

HOME RANGE AND GENETICS OF TEXAS HORNED LIZARDS
(*PHRYNOSOMA CORNUTUM*) IN TWO SMALL TOWNS IN SOUTH TEXAS

by

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Introduction

Urbanization converts natural habitats into human-modified ecosystems, often resulting in habitat degradation and fragmentation (Czech et al. 2000; Gardner et al. 2007; Hamer and McDonnell 2010). Physical structures (like roads, fencerows, and unsuitable habitat) divide habitat and can create suitable areas surrounded by poor-quality, unfavorable areas. These, along with other characteristics brought about by urbanization (like exotic species and vegetative homogenization), can inhibit movement by acting as barriers or by negatively affecting biotic requirements of individuals (MacPherson et al. 2011, Wofford et al. 2005). Consequently, movement of individuals in urban areas can be curtailed. Restricted gene flow between fragmented areas creates small, isolated populations with reduced genetic variability, which is critical for the long-term survival of a population (Hitching and Beebee 1997, Anderson et al. 2004, Stow and Sunnucks 2004, Jump and Pañuelas 2006).

For example, Delaney et al. (2010) compared three lizard species and one bird species in a highly urban area in California and found that the presence of one highway produced large genetic differences between populations of the same species on either side of the highway, indicating that it had essentially eliminated movement of these species. In the widely distributed red-backed salamanders (*Plethodon cinereus*), allelic richness and heterozygosity were significantly lower in urban populations than in continuous habitat, and genetic differentiation was significantly high between individuals on either side of an interstate highway while individuals on either side of smaller roads were not significantly differentiated (Noel et al. 2007, Marsh et al. 2008).

Interestingly, barriers significantly altered the genetic structure of these species despite the fact that they are relatively common. Rare species with limited dispersal can be at greater risk to the deleterious effects of urban barriers on genetic diversity and survival (Delaney et al. 2010).

The Texas horned lizard (*Phrynosoma cornutum*) was once abundant throughout Texas, with its native range encompassing much of Texas and Oklahoma, parts of Kansas, Arizona, New Mexico, Louisiana, and northern Mexico (Price 1990, Conant and Collins 1991). The species has declined through much of its range, including a virtual disappearance from eastern and central Texas (Price 1990, Dixon 2000; Henke 2003) and is considered threatened in Texas (Donaldson et al. 1994). Texas horned lizards have remained a wildlife component of some small towns throughout Texas, yet no studies have investigated whether lizards located in these areas may be isolated from outside gene flow or have curtailed movement due to physical structures like roads, buildings, and unsuitable habitat.

An earlier study of genetic diversity in Texas horned lizards revealed that lizards residing within Matagorda Island and the small town of Bastrop, Texas, exhibited significantly lower levels of allelic richness and average heterozygosity than lizards living in the large, protected habitats of the Matador Management Area (WMA) and Yoakum Dunes Wildlife Preserve (Williams and Hale 2010). This suggests that small towns may effectively function as islands in that individuals may be isolated from nearby subpopulations, ultimately reducing their ability to survive and adapt to a changing environment.

A common theme of urbanized areas is the introduction of exotic species and subsequent disruption of native species. In addition to habitat loss, the decline of Texas horned lizards is hypothesized to have arisen due to the introduction of the exotic red fire ant (*Solenopsis invicta*). While these lizards have developed defensive responses to fire ants (sprinting 2.5 – 7 m away from the attack site and rapidly burying themselves), the successive use of insecticides against fire ants has affected multiple ant species, killing the lizard's primary food source, the harvester ant (*Pogonomyrmex* sp.) (Webb and Henke 2003, Price 1990, Donaldson et al. 1994, Dixon 2000). The introduction of fire ants has also been suggested to decrease the abundance of harvester ants due to competitive exclusion (Allen et al. 1994, Porter and Savignano 1990, Wilder et al. 2013). Nonnative prey species from the urban matrix can also negatively affect horned lizards. For example, consumption of invasive Argentine ants (*Linepithema humile*) and arthropods decreased or even ceased growth rates in coastal horned lizards (*P. coronatum*) (Suarez and Case 2002).

Homogenization of vegetation in urban areas can produce large areas that are unfavorable to Texas horned lizards, such as the widespread establishment of nonnative vegetative communities (Gustafson and Gardner 1996, Hamer and McDonnell 2010). Several studies have found that nonnative vegetation can negatively impact thermoregulation, mobility, and sprint velocity of horned lizards (*P. platyrhinos*) (Newbold 2005, Rieder et al. 2010) while McIntyre (2003) found that exotic grasses did not limit harvester ant abundance and may therefore still provide suitable habitat for Texas horned lizards.

In two small towns within Karnes County, Texas, we used radio-telemetry and genetics to determine if small towns are characterized by 1) restricted movement, 2) low genetic diversity, and 3) high genetic differentiation between and within towns. Data on habitat characteristics, including harvester ant abundance and vegetation, were collected to understand habitat features that allow the species to persist in an area. Insight into the movement of urban populations will allow the creation and implementation of management strategies to preserve Texas horned lizards and other urban herpetofauna for future generations.

