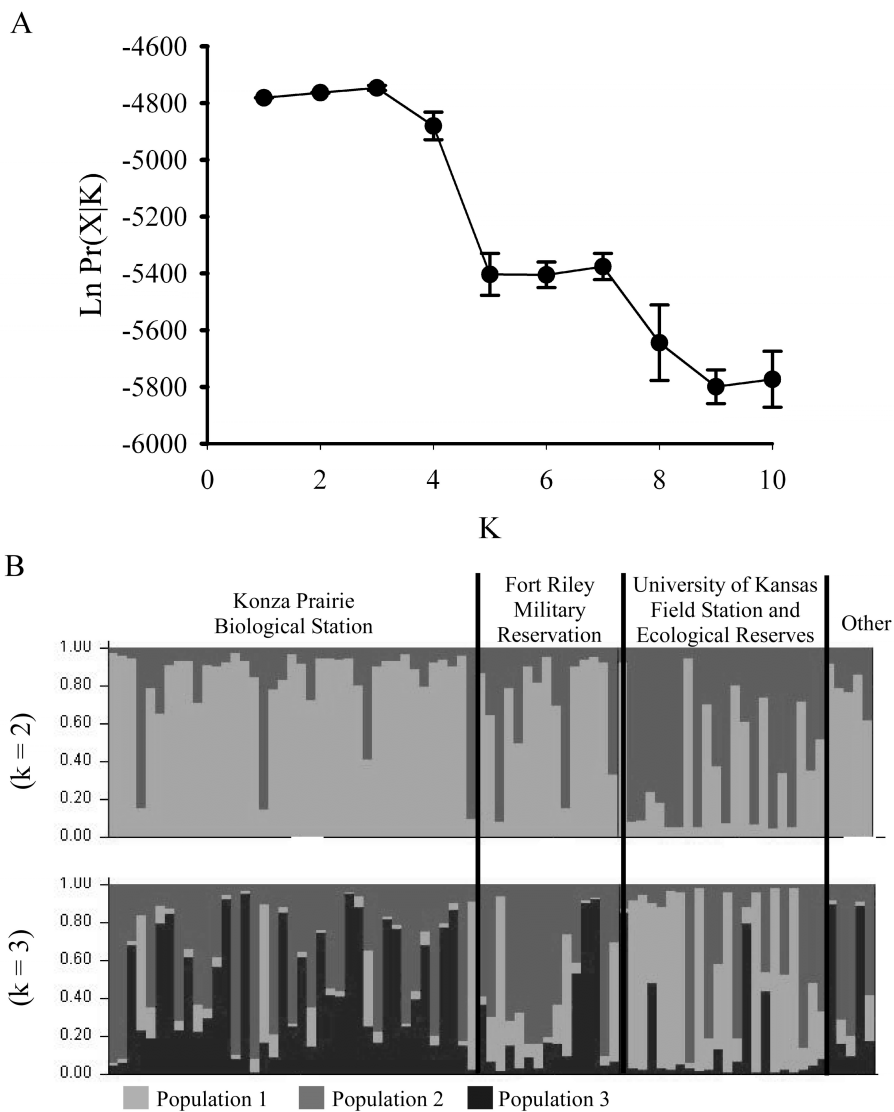
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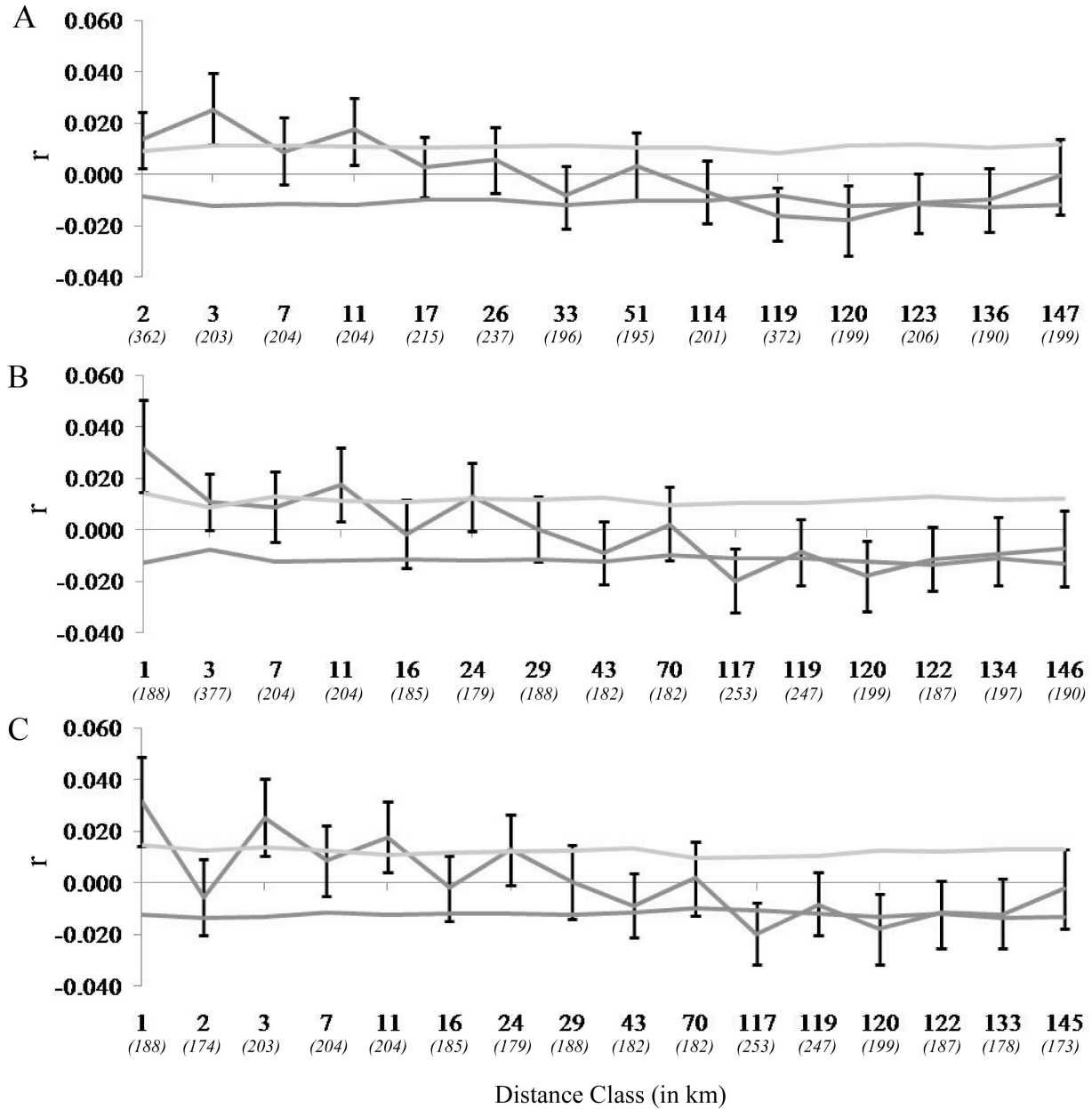
**Figure 2.1 Site map of the study region showing land-cover types from the Kansas GAP project that could potentially impact the connectivity of the landscape for eastern yellowbelly racers (*Coluber constrictor flaviventris*) The outlines represent the counties in which sampling occurred: Riley/Geary (n = 54), Pottawatomie (n = 3), Marshall (n= 1), Leavenworth (n = 1), Douglas/Jefferson (n = 22). For each of the six habitat types illustrated, no habitat = gray and habitat = black.**



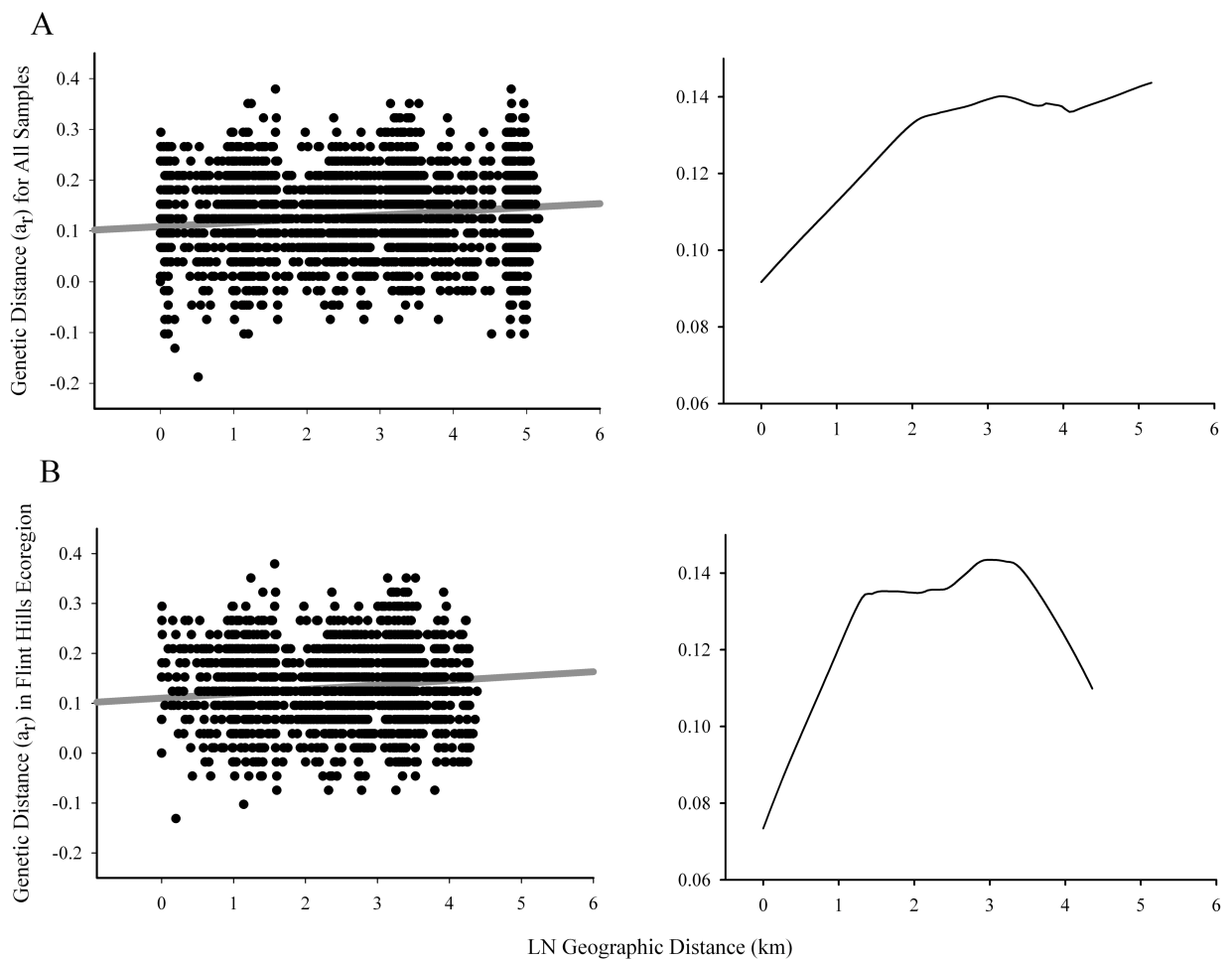
**Figure 2.2 A) Plot of the likelihood of each value of  $\ln\text{Pr}(X|K)$  for the number of subpopulations in the eastern yellowbelly racer samples. In STRUCTURE we used the average of 5 iterations for  $K = 1-10$ .  $\ln\text{Pr}(X|K)$  was maximized when the value of  $K = 1-3$  (i.e., asymptote of the curve). As the value of  $\ln\text{Pr}(X|K)$  remains constant with increasing variance between independent runs we would select the  $K$  with the least amount of variation but the largest  $\ln\text{Pr}(X|K)$  which was  $K = 1$ . B) The plots of ancestry estimates for  $K = 2$  and  $K = 3$ , which represents the estimated membership for each racer in each of the  $K$  inferred clusters. The actual location where the tissue sample was collected is also listed.**



**Figure 2.3 Correlograms of the correlation coefficient ( $r$ ) for genetic distance as a function of geographic distance for yellowbelly racers in northeastern Kansas. The permuted 95% confidence intervals (upper = dark gray; lower = light gray) and bootstrapped 95% error bars (black) are illustrated. The number of pairwise comparisons within each distance class is listed below each distance class in parentheses and italics.**



**Figure 2.4** Patterns of isolation-by-distance for yellowbelly racers in the tallgrass prairie of Kansas A) with all snake samples ( $n = 81$ ) and B) for the Flint Hills ecoregion only ( $n = 58$ ). Pairwise genetic differentiation ( $a_r$ ) between individuals are plotted against logarithm of distance (in km). All estimates for pairs of individuals are shown, as well as the linear regression line computed from these estimates. The local polynomial regression line is shown to identify trends in noisy data. We used the lowess smoothing method with 0.6 sampling proportion and a one-degree polynomial.



**Table 2.1 Microsatellite loci in eastern yellowbelly racers (*Coluber constrictor flaviventris*) with allele number and expected and observed heterozygosity for all samples combined (Total), and for the Flint Hills ecoregion (Riley and Geary Counties) and Central Irregular Plains ecoregion (Douglas and Jefferson Counties) of northeastern Kansas.**

GenBank Accession #	Allele No.	Total H <sub>E</sub> /H <sub>O</sub> (n= 81)	Flint Hills Ecoregion H <sub>E</sub> /H <sub>O</sub> (n= 54)	Central Irregular Plains Ecoregion H <sub>E</sub> /H <sub>O</sub> (n= 22)
GQ371177	13	0.75/0.75	0.77/0.78	0.72/0.73
GQ371178	21	0.89/0.54*	0.86/0.59*	0.93/0.45*
GQ371179	20	0.87/0.81	0.88/0.83	0.84/0.73
GQ371180	21	0.92/0.86	0.92/0.85	0.91/0.95
GQ371181	14	0.90/0.86	0.87/0.81	0.89/0.95
GQ371182	15	0.90/0.72*	0.92/0.80	0.77/0.50
GQ371183	29	0.92/0.67*	0.93/0.57*	0.91/0.86
GQ371184	8	0.29/0.31	0.29/0.30	0.21/0.23
GQ371185	14	0.86/0.88	0.88/0.89	0.76/0.82
GQ371186	51	0.97/0.85	0.97/0.87	0.96/0.82
GQ371187	18	0.80/0.74	0.83/0.76	0.78/0.73
GQ371188	27	0.93/0.81	0.93/0.81*	0.91/0.77

\*locus deviated significantly from Hardy-Weinberg equilibrium

**Table 2.2 The resistance values for each land-cover type used to create friction grids for calculating resistance distances between individual yellowbelly racers. The resistance distances (RD) derived from these eight friction grids were then used to correlate geographic distance (Euclidean or RD) and genetic distance ( $a_r$ ).**

RESISTANCE MAP VALUES	
<u>NATIVE PRAIRIE:</u>	<u>GRASSLAND:</u>
Native prairie = 10	Native prairie = 10
CRP/Restored = 100	CRP/Restored = 10
Gallery forests = 100	Gallery forests = 100
Wetlands/marshes = 100	Wetlands/marshes = 10
Cultivated fields = 100	Cultivated fields = 100
Water bodies = 100	Water bodies = 100
Urban centers = 100	Urban centers = 100
<u>GALLERY FOREST:</u>	<u>CULTIVATED:</u>
Native prairie = 100	Native prairie = 100
CRP/Restored = 100	CRP/Restored = 100
Gallery forests = 10	Gallery forests = 100
Wetlands/marshes = 100	Wetlands/marshes = 100
Cultivated fields = 100	Cultivated fields = 10
Water bodies = 100	Water bodies = 100
Urban centers = 100	Urban centers = 100
<u>WATER BODIES:</u>	<u>GRADIENT:</u>
Native prairie = 100	Native prairie = 10
CRP/Restored = 100	CRP/Restored = 10
Gallery forests = 100	Gallery forests = 10
Wetlands/marshes = 100	Wetlands/marshes = 10
Cultivated fields = 100	Cultivated fields = 50
Water bodies = 10	Water bodies = 100
Urban = 100	Urban = 100
<u>URBAN:</u>	<u>BARRIER:</u>
Native prairie = 100	Native prairie = 10
CRP/Restored = 100	CRP/Restored = 10
Gallery forests = 100	Gallery forests = 10
Wetlands/marshes = 100	Wetlands/marshes = 10
Cultivated fields = 100	Cultivated fields = 50
Water bodies = 100	Water bodies = barrier
Urban centers = 10	Urban centers = barrier

**Table 2.3 Correlation coefficient and corresponding significance for Mantel test and partial Mantel test comparing the relationship between genetic and geographic distance for yellowbelly racers assuming either the Euclidean model or one of the eight resistance distance models (cf. Table 2.2). A) all 81 samples used and B) only samples from the Flint Hills ecoregion.**

A ALL SAMPLES	Mantel Test		Partial Mantel Test	
	r	P-value	r	P-value
Euclidean	0.075	0.006	.	.
Barrier	0.084	0.013	0.041	0.139
Gradient	0.084	0.009	0.040	0.127
Prairie	0.076	0.014	0.029	0.212
Forest	0.073	0.002	0.026	0.149
Urban	0.070	0.002	0.022	0.161
Grassland	0.071	0.033	0.021	0.305
Water	0.069	0.001	0.021	0.183
Cultivated	0.059	0.010	0.013	0.302

B RILEY/GEARY	Mantel Test		Partial Mantel Test	
	r	P-value	r	P-value
Euclidean	0.042	0.286	.	.
Gradient	0.051	0.198	0.030	0.326
Prairie	0.046	0.246	0.022	0.369
Barrier	0.038	0.272	0.016	0.422
Grassland	0.039	0.301	0.011	0.412
Forest	-0.002	0.530	-0.031	0.763
Water	-0.003	0.517	-0.040	0.828
Urban	-0.006	0.555	-0.042	0.840
Cultivated	-0.016	0.615	-0.047	0.875

# **CHAPTER 3- Ecology, Movement, and Space Use of the Eastern Yellowbelly Racer and the Great Plains Ratsnake in the Tallgrass Prairie**

## **Abstract**

The Flint Hills of Kansas and Oklahoma encompass the largest, contiguous tallgrass prairie in North America and are an important conservation area for tallgrass prairie species. We conducted a radio-telemetry study to address the landscape ecology of two snake species within this contiguous grassland while qualitatively examining the influence of grazing and burning. We monitored 12 eastern yellowbelly racers (*Coluber constrictor flaviventris*) and 12 Great Plains ratsnakes (*Pantherophis emoryi*) at Konza Prairie Biological Station. Although racers (mean = 66.7 m, SE = 10.7) and ratsnakes (mean = 40.0 m, SE = 4.8) differed in the distance moved per day, they did not differ significantly in home range size. The minimum convex polygon (MCP) home range averaged 11.45 ha (SE = 3.06) for racers and was significantly different between males (mean = 21.6 ha, SE = 6.4) and females (mean = 6.4 ha, SE = 1.5). For ratsnakes, MCP averaged 15.06 ha (SE = 2.48) and was not significantly different between males (mean = 15.2 ha, SE = 3.2) and females (mean = 14.5 ha, SE = 3.9). By analyzing the autocorrelation of locations (Mantel correlograms), we found racers had irregular movements whereas ratsnakes exhibited periods of inactivity between directed movements. The body temperature (BT) of racers was significantly greater in woody (BT = 30.7°C, SE = 0.4) compared to grassy habitat (BT = 29.8°C, SE = 0.3). Our study benefits the conservation of native snakes by elucidating space requirements and provides a baseline for comparisons between contiguous and fragmented landscapes.



