AN ASSESSMENT OF THE GENETIC STRUCTURE OF A STRIPED SKUNK (*MEPHITIS MEPHITIS*) POPULATION ACROSS AN URBAN LANDSCAPE

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ABSTRACT

Striped skunks act as reservoirs of many zoonotic diseases and are highly adept at persisting in urbanized landscapes in relatively high densities. A better understanding of the interaction between skunks and the urban environment could aid in the development of management techniques for epidemiological outbreaks, as well as provide information on wildlife responses to urbanization-induced habitat fragmentation. We studied the genetic structure of a population of striped skunks in an urban environment, assessing the presence of subpopulation structuring, sex-biased philopatry, and natal habitat-biased dispersal. We failed to detect any significant population clustering or evidence of natal habitat-biased dispersal, but spatial autocorrelation analyses did reveal patterns of limited dispersal (< 2 km) and female-biased philopatry. Implications for wildlife and disease management personnel involve the implementation of geographically expansive protocol in wildlife population and disease monitoring and management.

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INTRODUCTION

Expanding human populations result in the continual degradation of natural habitats and landscapes; the development of urban and suburban areas results in changes in climate, hydrology, light, soil, noise levels, and natural community demographics (Gehrt 2010). These processes typically result in the homogenization of animal diversity (Chace and Walsh 2006; McKinney 2006) with relatively few species accruing benefit from urban areas and thriving (i.e., synanthropic species – Gehrt et al. 2009; Johnston 2001), and the resulting extirpation of many less tolerant species. One such synanthropic species is the striped skunk, *Mephitis mephitis* (Rosatte and Larivière 2003).

Striped skunks range from northern Mexico, throughout the United States, and well into Canada, inhabiting a variety of habitats, including urban environments (Rosatte and Larivière 2003; Wade-Smith and Verts 1982). Anthropogenic food sources and readily available den sites allow urban habitats to support high densities of skunks (Broadfoot et al. 2001; Rosatte and Larivière 2003; Rosatte et al. 1991; Rosatte et al. 2010). Striped skunks act as reservoirs for many zoonotic and interspecific transmissible diseases, including rabies, West Nile virus, and canine distemper (Bentler et al. 2007; Rosatte et al. 2010; Wade-Smith and Verts 1982). The high densities supported by urban environments and the inherent proximity to humans and pets leads to an increase in the likelihood for transmission of pathogens (Rosatte 1988). A better understanding of the interaction between skunks and the urban environment could aid in the development of management techniques for disease control and response protocol for epidemiological outbreaks (Broadfoot et al. 2001; Santonastaso et al. 2012; Talbot et al. 2012), as well as provide general information on wildlife responses to urbanization-induced habitat fragmentation. The latter being valuable

given that fragmentation and loss of habitat are key threats to biodiversity (Mcdonald et al. 2008; McKinney 2002; Ordeñana et al. 2010).

Gathering these data through field-based research can be difficult, however, given behavioral traits of the species of study (e.g. nocturnality) and logistical problems inherent in these types of studies (Cullingham et al. 2008; Santonastaso et al. 2012). Advances in molecular techniques, statistical methodologies, and geographic information software allow researchers to address what were traditionally field-studied ecological events (Manel et al. 2003; Santonastaso et al. 2012; Selkoe and Toonen 2006). Utilization of these techniques allows for the inference of population genetic structure, dispersal patterns, and gene flow, as well as those processes and landscape features by which they are governed (Manel et al. 2003; Cushman et al. 2006).

The genetic structure of a population is influenced by a variety of factors, including demographic process, social organization, natural selection, barriers to gene flow, and habitat quality (Broquet et al. 2006; Jones et al. 2004; Moyer et al. 2006; Riley et al. 2006; Slatkin 1994; Watts et al. 2011). Past research examining striped skunk populations have revealed little genetic structure. Hansen et al. (2003) failed to detect genetic structure of a population across the scale of their study (a linear distance of approximately 60 km), but did find evidence for female-biased philopatry and male-biased dispersal. This phenomenon is frequently exhibited by mammals and has most commonly been attributed to inbreeding avoidance and processes involved in kin selection (Lawson Handley and Perrin 2007). Talbot et al. (2012) also failed to find genetic structure within a population of striped skunks on a much larger scale (22,000 km^2) in eastern Canada. They did, however, find a female-specific inverse correlation between genetic relatedness and the number of major rivers

separating samples. They also reported a weaker inverse correlation between female genetic relatedness and the number of highways between sampled skunks.

While past studies have indicated panmictic populations of striped skunks over large areas, different methodologies could provide insight into processes governing gene flow and dispersal in striped skunk populations. Past studies have also been conducted in rural environments and population responses to urbanization could yield genetic partitioning. These patterns could be exhibited at a smaller scale than those previously studied given the reduced home range sizes and movements of urban skunks compared to those studied in rural areas (Rosatte et al. 1991; Weissinger et al. 2009). Urban environments also produce unique challenges to wildlife inhabitants. For example, Riley et al. (2006) found that a major thoroughfare presented a barrier to gene flow to two carnivores [bobcats (*Lynx rufus*) and coyotes (*Canis latrans*)] in southern California.

Unique individual responses to habitat type can also lead to genetic population structuring, even in a generalist species (Sacks et al. 2008). The tendency for dispersing individuals to occupy an area with similar habitat characteristics to that in which they were reared, or natal-habitat biased dispersal, has been documented for a number of species (Davis and Stamps 2004), including the medium-sized carnivore *Bassariscus astutus* (Lonsinger 2010) and the generalist carnivore *C. latrans* (Sacks et al. 2004). Broadfoot et al. (2001) reported increasing densities of striped skunks in urban areas with decreasing distance from industrial areas and increasing distance from tree cover – a result of preference for more open habitat. Ordeñana et al. (2010) reported increasing skunk occurrence associated with an increase in urban proximity, but decreasing occurrence in association with urban intensity. Given these different responses to landscape characteristics, an urban environment might

provide a sufficiently heterogeneous area to produce habitat-dependent genetic structure in striped skunks.

In this study, we examined the genetic structure of a population of striped skunks in an urban environment in west-central Texas. Specifically, we tested the hypotheses that (1) the striped skunk population within the urban study area exhibits patterns in genetic structure, (2) sex-biased dispersal patterns affect this genetic structure, and (3) natal-habitat dispersal also affects this genetic structure.

MATERIALS AND METHODS

Study area. – The study area was comprised of the city of San Angelo located in Tom Green County in west-central Texas. San Angelo covers an area of approximately 147 km^2 and has a population of 93,200 (U.S. Census Bureau, 2010). The climate is warm and semi-arid with an average rainfall of 51 cm per year, an average monthly low temperature in January of 0°C, and an average monthly high temperature in July of 36°C (Larkin and Bomar 1983). San Angelo provides a heterogeneous study site: several rivers and major thoroughfares could act as potential barriers to gene flow and contiguous stretches of undeveloped lands and natural areas might provide corridors for dispersal and gene flow.