Methods

Study Sites

We studied Texas horned lizards in two small towns in Karnes County, Texas: Kenedy and Karnes City (Fig. 1). These towns are characterized by recent development and rapid population growth. In 2001, the 77th Texas Legislature declared Kenedy the Horned Lizard Capital of Texas. Since that time, citizen reports and surveys indicate a noticeable decline of the lizards, primarily within the past six years. From June through September 2013, we searched public property areas (including alleyways, fields, parks, schoolyards) and private property lots (when permission from residents was acquired) for Texas horned lizards and their scat.



Figure 1. Karnes County, Texas relative to Bexar County and Travis County.

Comparison Sites

We compared data from Karnes County with three large, protected areas: Rolling Plains Research Ranch (west Texas), the Chaparral Wildlife Management Area (CWMA, Dimmit and La Salle Counties, Texas), and the Marvin and Marie Bomer WMA (Duval County, Texas). These areas are managed for game species. We also compared data from Kenedy and Karnes City with Tinker Air Force Base (AFB). Tinker AFB is located in southeast Oklahoma County and contains suitable habitat surrounded by housing development (Endriss et al. 2007, Wolf et al. 2013).

Abundance, sex ratio, and morphometrics

Searches were conducted when lizards were most active, typically between the hours of 8:00 AM to 11:00 AM and 5:30 PM to 8:00 PM (Moeller et al. 2005). Texas

horned lizards are relatively slow moving and rely on camouflage to avoid predator detection. This typically allows easy capture once visually detected, so we systematically searched areas repeatedly to avoid overlooking individuals. After capture, we measured the mass of individuals in bags to the nearest 0.5 g using a hand-held Pesola® scale and a small bag, measured snout-vent length (SVL) in mm, and identified the sex when possible. Individuals were then marked with a red nail polish dot on the side of the tail in an attempt to avoid recapture. However, the paint dots were not retained following shedding events and some individuals were recaptured and sampled on more than one occasion. Genetic matching allowed identification of these individuals to avoid over-estimation of population size. Individuals were returned at the point of capture.

Deviation from an expected 1:1 sex ratio was tested using a chi-square test. We used t-tests to test whether weight or SVL differed between males and females. All means are presented as mean \pm SE (standard error).

Radio-telemetry

During the 2013 summer active season, we fit individuals with A1065 beaded transmitters (1.4 g) (ATS - Advanced Telemetry Systems). Selection of individuals depended on weight (individuals under 20 g were not tagged), health (lizards with missing body parts were not tagged), and gender (equal numbers of tagged males and females was attempted at each study area). Transmitters were attached to the upper dorsal side using Mega Pro bonding glue (JB Cosmetics) and further secured with a collar made with fishing line covered with IV tubing. Individuals were released at the location of capture. In most cases, the collar prevented detachment of the transmitter from the

individual following shedding events. In these cases, we reattached the transmitter dorsally. When individuals lost their transmitter, we attached the transmitter to another lizard. Using 14 transmitters, we tagged a total of 19 lizards (ten lizards in Karnes City (5F, 5M), six in Kenedy (4F, 2M), and three in a continuous, protected habitat outside of town (2 F, 1M).

We located lizards daily from June through September 2013 using an R-1000 telemetry receiver (Communications Specialists, Inc.) with a Yagi directional antenna (RA-150) and recorded the GPS coordinates of each location. Locations were visually confirmed when possible (92% of locations), and we attempted to observe them from a distance to minimize disturbance to movement. We located lizards at different times each day (from 8:00 AM to 12:00 AM, 12:00 PM to 4:00 PM, or 4:00 PM to 8:00 PM) to reduce any bias that might result from lizards preferring to be in a certain part of their home range during a particular time of day. Lizards were recaptured and transmitters were removed before the end of their expected lifespan (~9 weeks) in September 2013 using Pro-grade gel remover.

Home ranges were constructed using the 95% and 100% Minimum Convex Polygon (MCP) methods calculated using ArcMET 10.1.11 (Wall 2013) in ArcGIS Desktop version 10.1. We calculated MCP areas for individuals that had >19 location points (i.e. 20 or more days of tracking) since home range area appeared to level off for most individuals after this threshold (Appendix 1 and 2). This value is similar to that used in other studies of horned lizards (Rose 1982, Endriss 2007, Wolf 2012, Burrow et al. 2010). We also included an individual for which 17 locations were gathered but whose

sampling curve showed the same pattern as those with 20 location points. We therefore compared a total of 11 individuals from Kenedy and Karnes City with estimates gathered from previous studies.