## Introduction

The spatial distribution and abundance of resources such as food, mates, and shelter are important factors regulating an organism's use of space (i.e., home range size, distance of movement, and frequency of movement; Gregory et al., 1987; Brown and Weatherhead, 1999; Roe et al., 2004). Space use by snakes is affected by habitat heterogeneity, which may afford a greater abundance of prey (Anderson et al., 2003), a greater range of thermoregulation sites (King and Duvall, 1990), and refugia from predators (Wilgers and Horne, 2007). Within species, space use has also been shown to differ depending on sex or reproductive condition (i.e., male, non-gravid female, or gravid female; Fitzgerald et al., 2002; Whitaker and Shine, 2002) and whether snakes are searching for mates, food, or cover, which varies by season (Waldron et al., 2006). Although movements in snakes are influenced by the need to mate, forage, and avoid predators, maintenance of an optimal body temperature also has a profound influence on their behavior (Huey, 1991). The body temperature of snakes is directly linked to environmental temperatures and greatly influences movement ability (i.e., daily and seasonal movement patterns), and thus ultimately contributes to foraging success and overall fitness (Greenwald, 1974; Whitaker and Shine, 2002; Blouin-Demers et al., 2003). All of these factors influence the movements of individual snakes and ultimately scale-up to influence population distributions and ecological interactions such as those between snakes and their prey (Roe et al., 2004).

Recent research has highlighted the importance of snakes as predators of bird nests (Weatherhead and Blouin-Demers, 2004). Thus, conservation efforts aimed at decreasing predation on bird nests will benefit from a study of snake activity patterns and space use (Sperry et al., 2008). Although there is a strong interest in understanding snakes in light of declining bird populations, snakes as a whole have shown regional and global declines and may be species of conservation concern in their own right (Gibbons et al., 2000; Cagle, 2008). As snakes are

difficult to monitor, long-term trends for snake populations are not available and even the snakes that appear common today may be experiencing declines (Brown et al., 2008). Therefore, any baseline ecological information on snake habitat use and home range requirements will benefit snake conservation by informing managers of the vulnerability of snakes to habitat fragmentation and management (Blouin-Demers and Weatherhead, 2001b), effectiveness of relocation to restored habitats (Webb and Shine, 1997) and the importance of reserve size, shape, and location (Fitzgerald et al., 2002).

Although much emphasis has been put on understanding snakes for their intrinsic values as well as their role as avian nest predators, the spatial ecology of snakes is still poorly understood (Diffendorfer et al., 2005; Carfagno and Weatherhead, 2008) especially for snakes that are not of immediate conservation concern. Understanding the spatial ecology of a generalist and relatively common snake will address the conservation goal of keeping common species common (Scott et al., 1993), while simultaneously contributing to understanding the impact of snakes on prey species of conservation concern (Weatherhead and Blouin-Demers, 2004).

We have chosen to study the eastern yellowbelly racer (*Coluber constrictor flaviventris*) and the Great Plains ratsnake (*Pantherophis emoryi*), hereafter referred to as racer and ratsnake. In the Flint Hills of Kansas, a decline in grassland snakes is hypothesized due to habitat degradation through intensive grazing and fire which denudes vegetation structure (Szaro et al., 1985; Cavitt, 2000a; Beever and Brussard, 2004) or through the lack of both which allows successional conversion to forests (Fitch, 2006; Wilgers et al., 2006). Areas within the Flint Hills, such as the Fort Riley Military Reservation that have maintained native vegetation through moderate use of grazing and burning, have not shown drastic changes in herpetological assemblages and many species have remained abundant on surveys conducted over the past 100

years (Busby and Parmelee, 1996). In other regions of the tallgrass prairie, the loss and fragmentation of grassland habitats by agriculture has resulted in drastic declines for snake populations (Cagle, 2008). Since extensive, long-term data do not exist to indicate if snake populations are currently stable, increasing or decreasing (Gibbons et al., 2000), effort should be made to better understand the spatial requirements of snakes that are not of immediate conservation concern.

Both racers and ratsnakes have wide ranges that encompass a variety of habitat types. Racers range from Montana to western North Dakota and from eastern Iowa to southern Texas (Conant and Collins, 1998). Ratsnakes range from southwestern Illinois to southeastern Colorado and from eastern New Mexico to northern Mexico (Conant and Collins, 1998). The ratsnake is mainly nocturnal during the warm summer months and uses rocky areas as daytime retreats (Fitch, 1999). The racer is strictly diurnal and is often found in tallgrass prairie and pastureland (Fitch, 1999). Both racers and ratsnakes have been documented to be important predators on birds of conservation concern through video-monitoring of nests (Thompson et al., 1999; Stake et al., 2004; Klug, 2005) and diets analyses (Fitch, 1999; Cavitt, 2000b).

We conducted a radio telemetry study to describe the landscape ecology of two snake species on the Konza Prairie Biological Station (KPBS), which is contained within the Flint Hills region of northeast Kansas. KPBS is divided into treatment units where tallgrass prairie habitat is managed with prescribed burning and grazing. By using the KBPS, we were able to address the spatial ecology of these two snake species in a contiguous grassland habitat while qualitatively examining the influence of grazing and burning on their ecology and space use. Our four main objectives were to (1) analyze the movement patterns and behavior of radio-tagged snakes; (2) analyze the autocorrelation of movement trajectories to better understand how locations of

individual snakes are correlated over time; (3) analyze the size and composition of home ranges for two tallgrass prairie snake species in the contiguous Flint Hills region; and (4) evaluate if body temperature of snakes varies with behavior and habitat by using temperature-sensitive radio-transmitters.

## **Methods**

### ***Study Sites***

The KPBS is a 3,487 ha National Science Foundation Long-Term Ecological Research site owned by the Nature Conservancy and operated by the Division of Biology at Kansas State University in the northern Flint Hills of Kansas (39°05'N and 96°35'W). Habitat at the study site consists of tallgrass prairie managed with or without native bison (*Bos bison*) and prescribed burning that occurs every 1-20 years. Our research was concentrated in eight watersheds subjected to the following treatments: four-year burn with no grazing (K4A, K4B), four-year burn with grazing (N4D, N4C), annual burn with no grazing (K1A, K1B), and annual burn with grazing (N1A, N1B), but we tagged and followed snakes opportunistically as encountered within a total of 16 watersheds of differing treatments across the KPBS. The dominant prairie grasses on KPBS include big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*). In addition to grasslands, the landscape contained gallery forests in the lowlands and shrub islands (groups of shrubs > 1 m in diameter) along limestone rock outcrops. Shrubs occurred within all watersheds but were more prevalent in areas that were not frequently burned (i.e., 4-20 year burn-interval). Shrub species found on the site include rough-leaved dogwood (*Cornus drummondii*), sumac species (*Rhus* spp.), prickly ash (*Zanthoxylum americanum*), and eastern red cedar (*Juniperus virginiana*).

### ***Snake Capture and Radio Tracking***

We captured snakes by setting out coverboards (60 x 180 cm plywood sheets), erecting drift fences equipped with funnel traps, and through opportunistic encounters (Parmelee and Fitch, 1995; Cavitt, 2000a). In January 2006, we set out 10 coverboards in each of the eight focal watersheds. In each watershed, one transect (250 m) consisting of five boards (> 50 m apart) was placed in the lowlands and another in the uplands. We constructed two drift fences in each of the four focal watersheds that were not grazed by bison. Drift fences consisted of 61-cm tall silt fences arranged in a Y-shape with three large funnel traps at each end and four small funnel traps placed in the middle of each 15-m arm. To capture snakes, we checked coverboards and drift fences daily prior to 1030 from April to June and overturned rocks along limestone outcrops from April to May. Racers and ratsnakes were the two most common snake species captured at KPBS (Klug et al., In revision).

We implanted radio-transmitters under the supervision of a veterinarian affiliated with the Kansas State University College of Veterinary Medicine following accepted procedures (Reinert and Cundall, 1982; Hardy and Greene, 1999). The temperature-sensitive radio-transmitters weighed 5 g and had a battery life of 6-12 months at 20 °C (model SI-2T, Holohil Systems Inc., Carp, Ontario, Canada). Transmitters were implanted into the body cavity of snakes with a mass >100 g (transmitters were thus <5% body mass). After successful implantation of the radio-transmitter, we released snakes at the location of capture within 48 h of surgery and tracked the snakes every 24-48 h from 28 May to 31 July and once every two weeks from 1 August to 30 November or until the snakes retreated to their hibernacula. Telemetry locations were taken between the hours of 0400 and 2400 using a radio-telemetry receiver (Challenger 2100, Advanced Telemetry Systems, Isanti, MN). We varied the time of day (i.e., alternating morning, afternoon, evening, and night) in which snakes were tracked to encompass

the potential periods of activity for each species (e.g., ratsnakes are primarily nocturnal and racers are strictly diurnal).

### ***Body temperature***

At each snake location, we recorded GPS coordinates, position (underground or aboveground), and behavior (moving or stationary). We calculated body temperature from correlation curves of temperature against the pulse interval designed for each transmitter. At each snake location, we used a stopwatch to measure the pulse interval by recording the time (nearest millisecond) between 11 pulses and dividing by ten. We used a handheld anemometer to record the ambient air temperature and wind speed within 20 min of locating each snake. We used an infrared thermometer (model OS530LE, Omega Engineering Inc., Stamford, CT) to record the shaded temperatures of four substrates including bare ground, litter, vegetation, and under rock within 50 m of the snake location.

We used a mixed-model analysis of variance (ANOVA) with the individual snake as a random factor to test for the effect of position (i.e., aboveground or underground), activity (i.e., moving or not moving), and habitat (i.e., grassy or woody) on body temperature. To analyze the relationship between snake body temperature and substrate temperatures, we conducted a multiple linear regression to understand which substrate temperature was best correlated with snake body temperature (Kapfer et al., 2008). All parametric statistics were performed in SAS 9.1.3 (SAS Institute, Cary, North Carolina).

### ***Movement Patterns***

We analyzed basic movement statistics to understand the differences in activity between the two snake species. We measured the total distance moved as the sum of all movements over the course of the season, the mean distance moved per day as the total distance moved divided by

the length of the monitoring period (i.e., number of days snake was observed), and the mean distance per move as the distance moved between successive locations divided by the number of days between locations. The length of the activity range was measured as the Euclidean distance between the two most divergent locations in the home range.

We computed the fractal dimension (D) of snake trajectories using the Fractal Mean Estimator in the Fractal program (Nams, 1996) to understand if snakes travel in a directed or tortuous manner (With, 1994a). We set the window range to 0.25 and the confidence interval to 95%. The Fractal Mean Estimator computed fractal dimension at multiple scales and gave a measure of variance to compensate for the non-independence of the log and path length measurement encountered in traditional fractal divider methods (Nams, 1996). Movements with lower fractal dimension could be interpreted as more directed movements with less searching whereas movements with higher fractal dimension indicate more intensive searching ( $D = 1$  indicates a straight line and  $D = 2$  indicates a tortuous line that completely fills a plane; With, 1994a). We computed the displacement ratio (DR) of the trajectory to understand foraging strategy (Phillips et al. 2004). The displacement ratio is a scale-independent measure of the distance from the start location to the end location ( $DR = 0$  when the start and end are the same location and  $DR = 1$  when the trajectory is a straight line; With, 1994b). Although the displacement ratio is related to the fractal dimension it is possible for two individuals to have the same displacement ratio but differing fractal dimensions. The displacement ratio is the net movement whereas fractal dimension is the shape of the trajectory.

We used the Mann-Whitney test to analyze differences in space use between species including total distance moved, distance moved per day, distance per move, maximum daily movement, activity range, fractal dimension, and displacement ratio. All non-parametric

statistics were performed in Minitab (Minitab, Inc. Pennsylvania, USA).

Autocorrelation has been considered both a predicament to be overcome in animal movement analyses and a useful ecological parameter describing the scale-dependency of animal movement patterns (Legendre, 1993; Cushman et al., 2005). Autocorrelation in radio tracking violates the assumption of independence necessary for most statistical tests and may cause bias in estimations of home range size, use of core areas, and habitat selection (Swihart and Slade, 1985). For example, autocorrelation of animal movements is a nuisance when too few locations are collected in too short of a time interval relative to the behavior of the organism, which results in an underestimation of home range size. We address the negative consequences of autocorrelation in the estimation of home ranges sizes (see *Home range size and composition* below) but in this section we focused on autocorrelation as a useful measure to describe the structure of movement pathways in snakes (Legendre and Fortin, 1989).

We analyzed the autocorrelation structure of the individual movement pathways of racers and ratsnakes to permit comparisons among individuals and species. For each individual, we used Mantel correlograms to compare the distance between locations in geographical space with distance between locations in time (Cushman et al., 2005). Mantel correlograms were created in Program R using modifications of the “ecodist” package (Goslee and Urban, 2007). For each snake, we calculated Mantel correlograms using the June to August monitoring period. Due to the structure of our radio tracking data, we used lag times of five days for racers and six days for ratsnakes. We compared patterns of autocorrelation within a species by computing pair-wise Pearson correlations between the Mantel  $r$  at each lag distance. If all individuals have similar Mantel correlograms (across all lag distances), the association will be high for the pair-wise Pearson correlation and will indicate that the relationship between time and geographical



distance is similar for all individuals. Due to different lag distances in racers and ratsnakes we were unable to compare correlograms between species using Pearson correlation coefficients.

### ***Home Range Size and Composition***

We estimated snake summer home ranges (referred to as home ranges hereafter) based on the minimum convex polygon (MCP) and fixed kernel density estimates (KDE) within the Animal Movement Extension of ArcView 3.3 (Hooge et al., 1999). We attained the minimum number of locations needed for calculating the home range of each individual by plotting the number of locations versus the area of the MCP home range. We computed the MCP home range starting with 10 locations and increased the number of locations by five up to the total number of locations for that individual. The number of locations needed was estimated by looking at the asymptote of the curve or where the area of the home range no longer increased.

We included MCP to allow comparison with other radio-tracking studies even though the MCP method only gives an outline of all locations and does not indicate areas within the home range with a higher density of locations. We thus used the KDE to construct 95% probability contours and 50% probability contours to highlight the core areas of use. The advantage of using a nonparametric statistical estimator such as KDE is that it incorporates the density of locations to give more weight to frequently used areas and can create home ranges of any shape. KDE requires the selection of a smoothing factor ( $h$ ), which is often obtained through arbitrary means such as least squares cross validation (LSCV) or selected based on biological significance. We report results from KDE-LSCV method because it is the most commonly used method and is often highly recommended (Seaman and Powell, 1996; Powell, 2000). Unfortunately, KDE-LSCV can result in a wide range of smoothing factor values because sample size and data structure influence the smoothing factor, and in turn, the size of the home range, thus

contributing to inaccuracies in the home-range estimates (Row and Blouin-Demers, 2006).

Therefore, in addition to the KDE-LSCV, we used the MCP (KDE-MCP) as the area of the home range and adjusted the smoothing factor until the area of the 95% probability contour equaled that of the MCP polygon (Row and Blouin-Demers, 2006). We used the KDE-MCP method when comparing the proportion of habitat within home ranges between species and sexes as it provides an objective method to selecting a smoothing factor (Row and Blouin-Demers, 2006).

We classified habitat found on KPBS and in snake home ranges as either grassy or woody through a remotely sensed Quickbird image (Geoeye <sup>TM</sup>) acquired on 13 August 2007. The Quickbird imagery had four multispectral bands at 4-m resolution and pan-sharpened to a 1-m accuracy. We used ENVI 4.3 software to run a supervised classification based on maximum likelihood to classify each pixel as grassy or woody habitat. We employed a KAPPA index to assess classification accuracy. Of the 245 pixels assessed, 97% were correctly classified with a KAPPA statistic of 0.81, which is sufficient for evaluation of land cover (Thomlinson et al., 1999). We assessed correct classification by visually analyzing the remotely sensed image to assign random pixels as grassy or woody habitat and comparing our results to the classified image. Woody cover was unlikely to change substantially within the three-year time period of this study, and therefore classification of a single image during this period is sufficient for capturing the distribution of woody cover across all years.

We compared habitats (grassy and woody) contained within the home ranges of snakes to the availability of those habitats across the study area. We defined the study area for each species by creating a MCP that included all of the individual snake home ranges (racers = 858 ha, ratsnakes = 1,239 ha). For each snake, we calculated the expected use as the proportion of each habitat type in the entire study area and observed use as the proportion of each habitat type

within the individual home ranges. We tested the hypothesis that the proportion of grassy and woody habitat used by snakes was in proportion to the availability of those habitats by using a compositional analysis (Aebischer et al., 1993). We took the log-ratios of the proportion of habitat used (i.e., home range) and the log-ratios of the proportion of available habitat (i.e., study site) for both racers and ratsnakes. We considered habitat use to be random if the log-ratios of available and used habitat were approximately equal.

We used the Wilcoxon signed-rank sum test to determine if the MCP home ranges were significantly different than the KDE-LSCV home ranges for each species. We used the Mann-Whitney test to analyze intraspecific differences in home range size and habitat composition (i.e., proportion of woody habitat within the KDE-MCP). All non-parametric statistics were performed in Minitab (Minitab, Inc. Pennsylvania, USA).

## **Results**

### ***Snake Capture and Radio Tracking***

Over three years, we captured 57 racers with snout to vent length (SVL) ranging from 22-81 cm (mean = 56.8 cm SE = 1.8) and a mass ranging from 14-256 g (mean = 80.4 g, SE = 7.2). Snakes were found between 30 April and 26 June under coverboards ( $n = 19$ ), in funnel traps/drift fences ( $n = 8$ ), sunning themselves on rocks ( $n = 8$ ), under rocks ( $n = 4$ ), dead on road ( $n = 8$ ), live on road ( $n = 7$ ), and active in the vegetation ( $n = 5$ ). Of the 57 racers, 13 (12 found alive) met the size criterion ( $>100$  g) for placement of a radio transmitter. We tagged a total of 12 racers, including eight females (mean SVL = 71.7 cm, SE = 1.9; mean mass = 160.3 g, SE = 15.8) and four males (mean SVL = 65.5 cm, SE = 0.5; mean mass = 113.0 g, SE = 2.8; Table 3.1). All racers ( $n = 12$ ) survived the surgery to implant transmitters. Tagged racers occurred in five watersheds consisting of three treatment types (Table 3.1).