Sampling. – Sampling of striped skunks occurred between November 2011 and October 2012. Samples were gathered with the aid of members of the San Angelo Animal Services Division, who salvaged road-killed and euthanized skunks. These samples were augmented by independent collection of road-killed and live-trapped skunks by several researchers. All capturing and handling techniques followed the guidelines set forth by the American Society of Mammalogists (Sikes et al. 2011; Sikes et al. 2012).

DNA extraction and gender determination. – Whole genomic DNA was isolated from heart, kidney, liver, or muscle tissue from collected skunks using the DNeasy Tissue Kit (QIAGEN Inc., Valencia California) following the manufacturer's protocol. We determined the gender of skunks by physical examination while collecting tissue samples; this was often not possible, however, given the degraded state and reduced dimensionality of many of our samples. We therefore conducted molecular sexing using the primer sequences LGL331 5'-CAA-ATC-ATG-CAA-GGA-TAG-AC-3' and LGL335 5'-AGA-CCT-GAT-TCC-AGA-CAG-TAC-CA-3' (Cathey et al. 1998), which are used to amplify an intron within the zinc-

finger x (*Zfx*) and zinc-finger y (*Zfy*) genes. Polymerase chain reactions were conducted and analyzed following modified protocol from Shaw et al. (2003) and Talbot et al. (2012), and consisted of 25-500 ng of DNA, 1 U of *Taq* polymerase, 0.2 µM of forward and reverse primer, 1.5 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates, and 1X reaction buffer for a total volume of 15.0 µl. Reaction cycles consisted of an initial denaturation of 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 53°C for 30 s, and72°C for 30 s, with a final extension of 72°C for 5 min. Products from the PCR were visualized using agarose gel electrophoresis following protocols from Pilgrim et al. (2005), Shaw et al. (2003), and Talbot et al. (2012).

Molecular sexing was also conducted using the primers 5'-CGA-GGT-AAT-TTT-TCT-GTT-TAC-T-3'and 5'-GAA-ACT-GAG-TCA-GAG-AGG-C-3' designed by Pilgrim et al. (2005) which amplify the amelogenin gene region found on both sex chromosomes. Reactions were carried out using the thermal profile as in Pilgrim et al. (2005) with 25-500 ng of DNA, 1 U of *Taq* polymerase, 1.0 µM of forward and reverse primer, 2.0 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates, and 1X reaction buffer for a total volume of 15.0 µl. Polymerase chain reaction products were visualized after 4 hours of electrophoresis at 100 volts on 4% agarose gels in a 1X sodium borate buffer using ethidium bromide staining. A midrange DNA ladder (New England Biolabs) was run along with amplified PCR products in order to facilitate the estimation of allele sizes.

The *Zfx* and *Zfy* and amelogenin primer sets amplify regions on both sex chromosomes with chromosome specific size disparities. Polymerase chain reaction products were visualized and assessed by agarose gel electrophoresis, with the presence of two bands indicating the sample came from a male and a single band from a female. The accuracy of

this method was assessed by amplification of target sequences from samples of known gender.

Because of disparities between gender determination upon physical examination and molecular sexing, we only felt confident identifying the gender of samples that produced two highly visible bands (i.e. males). Electrophoresis of samples that produced a single band but were determined to be males upon physical examination were considered males for this study. Preferential amplification of only a single band is a well-documented artifact of PCR, and could easily explain the single visualized band (Chapuis and Estoup 2007; Gagneux et al. 1997). Samples that produced only a single size fragment and for which gender was not determined upon physical examination were conservatively classified as being of unknown gender for this study.

Microsatellite analyses. – Thirteen microsatellite loci were amplified via polymerase chain reaction using primers designed by Dragoo et al. (2009) and Munguia-Vega et al. (2009; Table 1). Forward primers were labeled with an additional sequence of nucleotides in order to facilitate the hybridization within the reaction to fluorescent WellRED oligos (Sigma-Aldrich Corporation) and allow for the detection of amplified products (Schuelke 2000). Polymerase chain reactions were carried out following protocol modified from Munguia-Vega et al. (2009; Tables 2 and 3). Amplified products were screened using a Beckman-Coulter CEQ 8000 automated capillary genotyping system and manually scored using Genetic Analysis System Software, version 9.0 (Beckman-Coulter, Inc.). We tested for Hardy-Weinberg equilibrium and linkage disequilibrium between loci using Fisher's exact tests in GENEPOP (http://genepop.curtin.edu.au/; Raymond and Rousset 1995; Rousset 2008) with Markov chain parameters set to a dememorization of 10,000 for 1,000 batches at

10,000 iterations per batch. We used the program CERVUS 3.0 (Kalinowski et al. 2007) to calculate the number of alleles, observed and expected heterozygosity of each locus, and to test for the presence of null alleles.

Sex-biased dispersal patterns. – The program GenAlEx 6.5 (Peakall and Smouse 2006, 2012) was used to calculate pairwise genetic and geographic distance, as well as to conduct spatial genetic analyses using the genetic spatial autocorrelation option (Banks and Peakall 2012; Peakall et al. 2003). This option uses pairwise comparisons to estimate an autocorrelation coefficient *r* for each specified distance class. We estimated *r* at 2 km intervals up to 16 km to test for spatial autocorrelation and to determine the geographic distance at which it is no longer significant. Distance classes were specified at this interval in order to produce results on as fine a scale as possible while maintaining enough samples in each distance class to have an adequate number of pairwise comparisons for statistical testing (Cullingham et al. 2008). Confidence limits of autocorrelation coefficients were calculated by permutation and bootstrapping (999 iterations). We conducted these analyses in four groups: all skunks, male skunks only, female skunks only, and female and unknown skunks. The last group was assessed because of the low number of confidently identified female skunks. These analyses allowed us to test for female-biased philopatry as female skunks should have higher spatial autocorrelation coefficients than males. This is based on the assumption that female-biased philopatry results in increased genetic similarity among neighboring females (Banks and Peakall 2012).

Population structure. – The program GENELAND version 4.0.3 (Guillot et al. 2005b) is a Bayesian clustering method that implements a Markov chain Monte Carlo (MCMC) algorithm to estimate the number of populations and their spatial boundaries based

on supplied spatial coordinates and multilocus genotypes. This program is similar to the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003), except for the additional incorporation of a spatial parameter. GENELAND has been shown to function well in the detection of populations with low genetic differentiation (Coulon et al. 2006), and we used this program to infer the number of population clusters (*K*) and to detect the presence of any physical barriers to dispersal and gene flow presented by the urban environment.