Home range estimates were compared to studies conducted within Tinker AFB and the Chaparral WMA using the 95% MCP method and to Marvin and Marie Bomer WMA using the 100% MCP method. Estimates for Tinker AFB were taken from Table 1 in Endriss et al. (2007) (May - August 2005) and Wolf et al. (2013) Table 6 for the active season (from April – October, 2003-2011) Estimates from the Chaparral WMA were taken from Burrow et al. (2010) Table 3 (inactive season 1 July to 15 August 1998 - 2000). Estimates for the Marvin and Marie Bomer WMA were taken from Fair and Henke (1999) Table 2 (total area of use column, May – October 1994). We calculated 95% confidence intervals around the means using estimates from Tinker AFB (Wolf et al. 2013), Chaparral WMA (Burrow et al. 2010), and Bomer WMA (Fair and Henke 1998) and the two towns in this study combined. Comparisons for which confidence intervals did not overlap were considered significantly different. We conducted an F test to compare variances between our data and the data from Tinker AFB (Endriss et al. 2007) and Bomer WMA (Fair and Henke 1999), which was provided as raw data. Means are presented as mean \pm SE.

Swab DNA analysis

We obtained DNA samples using a minimally invasive sampling method developed by Williams et al. (2012) using small cotton swabs to gather cells from the cloaca. Swabs were preserved in 300 μ l lysis buffer (75 mM NaCl, 25 mM EDTA, 1% SDS)

until extraction. Cloacal swabbing was not attempted on individuals below 37 mm SVL. When possible, cheek swabs were obtained from individuals between 21 mm and 36 mm SVL. DNA was extracted by adding 10 μ L Proteinase K (20 mg/ml), to the swab-lysis buffer mix then incubated at 55°C for 2-3 hours. The swab was then removed and 1.5 volumes of 7.5 M ammonium acetate was added and placed on ice for 10 minutes to precipitate proteins which were pelleted by centrifugation for 15 minutes. We then added 0.7 volume isopropanol to the supernatant and placed the samples in the freezer overnight to precipitate the DNA. The sample was then centrifuged the next morning for 20 minutes to pellet the DNA. The DNA pellet was washed with 70% ethanol and then allowed to dry before resuspending in 100 μ L 10 mM Tris-HCl pH 8.5.

We genotyped individuals at 14 microsatellite loci developed using methods described in Williams and Hale (2010) and Williams et al. (2012) and scored the genotypes using GeneMapper 5.0 (Life Technologies). We also amplified a 454 bp section of the mitochondrial control region (d-loop) using the primers HLCR_F: 5'-CTTATGATGGCGGGTTGCT-3' and HLCR_R: 5'-GGCTGTAAATTTATCCTCTGGTG-3'. Polymerase chain reactions (PCR) (10 μ L) contained 10-50 ng DNA, 0.5 μ M of each primer, 1X Qiagen Multiplex PCR Master Mix with HotStarTaq, Multiplex PCR buffer with 3mM MgCl₂ pH8.7, and dNTPs. Reactions were cycled in an ABI 2720 thermal cycler. The cycling parameters were one cycle at 95°C for 15 min, followed by 30 cycles of 30s at 94°C, 90s at 55°C, 90s at 72°C, and then a final extension at 72°C for 5 minutes. Products were sequenced using ABI Big Dye Terminator Cycle Sequencing v3.1 Chemistry (Life Technologies) using the PCR primers. Sequences were electrophoresed on an ABI

3130XL Genetic Analyzer (Life Technologies); edited, contiged, and trimmed using Sequencher v5.0 (Gene Codes USA); and then aligned in MEGA 6.0 (Tamura et al. 2013) using Muscle (Edgar 2004). Haplotypes were identified using GenAIEx v6.5 (Peakall and Smouse 2012), using a 359 bp sequence.

Fecal DNA analyses

We also collected fresh fecal pellets of Texas horned lizards (easily recognized by the cylindrical shape of the feces) and preserved the samples in 1.0 mL 8M Urea buffer (10 mM Tris pH 7.5, 125 mM NaCl, 10 mM EDTA, 1% SDS, 8 M urea) (Asahida et al. 1996) until extraction. DNA extraction followed the protocol outlined in the QIAamp DNA Stool Mini-kit (Qiagen Genomics, Valencia, CA). A negative control was made with each round of extraction to ensure non-contamination of reagents. Extractions were conducted in an extraction dedicated AirClean® 600 PCR workstation.

For fecal DNA samples PCR reactions were conducted in a separate room from DNA extractions in a PCR dedicated AirClean® 600 PCR workstation. Negative controls were used in all PCR reaction batches. PCR cycling parameters were the same as for swabs except they were run for 40 cycles instead of 30. All samples were initially run in triplicate for all microsatellite loci. We then created consensus genotypes for each individual using the comparative method (Hájková et al. 2009). We accepted homozygotes if we observed that genotype at least 3X and a heterozygote if we observed those alleles at least 2X. If a sample did not meet one of these criteria then we amplified it again 3X to determine the consensus genotype. Loci that failed to have a consensus after 6X were scored as missing. Consensus genotypes were constructed

manually for each individual. Genotypes were discarded if the consensus had only six or fewer loci. Genotypes were considered identical when they matched at all loci or all but one locus. We used the multilocus analysis in GenAEx v6.5 to find genotyping matches. A smaller section of the control region was also amplified using the primers HLCR_F: 5'-CTTATGATGGCGGGTTGCT-3' and HLCRr446b: 5'-CGGCTGTTAAATTTATCCTCTGGT-3' and then sequenced.