We found 34 Great Plains Ratsnakes with snout to vent length (SVL) ranging from 32-105 cm (mean = 74.3 cm, SE = 3.7) and mass ranging from = 12-431 g (mean = 174.3 g, SE = 17.3). Across all three years, snakes were found between 7 May and 9 June under rocks ( $n = 21$ ), under coverboards ( $n = 9$ ), and live on the road ( $n = 5$ ). Of the 34 ratsnakes, 25 met the size criterion ( $>100$  g) for placement of a radio transmitter. We tagged a total of 16 ratsnakes, including four females (mean SVL = 90.7 cm, SE = 7.1; mean mass = 295.3 g, SE = 73.0) and 12 males (mean SVL = 89.3 cm, SE = 3.0; mean mass = 236.4 g, SE = 19.5; Table 3.1). One female ratsnake died during surgery most likely due to an overdose of Isoflurane. Three male ratsnakes were preyed upon before a sufficient number of locations could be acquired and were removed from the analysis. The remaining ( $n = 12$ ) ratsnakes (mean SVL = 88.5 cm, SE = 2.9; mean mass = 256.7 g, SE = 25.4) occurred in six watersheds consisting of four treatment types (Table 3.1).

### ***Body Temperature***

Summarizing across all observations, body temperature in racers ranged from 16.5 to 40.2 °C (mean = 29.81 °C, SE = 0.20). Ambient air temperature and litter temperature were significant predictors of racer body temperature, but the temperature of the vegetation, bare ground, and under rocks were not ( $F_{5, 375} = 133.1$ ,  $P < 0.0001$ ,  $R^2 = 0.67$ ). Although racers were found underground in 18% of observations, body temperature did not differ significantly with position above or below ground ( $F = 2.38$ ,  $P = 0.12$ ; Fig. 3.1A). Body temperature was significantly greater ( $F = 32.34$ ,  $P < 0.0001$ ) when moving (mean = 32.31 °C, SE = 0.70) compared to not moving (mean = 28.73 °C SE = 0.57; Fig. 3.1A). Body temperature was ~1.5 °C greater ( $F = 6.42$ ,  $P = 0.01$ ) when racers were located in woody habitat (mean = 30.59 °C, SE = 0.46) as opposed to grassy habitat (mean = 29.07 °C, SE = 0.44; Fig. 3.1A).

Summarizing across all observations, we found that the body temperature in ratsnakes ranged from 16.5 to 37.5 °C (mean = 26.40 °C, SE = 3.39). Ambient air temperature and the temperature under rocks were significant predictors of ratsnake body temperature, but temperature of the litter, vegetation, or bare ground was not ( $F_{5, 375} = 99.4$ ,  $P < 0.0001$ ,  $R^2 = 0.57$ ). Ratsnakes were found underground in 67% of the locations, and body temperatures were significantly greater when snakes were aboveground compared to underground ( $F = 5.16$ ,  $P = 0.02$ ; Fig. 3.1B). Body temperatures of ratsnakes were significantly greater ( $F = 4.30$ ,  $P = 0.04$ ) when moving (mean = 26.32 °C, SE = 0.27) compared to not moving (mean = 27.60 °C, SE = 0.63; Fig. 3.1B). Body temperature was not significantly different when ratsnakes were located in woody habitat than in grassy habitat ( $F = 0.23$ ,  $P = 0.63$ ; Fig. 3.1B).

### ***Movement Patterns***

We monitored racers between 29 May and 1 August 2006-2008. Individual snakes were tracked 32-59 days (mean = 49.8, SE = 2.7,  $n = 12$ ), with the number of locations per snake ranging from 18 to 43 (mean = 30.3, SE = 2.0) (Table 3.1). The distance per move for racers was skewed to short-range distances with 20% of relocations within 10 m of the previous location (Fig. 3.2A). Racers were observed on the move 28% of the time. On average, racers moved 67.4 m per move, for a total of nearly 3.5 km per season (Table 3.1). Their average activity range was 682.5 m with a maximum distance moved of 260.2 m. Male and female racers did not differ significantly in total distance moved ( $U = 43$ ,  $P = 0.11$ ), activity range ( $U = 44$ ,  $P = 0.20$ ), maximum distance per move ( $U = 50$ ,  $P = 0.80$ ), mean distance per move ( $U = 43$ ,  $P = 0.15$ ), or mean distance moved per day ( $U = 43$ ,  $P = 0.15$ ). Male and female racers also did not differ significantly in the fractal dimension ( $U = 60$ ,  $P = 0.20$ ) or displacement ratio ( $U = 56$ ,  $P = 0.55$ ) of their movement pathways.

We monitored ratsnakes for 47-63 days (mean = 58.4, SE = 1.3) between 29 May and 1 August, with the number of locations per snake ranging from 23 to 32 (mean = 29.6, SE = 0.7) (Table 3.1). The distance per move was skewed to short-range distances with 50% of relocations within 10 m of the previous location (Fig. 3.2B). Ratsnakes were on the move 8% of the time. On average, ratsnakes moved 37.5 m per move, for a total of nearly 2.4 km per season (Table 3.1). Their average activity range was 820.0 m with a maximum distance moved of 285.4 m (Table 3.1). Male and female ratsnakes did not differ significantly in total distance moved ( $U = 61, P = 0.71$ ), activity range ( $U = 56, P = 0.71$ ), maximum distance per move ( $U = 52, P = 0.23$ ), mean distance per move ( $U = 60, P = 0.85$ ), or mean distance moved per day ( $U = 61, P = 0.71$ ). Male and female ratsnakes also did not differ significantly in the fractal dimension ( $U = 60, P = 0.85$ ) or displacement ratio ( $U = 60, P = 0.93$ ) of their movement pathways.

As male and females did not differ for either racers or ratsnakes, we pooled sexes when comparing interspecific differences. Racers and ratsnakes did not differ significantly in the fractal dimension ( $U = 151, P = 0.98$ ) or displacement ratio ( $U = 146, P = 0.82$ ) of their movement pathways. Movement pathways of both species had fractal dimensions ranging from 1.09 to 1.42 (racer: mean = 1.219, SE = 0.022; ratsnake; mean = 1.215, SE = 0.026), indicating a slightly tortuous line. Racers and ratsnakes also did not differ significantly in the total distance moved ( $U = 167, P = 0.34$ ), activity range ( $U = 131, P = 0.29$ ), or maximum daily movement ( $U = 144, P = 0.75$ ). The average distance moved per day ( $U = 186, P = 0.04$ ) was 1.7x greater and the average daily movement ( $U = 188, P = 0.03$ ) was 1.8x greater for racers than ratsnakes perhaps a consequence of the greater observed activity of racers (i.e., ratsnakes were observed moving only 8% of the time vs. 28% of the time for racers therefore racers were 3.5x more active than ratsnakes).

Racer locations were no longer spatially autocorrelated at an average lag time 9.0 days (SE = 3.9;  $n = 10$ ; see Fig. 3.3A for examples). Weak similarities between the Mantel correlograms (i.e., average pair-wise Pearson's correlation = 0.36) indicates that individual variation exists in the movement patterns and individual racers do not exhibit the same behavior over space and time. The similarity was also weak when we examined sexes separately (average Pearson's correlation for males = 0.21, and females = 0.34). Racers appear to have a great deal of individual variation and random movements within the summer home range based on the shape of the Mantel correlograms (i.e., irregular fluctuations around a Mantel  $r = 0$ ; see Fig. 3.3A for an example).

Ratsnake locations were not autocorrelated at an average lag time of 14.5 days (SE = 4.8;  $n = 12$  (see Fig. 3.3B for examples). Moderate to strong similarities between the Mantel correlograms (i.e., average pair-wise Pearson's correlation = 0.62) indicates that individual ratsnakes are similar in their movement patterns and behavior across time and space. The similarity in the shape of the Mantel correlograms was also strong when we compared sexes (average Pearson's correlation: males = 0.62, females = 0.41). Ratsnakes appear to have long periods of inactivity between directed movements based on the shape of the Mantel correlograms (i.e., a gradual decrease from positive spatial autocorrelation to negative spatial autocorrelation as lag time increases; see Fig. 3.3B for an example).

### ***Home Range Size and Composition***

Racers appeared to reach the maximum MCP home range around 20 locations (Fig. 3.4A). Thus, all racers with  $> 20$  locations were included in the home range analyses, and had monitoring periods ranging from 45 to 93 days (mean = 75.8, SE = 4.6,  $n = 12$ ; Table 3.1). Data were analyzed by sex but not reproductive condition because we were uncertain about

reproductive status throughout the season, even though we initially palpated females to check for gravidity. Only one of the eight female racers was gravid upon initial palpation ( $U_{\text{♀}}$ ; Table 3.1). MCP home range sizes were estimated to be 1.2-33.5 ha (mean = 11.45, SE = 3.06,  $n = 12$ ) and KDE-LSCV home range sizes were estimated to be 1.4-69.4 ha (mean = 20.48, SE = 5.96,  $n = 12$ ; Table 3.1). Home range estimates were significantly larger for KDE-LSCV when compared to MCP (Wilcoxon matched-pairs test,  $W_{+} = 37$ ,  $P = 0.002$ ). Home range sizes for males (mean = 21.64, SE = 6.41,  $n = 4$ ) were 3.5x greater than females (mean = 6.36, SE = 1.46,  $n = 8$ ) and significantly different ( $U = 38$ ,  $P = 0.02$ ) when estimated with the MCP method but not with the KDE-LSCV method (male: mean = 29.37, SE = 8.02 ha and female: mean = 16.03, SE = 7.85 ha;  $U = 43$ ,  $P = 0.15$ ). The greater difference in MCP and KDE-LSCV estimates for females most likely is due to the density of locations especially concerning the one gravid female whose long-distance migration of 815 m (possibly to lay eggs) greatly increased the KDE-LSCV estimate (MCP = 13.2 ha and KDE-LSCV = 69.4; Table 3.1).

Woody habitat composed 1-39% of the 95% KDE-MCP home range for racers. For the 50% KDE-MCP home ranges, woody habitat composed 1-54%. The proportion of grassy and woody habitat in racer home ranges did not differ significantly from what was available in the landscape ( $t = 0.70$ ,  $P = 0.48$ ). The proportion of woody habitat within home ranges did not differ significantly between males and females ( $U = 62$ ,  $P = 0.11$ ).

Ratsnakes appeared to reach the maximum MCP home range around 20 locations (Fig. 3.4B). Thus, all ratsnakes with  $> 20$  locations were included in the home range analyses, and had monitoring periods ranging from 56 to 93 days (mean = 83.7, SE = 3.0,  $n = 12$ ; Table 3.1). Data were analyzed by sex but not reproductive condition because we were uncertain about reproductive status throughout the season, even though through initial palpation we did not find



any of the females to be gravid. The MCP home ranges were estimated to be 1.7-29.9 ha (mean = 15.06, SE = 2.48) whereas the KDE-LSCV home ranges were estimated to be 0.2-103.2 ha (mean = 29.33, SE = 9.17; Table 3.1). Home range estimates were significantly larger for KDE-LSCV when compared to MCP ( $W_+ = 37, P = 0.002$ ). Male home ranges (mean = 15.24, SE = 3.16,  $n = 9$ ) were not significantly different from female home ranges (mean = 14.52, SE = 3.85,  $n = 3$ ) when estimated as MCP ( $U = 58, P = 1.00$ ) or KDE-LSCV (female: mean = 28.52, SE 19.32,  $n = 3$ ; male: mean = 29.59, SE = 11.09  $n = 12$ ;  $U = 59, P = 1.00$ ).

For ratsnakes, woody habitat comprised 1-29% of the 95% KDE-MCP home range. For the 50% KDE-MCP home ranges, woody habitat comprised 0-74% of the area. The composition of grassy or woody habitat within home ranges was not significantly different from what was available in the landscape ( $t = 1.31, P = 0.22$ ). The proportion of woody habitat within home ranges was not significantly different between males and females when estimated as MCP ( $U = 60, P = 0.85$ ) or KDE-MCP ( $U = 61, P = 0.71$ ).

The MCP home range size was not significantly different between racers and ratsnakes (Mann-Whitney U test,  $U = 128, P = 0.21$ ). The proportion of woody habitat within racer and ratsnake home ranges did not differ for either the MCP ( $U = 160, P = 0.58$ ) or KDE-MCP home range estimates ( $U = 159, P = 0.64$ ).

## Discussion

An analysis of the movement behavior and space use of two grassland snake species has contributed a more detailed understanding of their spatial ecology, which is important for understanding snake populations in the largest, contiguous tallgrass prairie remaining in North America. The main difference in movement behavior between the racer and the ratsnake was daily distance moved and average distance per move (Table 3.1). Racers had a higher mean

distance moved per day because they are most often found actively foraging aboveground and use their high body temperatures to chase down prey (Fitch, 1999). Ratsnakes have a combination of active foraging and sit-and-wait behavior and are often found while taking refuge under large rocks or underground (Fitch, 1999). Our observed differences in the average daily movement and distance moved between racers and ratsnakes were likely conservative. Active snakes (e.g., racers) were more likely to have moved further between re-sightings than what was actually recorded but inactive snakes (e.g., ratsnakes) were less likely to have exhibited movements that were not captured as they likely did not move between subsequent relocations.

Movement pathways may reveal how organisms are interacting with heterogeneity by providing a spatial record of their response to habitat features on the landscape, and the fractal dimension of movement pathways has been proposed as a scale-independent measure to characterize species' perceptions of landscape structure (With 1994a). Both racers and ratsnakes had fractal dimensions that indicated a slightly tortuous pathway ( $D = 1$  directed straight line  $D = 2$  complex search path). The movement patterns may indicate how species are interacting with heterogeneity, and the apparent linear pathways of these two species would suggest that racers and ratsnakes may have been directing their movement to locate patchily distributed resources such as thermoregulation sites (e.g. shrubs or rock outcrops). In this study, we located individuals every 48 h during the summer season (i.e., June-August). While this was adequate for characterizing the general movement behavior, space use and home range sizes of these two snakes, more intensive tracking (i.e., every hour) might have uncovered more detailed information on daily foraging behavior, perhaps revealing different (finer) scales of response to habitat heterogeneity, especially in the active racer. For example, we may have seen a switch in spatial domains where snakes begin to exhibit an area-restricted search behavior and would

therefore have a higher fractal dimension (i.e., more random or plane-filling movement). The switch in spatial domain could possibly be more evident in racers as they are active foragers whereas ratsnakes also use sit-and-wait strategies and thus should maintain less complex movement paths of low fractal dimension. As with any study, there is a tradeoff between the frequency of sampling and the duration of study or number of individuals that can be studied. More intensive tracking would thus have limited the number of snakes we could have feasibly studied over the season, which would have compromised our other study objectives. Future studies on the movement behavior of snakes would thus benefit from an automated radio-telemetry system where multiple individuals can be tracked simultaneously, allowing for more frequent positional fixes per individual (Crofoot et al., 2008; Weatherhead et al., in press).

Although the fractal dimension does not elucidate how snake movements differed between racers and ratsnakes, the movement trajectory and Mantel correlograms better illustrated differences in the respective active and sit-and-wait foraging strategies (Fig. 3.3). When compared to the fractal dimension, the autocorrelation analyses (i.e., Mantel correlograms and movement trajectories) are more sensitive to periods of inactivity, which is reflected in the structure of the correlograms. For example, 39% of all ratsnake locations were in the exact same spot as opposed to racers who were only found in the exact same position 9% of the time (Fig. 3.2). This behavior was reflected in the temporal autocorrelation of snake locations, where racers showed shorter periods and ratsnakes showed longer periods of limited movement. Locations were no longer autocorrelated (i.e., open circles) at an average of 9.0 days for racers and an average of 14.5 days for ratsnakes (see Fig. 3.3 for examples). Differences between the active foraging strategy of racers and the sit-and-wait strategy of ratsnakes were more evident when using analyses sensitive to locations being in the same exact location as previous. For example,

racers show interspecific differences with ratsnakes in the shape of the Mantel correlograms and movement trajectories (i.e., random movements vs. directed movements, respectively).

Individual variation in movement behavior within racers is more prevalent than within ratsnakes. This highlights the possible influence of sex and environment. For example, in one male racer (N♂), it was evident that movement distances decreased as the season progressed, possibly due to decreased mate searching occurring later in the season (Fig. 3.3A). In contrast, one female racer (J♀), inhabiting an annually burned watershed, showed an increase in movement distances as the season progressed, possibly due to increased vegetation biomass and therefore decreased predation risk throughout the growing season (Wilgers and Horne, 2007). On the contrary, movement trajectories for ratsnakes were all similar regardless of sex or environment. All ratsnakes showed extended periods of inactivity under refuge sites (i.e., under rocks) interspersed with longer directed movements before returning to the refuge site (Fig. 3.3B). Although we are not able to determine statistically the differences between sex and habitat treatment due to small sample sizes, we can provide anecdotal evidence for possible individual differences based on sex or habitat management in racers but not ratsnakes.

For racers, individual differences occurred at the local scale (i.e., within KPBS) and may explain why the home range size of racers also varies across habitat types and ecoregions. Our average estimate of racer MCP home range was 11.45 ha, which is similar to home range sizes reported for racers in South Carolina [mean = 12.2 ha; (Plummer and Congdon, 1994)] and Illinois [mean = 15.1 ha; (Carfagno and Weatherhead, 2008)], but greater than home ranges previously reported for racers in the Central Irregular Plains (e.g. Osage Cuestas) of Kansas [2.5 ha; (Fitch and Shirer, 1971)]. The previous studies on racers were conducted in old-field and forest habitats, including the study in Kansas, which was located in the Central Irregular Plains

ecoregion described by a mosaic of grasslands and forests. Our study occurred in the Flint Hills ecoregion, which contains limestone and shale hills with steep narrow valleys and a greater proportion of native tallgrass prairie than the Central Irregular Plains. The differences in ecoregions could explain the differences between the Kansas studies because racers prefer open grassland and might be constrained by the mosaic of forests and cropland in the Central Irregular Plains. The grasslands in the Central Irregular Plains are mainly old-fields, which are a preferred habitat of racers and may fulfill necessary habitat requirements within a smaller space. The differences could also be due to the fact that the previous study force-fed transmitters to the snakes, which resulted in shorter monitoring periods and may have altered behavior by simulating a feeding event.

In our study, male racers had significantly larger home ranges than females, but individual differences were also apparent in space use. The two largest home ranges (~3x larger than average) for racers belonged to males (N♂ and T♂) residing in areas subjected to four-year burns and bison grazing (Table 3.1). The other male racers (F♂ and G♂) had smaller home ranges closer to the average and resided in areas that were burned annually with or without grazing. The females all had small home ranges when compared to males, except a gravid female (U♀) whose larger home range was driven by a long-distance movement of 815 m in which we hypothesize she laid eggs. We expect that individual differences may have been due to environmental variability, such as the distribution of resources within treatments, because snakes observed for similar lengths of time and of the same sex and body mass exhibited drastically different trajectories (see Fig. 3.3 for examples).