As suggested by Guillot et al. (2005a), we first ran the algorithm to infer *K* with eight independent runs with 1,000,000 MCMC iterations with a thinning of 100, a maximum rate of Poisson process fixed to 100, an uncertainty radius on the spatial coordinates of 100 m, *K* allowed to vary between 1 and 15, and the maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 300. This was done using the F-model of allelic frequency (allele frequencies assumed to be correlated) as Guillot (2008) showed this model to be most efficacious in the detection of population structure in the presence of low genetic differentiation, as well as the Dirichlet model (allele frequencies assumed to be independent). The number of populations was inferred from the run with the highest posterior probability after a burn-in period of the first 25% of the runs.

For the second step, we ran another eight independent runs of the MCMC with *K* fixed and the same parameters as above in order to infer population membership. The run with the highest posterior probability was selected for further analyses. Maps of posterior probability of population membership were generated with a burn-in of the first 25% of the runs and pixels in the spatial domain for the x and y axes were set to 270 and 300, respectively, so as to avoid having two individuals in the same pixel and to have the same resolution on both axes relative to the study site.

Natal habitat-biased dispersal. – We tested for the presence of natal-biased habitat dispersal through comparisons of land cover at collection sites and pairwise genetic distance. In order to assess land cover, we obtained a digitized map of the city of San Angelo outlining the zoning codes for each lot throughout the city (City of San Angelo Geographic Information Systems Division). These maps were updated by referencing 2010 1-m resolution digital orthophoto quarter-quad grids in ArcGIS (version 10.0, Environmental Systems Research Institute Inc. [ESRI], Redlands, California). Land cover was defined as belonging to one of six classes: (1) Commercial – including offices, retail and personal services, off-site parking lots, and public/semi-public lands with similar patterns of human use (e.g. schools); (2) Industrial – including manufacturing sites and caliche pads with heavy machinery and construction materials; (3) Open – including stretches of undeveloped land, natural areas, vacant lots, agricultural areas, and parks; (4) Residential – including low, medium, and high density housing and mobile homes/parks; (5) Roads – including two-lane residential and commercial streets to major four-lane highways; and (6) Water (Fig. 1). We then created 500-m and 1000-m buffered areas around each collection site and used the software extension Patch Analyst version 5.1 (Rempel et al. 2012) for ArcGIS to calculate percent land cover of each cover class for each of the buffered areas (Fig. 2). A 100% Minimum Convex Polygon (MCP) was constructed around the 1000-m buffered sample locations and the amount of each type of land cover within the MCP was calculated. This was done in order to facilitate comparisons and characterizations of the total available land cover and that surrounding collection sites. We used the MCP to examine land cover availability in order to reduce the bias of areas not sampled during the study (i.e. those areas included in the digitized map in which skunks were not collected).

We calculated Bray-Curtis dissimilarities (Bray and Curtis 1957) between each buffered collection site using the program R version 2.15 (R Development Core Team 2008). We then conducted a Mantel test using Pearson correlation method with 9999 permutations using the matrix of Bray-Curtis dissimilarities and a matrix of pairwise genetic distances in order to elucidate any correlation with habitat similarity and genetic relatedness (Legendre and Legendre 1998; Mantel 1967).

RESULTS

We collected a total of 93 striped skunks between November 2011 and October 2012. The degraded nature of many of the specimens led to inclusion of 64 individuals in the study collected across the sampling area (Fig. 3). Four individuals were collected through live-trapping techniques, and 89 through the salvaging of road-killed animals and collection of euthanized skunks.

We were able to confidently determine the gender of 41 striped skunks, yielding 26 males, 15 females, and 23 unknown. Amplification of the *Zfx* and *Zfy* introns (Shaw et al. 2003) failed to yield detectable size differences in amplified fragments and results from this sexing technique were therefore not useful for gender determination. Amplification of the amelogenin region (Pilgrim et al. 2005) yielded PCR products with detectable size disparities after agarose gel electrophoresis.

Microsatellite amplification of 13 loci resulted in greater than 95% total coverage among all samples across all sites. Four loci were found to deviate from Hardy-Weinberg equilibrium (Table 4), and linkage equilibrium was found among 3 pairs of loci: *Meme5* and *Meme20*, *Meme2C* and *Meph42-15*, and *Meme15* and *Meph22-14*. Null allele frequency estimates suggesting the presence of null alleles (>5%) were found at 4 loci (Table 4). Because the presence of null alleles can lead to an overestimation of genetic distance and subsequent erroneous conclusions regarding population structure (Chapuis and Estoup 2007), these loci were not used in any of the further analyses.

Results for the spatial genetic analyses revealed positive spatial autocorrelation among all samples (Fig.4a). Results from the bootstrap and permutation analyses reveal significant autocorrelation among samples collected < 2km apart (Table 5). Autocorrelation

values from permutation were significant for males collected between 2 and 4 km apart (Fig. 4b, Table 5), but in no other distance class. Permutation values for female and unknown samples indicated significant autocorrelation among samples collected < 2km apart (Fig. 4c, Table 5). Significant values were not obtained when analyses were conducted using only known female samples (Fig. 4d, Table 5).

GENELAND analyses conducted using the F-model (allele frequencies assumed to be correlated) resulted in an inferred *K* of 3. Subsequent MCMC runs with *K* set to 3 resulted in the assignment of all individuals to a single population and the presence of two 'ghost' populations (Guillot et al. 2005a), an artifact of the initial MCMC algorithm. GENELAND analyses conducted using the Dirichlet model (allele frequencies assumed to be independent) failed to detect any genetic clusters and returned an inferred *K* of 1. This precluded the need for additional MCMC runs to assign individuals to populations.

The 500-m and 1000-m buffered areas were similar in land cover composition and differed little from the total available land cover (Fig. 5). Mantel tests conducted to assess similarities between genotypes and the habitat in which samples were collected failed to yield significant results for either the 500-m buffered areas (r=0.04856, p= 0.183) or the 1000-m buffered areas (r=0.03332, p= 0.267).

DISCUSSION

The results of this study indicate a lack of genetic structure across this urban landscape for striped skunks. However, we did detect the presence of genetic spatial autocorrelation at relatively short distances (0-2 km) and among all samples suggesting relatively short dispersal and movement distances for striped skunks in this urban environment. While some skunks have been reported to disperse over great distances (e.g. Andersen 1981; Sargeant et al. 1982), the distance class in which we detected significant autocorrelation is in consensus with purported movement distances of striped skunks in an urban environment (Rosatte et al. 1991, 1992). Talbot et al. (2012) reported that the effect of geographic distance on genetic relatedness they detected in striped skunks in southeastern Canada could be the result of barriers to gene flow rather than geographic distance. We did not specifically test for the effect of any given landscape feature on gene flow or dispersal, however, and this type of analysis could offer further insight into the striped skunk's response to the urban environment (e.g. Magle et al. 2010; Munshi-South 2012).