We used GIMLET v. 1.3.2 to determine error rates for each locus including the proportion of successful PCRs across all replicates, the proportion of allele dropout (ADO) which occurs when an allele of a heterozygous individual does not amplify in a PCR that is positive for the other allele, and the incidence of false alleles (FA) which occurs when a homozygote from the consensus genotype is typed as a heterozygote from repeated genotypes (Broquet and Petit 2004).

We tested for Hardy-Weinberg and genotypic linkage equilibrium using GENEPOP v4.2 (Rousset 2008). We calculated the number of alleles, allelic richness, observed (H_O) and expected heterozygosity (H_E) for populations in Kenedy, Karnes City, Rolling Plains, and Tinker AFB using FSTAT 2.9.3.2 (Goudet <http://www2.unil.ch/popgen/softwares/fstat.htm>). We tested for differences in allelic richness and heterozygosity between populations using t- tests for unequal variance. Heterozygosity was arcsin transformed before statistical testing.

We used GenAEx v6.5 to conduct AMOVAs and to estimate mitochondrial haplotype frequencies and diversity. We used STRUCTURE (Pritchard et al. 2000, Pritchard and Wen 2003) to cluster individuals without location data. We ran the Monte

Carlo Markov Chain (MCMC) for 500,000 iterations following a burn-in period of 50,000 iterations for $K = 1 - 8$ using the correlated allele frequencies model and assuming admixture (the default values) 10 times. The most likely K was then estimated using the method of Evanno et al. (2005).

We also calculated the probability of identity (PI) and probability of identity for siblings (PIsibs) using GenAlEx v6.5 for the swab samples. PI is the probability that two individuals drawn at random from a population will have identical multilocus genotypes. The PIsibs is the probability that a parent or offspring of a particular individual or their siblings would have the same genotype (Hájková et al. 2009, Wood et al. 1999).

We used the program BOTTLENECK (Cornuet and Luikart 1996, Piry et al. 1999) to test for the genetic signature of a recent reduction in the effective population size (N_e) in both localities. Populations that have experienced a reduction in N_e are expected to have excess heterozygosity (H_E) relative to that expected under mutation-drift equilibrium (H_{eq}). This occurs because allelic richness is lost at a significantly faster rate than heterozygosity after a population reduction. As recommended by Piry et al. (1999), H_{eq} was calculated using the two phase mutation model (TPM) with a probability of 95% for single step mutations (SMM) and 5% multi-step mutations since this model is believed to better approximate mutations at microsatellite loci than a pure stepwise mutation model (SMM) (Di Renzo et al. 1994). A Wilcoxon sign-rank test was then used to determine if a significant number of loci exhibited excess heterozygosity. We also used a graphical method to look for evidence of a bottleneck by plotting the number of alleles in seven allele frequency categories (Luikart et al. 1998). A population that has

not experienced a bottleneck is expected to show an L-shaped distribution (many low frequency alleles and few high frequency alleles), while bottlenecked populations will exhibit a mode shift.

Habitat characteristics

For all areas searched, we recorded the GPS location of all harvester ant mounds with evidence of activity. On September 27 and October 14, 2013, we conducted vegetation sampling in areas currently with and without evidence of Texas horned lizards using the step-point method (Bonham 2013) (31 plots total). We purposely avoided areas in which Texas horned lizards had not been detected in past surveys such as manicured lawns planted with St. Augustine grass (*Stenotaphrum secundatum*) or in areas that had been cleared for construction. All sites with and without horned lizards were mowed at irregular intervals. A transect was placed diagonally across the plots and data were collected by placing an aluminum pin (~1 cm dia) every 1 m along the transects. All vegetation that touched the pin at each point was recorded to species (category L1), or if no vegetation was present we categorized the ground as bare soil, gravel, organic litter, or cement. We also recorded any tree cover above each point (category L2). We collected data for 1,354 points in areas with and 1,177 points in areas without Texas horned lizards. We categorized species as native and exotic using the Native Plants Database (<http://www.wildflower.org/plants/>) and the USDA PLANTS Database (<http://plants.usda.gov/java/>). We then compared the proportion of exotic and native grasses, litter, bare ground, L1 and L2 species between areas with and without horned lizards. We also used EstimateS 9.1.0 (<http://purl.oclc.org/estimates>)

(Colwell 2013) to calculate diversity indices of vegetation in areas with and without evidence of horned lizards using the Chao 1, Shannon, and Simpsons Inverse diversity indices.