For ratsnakes, movement patterns and space use did not show drastic differences between individuals, as we hypothesize the limiting resource for ratsnakes was refuge sites, which were

mainly large limestone outcrops present in all watershed treatments on KPBS. Our average estimate of ratsnake MCP home range was 14.52 ha for females and 15.24 ha for males, which was slightly higher than the MCP home range of 10.2 ha reported for male ratsnakes in Texas (Sperry and Taylor, 2008). The influence of sex on MCP home range was not important in ratsnakes, where the largest home ranges included five males (D♂, I♂, O♂, X♂, Y♂) and one female (H♀) residing in a variety of treatment types (Table 3.1). The smallest home range belonged to a male (R♂) who was often found under the remains of a dilapidated, concrete bridge. Previous studies on ratsnakes have also shown that they have a propensity for human-made structures such as concrete gully plugs (Sperry and Taylor, 2008), but in contrast to previous studies, we found spatial overlap between the home ranges of individual ratsnakes. In particular, two of the ratsnakes (R♂ and H♀) were repeatedly found together under a dilapidated bridge in a watershed that otherwise had minimal rock cover. We therefore hypothesize those individual differences that did occur in ratsnake home ranges may have been due to the availability of or preferences for refuge sites.

Although, habitat composition of snake home ranges did not differ significantly from what was available across the entire study site (racers = 12% woody; ratsnakes = 11% woody), previous work found that racers were located in woody habitat (i.e., 49%) more often than would be expected based on random locations (i.e., 25%; Klug et al. In revision). The different response to woody habitat is due to the different scales being assayed. The overall proportion of woody habitat in the home range was in proportion to what was available at the study site, but within home ranges, racers were located in woody habitat more than would be expected based on its availability at the home-range scale, which indicated a selection for woody vegetation. In the current study, we found that racers experienced higher body temperatures (+1.5°C) when located

in shrubs than when found in grass/forb habitat (Fig. 3.1A). Shrubs allow racers to get off the ground and above the grass canopy to bask in the sun while maintaining a degree of protection from predators. In one instance, we observed a racer elevating itself in a large shrub after consuming a meal of Dickcissel (*Spiza americana*) nestlings. This anecdote suggests that racers may use shrubs to increase body temperatures to aid in digestion, which has been shown in many other snake species (Regal, 1966; Peterson et al., 1993; Blouin-Demers and Weatherhead, 2001a; Blouin-Demers and Weatherhead, 2002).

Understanding the factors influencing movements of individual snakes and how these may ultimately scale up to influence population distributions are vital for the conservation of snake populations that are experiencing regional declines. Although we are uncertain if racers or ratsnakes are experiencing population declines, the results from studies on “common snakes” can be extrapolated to inform the conservation of snakes that are rare and known to be declining. The loss of grassland habitat along with its degradation and fragmentation threaten the sustainability of native snake populations throughout North America where < 4% of tallgrass prairie remains (Cagle 2008). The largest contiguous tallgrass prairie remaining is located in the Flint Hills of Kansas and Oklahoma (Knapp & Seastedt 1998); therefore, our study also provides a baseline for future comparisons between contiguous and fragmented tallgrass prairie landscapes.

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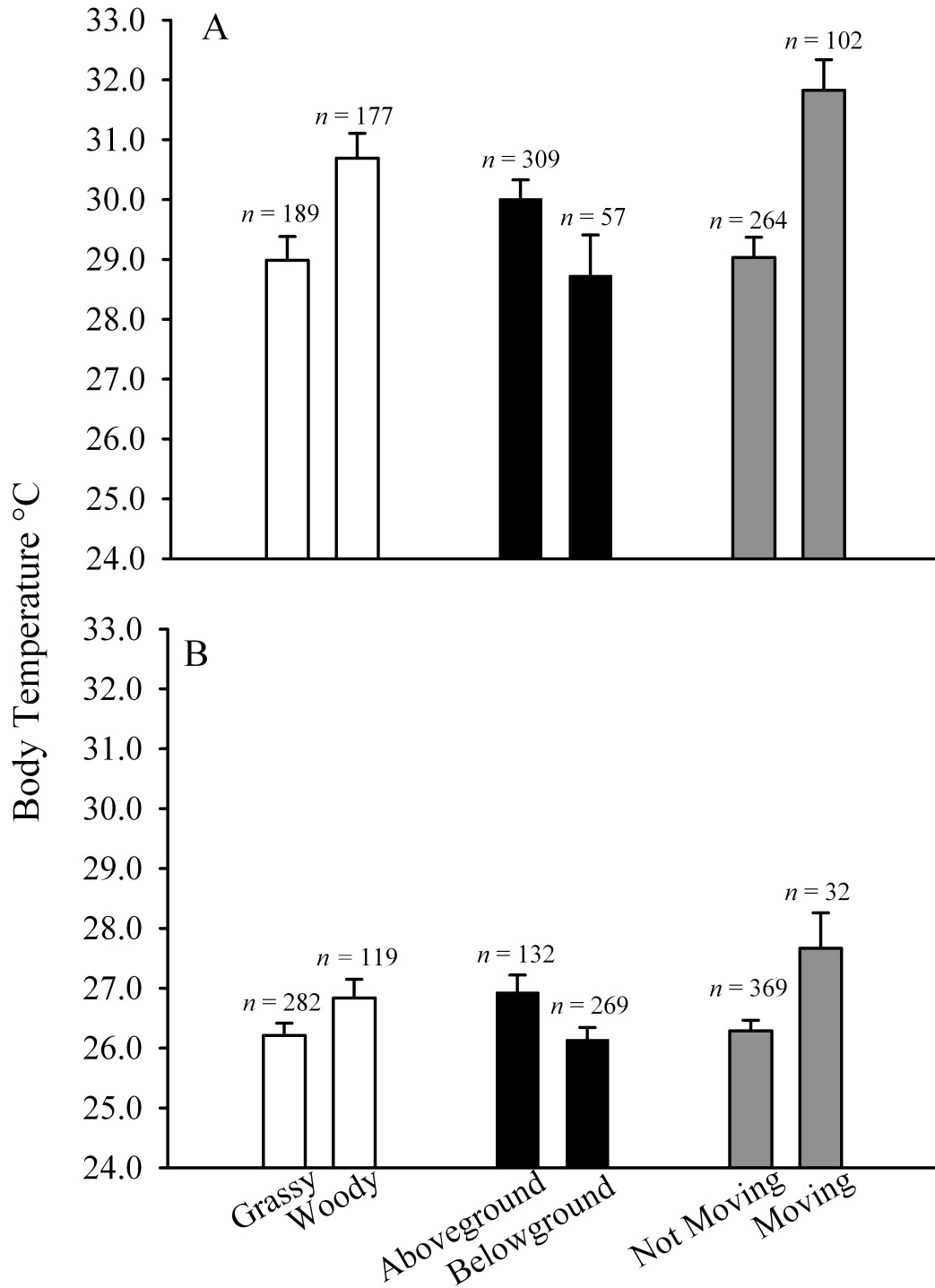
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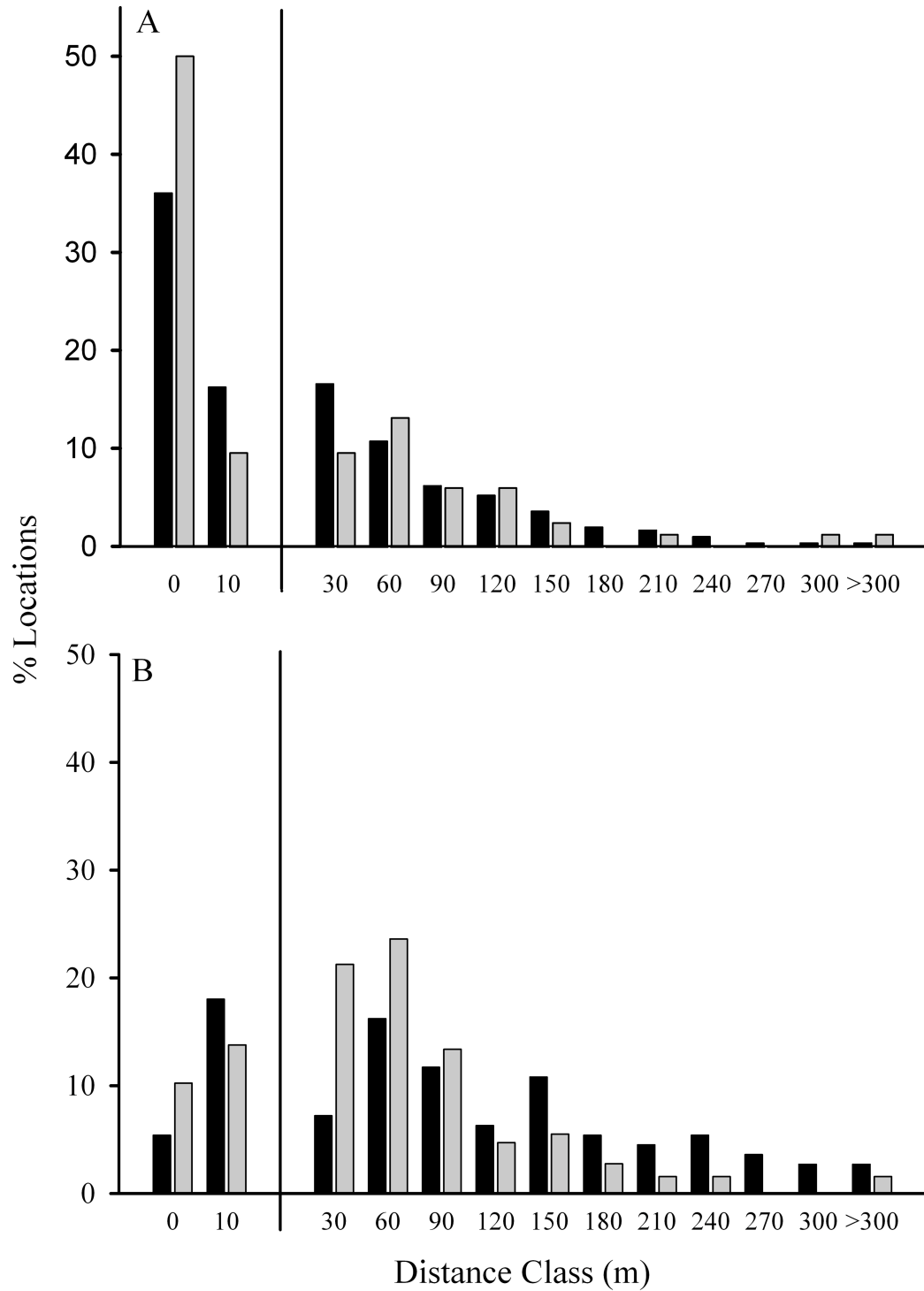
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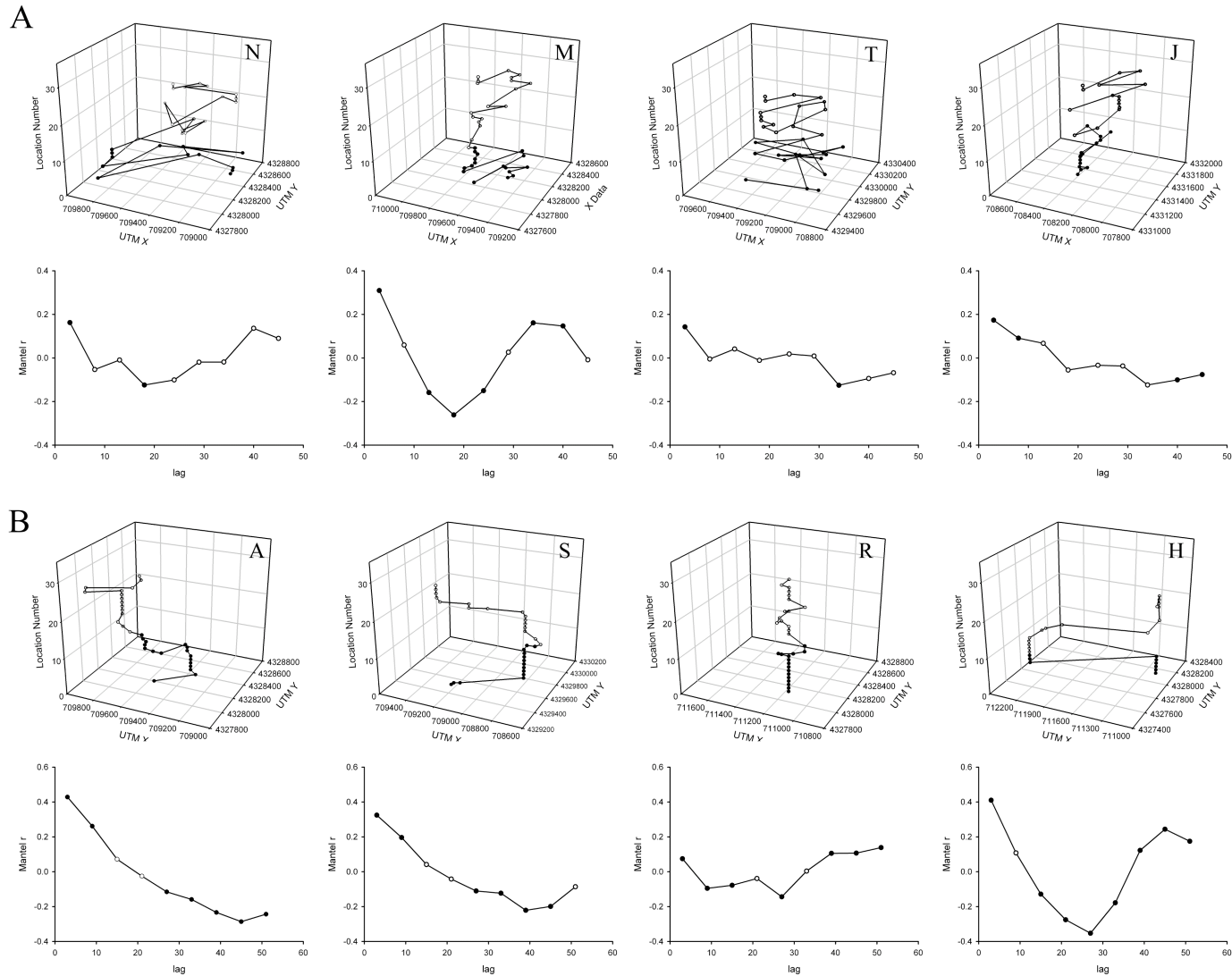
**Figure 3.1** Body temperature of a) racers and b) ratsnakes in different habitats (white bars), positions (black bars), and activities (gray bars). Sample sizes are the number of snake locations including all tagged racers ( $n = 12$ ) and ratsnakes ( $n = 12$ ).



**Figure 3.2** Frequency of movements in each distance class for a) ratsnakes and b) racers (female = black shading and males = gray shading). The vertical line represents a break where distance classes are composed of 30 m as opposed to 0-10 m increments.

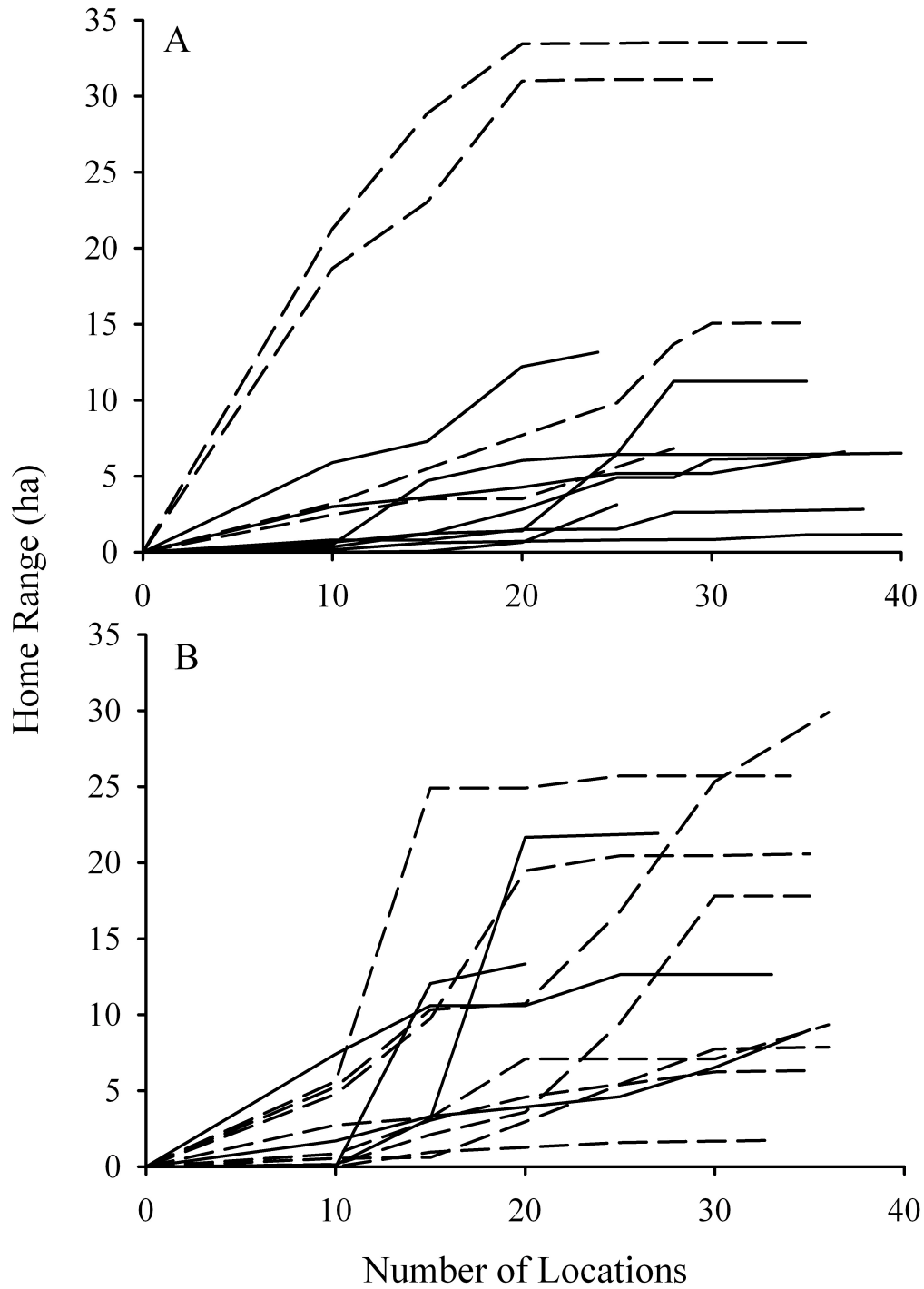


**Figure 3.3 Examples of Mantel correlograms and movement trajectories for a) racers (N-♂, M-♀, T-♂, J-♀) and b) ratsnakes (A-♂, S-♀, R-♂, H-♀).**





**Figure 3.4** The number of locations versus the size of the home range for a) racers ( $n = 12$ ) and b) ratsnakes ( $n = 12$ ). The curve asymptotes between 25 and 30 for most racers and ratsnakes (females = solid line and males = dashed line).