Evidence for the presence of female-biased philopatry has been reported in striped skunks in a number of studies using both molecular and field-based techniques (Hansen et al. 2003; Sargeant et al. 1982; Talbot et al. 2012), but has not been detected in others (Bjorge et al. 1981; Rosatte and Gunson 1984). We divided samples by gender for further analyses of genetic spatial autocorrelation. In addition to combining all known females, we included analyses on a combined set of female skunks and samples of unknown gender. We feel that this is valid given the ability to confidently assess the gender of male skunks via molecular sexing, but not females. Any differences between males and females would only be more difficult to detect given our methodologies. We were able to detect patterns in genetic spatial

correlation values that suggest female-biased philopatry (Table 5). Significant autocorrelation values were obtained among females and unknown samples within the shortest distance class (0-2 km) and within the second distance class (2-4 km) for males. These values were only supported by permutation statistics and not bootstrapping techniques, and could possibly be an artifact of the small sample sizes that resulted from partitioning by gender. These results merit further investigation given that restricted sex-biased dispersal can have important implications for management strategies. For example, Quaglietta et al. (2013) found limited male dispersal and female-biased philopatry in the Eurasian otter (*Lutra lutra*), and reported that the resulting genetic structure of the population could lead to an increase in regional extirpations and a subsequent decrease in genetic diversity. While there are limited conservation concerns for the ubiquitous striped skunk, understanding these types of population processes is nevertheless important.

The GENELAND analyses failed to detect the presence of any subpopulation structuring across the urban study site. Other studies conducted on striped skunks have found similar results in rural populations over varying spatial scales. In a study conducted in southeastern Canada, Talbot et al. (2012) found no genetic clustering in an area 22,000 km^2. A lack of genetic structure was also found across an area of 61.6 km^2 in northern Texas (Hansen et al. 2003) and Barton et al. (2010) found no genetic sub-structuring and high levels of gene flow across five states in the central Great Plains. Using methods similar to this study, Santonastaso et al. (2012) found genetic structuring in another synanthropic species, the raccoon (*Procyon lotor*) across a much larger metropolitan landscape. They found two major clusters separated by the most densely populated and urbanized area of the study site. Our study did not reveal a similar pattern, but this could be due to the smaller area covered

by our study as well as the decreased extent of urbanization. Future studies should examine the effect of urbanization on population genetic structure on a larger scale, as it is highly possible that striped skunks could exhibit a similar pattern given that they exhibit more negative response to increased urbanization relative to that displayed by raccoons (Ordeñana et al. 2010).

The availability of different land cover types and the average composition of buffered areas around collection sites showed only minor disparities. Furthermore, these values carry little weight given the coarse-grained nature of the habitat quantification used in this study and several sampling biases for which we did not control. A majority of our samples were salvaged road-killed skunks, and were typically found along thoroughfares with relatively high traffic-volumes and high speed limits. These types of roads are likely not found evenly distributed through the study site and are a likely source of bias.

We did not detect the presence of natal habitat-biased dispersal among our samples. This phenomenon has been detected in a number of mammals (Lonsinger 2010; Sacks et al. 2004; Selonen et al. 2007; Wecker 1963), but to our knowledge has never been examined in an urban landscape. Our failure to detect the presence of habitat-dependent genetic structuring could be the result of the methodologies employed rather than the absence of this process. Our characterization of the landscape was a simplified version of the environment encountered by the striped skunks and could quite possibly have not included those landscape factors to which skunks most strongly respond. A more fine-scaled, multivariate approach should be employed to assess the presence of this phenomenon in synanthropic species within an urban environment. As natal habitat-biased dispersal can lead to the propagation of genetic variability via disparities in local selection pressures, it is possible that

as a result of this process species could become more resilient to environmental change (Hedrick 1986; Maynard-Smith and Hoekstra 1980; Sacks et al. 2008; Spichtig and Kawecki 2004). Therefore, a better understanding of this process could have important implications for conservation and wildlife management.

Understanding patterns in genetic structure, dispersal, and wildlife responses to landscape features is necessary for the development of efficacious disease management and control protocols and to the modeling of epidemiological outbreaks (Cullingham et al. 2008; Broadfoot et al. 2001; Santonastaso et al. 2012; Talbot et al. 2012). Our study failed to detect any genetic structure that would aid in the development of these techniques (e.g. identification of anthropogenic barriers to gene flow), but patterns in dispersal distances recovered from this study can help in the parameterization of epidemiological models. Future work should further examine the effects of urbanization on the genetic structure of striped skunk populations as different methodologies could reveal patterns undetected by this study.

Table 1. Thirteen microsatellite loci (Dragoo et al. 2009; Munguia-Vega et al. 2009) amplified for an assessment of the genetic structure of an urban population of striped skunks, *Mephitis mephitis*. Locus name, GenBank accession number, repeat motif, and primer sequences (5'-3') are given. Forward primers were labeled with an additional sequence of 5'-TGTAAAACGACGGCCAGT-3' to facilitate hybridization with a fluorescent probe.

Locus	GenBank	Primer sequences	Repeat Motif
Meme2C	GQ453408	F: CTATGACCCTGGATAAAATGCTT R: CCTGCTTGTGTTCCCTGTCT	(CTTT)14
Meme5	GQ453409	F: CCTGAATGCAGGAGATGGAT R: GATGACTGATTAAAGCAGTCTGCC	(CA)26
Meme15	GQ453410	F: CCAGTCCTGAATGCAGGAGATGGAT R: TCCTTACACGCTCCTTCTGC	(GT)26
Meme20	GQ453411	F: CATGAGCCCTGACAGGTGTA R: TCTTGGAACACTGCATCAAAA	(GT)29
Meme28	GQ453412	F: TCTGCTCTTCGGGAGCTTAG R: CCTGCTCTGGCTTTGTTTTT	(CA)17
Meme75	GQ453413	F: GTGTAGCTCTTCAGAGATGGATAGG R: TTCCAGGATGAACCAGGATG	(GT)22
Meme82	GQ453415	F: TACCCGCTAGTTCCATCCAC R: GAGCCTATATGCCCATCAACA	(CA)15
Meme84	GQ453416	F: GCAAAGGATATATTTGATAAGGGATT R: AATGGCTTTGTTTCCAGCAG	(CA)15
Meme88	GQ453417	F: TAGCAGCAATGCCCACAATA R: CATTCTTTCTGATGGCTGCAT	(CA)24
Meph22-14	EU623423	F: CTTTTGGGTCATTAGTGCATTTATG R: GGAAAGAGGAAAGAAAACCCATG	(GT)24
Meph42-15	EU623429	F: CATTTGAACGATATTCTCCCCATCC R: CTGACGTTTCTCAGCTGTTTAGGAAG	(CA)15
Meph42-67	EU623431	F: CAATATTGTACCCCCTCAAAATCC R: CAACTGTTACAGGGCATTTTATAATTG	(CA)24
Meph42-73	EU623432	F: AAAGGACAATCCCACAGGTCT R: TGGACATGGAATTCTGGTTG	(CA)14