Results

Morphometrics and Sex Ratio

We captured 125 individuals in Kenedy (22 total; 12 females: 5 males; 5 unknown), Karnes City (98 total; 45 females: 28 males; 22 unknown; 3 dead), and the ranch (5 total; 3 females; 1 male; 1 unknown). Females were significantly heavier than males (33.32 ± 1.70 SE and 28.32 ± 1.59 g, respectively; $p = 0.03$) and had a greater SVL than males (75.96 ± 1.14 and 69.43 ± 1.32 mm, respectively; $p < 0.001$; Fig. 2). The sex ratio in Kenedy and Karnes City was female-biased, 57:34 (39 unknown) ($\chi^2_1 = 5.8$. $p = 0.016$).

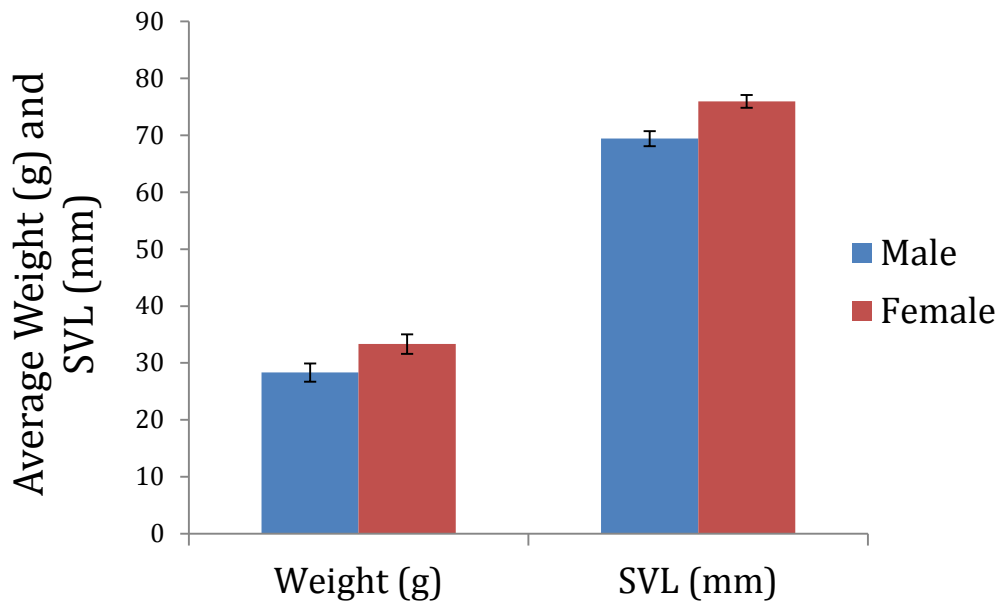


Figure 2. Average weight and snout-vent length of male and female lizards in Karnes County. Bars are means \pm SE.

Home Range

In Kenedy and Karnes City, the average home range size was 0.24 ha using 95% MCP and 0.40 ha using 100% MCP (Appendix 3); and the two towns did not differ significantly from each other ($t = 0.005$, $N = 11$, $df = 9$, $p = 1.00$ using 95% MCP, and $t = 0.04$, $N = 11$, $df = 9$, $p = 0.97$ using 100% MCP). Within town, males (0.24 ± 0.10 ha using 95%, 0.33 ± 0.13 ha using 100% MCP) and females (0.24 ± 0.09 ha using 95% and 0.44 ± 0.21 ha using 100% MCP) did not differ significantly in home range size ($t = 0.04$, $N = 11$, $df = 9$, $p = 0.97$ using 95% MCP, and $t = -0.43$, $N = 11$, $df = 9$, $p = 0.67$ using 100% MCP).

Average home range size of lizards in Kenedy and Karnes City did not differ significantly from those within Tinker AFB (0.50 ± 0.09 ha using 95% MCP, $t = 1.76$, $p = 0.09$) (Endriss 2007), however the 95% confidence limits around estimates for home range size within towns (0.13-0.35 ha) did not overlap with those for the larger data set from Tinker AFB (0.55-1.33 ha) (Fig. 3) (Wolf 2013). Confidence limits did overlap with those of the Chaparral WMA (0.23-0.83 ha) (Fig. 3). Average home range size of lizards in Kenedy and Karnes City also did not differ significantly from those within Bomer WMA (0.73 ± 0.21 ha using 100% MCP, $t = 1.42$, $p = 0.18$, Fig. 4). While variance in home range size of individuals within the two small towns did not differ significantly from each other ($df = 3$, $F = 0.48$, $p = 0.29$ using 95% MCP and $df = 3$, $F = 0.29$, $p = 0.17$ using 100% MCP) or from Bomer WMA ($df = 8$, $F = 2.14$, $p = 0.13$ using 100% MCP), they possessed significantly lower variance in home range size than Tinker AFB ($df = 23$, $F = 4.37$, $p = 0.01$ using 95% MCP).