**Table 3.1 Movement and home range statistics for snakes monitored with radio-telemetry.**

Snake ID	Year	Trt. <sup>1</sup>	Mass (g)	SVL (cm)	Monitor. Period <sup>2</sup>	No. of Locations <sup>2</sup>	Total Dist. Moved (m)	Dist. Moved per Day (m)	Dist. per Move (m)	Max. Dist. per Move (m)	Activity Range (m)	Fractal Dimen.	Displac. Ratio	MCP (ha)	95% KDE-LSCV (ha)	50% KDE-LSCV (ha)
<b>Racer</b>																
F-♂	2007	G01-Y	111	65	47/80	23/28	1767	35	33	133	460	1.10	0.14	6.8	10.2	2.8
G-♂	2008	U01-Y	117	65	59/92	29/35	4622	78	79	401	830	1.21	0.04	15.1	24.6	4.3
N-♂	2008	G04-N	118	67	58/85	29/34	7983	138	135	390	1020	1.20	0.08	31.1	48.1	6.7
T-♂	2008	G04-N	106	65	58/93	29/35	7637	132	125	265	770	1.18	0.01	33.5	34.6	3.3
B-♀	2006	U01-Y	123	67	52/86	43/47	3483	66	71	195	610	1.31	0.09	6.5	10.7	1.6
E-♀	2006	G01-Y	140	72	32/45	24/25	956	30	28	138	570	1.19	0.37	3.1	6.3	0.9
J-♀	2008	U01-Y	256	81	59/84	30/35	4542	77	77	312	700	1.21	0.02	11.3	16.2	1.9
L-♀	2006	G04-N	154	67	51/57	39/40	1908	37	42	142	165	1.37	0.05	1.2	1.4	0.3
M-♀	2006	G04-N	192	76	46/79	35/37	3708	81	84	519	620	1.18	0.10	6.6	14.2	2.5
P-♀	2006	U01-Y	140	76	45/78	35/38	2043	45	46	141	300	1.24	0.02	2.8	2.1	0.2
Z-♀	2008	U01-Y	122	66	57/81	30/35	1666	28	28	80	355	1.30	0.07	6.2	7.9	1.0
U-♀	2008	G01-Y	155	69	34/50	18/24	1918	53	62	409	1790	1.14	0.17	13.2	69.4	11.1
<b>Mean</b>			<b>144.5</b>	<b>69.7</b>	<b>49.8/75.8</b>	<b>30.3/34.4</b>	<b>3519.4</b>	<b>66.7</b>	<b>67.4</b>	<b>260.2</b>	<b>682.5</b>	<b>1.219</b>	<b>0.097</b>	<b>11.45</b>	<b>20.48</b>	<b>3.06</b>
<b>SE</b>			<b>12.3</b>	<b>1.5</b>	<b>2.7/4.6</b>	<b>2.0/1.8</b>	<b>671.9</b>	<b>10.7</b>	<b>10.2</b>	<b>41.2</b>	<b>121.9</b>	<b>0.022</b>	<b>0.029</b>	<b>3.06</b>	<b>5.96</b>	<b>0.91</b>
<b>Ratsnake</b>																
A-♂	2007	G04-N	348	102	63/93	32/36	1794	25	29	244	710	1.21	0.05	7.9	15.9	3.8
C-♂	2008	U04-Y	235	86	58/84	30/35	1836	32	29	165	365	1.23	0.10	6.3	7.7	0.79
D-♂	2008	U01-Y	148	76	57/84	29/35	3580	63	54	213	870	1.21	0.07	20.6	29.1	5.66
I-♂	2008	U01-Y	219	86	59/76	31/35	3403	55	55	220	655	1.27	0.06	17.8	14.5	1.0
O-♂	2008	U04-Y	225	85	58/84	28/34	2721	47	40	203	820	1.25	0.17	25.7	22.7	3.7
Q-♂	2007	G04-N	226	84	61/93	31/36	1842	29	31	237	545	1.19	0.07	9.3	8.8	1.3
R-♂	2007	U01-Y	336	99	61/93	30/33	1118	18	17	78	255	1.42	0.00	1.7	0.2	0.1
X-♂	2007	G20-N	320	99	62/92	32/36	3882	62	55	650	1900	1.11	0.14	29.9	103.2	20.0
Y-♂	2007	G20-N	137	74	56/56	28/28	2246	34	36	136	1145	1.09	0.26	18.0	64.2	15.7
H-♀	2007	U01-Y	431	104	47/78	23/27	2977	62	49	424	1190	1.12	0.00	21.9	66.7	18.0
S-♀	2008	G04-N	274	88	58/87	30/35	1402	24	23	206	635	1.18	0.07	9.0	14.6	1.7
V-♀	2007	U01-Y	181	80	61/85	31/33	1926	31	33	650	750	1.30	0.31	12.6	4.3	0.7
<b>Mean</b>			<b>256.7</b>	<b>88.5</b>	<b>58.4/83.7</b>	<b>29.6/33.6</b>	<b>2393.9</b>	<b>40.0</b>	<b>37.5</b>	<b>285.4</b>	<b>820.0</b>	<b>1.215</b>	<b>0.108</b>	<b>15.06</b>	<b>29.33</b>	<b>6.04</b>
<b>SE</b>			<b>25.4</b>	<b>2.9</b>	<b>1.3/3.0</b>	<b>0.71/0.87</b>	<b>259.9</b>	<b>4.8</b>	<b>3.7</b>	<b>54.5</b>	<b>125.9</b>	<b>0.026</b>	<b>0.028</b>	<b>2.48</b>	<b>9.17</b>	<b>2.12</b>

<sup>1</sup> G = grazed, U = ungrazed, # = years between burn, Y = burned that year, N = not burned that year and <sup>2</sup> a/b: a is for movement statistics and b is for the home range statistics

## CHAPTER 4- Linking Snake Habitat Use to Nest Predation Risk in Grassland Birds: the Dangers of Shrub Cover

### Abstract

Extremes in rangeland management, varying from too-frequent fire and intensive grazing to the suppression of both, threaten rangeland ecosystems worldwide. Intensive fire and grazing denude and homogenize vegetation whereas their suppression increases woody cover. Although habitat loss is implicated in grassland bird declines, degradation through intensive management or neglect also decreases breeding habitat and may reduce nesting success through increased rates of nest predation. Snakes are important nest predators, but little is known about how habitat use in snakes relates to predation risk for grassland birds nesting within tallgrass prairie subjected to different grazing and fire frequencies. We evaluated nest survival in the context of habitat used by nesting songbirds and two bird-eating snakes, the eastern yellowbelly racer *Coluber constrictor flaviventris* and Great Plains ratsnake *Pantherophis emoryi*. Daily nest survival rates decreased with increasing shrub cover and decreasing vegetation height, which characterize grasslands that have been neglected or intensively managed, respectively. Discriminant function analysis revealed that snake habitats were characterized by higher shrub cover, whereas successful nests were more likely to occur in areas with tall grass and forbs but reduced shrub cover. Because snakes often use shrub habitat, birds nesting in areas with increased shrub cover may be at higher risk of nest predation by snakes in addition to other predators known to use shrub habitat (e.g., mid-sized carnivores and avian predators). Depredated nests also occurred outside the discriminant space of the snakes, indicating that other predators (e.g., ground squirrels *Spermophilus* spp. and bullsnakes *Pituophis catenifer*) may be

important in areas with denuded cover. Targeted removal of shrubs may increase nest success by minimizing the activity of nest predators attracted to shrub cover

## **Introduction**

At a global scale, temperate grasslands have experienced severe habitat loss, fragmentation, and degradation (White et al. 2000). In North America, the estimated loss of grassland ecosystems has exceeded 80% (Samson and Knopf 1994). Less than 4% of tallgrass prairie remains, most of which is located in the Flint Hills of Kansas and Oklahoma (Knapp and Seastedt 1998). As a result of such extensive habitat loss, grassland birds have suffered the most widespread decline of any North American bird group (Brennan and Kuvlesky 2005; Sauer et al. 2004). While the effect of habitat loss on grassland birds is clear, habitat degradation through the intensive management of remaining grasslands is an additional concern, given that the conversion of grasslands to agriculture was mostly completed by the 1940s in the Midwestern United States (Waisanen & Bliss 2002). Degradation of grasslands results from extremes in management ranging from intensive fire and grazing to the suppression of both (Briggs et al. 2005; With et al. 2008). For example, the majority of the Flint Hills is privately owned with management aimed at increasing livestock production, often to the detriment of native wildlife (Fuhlendorf and Engle 2001). In the Flint Hills, annual burns and early-intensive grazing were adopted in the 1980s and make up 25% of managed grasslands in the region (With et al. 2008). Intensive use of fire and grazing dramatically reduces vegetation cover and homogenizes the landscape, thereby reducing breeding habitat for grassland birds (Robbins et al. 2002; With et al. 2008). At the other extreme, neglected grasslands rapidly become shrublands or savannas (Archer et al. 1995; Briggs et al. 2002), again rendering sites unsuitable for grassland obligates (Grant et al. 2004). Current land management thus does not resemble the historical disturbance

regime where tallgrass prairie experienced two to three fires every five years followed by ungulates that would graze recently burned areas, producing a heterogeneous grassland mosaic (Collins and Gibson 1990).

Extremes in land management may increase the risk of nest predation by increasing the numerical or functional response of predators by altering habitat and nesting cover. For example, increased encounters between predators and nests may occur through increased abundance of predators or by changes in nest concealment that make nests easier to locate. Nest predation accounts for the majority of nest losses in many ecosystems (Martin 1993a; Ricklefs 1969), and in the Flint Hills >80% of all nest failures were attributed to predation (Rahmig et al. 2009; Sandercock et al. 2008). In particular, shrub cover may increase the abundance and activity of species known to depredate grassland bird nests, such as snakes (Blouin-Demers and Weatherhead 2001a). Snakes have been documented as responsible for up to 90% of predation events in California sage scrub (Morrison and Bolger 2002) and 38-72% of depredated nests in Missouri (Thompson et al. 1999; Thompson and Burhans 2003) and in Nebraska and Iowa (Klug 2005). Although snakes have been shown to be important predators of bird nests in many habitat types (Weatherhead and Blouin-Demers 2004), few studies have examined the habitat use of snakes in relation to nest-predation risk in birds (Sperry et al. 2009). Whether snakes actively search for nests or take nests through incidental encounters, nests in areas of high snake activity will be at greater risk of predation (Zimmerman 1984).

We compared habitat use of two snake species and five species of nesting songbirds within a landscape subjected to different fire and grazing treatments. The three main questions we addressed were: (1) Do snakes use areas of shrub cover within the tallgrass prairie disproportionate to the availability of shrubs on the landscape? (2) Is nest survival lower for

grassland birds nesting in or near shrub cover? and (3) What degree of habitat overlap exists between nesting grassland birds and their snake predators? Our aim is thus to achieve a better understanding of predation risk for grassland birds through an analysis of how snakes respond to habitat shaped by different management practices. Insights into snake habitat use can inform conservation efforts for grassland birds by gauging the plausibility of managing habitat features intensively used by predators.

## **Methods**

### ***Study Site***

Our research was conducted at Konza Prairie Biological Station (KPBS) (3,487 ha) in the northern Flint Hills of Kansas (39°05'N, 96°35'W). Data collection occurred during the avian breeding season from June to August in 2006, 2007, and 2008. The KPBS is an experimental landscape designed to address the effects of grazing and fire on tallgrass prairie. Data collection for both snakes and bird nests initially occurred in eight focal watersheds that were either burned annually or every four years, and were either grazed or not by bison (*Bos bison*). Radio-tracked snakes would occasionally move into other watersheds, however, and thus we ended up working in an additional eight watersheds, where we also encountered bird nests. In total, our study area covered 16 watersheds that encompassed a range of burn frequencies (1-20 years) and were either ungrazed or grazed by bison.

The KPBS exhibits a range of grassland habitats found in the Flint Hills including areas with shrub cover, areas dominated by C<sub>4</sub> prairie grasses, areas of increased forb density, and grazing lawns denuded of vegetation. Prairie grasses are found across the landscape with increased productivity in lowlands and areas that are not heavily grazed. The dominant grasses include big bluestem *Andropogon gerardii*, little bluestem *Schizachyrium scoparium*, Indian

grass *Sorghastrum nutans*, and switchgrass *Panicum virgatum*. Shrubs include rough-leaved dogwood *Cornus drummondii*, sumac *Rhus* spp., prickly ash *Zanthoxylum americanum*, and eastern red cedar *Juniperus virginiana*. Although burning and grazing influence vegetation structure, a variety of habitats can still be found within individual watersheds on KPBS. For example, shrubs occur in lowlands and along rock outcrops within all watersheds as a function of variation in soil moisture, but are more prevalent in areas that are infrequently burned. Thus, habitat heterogeneity occurs at scales finer than the watershed.

### ***Bird Nests***

We located nests of grassland birds by dragging a 30-m rope over the ground to flush incubating or brooding females, through behavioral observations of females, and via opportunistic encounters. Nest contents (number of eggs or young) were monitored every third day until the nest was successful or failed. We considered nests successful if they fledged at least one young, including young of the brood-parasitic brown-headed cowbird *Molothrus ater*, because our focus was on nest predation and not host productivity per se (i.e., it was unlikely to matter to the predator whether it consumed a cowbird or host chick). We considered nests successful if we observed parents alarm-calling and feeding fledglings nearby or found fecal droppings on the nest rim (where chicks perch prior to fledging). Only nests that were depredated were included in the analysis whereas nests that failed due to weather or abandonment were excluded ( $n = 14$ ).

Of the 156 nests that were depredated (70.3% of 222 total nests), 115 nests (73.7%) were found prematurely empty (before young could have fledged) but were otherwise completely intact. Although this evidence is consistent with snake predation, it does not rule out other

species of predators that also cause minimal disturbance to the nest (Pietz and Granfors 2000). For example, brown-headed cowbirds have been known to remove both eggs and nestlings from a nest without laying eggs (Klug 2005). Although brown-headed cowbirds are abundant on KPBS, previous research did not find cowbirds to be a major source of nest loss (Sandercock et al. 2008). Cricetid rodents are ubiquitous on KPBS but usually leave eggshell fragments or nestling carcasses behind as evidence (Pietz and Granfors 2000). Long-term data sets on the abundance of vertebrates on KPBS have also shown that ground squirrels are rare (KPBS-LTER, CSM04; <http://www.konza.ksu.edu/>) and therefore not likely to have a significant impact on avian reproductive success. Mid-sized carnivores such as raccoons also occur on KPBS, but at low densities and are mainly found in lowland gallery forests (Kaufman et al. 2005).

Because of the limitations of inferring the identity of predators from evidence left at the nest, we ran the analyses with all depredated nests regardless of predator type (Pietz and Granfors 2000). We present results from the analyses with all depredated nests to illustrate the full-range of habitat conditions characterizing depredated nests, thus enabling us to pinpoint the habitat domain of the two most abundant snakes versus those of other potential predators. Although a high degree of habitat overlap between snakes and depredated bird nests does not prove that snakes are the culprit, it does provide a strong degree of association and puts them at the scene, thus implicating them as likely nest predators.

### ***Snake Predators***

Snakes were captured using coverboards (60 x 180 cm plywood sheets), drift fences with funnel traps, and through opportunistic encounters (Cavitt 2000a; Parmelee and Fitch 1995). We have focused on the eastern yellowbelly racer *Coluber constrictor flaviventris* and the Great Plains ratsnake *Pantherophis emoryi* given their abundance on KPBS and known association



with open grassland habitats (Wilgers and Horne 2006). *Coluber constrictor* and *P. emoryi* made up 47% and 27% of the captures, respectively, representing 74% of the snakes we captured ( $n = 146$ ). Among the other snakes encountered, 10% were red-sided garter snakes *Thamnophis sirtalis*, 9% common kingsnakes *Lampropeltis getula*, 6% bullsnakes *Pituophis catenifer*, and 1% black ratsnakes *Elaphe obsoleta*. Although these snake species might also be nest predators, we did not find them in sufficient numbers for study, and thus assume their impact on grassland birds was minimal in comparison to *C. constrictor* and *P. emoryi*. *Coluber constrictor* has been documented depredating dickcissel *Spiza americana* nests on KPBS (Sandercock et al. 2008; pers. obs.), in old fields located in Missouri (Thompson et al. 1999; Winter 1999; Thompson and Burhans 2003) and restored grasslands in Nebraska and Iowa (Klug 2005). *Pantherophis emoryi* has been identified consuming golden-cheeked warbler *Dendroica chrysoparia* nests in the oak savannas of Fort Hood, Texas. In addition to video-documentation, the diets of *C. constrictor* and *P. emoryi* in Kansas (Fitch 1999) and on the KPBS (Cavitt 2000b) have been shown to include birds.

We assessed habitat use by *C. constrictor* and *P. emoryi* by radio-tracking individual snakes. We fitted adult snakes weighing at least 100 g with temperature-sensitive radio-transmitters (model SI-2T, Holohil Systems Inc., Carp, Ontario, Canada, 5.0 g, 6-12 month battery life at 20 °C). Radio-transmitters were implanted under the supervision of a veterinarian affiliated with the Kansas State University College of Veterinary Medicine, following standard procedures (Hardy and Greene 1999; Reinert and Cundall 1982). Other than one female *P. emoryi* that did not recover from surgery, implantation did not cause any major physiological or behavioral changes in the snakes. After implantation, we released snakes at the location of capture and tracked individuals every 24-48 h during the peak of the avian breeding season

(June-August). We used a portable, radio-telemetry receiver (Challenger 2100, Advanced Telemetry Systems, Isanti, MN) to locate snakes between 0400 and 2400 hours to allow locations to be taken in the coolest and warmest parts of the day to fully encompass the activity range of both a nocturnal (*P. emoryi*) and a diurnal (*C. constrictor*) snake. For each location, we recorded UTM coordinates with a handheld GPS, and flagged all locations for when we returned to measure local vegetation structure after the snake had moved (>100 m). We included locations that were used repeatedly by a single snake only once in the habitat overlap analysis.

### ***Habitat Categorization***

To evaluate the use of shrub and grass habitat by snakes, we categorized the area surrounding each location (within 4 m) as either predominantly grass or shrub. We also selected a random point within 200 m (i.e., maximum breadth of summer home range for both *C. constrictor* and *P. emoryi*; Klug et al. In review) of each snake location to measure habitat availability. We classified habitat at snake and random points as either predominantly grass or shrub based on visual observation in ArcMap 9.0 by referencing a remotely-sensed Quickbird image of the KPBS taken on 13 August 2007, which was pan-sharpened for a 1-m resolution. Shrub cover was unlikely to change substantially over the course of this study (i.e., 3-yr); thus, the single image was sufficient for capturing the presence of shrub cover. Habitat characterization was carried out to identify habitat use of snakes using compositional analysis (see Statistical Analyses).