Table 2. Polymerase chain reaction conditions for 13 amplified microsatellite loci used in an assessment of the genetic structure of an urban population of striped skunks (*Mephitis mephitis*). Sets (A-C) indicate different PCR conditions. Fluorescent probes were custom ordered WellRed oligos from Sigma-Aldrich, with sequences matching those attached to the 5' end of forward primers (Table 1). Final reaction volumes were 15 µl with 25-500 ng DNA. Protocol modified from Dragoo et al. (2009) and Munguia-Vega et al. (2009).
Set A: *Meme2C, Meme5, Meme15, Meme20, Meme28, Meme75, Meph42-67*
Set B: *Meme82, Meme84*
Set C: *Meme88, Meph42-73, Meph22-14, Meph42-15*

Reagent	Set A	Set B	Set C
Reaction Buffer	1X	1X	1X
Forward Primer (µM)	0.02	0.04	0.025
Reverse Primer (µM)	0.2	0.2	0.25
Fluorescent Probe (µM)	0.2	0.2	0.25
$MgCl_2$ (mM)	1.5	1.5	1.5
dNTP (mM)	0.8	0.8	0.8
Taq polymerase(unit)	0.1	0.1	0.1

Table 3. Temperature profiles for touchdown polymerase chain reactions used to amplify 13 microsatellite loci used in an assessment of the genetic structure of an urban population of striped skunks, *Mephitis mephitis*.

PCR	Temperature Profiles	Loci
1	94°C for 5 min, **10 cycles** at 94°C for 30s, annealing at **65-56°C** (1°C decrease each cycle), 72°C for 30s, followed by **25 cycles** at 94°C for 30s, 57°C for 30s 72C° for 30s, and a final extension of 72° for 5 min	*Meme2C*, *Meme5*, *Meme15*, *Meme20*, *Meme28*, *Meme75*, *Meph42-67*
2	94°C for 5 min, **10 cycles** at 94°C for 30s, annealing at **65-56°C** (1°C decrease each cycle), 72°C for 30s, followed by **35 cycles** at 94°C for 30s, 57°C for 30s 72C° for 30s, and a final extension of 72° for 5 min	*Meme82*, *Meme84*
3	94°C for 5 min, **15 cycles** at 94°C for 30s, annealing at **65-51°C** (1°C decrease each cycle), 72°C for 30s, followed by **40 cycles** at 94°C for 30s, 55°C for 30s 72C° for 30s, and a final extension of 72° for 5 min	*Meme88*
4	94°C for 5 min, **15 cycles** at 94°C for 30s, annealing at **65-51°C** (1°C decrease each cycle), 72°C for 30s, followed by **30 cycles** at 94°C for 30s, 55°C for 30s 72C° for 30s, and a final extension of 72° for 10 min	*Meph22-15*, *Meph42-15*, *Meph42-73*

Table 4. Characteristics of 13 microsatellite loci (Dragoo et al. 2009; Munguia-Vega et al. 2009) amplified for 64 striped skunks (*Mephitis mephitis*) from San Angelo, Texas. The size range of alleles, number of different alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, estimated frequency of null alleles (F_{NA}), and *p*-values from Hardy-Weinberg probability test are given. Deviation from Hardy-Weinberg Equilibrium is indicated by an asterisk (*).

Locus	Size range	N_A	H_O	H_E	F_{NA}	*p*-value
Meme2C	188-216	8	0.578	0.798	+0.154	0.0000*
Meme5	200-234	11	0.812	0.840	+0.015	0.5518
Meme15	158-184	13	0.867	0.830	-0.029	0.5385
Meme20	174-196	10	0.188	0.773	+0.607	0.0000*
Meme28	154-182	13	0.825	0.869	+0.025	0.0110*
Meme75	152-180	11	0.778	0.757	-0.023	0.3167
Meme82	166-172	4	0.127	0.458	+0.555	0.0000
Meme84	162-194	11	0.793	0.790	-0.003	0.3826
Meme88	155-191	12	0.778	0.752	-0.020	0.2639
Meph22-14	260-288	15	0.841	0.906	+0.031	0.3639
Meph42-15	193-215	12	0.746	0.835	+0.056	0.0269*
Meph42-67	237-271	11	0.797	0.845	+0.038	0.2689
Meph42-73	163-183	8	0.750	0.779	+0.012	0.1390

Table 5. Permutation and bootstrap statistics from genetic spatial autocorrelation analyses among striped skunks (*Mephitis mephitis*) collected between November 2011 and October 2012. The distance classes specified for pairwise comparisons across the study site, number of pairwise comparisons for each of the distance classes (*n*), estimated autocorrelation values (*r*), upper (*U*) and lower (*L*) 95% confidence limits about the null hypothesis of no spatial structure as determined by permutation, probability of significance (*P*), mean bootstrap autocorrelation values, and Upper (*Ur*) and lower (*Lr*) 95% confidence interval about (*r*) as determined by bootstrap resampling are given. Significant *P*-values are indicated by an asterisk (*)

	Distance Class	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16
All samples (n = 64)	*n*	162	389	500	386	308	162	81	23
	r	0.031	0.006	-0.013	-0.011	-0.002	0.015	0.015	0.016
	U	0.020	0.012	0.010	0.012	0.014	0.020	0.029	0.053
	L	-0.018	-0.011	-0.010	-0.013	-0.014	-0.020	-0.029	-0.051
	P	0.004*	0.157	0.993	0.943	0.602	0.062	0.160	0.273
	Mean Bootstrap r	0.030	0.006	-0.013	-0.011	-0.002	0.015	0.014	0.017
	Ur	0.057	0.019	-0.002	0.003	0.012	0.034	0.040	0.066
	Lr	0.005	-0.007	-0.025	-0.026	-0.017	-0.005	-0.011	-0.033
Males only (n=26)	*n*	30	63	92	54	36	25	15	9
	r	-0.016	0.027	-0.012	-0.017	-0.009	0.052	-0.007	0.001
	U	0.051	0.031	0.023	0.033	0.043	0.051	0.068	0.081
	L	-0.044	-0.031	-0.025	-0.036	-0.041	-0.049	-0.072	-0.078
	P	0.751	0.041*	0.836	0.813	0.687	0.024	0.562	0.493
	Mean Bootstrap r	-0.015	0.027	-0.012	-0.017	-0.010	0.052	-0.007	0.001
	Ur	0.053	0.064	0.016	0.030	0.030	0.101	0.053	0.094
	Lr	-0.077	-0.011	-0.045	-0.059	-0.046	0.004	-0.065	-0.085
Female and Unknown Samples (n=38)	*n*	60	118	181	132	126	60	21	5
	r	0.039	-0.002	-0.010	-0.009	0.008	-0.010	0.005	0.073
	U	0.034	0.022	0.016	0.020	0.021	0.032	0.052	0.113
	L	-0.032	-0.022	-0.017	-0.020	-0.022	-0.033	-0.051	-0.124
	P	0.015*	0.576	0.880	0.806	0.225	0.719	0.413	0.101
	Mean Bootstrap r	0.040	-0.002	-0.010	-0.008	0.008	-0.011	0.005	0.070
	Ur	0.086	0.020	0.010	0.016	0.032	0.019	0.048	0.138
	Lr	0.002	-0.024	-0.030	-0.035	-0.018	-0.039	-0.037	0.011
Females Only (n=15)	*n*	14	22	34	17	11	7	-	-
	r	0.020	0.002	-0.007	0.009	-0.051	0.049	-	-
	U	0.062	0.045	0.034	0.055	0.063	0.082	-	-
	L	-0.055	-0.048	-0.034	-0.053	-0.070	-0.076	-	-
	P	0.252	0.467	0.671	0.370	0.923	0.106	-	-
	Mean Bootstrap r	0.020	0.005	-0.009	0.010	-0.050	0.049	-	-
	Ur	0.112	0.043	0.035	0.075	0.044	0.022	-	-
	Lr	-0.063	-0.053	-0.061	-0.037	-0.026	-0.078	-	-