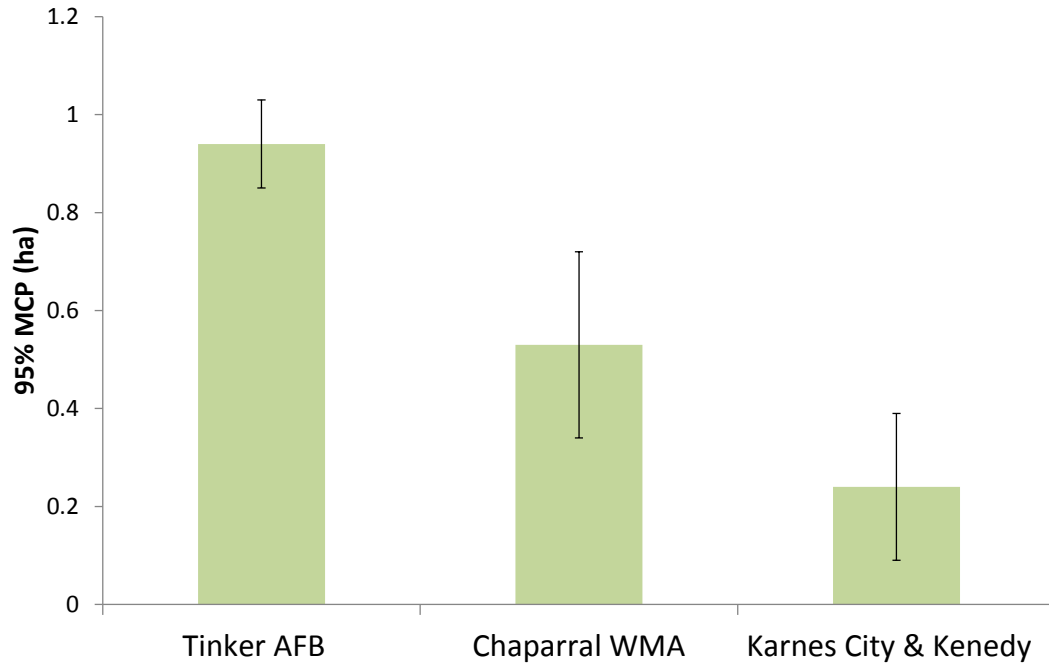


Figure 3. Home range size of lizards in Kenedy and Karnes City (N = 11) compared to those in Tinker AFB (N = 31) during two study periods and the Chaparral WMA (N = 35) using 95% MCP method. Bars are means \pm SE.

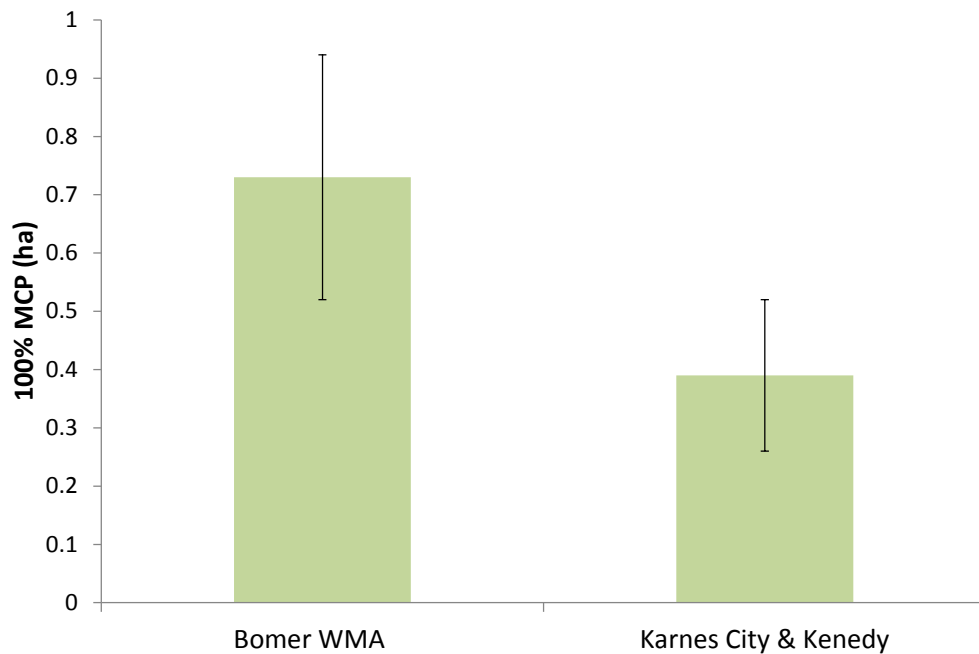


Figure 4. Home range size of lizards Karnes County (N = 11) compared to those in Bomer WMA (N = 9) using the 100% MCP method. Bars are means \pm SE.

Out of the 11 lizards for which at least 17 locations were observed, about half (55%) had home ranges that did not cross a road. Three lizards crossed a road once, one lizard crossed a road twice, and one lizard crossed at least five times. The majority (98%) of all telemetry points (N = 421) were within the street block that surrounded a horned lizard's territory and only 2% were located across a street in another block.

Habitat Characteristics

All lizards were located within 39.10 ± 1.31 m and 14.51 ± 1.65 m of an ant mound in Kenedy and Karnes City, respectively (Fig. 5 and 6).