### ***Habitat Structure***

To evaluate overlap between nesting songbirds and snakes, we measured habitat at bird nests ( $n = 222$ ), *C. constrictor* locations ( $n = 155$ ), and *P. emoryi* locations ( $n = 128$ ). At each location, we measured vegetation structure within 30 m of the nest or snake location by

averaging the readings at 10 sampling points. We chose a maximum distance of 30 m to represent the foraging neighborhood of snakes, based on the estimated daily distance moved by *C. constrictor* (Fitch and Shirer 1971). Heterogeneity of habitat structure within grasslands has been shown to influence nest predation risk for grassland birds (Winter 1999; Churchwell et al. 2008). Thus, our analysis was conducted at the local scale (within 30 m of snake and bird locations).

We took one reading directly at the nest or snake location and nine additional readings at 3, 15, and 30 m from the location. The three points taken at each distance were 120-degrees apart from the first randomly chosen direction. We surveyed habitat an average of 7 days ( $n = 222$ , range = 0-30 days, SE = 0.4) after nest completion and an average of 13 days ( $n = 283$ , range = 1-30 days, SE = 0.6) after the snake had moved (i.e., > 100 m from previous location).

We measured 12 structural variables at each point. Horizontal structure was measured with a Daubenmire frame (0.5 m X 0.5 m) to record percent vegetation cover including grass, forbs, and shrubs and to record percent ground cover including litter, bare ground, and rock. Vertical structure was assayed as both the height of the tallest vegetation within the Daubenmire frame, and by the visual obstruction reading of vegetation (i.e., biomass) in front of a 100-cm Robel pole placed perpendicular to the ground, with measurements taken in the four cardinal directions. We recorded litter depth as the average of four measurements taken at the corners of the Daubenmire frame. We used the coefficient of variation (CV) to capture heterogeneity in vegetative height, biomass, and litter depth. We tested for correlations among variables and dropped any variables that were highly correlated ( $r > 0.6$ ). We excluded biomass (mean and CV) as it was highly correlated with vegetation height (mean:  $r = 0.8$ ; CV:  $r = 0.6$ ). We excluded percent bare ground as it was negatively correlated with both percent litter ( $r = -0.9$ ) and litter

depth ( $r = -0.7$ ). We also excluded litter depth as it was positively correlated with percent litter ( $r = 0.7$ ). Habitat structure was quantified to evaluate the daily survival rate (DSR) of nests as well as the overlap between bird nests and snake locations using discriminant function analysis (see Statistical Analyses).

### ***Statistical Analyses***

Our first objective was to evaluate if snakes occurred in grass or shrub habitat disproportionate to the availability of these habitats on the landscape. For each snake ( $n = 12$  *C. constrictor* and  $n = 15$  *P. emoryi*), we calculated habitat use as the proportion of snake locations in grass versus shrub habitat, where habitat availability was the proportion of random locations in grass or shrub habitat. We used compositional analysis to compare habitat use to availability (Aebischer et al. 1993). We compared the log-ratios of the proportion of habitat used and the log-ratios of the proportion of available habitat in separate analyses for *C. constrictor* and *P. emoryi* in SAS (SAS Institute, Cary, North Carolina). We considered habitat use to be random if the log-ratios of available and used habitat were approximately equal. If habitat use was nonrandom, we ranked habitat by comparing the pair-wise differences between matching log-ratios.

To address our second objective as to which habitat variables best explained DSR, we used the review of the effects of management on grassland birds by Johnson et al. (2004) to identify habitat variables considered *a priori* to be important in explaining the reproductive success of grassland birds. We considered eight habitat variables using both the mean (%shrub, %grass, %forb, %litter, %rock, and vegetation height) and CV (vegetation height and litter depth) after excluding biomass, CV biomass, and litter depth due to correlations with other variables as mentioned previously. We estimated DSR with the design matrix tools and the logit-link function in the nest survival model of Program MARK (Dinsmore et al. 2002). We

calculated the variance in survival rates projected to a 20-day nesting cycle according to the delta method (Powell 2007). The encounter days were the number of days between the first and last day of nest monitoring. The variance inflation factor ( $\hat{c}$ ) cannot be identified in the nest survival model in Program MARK and therefore overdispersion could not be tested (Dinsmore et al. 2002). For model selection, we used Akaike Information Criterion adjusted for small sample sizes ( $AIC_c$ ) and evaluated model fit based on differences in deviance,  $AIC_c$  values ( $\Delta AIC_c$ ) and Akaike weights ( $w_i$ ) (Guthery et al. 2005). After running the global model (all eight variables), we evaluated the effect of each habitat variable by assessing if the slope coefficient ( $\beta$ ) had a 95% CI overlapping zero. We used the variables whose 95% CI for  $\beta$  did not overlap zero as a base for constructing models containing two habitat variables (13 models). We also included a constant model (i.e., nest survival alone), single-variables (8 models), species model, treatment model, and year model for a total of 26 models. We also compared survival rates between year, treatment, and species with Program CONTRAST (Hines and Sauer 1989).

To evaluate our third objective as to the degree of habitat overlap between nesting grassland birds and snakes, we performed a multivariate analysis to compare the habitats associated with *C. constrictor* locations, *P. emoryi* locations, successful bird nests, and depredated bird nests. We used SAS (SAS institute, Cary, North Carolina) to run a multivariate analysis of variance (MANOVA) to determine if any significant differences existed among the four groups followed by a discriminant function analysis (DFA) to identify the environmental variables contributing to differences among the groups. The DFA is a gradient analysis that allowed us to visualize the separation of depredated and successful nests in the context of snake habitat use.

Multivariate statistics come with assumptions that we considered in the analysis. Bartlett's modification of the likelihood ratio test indicated heterogeneity of the within-group covariance matrices ( $\chi^2 = 487.7, P < 0.0001$ ). Although the homogeneity of covariance assumption is rarely met with ecological data, multivariate analyses can still have descriptive value (Blouin-Demers and Weatherhead 2001b). Numerous variables exhibited heteroscedacity and were log-transformed to meet the assumption of normality. The conclusions from the log-transformed analysis were not different from the analysis with the original data; we therefore report only the analyses based on non-transformed data. Finally, repeated measures on individual snakes violate the assumption of data independence in analysis of variance, and radio-telemetry data are unlikely to represent a random sample. We could have taken the means of individual snakes to avoid potential bias, but this would have greatly reduced our sample sizes (i.e., 12 *C. constrictor* and 15 *P. emoryi*) and would not have captured the heterogeneity found within individuals, thus resulting in the loss of vital information about snake habitat use. The snakes with the most observations accounted for 11% and 10% of the total locations for *C. constrictor* and *P. emoryi*, respectively; thus, we view potential bias as minimal.

## Results

### *Snake Telemetry and Habitat Use*

We monitored 27 snakes over three field seasons. For *C. constrictor*, we tagged eight females (SVL: range = 66-81 cm, mean = 72 cm, SE = 1.9; MASS: range = 122-256 g, mean = 160 g, SE = 15.8) and four males (SVL: range = 65-67 cm, mean = 66 cm, SE = 1.0; MASS: range = 106-118 g, mean = 113 g, SE = 2.8). For *P. emoryi*, we tagged four females (SVL: range = 80-104 cm, mean = 91 cm, SE = 7.1; MASS: range = 181-431 g, mean = 295 g, SE = 72.9) and 12 males (SVL: range = 74-105 cm, mean = 89 cm, SE = 3.0; MASS: range = 137-348 g, mean =

236 g, SE = 19.5). We collected a total of 403 locations for *P. emoryi* and 374 locations for *C. constrictor*.

The use of habitats differed significantly from percent available for *C. constrictor* ( $t = -2.67$ ,  $P = 0.004$ ), which used shrub habitat more often than expected based on availability (Fig. 4.1). The proportion of *C. constrictor* locations found in shrub habitat averaged 46% ( $n = 12$ , SE = 0.09), which was significantly greater than 23% (SE = 0.05), the average proportion of random locations found in shrub habitat. For *P. emoryi*, the use of either habitat was not significantly different from percent available ( $t = 0.328$ ,  $P = 0.75$ ). The proportion of *P. emoryi* locations found in shrub habitat averaged 27% ( $n = 12$ , SE = 0.07), which was similar to 21% (SE = 0.04), the average proportion of random locations found in shrub habitat (Fig. 4.1).

### ***Avian Nesting Success***

Over three years, we found 222 nests of five species of grassland songbirds: dickcissel (DICK;  $n = 156$ ), grasshopper sparrow *Ammodramus savannarum* (GRSP;  $n = 38$ ), lark sparrow *Chondestes grammacus* (LASP;  $n = 19$ ), eastern meadowlark *Sturnella magna* (EAME;  $n = 7$ ), and field sparrow *Spizella pusilla* (FISP;  $n = 2$ ). Of the 222 nests, 66 fledged at least one young for an apparent nest survival rate of 29.7%. The DSR of nests was  $0.915 \pm 0.007$ , for an overall survival of  $16.8 \pm 2.0\%$  if we extrapolated a 20-day nesting cycle for all five species. We monitored 90 nests in 2006, 56 in 2007, and 76 in 2008. Variation in DSR could not be explained further by year (2006 = 0.906, SE = 0.0107; 2007 = 0.929, SE = 0.0118; 2008 = 0.914, SE = 0.0115;  $\chi^2 = 2.04$ ,  $df = 3$ ,  $P = 0.36$ ). The nests were found in nine treatments including annual burn and ungrazed (nests: DICK = 62, GRSP = 4, LASP = 6, FISP = 1, EAME = 1), annual burn and grazed (nests: DICK = 21, GRSP = 9, LASP = 7), two-year burn and grazed (nests: DICK = 6), two-year burn and ungrazed (nests: DICK = 2), four-year burn and grazed (nests: DICK = 29,

GRSP = 14, LASP = 3), four-year burn and ungrazed (nests: DICK = 31, GRSP = 7, LASP = 1, EAME = 4, FISP = 1), 20-year burn and grazed (nests: DICK = 1, GRSP = 4), 20-year burn and ungrazed (nests: EAME = 2), and areas of ungrazed and no set burning treatment (nests: DICK = 4, LASP = 2). When considering the four focal treatments ( $n = 201$ ), variation in DSR could not be explained further by treatment type (annual burn/grazed = 0.930, SE = 0.0141; 4-year burn/grazed = 0.890, SE = 0.0171; annual burn/ungrazed = 0.908, SE = 0.01171; 4-year burn/ungrazed = 0.926, SE = 0.0130;  $\chi^2 = 4.27$ ,  $df = 3$ ,  $P = 0.23$ ). Variation in DSR also could not be further explained by species (DICK = 0.913, SE = 0.0080; EAME = 0.964, SE = 0.0176; FISP = 0.944, SE = 0.0542; GRSP = 0.898, SE = 0.0177; LASP = 0.918, SE = 0.0217;  $\chi^2 = 8.66$ ,  $df = 4$ ,  $P = 0.07$ ; Table 4.1). We combined nests from all three years across species for the entire site to analyze the influence of local habitat on nest-survival rates of the grassland community. Out of the final habitat models, the best model indicated that birds nesting in areas of increased vegetation height but decreased shrub cover had higher nest success (Table 4.1). The top model showed that the effect of percent shrub cover on nest survival was negative ( $\beta = -0.30$ , 95% CI = -0.48 to -0.12) and vegetation height was positive ( $\beta = 0.32$ , 95% CI = 0.03 to 0.41). As dickcissel nests made up 70% of the nest data set, we ran the DSR analysis in Program MARK with dickcissel nests only and the results did not change. Therefore, we present the results including all grassland bird species because we are interested in nest predation by snakes on the entire community of grassland birds.

### ***Habitat Overlap***

When testing all four groups together, we found significant differences between the habitat associations of *C. constrictor*, *P. emoryi*, depredated nests, and successful nests (Wilk's  $\lambda = 0.677$ ,  $F_{45, 1447.5} = 4.52$ ,  $P < 0.001$ ). The DFA produced two statistically significant



discriminant functions that collectively accounted for 97% of the variation (Table 4.2). Although the discriminant functions are multivariate, we base our interpretation on the strength of the loadings (eigenvalue > 0.4) on particular variables and present the univariate differences among groups for comparative purposes. The first discriminant function ( $F_{24, 1433.4} = 6.60, P < 0.001$ ) accounted for 59% of the variation and was loaded on percent shrub cover, vegetation height, heterogeneity of vegetation height, and percent litter cover (Table 4.2). The first discriminant function could be interpreted as a gradient from open grassland to increased shrub cover and separated habitat used by snakes from that used by nesting songbirds (Table 4.3; Fig. 4.2). We did not expect complete overlap between snakes and birds because snakes use a variety of habitats and are not just concentrated at bird nest locations. The second discriminant function ( $F_{24, 1433.4} = 6.60, P < 0.001$ ) accounted for 38% of the variation and was loaded on vegetation height and rock cover (Table 4.2). We interpreted the second discriminant function as a gradient from short vegetation with little rock cover to tall vegetation with abundant rock cover, which separated successful nests from depredated nests (DF2:  $F_{1, 221} = 16.3, P < 0.001$ ), as well as the two snake species (DF2:  $F_{1, 282} = 43.3, P < 0.001$ ) (Table 4.3; Fig. 4.2).

Habitat used by *C. constrictor* was significantly different from successful nests along both discriminant functions, with snake locations characterized by increased heterogeneity and taller vegetation due to shrubs (DF1:  $F_{1, 220} = 49.5, P < 0.001$ ), and successful nests having taller vegetation related to grass and forbs (DF2:  $F_{1, 220} = 31.9, P < 0.001$ ) (Table 4.3; Fig. 4.2). Habitat used by *C. constrictor* was not significantly different from depredated nests along the second discriminant function, as both were characterized by lower vegetation and rock cover (DF2:  $F_{1, 310} = 3.5, P = 0.06$ ) (Table 4.3). Habitat used by *P. emoryi* was significantly different from depredated nests along both discriminant functions, having taller, shrubbier vegetation and more

rock cover than at depredated nest sites (DF1:  $F_{1,283} = 47.1$ ,  $P < 0.0001$  and DF2:  $F_{1,283} = 25.0$ ,  $P < 0.001$ ) (Table 4.2 & 4.3). *Coluber constrictor* and *P. emoryi* locations both had about 3x the shrub cover of successful nests (Table 4.2). Depredated nests, in turn, had 62% more shrub cover than successful nests (Table 4.2). Thus, nests in areas of increased shrub cover overlapped with habitat used by these two snake predators and suffered a higher rate of failure (Table 4.2).

Generalized linear distances were used to classify discriminant scores as belonging to one of the four groups through resubstitution (Table 4.3). Although groups may have been significantly different on average, individual discriminant scores may be classified into another group based on habitat characteristics. Only 9.1% of successful nests were misclassified as depredated, whereas 31.4% of depredated nests were misclassified as successful. For depredated nests, 25% were misclassified as *C. constrictor*, whereas only 16.7% of successful nests were misclassified as such. Of the depredated nests, 35.9% were misclassified as *P. emoryi* but only 4.6% of the successful nests were misclassified as *P. emoryi* (Table 4.3). Thus, 61% of depredated nests were misclassified as snake habitat, whereas only 21.3% of successful nests were misclassified as such, which indicates that substantial overlap occurs between habitats used by snakes and depredated nests.

## Discussion

Nest predation is the major factor limiting reproductive success in grassland birds (Martin 1993b). A simultaneous assessment of habitat use by nesting songbirds and their nest predators thus contributes to a more comprehensive understanding of how habitat mediates nest predation risk in grassland birds. In this study, we were able to relate nest predation risk for grassland birds in tallgrass prairie to shrub habitat, which is also used extensively by snakes. We found that grassland birds achieved higher nesting success in areas with decreased shrub cover but

increased vegetation height from grass and forbs. Other studies of nest predation in grassland birds have found a positive correlation between vegetation height and survival rate (Davis 2005; Winter 1999) possibly due to greater nest concealment or interference with predator search strategies. Although shrubs can provide cover for nests, we have shown that shrubs are heavily used by snakes (i.e., *C. constrictor*) and may contribute to increased predation pressure.

Snakes may use shrub habitat because it affords cover and increased structural heterogeneity, which may provide protection from predators (Wilgers and Horne 2007), a favorable thermal environment (Blouin-Demers and Weatherhead 2001a), or higher prey densities (Carfagno et al. 2006). Studies in the tropics have shown that snakes are attracted to shrubby edges because of an increased density of small mammals (Heard et al. 2004), but studies in temperate regions have not shown the same relationship possibly due to greater prey diversity or because thermoregulatory needs place a greater constraint on habitat selection (Carfagno et al. 2006). We have anecdotal evidence that snakes may be using shrubs to reach higher body temperatures to aid in digestion. For example, we observed a *C. constrictor* consuming a large meal of dickcissel nestlings and subsequently elevating itself in a dogwood shrub. We also found that snakes had higher body temperatures (+1.5 °C) when elevated in shrub habitat compared to grass habitat (Klug et al. In review), which again suggests that snakes may use shrubs for thermoregulation. Regardless of why snakes use shrubs, birds nesting in or near shrubs will have a higher likelihood of being encountered by a snake.

Other potential nest predators also occur in the system. For example, raccoons *Procyon lotor* may also preferentially forage in shrubby habitats (Newbury and Nelson 2007), thus compounding predation pressure on bird nests in shrubby areas if the effect of additional predators is additive. Depredated nests outside the snake habitat space may be attributable to

another type of predator, such as bullsnakes or ground squirrels *Spermophilus* spp., which prefer short vegetation, as might be found in intensively grazed and burned sites (Kaufman and Fleharty 1974; Fitch 1999). The region in discriminant space consisting only of successful nests could be viewed as habitat with a low risk of predation. The low-risk area was characterized by tall vegetation but low shrub cover. On KPBS, this type of vegetation is found in watersheds with annual burning and low-density grazing. Elsewhere in the Flint Hills, tall vegetation and low shrub cover is likely to be attained in native prairie hayfields, which have late-season haying, and in season-long grazed pastures that are stocked at half the density of pastures that are annually burned and double-stocked (Rahmig et al. 2009). Several grassland birds (dickcissel, grasshopper sparrow and eastern meadowlark) were found to have higher reproductive success in hayfields than in grazed grassland in the Flint Hills (Rahmig et al. 2009; With et al. 2008), perhaps as a consequence of lower predation pressure in hayfields relative to other managed grasslands.

Rangeland management in the Flint Hills, which involves widespread grazing and annual burning, has reduced tallgrass vegetation to a stature akin to that of the shortgrass steppe of the western Great Plains. At the other extreme, fire suppression, particularly around urban growth centers (exurban development), is spurring woody encroachment. In both cases, reductions in vegetative cover and the promotion of woody invasives is predicted to lead to increased predation risk for nesting grassland birds by predators such as snakes, which may explain the inordinately high rates of nest predation and reproductive failure experienced by grassland birds in the Flint Hills region (Rahmig et al. 2009; With et al. 2008). Therefore, any increase in the survival rate of nests through reductions in predation could help increase reproductive success

and alleviate declines in grassland bird populations, which are estimated to be declining by 3-29% annually in the Flint Hills (With et al. 2008).