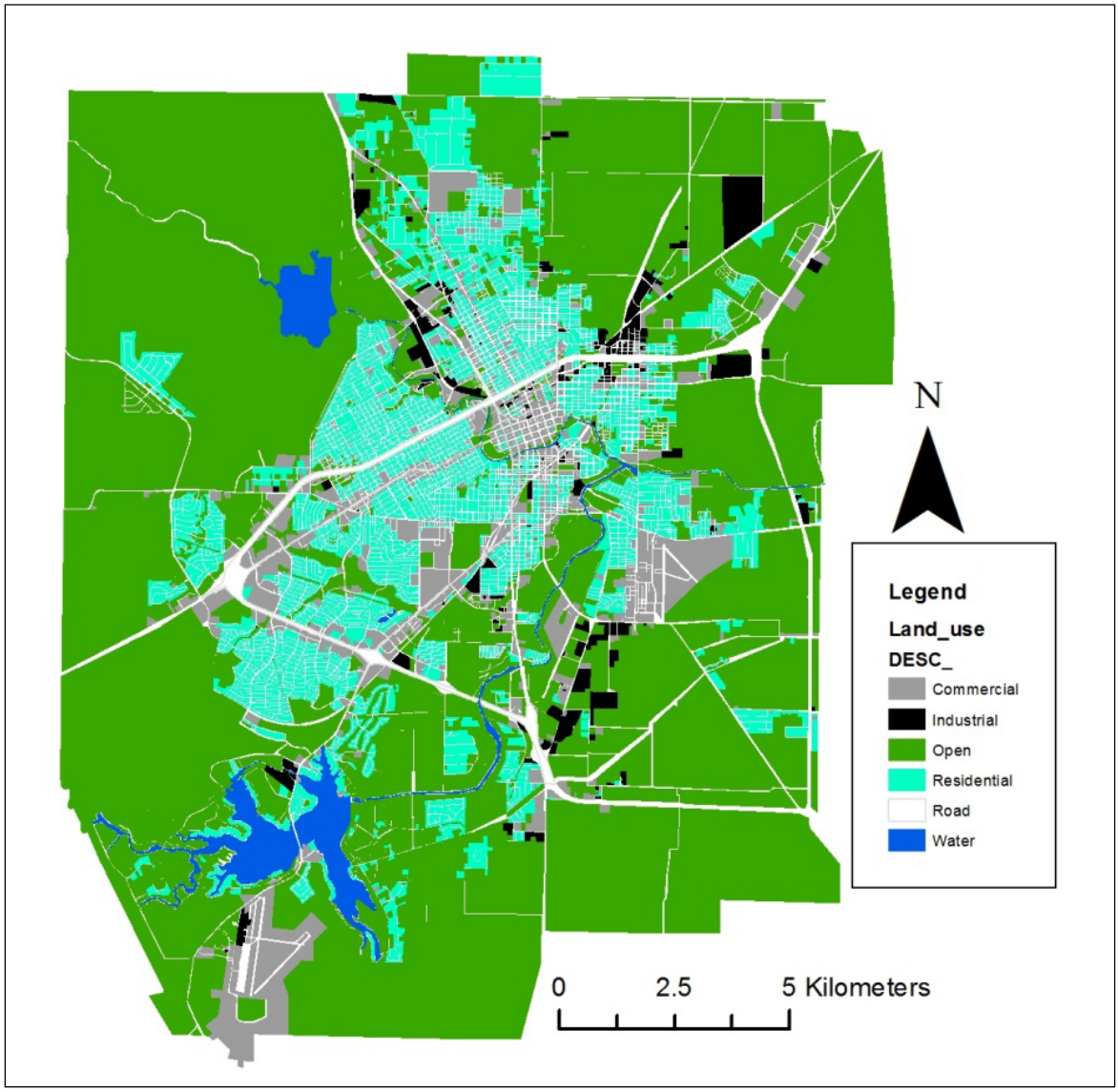

Figure 1. Map depicting land cover categories for San Angelo, Texas used in an assessment of the genetic structure of a striped skunk population in an urban environment between November 2011 and October 2012.