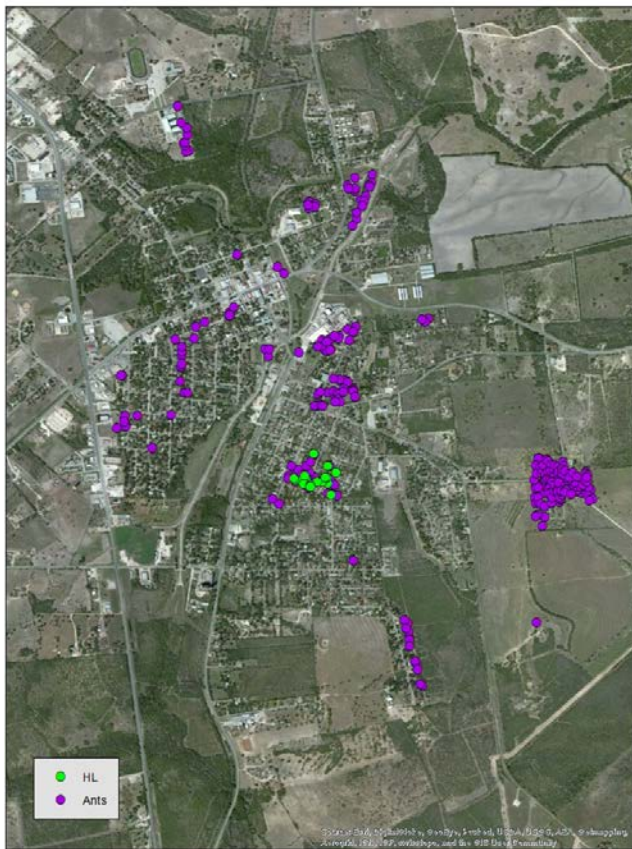


Figure 5. Kenedy, Texas. Each green dot indicates the presence of a Texas horned lizard while each purple dot indicates the presence of a harvester ant nest.

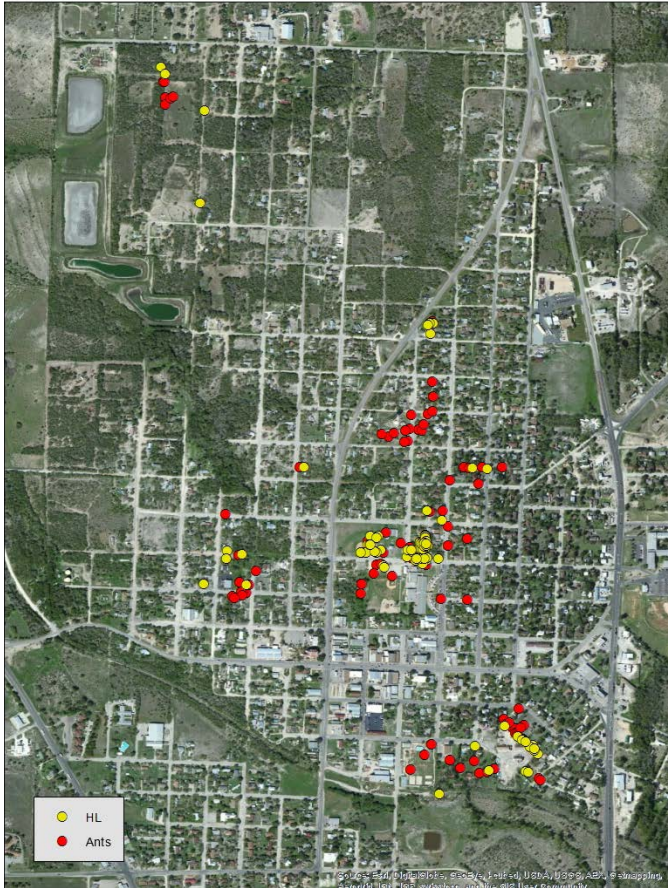


Figure 6. Karnes City, Texas. Each yellow dot indicates the presence of a Texas horned lizard while each red dot indicates the presence of a harvester ant mound.

Areas with horned lizards had a higher number of species with single occurrences (uniques) in all categories. The most common native grass species was plains bristle grass (*Setaria vulpisetia*; N = 63) followed by tumble windmill grass (*Chloris verticillata*; N = 47) in plots with horned lizards and tumble windmill grass (N = 60) followed by buffalo grass (*Bouteloua dactyloides*; N = 58) in plots without horned lizards (Fig. 7). In areas both with and without horned lizards, the most common exotic grass was Bermuda grass (*Cynodon dactylon*; N = 221 and N = 287, respectively; Fig. 8).