The primary focus of grassland bird conservation is usually on either the restoration of grasslands or the management of existing grasslands to improve habitat quality. In regions such as the Flint Hills, where much of the grassland is under private land ownership and management is mainly commodity driven (e.g., for the benefit of livestock production), opportunities for altering land-management practices are limited. Elsewhere, such as in areas of exurban development, it may not be possible to restore burning and grazing to halt shrub invasion. Once shrubs become established, however, burning and grazing may no longer be sufficient in any case, and mechanical removal is necessary (Briggs et al. 2005). Thus, one alternative might be the strategic removal of shrubs and trees to mediate nest predation risk in areas where woody invasion is of particular concern. In our system, shrub cover should be reduced or maintained to encompass no more than 5% of the total area (i.e., the average shrub cover at successful nests), which may require only a small reduction in existing shrub cover, given that the average shrub cover at depredated nests was ~10% (Table 4.2). Thus, relatively small changes in the amount of shrubs might significantly reduce predation risk on nests of grassland birds, although this needs to be tested through future experimental research.

Although removal of shrub habitat may aid in the recovery of grassland birds, it could also remove habitat that may be necessary for sustaining snake populations, which are equally valuable components of the endangered tallgrass prairie. Conversely, if snake populations can be sustained without shrub cover, the removal of shrubs may simply spread snake activity across the landscape endangering a broader range of nests. Future research should thus examine the effect of shrub removal on snake habitat use and density in addition to nest survival (a before-after

comparison), especially given the labor and expense of manually removing shrubs and trees. In addition, the removal of shrubs not only impacts individual predators but interactions between the suite of predators known to use shrubs (e.g. mid-sized carnivores, snakes, and brown-headed cowbirds). Therefore future studies analyzing the experimental removal of shrubs should consider the impact on the abundance and activity of multiple predators as well as their interactions (Klug et al. 2009).

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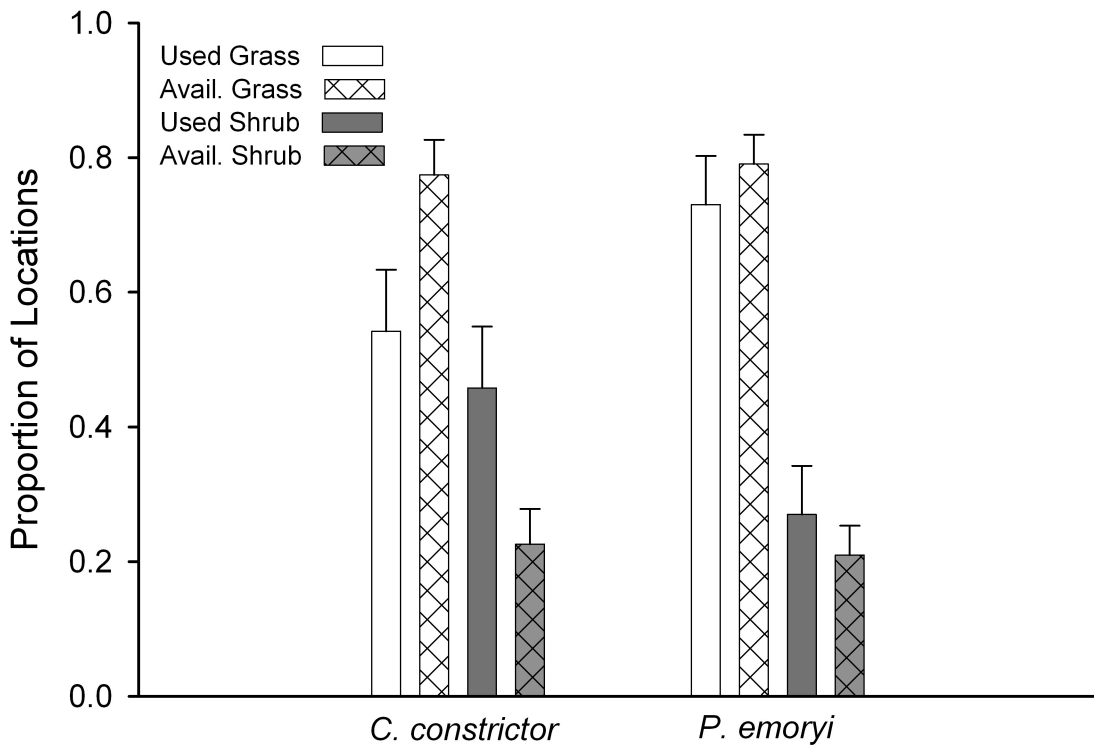
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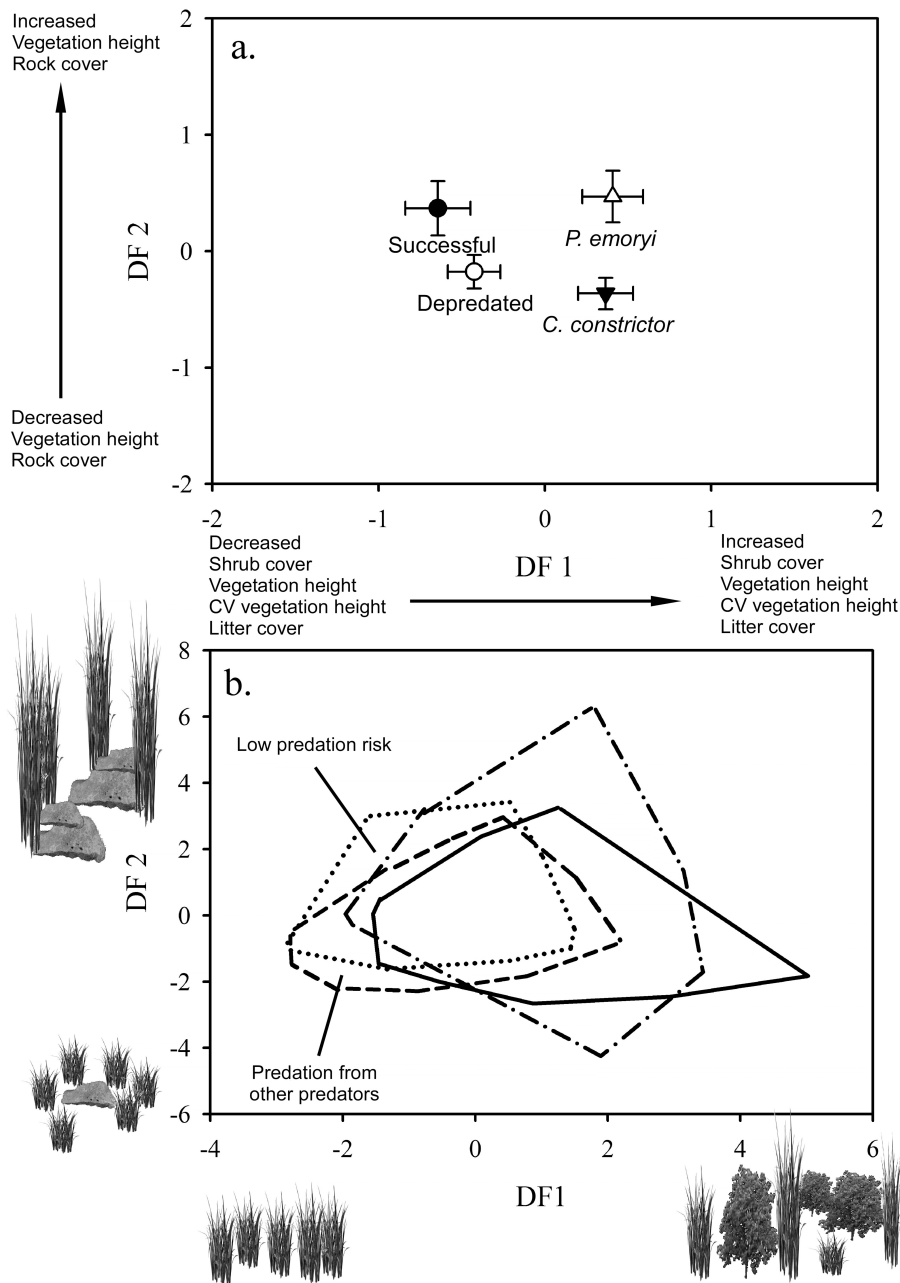
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**Figure 4.1** The proportion of locations for *C. constrictor* and *P. emoryi* in grass habitat and shrub habitat as compared to the proportion of random locations in each habitat type. The mean and SE were calculated by averaging the proportions for *C. constrictor* ( $n = 12$ ) and *P. emoryi* ( $n = 12$ ). The total number of used and random locations was 374 and 403 for *C. constrictor* and *P. emoryi*, respectively. For *C. constrictor*, the number of random locations in shrubs was 94 (25.1%), and the number of used locations in shrubs was 183 (48.9%). For *P. emoryi*, the number of random locations in shrubs was 87 (21.5%), and the number of used locations in shrubs was 119 (29.5%).



**Figure 4.2 Habitat gradients in the analysis of habitat use by grassland birds and two species of snake predators on the Konza Prairie Biological Station from 2006-2008. (a.) Group centroids and standard errors on the significant discriminant axes for successful nests, depredated nests, and locations of *C. constrictor*, and *P. emoryi*. (b.) Outline of discriminant scores for each group: successful nests (dotted), depredated nests (dashed), *C. constrictor* locations (solid), and *P. emoryi* locations (dashed/dotted).**



**Table 4.1 Habitat models tested in Program MARK to predict daily survival rates of grassland birds on Konza Prairie Biological Station from 2006-2008 ( $n = 222$ ). Ascending  $\Delta AIC_c$  ranks the candidate models.  $\Delta AIC_c$  is the scaled value for  $AIC_c$  (Akaike's information criterion for small sample sizes),  $w_i$  is the Akaike weight representing the support for each model, and  $k$  is the number of parameters.**

Model	Deviance	k	$AIC_c$	$\Delta AIC_c$	$w_i$
{% Shrub, Veg. Height}	721.66	3	727.67	0.000	0.517
{% Shrub}	727.04	2	731.05	3.375	0.096
{% Shrub, % Litter}	725.56	3	731.58	3.905	0.073
{% Shrub, CV Veg. Height}	725.72	3	731.73	4.059	0.068
{% Shrub, % Grass}	726.92	3	732.93	5.261	0.037
{% Shrub, CV Litter Depth}	726.96	3	732.97	5.304	0.036
{% Shrub, % Rock}	726.97	3	732.99	5.318	0.036
{% Shrub, % Forb}	726.98	3	732.99	5.324	0.036
{Constant}	732.82	1	734.83	7.157	0.014
{GLOBAL}	717.51	9	735.61	7.943	0.010
{Veg. Height}	731.74	2	735.74	8.072	0.009
{% Litter}	732.58	2	736.59	8.916	0.006
{% Rock}	732.80	2	736.81	9.138	0.005
{CV Veg. Height}	732.81	2	736.82	9.149	0.005
{% Grass}	732.82	2	736.83	9.158	0.005
{CV Litter Depth}	732.82	2	736.83	9.161	0.005
{% Forb}	732.82	2	736.83	9.161	0.005
{Year}	730.89	3	736.90	9.231	0.005
{Species}	727.20	5	737.23	9.564	0.004
{Veg. Height, % Forb}	731.58	3	737.60	9.926	0.004
{Veg. Height, % Grass}	731.61	3	737.62	9.951	0.004
{Treatment}	721.63	8	737.71	10.042	0.003
{Veg. Height, CV Litter Depth}	731.71	3	737.72	10.049	0.003
{Veg. Height, %Rock}	731.73	3	737.74	10.073	0.003
{Veg. Height, %Litter}	731.74	3	737.75	10.079	0.003
{Veg. Height, CV Veg. Height}	731.74	3	737.75	10.080	0.003

**Table 4.2 Univariate statistics (mean and SE) of each habitat variable used in the DFA to separate the four groups and the pooled within-group correlations between measured variables to indicate the weighting of each variable on the discriminant functions (eigenvalues > 0.4 are in italics).**

Variable	Successful nests ( <i>n</i> = 66)	Depredated nests ( <i>n</i> = 156)	<i>C. constrictor</i> ( <i>n</i> = 155)	<i>P. emoryi</i> ( <i>n</i> = 128)	DFA 1	DFA 2
					Eigenvalue	
% Shrub	5.81 (1.08)	9.40 (1.00)	18.71 (1.68)	17.64 (1.80)	<i>0.660</i>	-0.111
% Litter	47.12 (3.92)	45.47 (2.59)	58.46 (1.91)	57.25 (2.44)	<i>0.457</i>	0.006
% Rock	12.35 (1.62)	10.79 (0.88)	8.67 (0.65)	14.09 (1.33)	-0.012	<i>0.486</i>
% Grass	44.41 (2.08)	45.99 (1.62)	46.24 (1.18)	48.01 (1.50)	0.110	0.063
% Forb	35.25 (1.59)	33.92 (1.07)	31.43 (1.10)	32.86 (1.20)	-0.192	0.130
Veg. height	77.61 (2.71)	72.06 (1.68)	77.38 (1.84)	87.63 (2.51)	<i>0.379</i>	<i>0.517</i>
CV Veg. height	0.25 (0.02)	0.28 (0.01)	0.36 (0.02)	0.36 (0.02)	<i>0.569</i>	-0.066
CV Litter depth	0.86 (0.09)	0.81 (0.05)	0.86 (0.03)	0.88 (0.04)	0.080	0.075

**Table 4.3 The generalized squared distances <sup>a</sup> between the four group centroids <sup>b</sup> and the associated statistical significance including the multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) for each discriminant function as well as the percentage of discriminant scores classified into each of the four groups <sup>c</sup> (successful bird nests, depredated bird nests, *C. constrictor* locations, and *P. emoryi* locations) on Konza Prairie Biological Station from 2006-2008.**

	Depredated					<i>C. constrictor</i>					<i>P. emoryi</i>				
	Distance	% Classified	F	df	P	Distance	% Classified	F	df	P	Distance	% Classified	F	df	P
Successful	S-D = 28.2	S-D = 9.1	11.0	2,219	<0.001	S-C = 27.7	S-C = 16.7	35.1	2,218	<0.001	S-P = 30.0	S-P = 4.6	25.7	2,191	<0.001
DF1	D-S = 27.5	D-S = 31.4	2.5	1,221	0.12	C-S = 31.8	C-S = 17.4	49.5	1,220	<0.001	P-S = 30.0	P-S = 17.2	51.4	1,193	<0.001
DF2			16.3	1,221	<0.001			31.9	1,220	<0.001			0.3	1,193	0.57
Depredated						D-C = 27.1	D-C = 25.0	24.9	2,308	<0.001	D-P = 27.6	D-P = 35.9	32.9	2,281	<0.001
DF1						C-D = 28.5	C-D = 9.7	46.7	1,310	<0.001	P-D = 28.8	P-D = 10.2	47.1	1,283	<0.001
DF2								3.5	1,310	0.06			25.0	1,283	<0.001
<i>C. constrictor</i>											C-P = 29.1	C-P = 9.7	22.2	2,280	<0.001
DF1											P-C = 27.5	P-C = 27.3	0.1	1,282	0.73
DF2													43.3	1,282	<0.001

<sup>a</sup> Generalized squared distances for each group (S-S = 26.51, D-D = 27.60, C-C = 26.23, P-P = 28.58)

<sup>b</sup> S = successful, D = depredated, C = *C. constrictor*, P = *P. emoryi*

<sup>c</sup> Percent of discriminant scores correctly classified into each group (S-S = 69.7, D-D = 35.9, C-C = 63.2, P-P = 45.3)

## CHAPTER 5- Conclusions and Synthesis

Grassland birds have suffered the greatest and most widespread decline of any North American bird group (Sauer et al. 2004) as a result of habitat loss, fragmentation, and degradation (Herkert 1994). The Flint Hills region should be a source for grassland bird populations because it is the largest, contiguous tallgrass prairie remaining in North America and has not suffered from widespread habitat conversion to row-crop agriculture. Although the Flint Hills may appear to be a well-connected, contiguous landscape, habitat degradation may result in effective habitat loss with serious negative consequences for grassland bird populations. Previous studies on grassland birds inhabiting the Flint Hills region have found an inordinately high rate of reproductive failure mainly due to high nest predation rates (With et al. 2008, Rahmig et al. 2009). Therefore, any reductions in predation rates could help increase reproductive success and alleviate declines in grassland bird populations, which are estimated to be declining by 3-29% annually in the Flint Hills (With et al. 2008).

Snakes are important predators of bird nests (Weatherhead and Blouin-Demers 2004), but little is known about how snakes in the tallgrass prairie may exacerbate predation risk in nesting grassland birds. Therefore, the overall goal of this dissertation was to understand nest predation in grassland birds through an evaluation of the predator ecology of snakes known to depredate bird nests. To address potential interactions between grassland birds and their snake predators, I first evaluated the landscape genetics of the eastern yellowbelly racer (*Coluber constrictor flaviventris*) population across the broader landscape (13,500 km<sup>2</sup>). The eastern yellowbelly racer is one of the most common snake species in this system, and has been implicated as a predator on grassland bird nests. Through the use of population genetics, I was able to quantify population



subdivision (or lack thereof) in yellowbelly racers using Bayesian clustering techniques, and identify the scale of genetic structure within racer populations using spatial autocorrelation. Additionally, through a combination of isolation-by-distance theory and geospatial mapping, I was able to identify the dispersal range and landscape connectivity of yellowbelly racers across this region, by evaluating different hypotheses as to how habitat resistance versus simple geographic distance related to the genetic distance of yellowbelly racers. Secondly, I radio-tracked individual yellowbelly racers and Great Plains ratsnakes (*Pantherophis emoryi*)—two of the most common snakes at the Konza Prairie Biological Station (KPBS)—to compare movement behavior and space use. Snakes may not seek out bird nests but encounter them incidentally while foraging. Therefore, the importance of a particular snake species as a nest predator will depend on movement and space use. By tracking individual snakes, I was able to discern how the two species may differentially influence avian nesting success. I also examined nest survival of grassland birds in the context of habitat used by both the Great Plains ratsnake and eastern yellowbelly racer in an effort to identify habitat overlap between nesting grassland birds and two of their snake predators.

Although I studied both the yellowbelly racer and the Great Plains ratsnake for various aspects of my dissertation research, I have more data on the yellowbelly racer across a range of scales and levels of biological organization (genetic, individual, and population), and therefore am in a better position to evaluate the potential of the yellowbelly racer to influence nesting success in grassland birds. In this concluding chapter, I therefore focus on how the results of my dissertation implicate the yellowbelly racer as a potentially high-impact predator on grassland birds, and how knowledge of this predator's ecology may contribute to management recommendations for declining bird populations in the tallgrass prairie of northeastern Kansas.