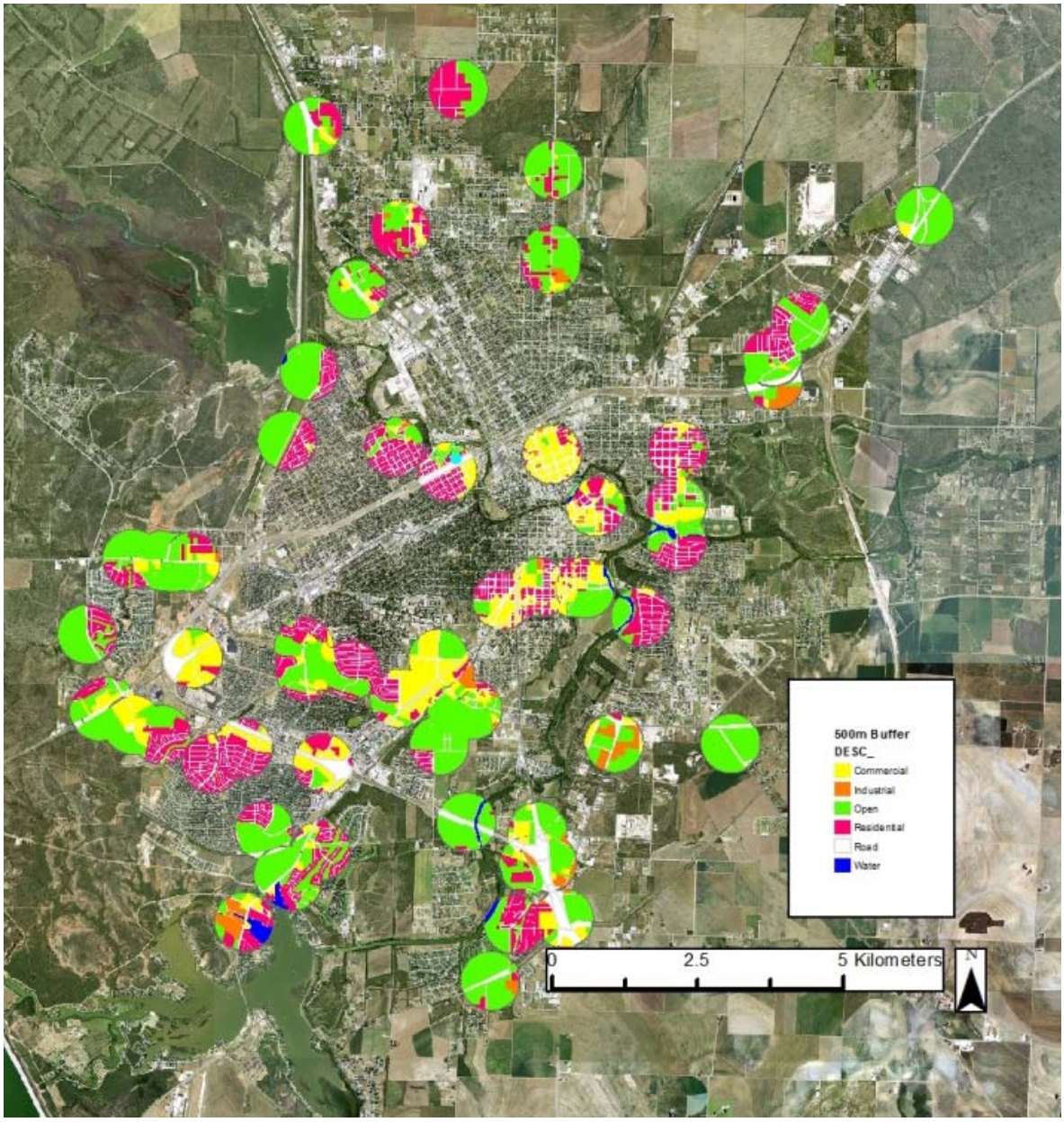

Figure 2. Land cover of 500m buffered area surrounding collection sites of 64 striped skunks in San Angelo, Texas.

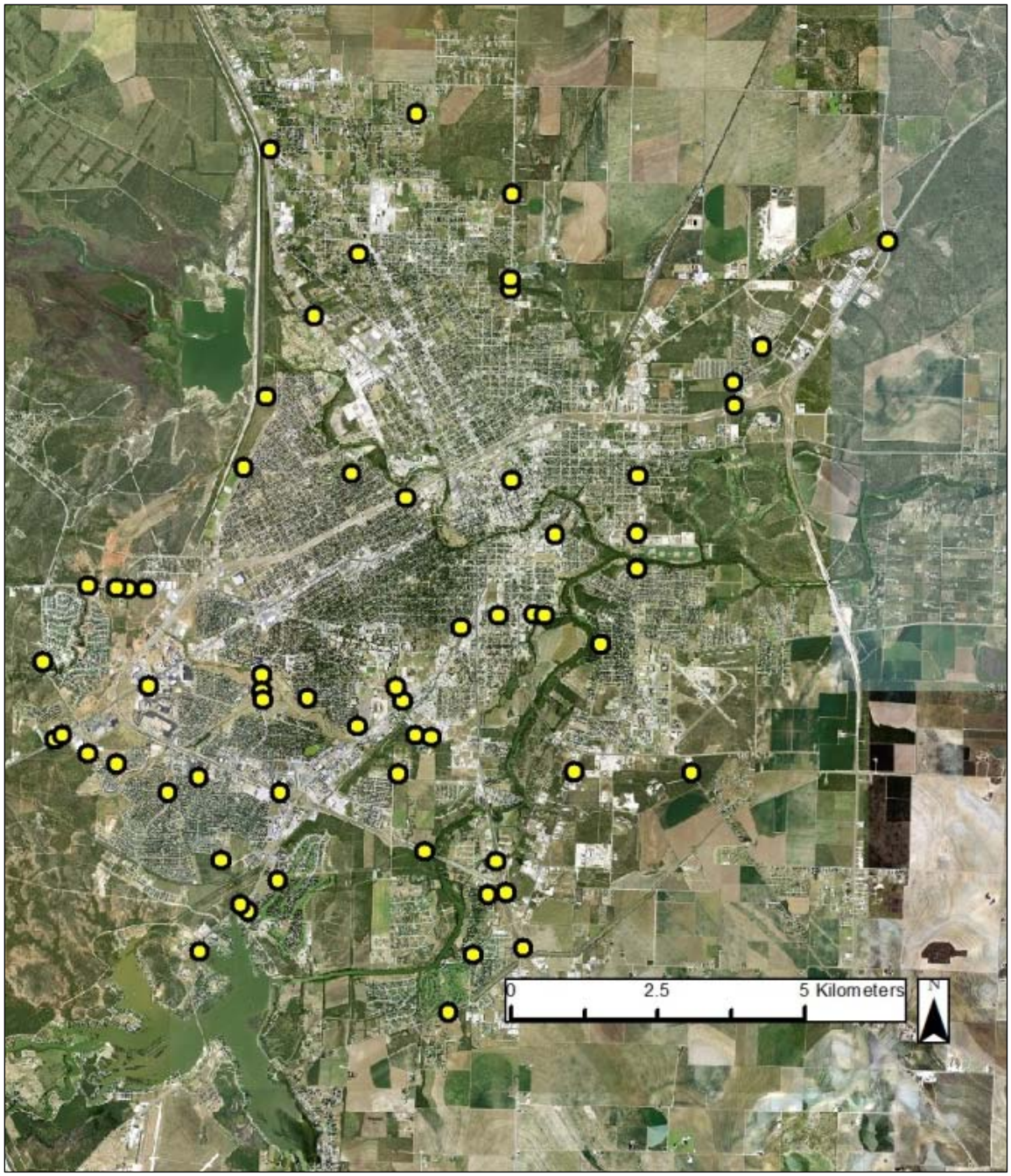

Figure 3. Locations for 64 striped skunks collected between November 2011 and October 2012 in San Angelo, Texas.