## Profile of a Predator: Eastern Yellowbelly Racer

The eastern yellowbelly racer has the profile of a predator capable of negatively impacting grassland bird nesting success. The characteristics that make the yellowbelly racer a potentially high-impact predator are:

- 1) Yellowbelly racers are abundant in northeast Kansas [e.g. Fort Riley Military Reservation (Busby and Parmelee 1996) and University of Kansas Nelson Environmental Study Area (Fitch and Echelle 2006)]. In my study at KPBS, the yellowbelly racer made up 47% of the snakes captured ( $n = 146$ ) and is therefore relatively abundant compared to other snake species. Evidence for large population numbers were also supported in this study through the observed population genetic structure. For example, the yellowbelly racer population was shown to be maintaining migration-drift equilibrium, indicating that no subpopulations were isolated from each other and genetic drift was continually offset by migration of new individuals. In addition, high heterozygosity was observed and therefore the loss of heterozygosity that often occurs with genetic drift in small populations was not evident. The yellowbelly racer population also exhibited a high number of alleles per locus (maximum = 51 alleles), which indicates that a large number of individuals must be contributing to the gene pool. This genetic evidence in conjunction with the snake survey data indicates that the yellowbelly racer is an abundant snake in northeastern Kansas.
- 2) Yellowbelly racers appear to have a near-continuous distribution across the landscape. Genetic spatial autocorrelation revealed that yellowbelly racers exhibit restricted dispersal within 3 km, likely due to limited dispersal between natal sites as well as limits to individual movements of adults between hibernacula and summer home ranges. Although individuals have limited dispersal ranges, substantial admixture occurred within 30 km, indicating that racers must be abundant and continuously distributed in order for gene flow to be fluid throughout the region. Significant isolation-by-distance eventually occurred at broad regional scales ( $> 100$  km) separating ecoregions (i.e., the Flint Hills from Central Irregular Plains populations east of the Flint Hills), but Bayesian clustering analyses were unable to define discrete subpopulations based on allele frequencies (F-

statistics). Thus, high gene flow occurs throughout the region despite restricted dispersal at local (km-wide) scales, which would be facilitated by a near-continuous distribution of snakes and the ability to use a variety of habitats across the landscape.

- 3) The yellowbelly racer is a habitat generalist (Fitch 1963, 1999). I found that the population genetic structure of yellowbelly racers was not heavily influenced by landscape composition, which suggests no natural (i.e., forest or bodies of water) or anthropogenic habitat barriers (i.e., row-crop agriculture and urban centers) currently limit snake dispersal (gene flow) across the landscape. I also conducted snake surveys and a radio-telemetry study examining local habitat use in yellowbelly racers. I found yellowbelly racers in almost every watershed treatment at KPBS (i.e., 1-20 year burns; grazed and ungrazed) as well as in gallery forests. Thus, yellowbelly racers are a ubiquitous predator in this system.
- 4) The yellowbelly racer is a prey generalist (Fitch 1963, 1999). Although, I did not study the diet of racers directly, previous studies have shown that 30% of regurgitated meals were either bird eggs or nestlings (Cavitt 2000b). High predation pressure on prey (i.e., grassland birds) may occur when a generalist predator (i.e., yellowbelly racer) is able to attain high abundances because of the widespread availability of abundant alternative prey (Cantrell et al. 2002). A generalist diet may thus enable yellowbelly racers to attain high densities in tallgrass prairie and to negatively impact bird populations even as bird populations are in decline.
- 5) The yellowbelly racer is an active snake (Fitch 1963, 1999). By analyzing the autocorrelation of radio-tracked locations (Mantel correlograms), we found yellowbelly racers had irregular, frequent movements that highlighted their active foraging strategy for locating prey. Therefore, yellowbelly racers may be especially likely to encounter bird nests, given that they are active on a daily basis (i.e., only 9% of locations were in the exact same position as the previous location) and traverse substantial distances throughout the day (i.e., ~ 67 m per day). While radio-tracking racers, I made anecdotal observations as to their activity and behavior that would increase interactions with

grassland birds. For example, one female racer, which inhabited an annually burned watershed, showed an increase in movement distances as the season progressed, possibly due to the increased vegetation biomass. Previous studies have also shown that yellowbelly racers were able to recolonize recently burned areas as the vegetation recovered throughout the season (Cavitt 2000a). Given that some grassland birds also appear to shift their nest-site locations to take advantage of increased vegetation cover later in the season (Frey et al. 2008), an increased use of more heavily vegetated areas by racers might therefore increase their incidental encounter with grassland bird nests.

- 6) Grassland birds appear to suffer higher rates of nest predation in habitats preferred by yellowbelly racers (Klug et al. In revision). The daily nest-survival rates of grassland birds decreased with increasing shrub cover and decreasing vegetation height. Discriminant function analysis revealed that successful nests were more likely to occur in areas with tall vegetation but reduced shrub cover, whereas higher shrub cover characterized yellowbelly racer habitats. In addition, 25% of the depredated nests were misclassified as yellowbelly racer habitat, whereas only 16.7% of successful nests were misclassified as such in the discriminant function analysis. Half of all yellowbelly racer locations were found to be in shrubby habitat during my radio-telemetry survey. This evidence suggests that birds nesting in areas with increased shrub cover may be at higher risk of nest predation by yellowbelly racers.

### **Management Recommendations for the Conservation of Grassland Birds**

Although predator removal is often proposed as a strategy for protecting bird populations (Côté and Sutherland 1997, Kosciuch and Sandercock 2008), it may not be an effective or feasible strategy in this instance, especially since snakes are a native and important component of the tallgrass prairie community (e.g., in terms of ecosystem services provided by snakes involving control of rodent populations). In addition, previous research by Cavitt (1998) on KPBS has shown that removing and relocating (> 15 km) bird-eating snakes (i.e., *C. constrictor*, *P. emoryi*, *Thamnophis sirtalis*, *Lampropeltis getula*, *Lampropeltis triangulum*, *Pituophis*

*catenifer*, and *Elaphe obsoleta*) neither decreased the relative abundance of snakes relative to reference sites nor increased the reproductive success of birds in the removal sites (i.e., brown thrashers *Toxostoma rufum*). In the study by Cavitt (1998), removal of snakes (i.e., 347 snakes removed over two years) may not have influenced nest-predation rates because the abundance of snakes in the area was large. Another possibility is that the snakes were simply capable of rapid recolonization after unoccupied space was made available once snakes were removed. In addition, nest-predation rates may not have been affected by this removal experiment because snake populations did not decrease or another predator may have become more important once snake populations decreased (i.e., compensatory predation; Schmidt, 2003). For example, small mammals (e.g., ground squirrels *Spermophilus* spp.) could have compensated for decreased snake abundance, especially if they were released from predation pressure. Thus, predator removal is unlikely to be a successful strategy to reduce nest predation in the tallgrass prairie.

The primary focus of grassland bird conservation is usually on the restoration of grasslands or the management of existing grasslands to improve habitat quality (Vickery et al. 2000). Although I did not find any natural or anthropogenic habitat barriers (i.e., forests or urban centers) limiting snake dispersal across the broader landscape (1,350,000 ha) in northeastern Kansas, I did find that the heterogeneity within grasslands (3,487 ha of KPBS) influenced habitat use in the yellowbelly racer (i.e., racers were found in shrub habitat more than would be expected based on the amount of shrubs in their home range). At the same time, I also found that bird nests surrounded by an average of 10% shrub cover within 30 m were more likely to be depredated than those nests surrounded by only 6% shrub cover (Klug et al. In revision). Therefore, evidence from the local scale (KPBS) suggests that removing shrubs from within grasslands may reduce nest predation by removing habitat that may be preferred by a major bird-

eating snake. The Dickcissel (*Spiza americana*), which made up 70% of our nest sample, is known to nest in or near shrubs (Winter et al. 2000) but has a broad association with forbs and other tall vegetation (Temple 2002). Presumably, this species would easily shift to nesting in other areas if shrub cover were reduced, possibly reducing predation risk by yellowbelly racers as a benefit. Unfortunately, I do not have data indicating how the removal of shrubs may influence the activity patterns of individual snakes nor the nest success of grassland birds, as the goal of this dissertation was to first identify habitat overlap between grassland birds and their snake predators. I propose that future research should examine the effect of shrub removal on snake habitat use in addition to nest survival, to evaluate if the labor and expense of removing shrubs will be worth the benefit to grassland birds.

Although we can manage grasslands in an attempt to minimize snake predation on grassland birds, snakes are a vital component of native grasslands and any functioning tallgrass prairie should include a diverse snake fauna. Therefore, future research should evaluate the efficiency of snakes at finding bird nests in different habitat treatments, especially in relation to the alteration of vegetation structure resulting from different grazing and burning regimes as practiced in the Flint Hills. Future studies attempting to understand fine-scale movements of snakes during foraging would benefit from an automated radio-telemetry system, which would allow for multiple individuals to be tracked simultaneously with frequent positional fixes per individual (e.g. every 30 minutes; Weatherhead et al. In press). An automated telemetry system would free up time and labor to simultaneously investigate the impact of vegetation structure, alternative prey, and bird nest distribution on snake space use. This type of fine-scale data on snake predators, in conjunction with data on nest success, would illuminate situations in which snakes are more likely to encounter nests, versus situations in which they are simply more

efficient at locating bird nests. For example, snakes may be better able to locate nests in shrubs because they can recognize this type of physical structure as a location for bird nests especially when the shrub is surrounded by limited vegetation structure from grass and forbs (e.g., With 1994). Another example would be decreased predation on bird nests when large densities of alternative prey (i.e., small mammals or invertebrates) are abundant. With more information on the mechanisms behind movement decisions, more complete recommendations can be made as to what management options might be useful for altering snake habitat use and foraging behaviors, so as to minimize predation on grassland birds.

The historical tallgrass prairie biome has not only suffered severe reductions in area and increased fragmentation, but is also experiencing habitat degradation in what grasslands remain. As these trends continue, a clear understanding of predator populations at multiple scales is necessary not only for understanding a native predator, but in making management recommendations for their prey species of conservation concern (i.e., grassland birds). In general, an understanding of how dispersal success and population connectivity influences the viability of predator populations at broad regional or landscape scales, combined with how movement behavior and habitat use at the individual level facilitates predator encounters with prey, will contribute to a better understanding of predation risk.

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# **Appendix A - Microsatellite Isolation and Development in the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) for Use in Landscape Genetic Studies<sup>1</sup>**

## **Abstract**

We isolated and optimized 12 microsatellite loci from the eastern yellowbelly racer (*Coluber constrictor flaviventris*). The loci were screened for polymorphism in 76 individuals from two populations in northeast Kansas, USA. The number of alleles per locus ranged from eight to 50 (mean = 20.7, SD = 11.0). Expected and observed heterozygosities ranged from 0.21 to 0.97 and from 0.23 to 0.95, respectively. No pairs of loci exhibited significant linkage disequilibrium. One locus deviated significantly from Hardy–Weinberg equilibrium in both populations (GQ371178) and two additional loci deviated significantly in one population (GQ371188 and GQ371183).

## **Introduction**

Little is known about the distribution, structure, or connectivity of snake populations. Snakes have extended periods of inactivity and are inconspicuous, which render visual surveys and mark-recapture methods ineffective for estimating population densities and delineating population structure (Parker & Plummer 2001). Although the study of snakes has been facilitated by the invention of small-bodied radio-transmitters, our ability to link movement to dispersal events, which ultimately influence population structure, is limited. We propose that genetic technologies could bridge this gap in our current understanding of individual dispersal and

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<sup>1</sup> This is the pre-peer reviewed version of the following article (Molecular Ecology Resources Primer Development Consortium et al 2009; Molecular Ecology Resources database accessions 37758–37769), which has been published in final form at <http://tomato.biol.trinity.edu/>.

population structure of snakes. Microsatellite markers have not yet been developed for *C. constrictor*, but sequences from the mtDNA cytochrome *b* gene have been used to construct phylogeographic structure, historical demography, and lineage age (Burbrink *et al.* 2008). The availability of microsatellite markers will allow estimates of effective populations sizes and complement previous studies of *C. constrictor* by elucidating how landscape structure shapes contemporary population structure for a generalist species adapted to a range of habitats.

## Methods and Results

We developed microsatellite markers for *C. constrictor*, a snake that inhabits a variety of ecosystems and has a transcontinental distribution across North America (Fitch 1999). We extracted DNA from scale clippings using the Qiagen Dneasy™ tissue kit. We digested the genomic DNA from one female specimen using *HaeIII*, *RsaI*, *XmnI*, and *HindIII*. We generated two microsatellite enriched plasmid libraries (TC<sub>10</sub> and a mix of AG<sub>12</sub>, TG<sub>12</sub>, AAC<sub>6</sub>, AAG<sub>8</sub>, AAT<sub>12</sub>, ACT<sub>12</sub>, ATC<sub>8</sub>) using the protocol of Hamilton *et al.* (1999) with a modified hybridization procedure (Glenn & Schable 2005). Transformed colonies positive for snake DNA inserts (n = 200) were sequenced and screened for microsatellite repeats. We found 42 sequences containing microsatellite loci with enough flanking region to design primers using Primer 3 ([http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi); Rozen & Skaletsky 2000).

The microsatellite primer sets were tested on 76 individuals from Kansas, USA. We used two sampling localities, which were 140 km apart and occupied different ecoregions. We collected samples from Riley and Geary Counties in the Flint Hills ecoregion (n = 54), which included Fort Riley Military Reservation and Konza Prairie Biological Station. We also obtained samples from Douglas and Jefferson Counties in the Central Irregular Plains ecoregion (n = 22), which included the University of Kansas Field Station and Ecological Reserves. The Flint Hills

ecoregion contains limestone and shale hills with steep narrow valleys and tallgrass prairie as the natural vegetation whereas the Central Irregular Plains is a mosaic of grasslands and forests.

The PCR conditions included the following: 2- $\mu$ L of template DNA (10-100 ng/ $\mu$ L) in 20- $\mu$ L total volume, 5x GoTaq® Flexi Buffer (Promega), 2-mM magnesium chloride, 0.2-mM dNTPs, 0.1  $\mu$ g/ $\mu$ L BSA, 0.8 M betaine, 0.1- $\mu$ M forward primer, 0.3- $\mu$ M reverse primer, 0.3- $\mu$ M universal M13-labelled primer, and 0.5 units of *Taq* DNA polymerase brought to volume with doubly-distilled water. An M13 sequence complement was added to the 5' end of the forward primer. The M13-primer was 5' end-labeled with ABI fluorescent dyes (HEX or FAM; Schuelke 2000). The thermal cycle profile began with a denaturation step of 5 minutes at 94°C, then 30 cycles at 94°C (30 s)/annealing temperature (45 s)/72°C (45 s), followed by 8 cycles of 94°C (30 s)/53°C (45 s)/72°C (45 s), and ending with an extension step of 30 minutes at 72°C. PCR products were visualized using an ABI PRISM™ 3730 DNA Sequencer with ROX labeled size standard, and analyzed using GeneMarker.

Twelve loci resulted in easily interpretable polymorphic banding patterns (Table A.1). We used GENEPOP (<http://wbiomed.curtin.edu.au/genepop>) to calculate diversity statistics and to test for deviations from HWE and linkage disequilibrium (Raymond & Rousset 1995). Allelic variation ranged from eight to 50 alleles per locus (mean = 20.7, SD = 11.0). Expected and observed heterozygosities from the Riley/Geary population ranged from 0.29 to 0.97 and 0.30 to 0.89, respectively. Expected and observed heterozygosities from the Douglas/Jefferson population ranged from 0.21 to 0.96 and 0.23 to 0.95, respectively. No pairs of loci exhibited linkage disequilibrium. We found a heterozygote deficit for GQ371178 in both populations and a heterozygote deficit for GQ371188 and GQ371183 in the Riley/Geary population. We used MICROCHECKER to statistically test for the presence of null alleles using the Brookfield-1

equation as the null estimator. We found evidence for null alleles for GQ371178 in both populations, GQ371182 in the Douglas/Jefferson population and GQ371183 in the Riley/Geary population ( $nf > 0.10$ ). The loci presented here will be useful in population and landscape genetic studies of *C. constrictor*.

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**Table A.1 Variable microsatellite loci in *Coluber constrictor flaviventris* with forward and reverse primer sequence (5'–3' direction without M-13 complement), repeat motifs, optimal annealing temperature, allele number, allele size range, and expected and observed heterozygosity for the Riley/Geary and Douglas/Jefferson county populations.**

GenBank Accession #	Primer sequences (5'-3')	Repeat Motif	T <sub>a</sub> (°C)	Allele No.	Size Range (bp)	Riley & Geary H <sub>E</sub> /H <sub>O</sub>	Douglas & Jefferson H <sub>E</sub> /H <sub>O</sub>
GQ371177	F:CTTTGATGAACAGCTTTCTGTG R:TCCAATCTTCAATCACATCC	(CA) <sub>14</sub>	56.7	13	154-178	0.77/0.78	0.72/0.73
GQ371178	F:TGTGCTGGAGGTGAGATGG R:AACGCAAAAGAAGTGCCAG	(TC) <sub>21</sub> (AC) <sub>13</sub>	59.9	21	160-244	0.86/0.59*	0.93/0.45*
GQ371179	F:TCCGTCTTCTAAGAGAGAGATAGC R:ACTAGGGCGTGAGTGACC	(AC) <sub>21</sub>	56.7	20	176-222	0.88/0.83	0.84/0.73
GQ371180	F:AAACTTCCCTTACCCCATGC R:CTTGCTAAGCGACCCTTG	(CA) <sub>26</sub>	57.9	21	182-228	0.92/0.85	0.91/0.95
GQ371181	F:GGAGGCGAGTCAAACAGTTG R:CAATCCAAGGCAGAAGAGG	(CA) <sub>17</sub>	58.8	14	138-180	0.87/0.81	0.89/0.95
GQ371182	F:CGAGATGTGTCCAGCTTCTG R:ACTTTGGGGGAATTCCAG	(AC) <sub>17</sub>	59.9	15	182-222	0.92/0.80	0.77/0.50
GQ371183	F:TTGAACCTGTCTTGGGGAAG R:GATCGGTTGACCTGGATTTG	(CT) <sub>12</sub> (CA) <sub>14</sub>	59.9	29	232-342	0.93/0.57*	0.91/0.86
GQ371184	F:AGCCAGGAGTACGGGATG R:CGCGCGTCTACTCTCC	(CA) <sub>16</sub>	59.9	8	222-238	0.29/0.30	0.21/0.23
GQ371185	F:GCCCATCCACAAGTGAATC R:ATAACGGAATGCTGGCAAAG	(AC) <sub>21</sub>	59.9	13	144-176	0.88/0.89	0.76/0.82
GQ371186	F:AACATTTTGGCACCCAGAAG R:ACTTCCCATGCAGTTCTG	(GTAG) <sub>03</sub> (CTAG) <sub>14</sub> (ATAG) <sub>13</sub>	59.9	50	188-420	0.97/0.87	0.96/0.82
GQ371187	F:CATCATGCTTAGCCCAGG R:TCCTTCCTTGGTGGTAGTGG	(CT) <sub>12</sub> (CA) <sub>14</sub>	59.9	17	162-202	0.83/0.76	0.78/0.73
GQ371188	F:CGGTTGACCTGGATTTGTTT R:TTGAACCTGACTTGGGGAAG	(TC) <sub>13</sub> (AC) <sub>15</sub>	54.8	27	226-334	0.93/0.81*	0.91/0.77

\*locus deviated significantly from Hardy-Weinberg equilibrium