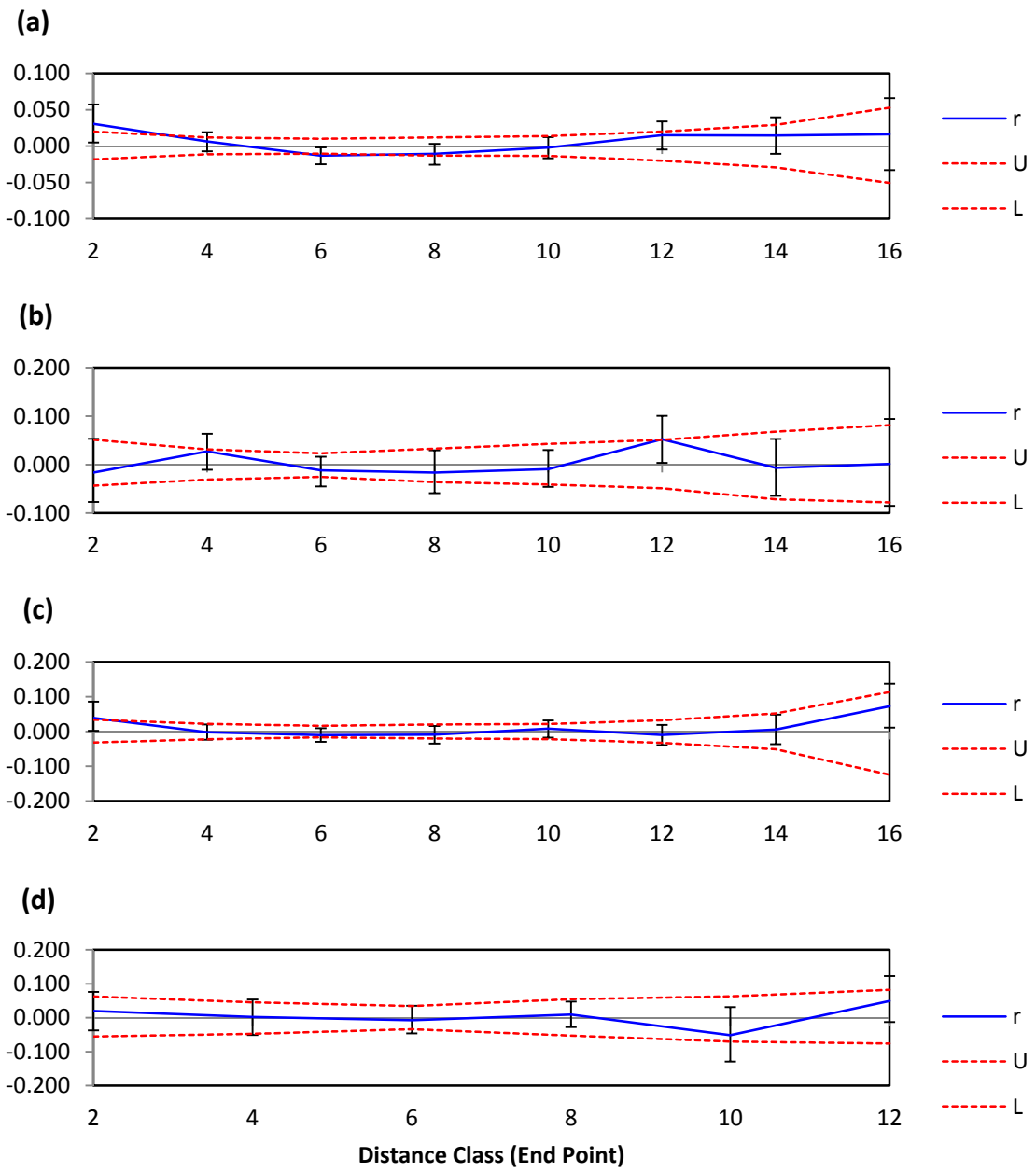

Figure 4. Spatial-autocorrelation values (*r*) calculated among (a) 64 male and female, (b) 26 male, (c) 38 female and unknown, and (d) 15 female striped skunks collected in San Angelo, Texas. Confidence limits for the *r*-values are indicated by vertical error bars and were estimated by bootstrap estimates (999 iterations). Upper (U) and lower (L) 95% confidence limits for the null hypothesis of no spatial autocorrelation ($r = 0$) as estimated by permutation. Distance classes range from samples collected 0-2 km apart (indicated by the value 2 on the x-axis of the correlograms) to samples collected 14-16 km apart (indicated by the value 16 on the x-axis of the correlograms).

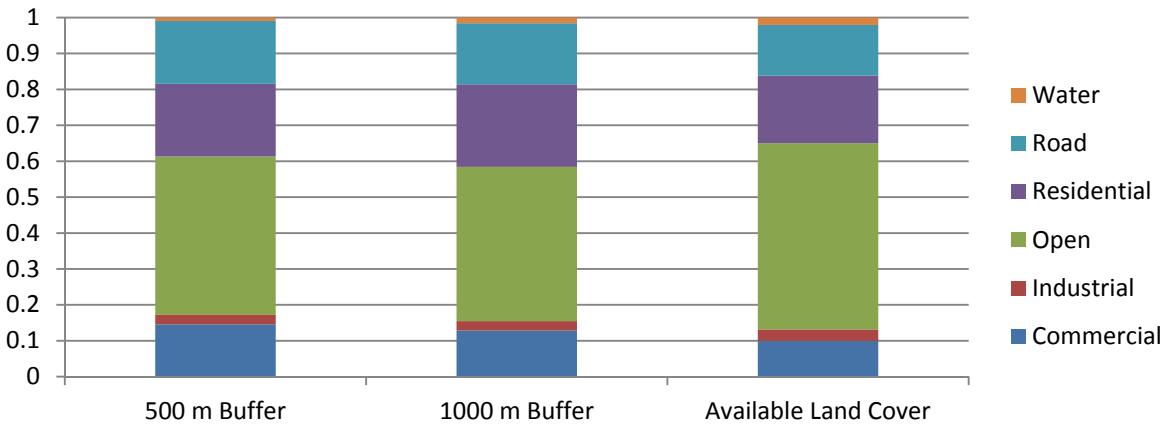

Figure 5. Average habitat composition of 500-m and 1000-m buffered areas around collection sites of 64 striped skunks (*Mephitis mephitis*) in San Angelo, Texas, as well as the habitat composition across the study site.

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VITA

Wesley Allen Brashear was born in San Angelo, Texas to Jimmy and Cindy Brashear. He graduated from Central High School in 2004 and began at Angelo State University the following fall. He graduated from ASU in 2010 with a B.S. in Biology, gaining research experience along the way while conducting an assessment of the home range and denning characteristics of American hog-nosed skunks. Presentation of this work at regional and national meeting led to several awards, including the Rollin H. Baker Award for best overall undergraduate presentation and the William B. Davis Award for best oral presentation in classical mammalogy at the Texas Society of Mammalogists. He was also chosen to present the results of this study for the Wilk's Award competition of the Southwestern Association of Naturalists.

Starting his graduate career at ASU in the fall of 2010, Wesley Brashear was involved in several research endeavors while earning an M.S. in Biology. He gained research experience in molecular techniques and phylogenetic analyses through investigations of the evolutionary history of several taxa. He conducted work on the rodent fauna of the Galapagos Islands as well as the genus *Cheiromeles* in the bat family Molossidae; presentations covering the latter of which earned him awards at the North American Symposium for Bat Research in Puerto Rico in 2012 and the Texas Society of Mammalogists meeting in 2013. Wesley Brashear was selected as the outstanding graduate student of the College of Arts and Sciences for 2013.

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