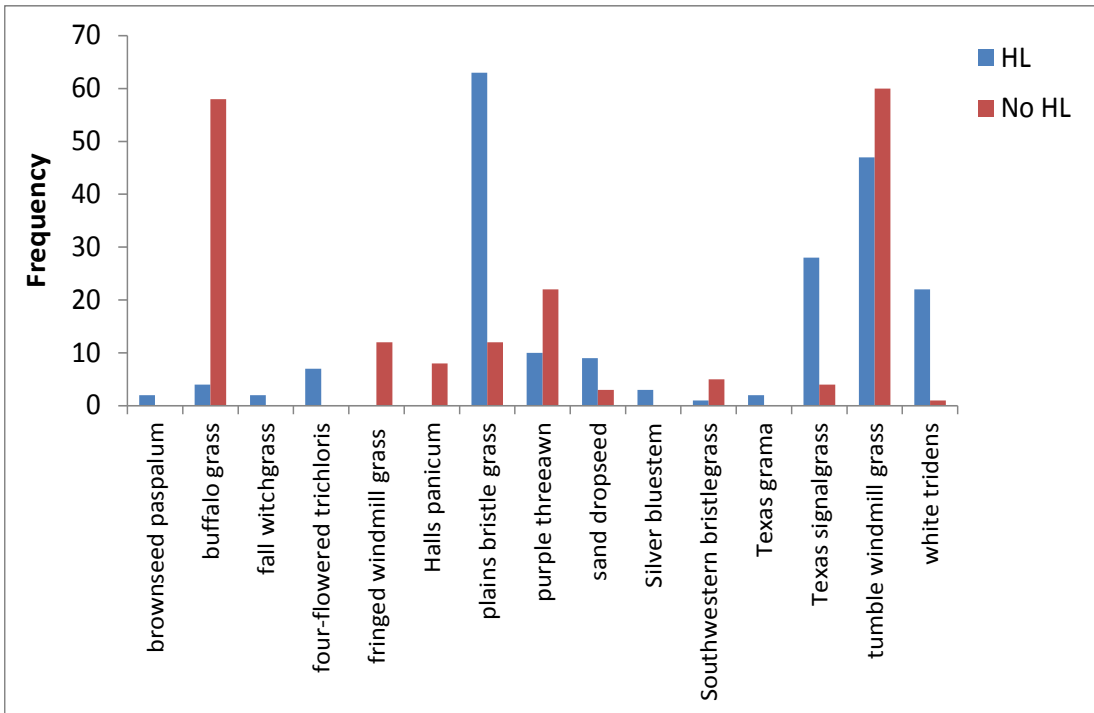


Figure 7. Frequencies of native grass species found in areas with (blue) and without (red) Texas horned lizards.

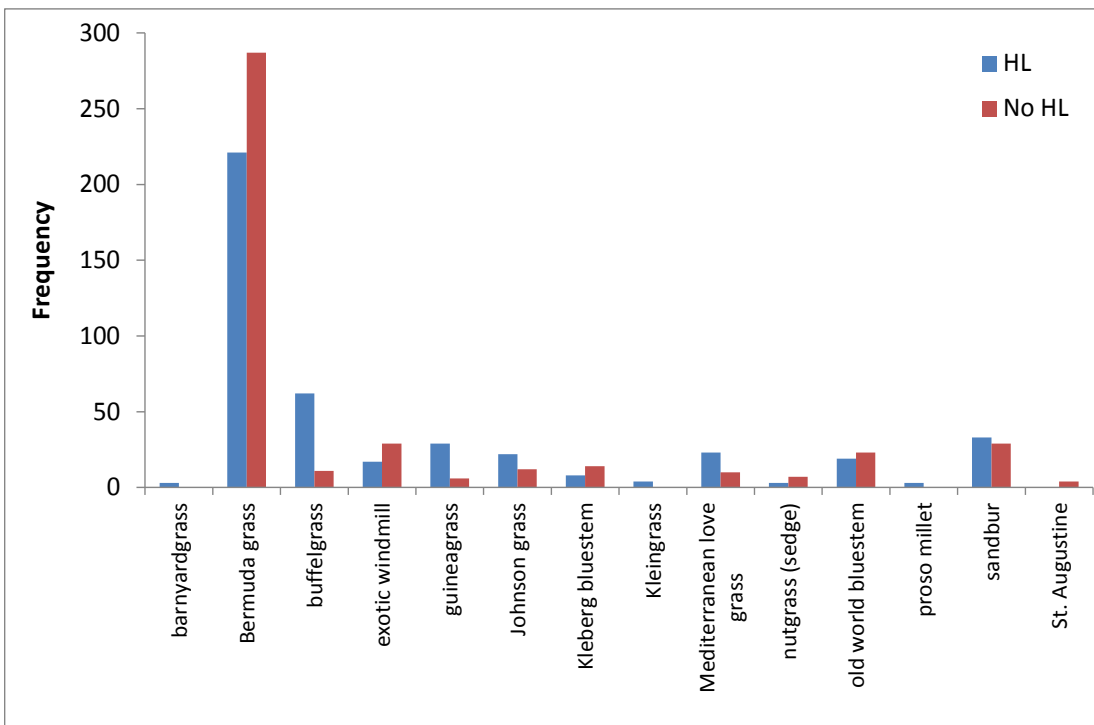


Figure 8. Frequencies of exotic grass species found in areas with (blue) and without (red) Texas horned lizards.

Diversity of L1 and L2 species was similar between the compared areas and diversity of grasses (native and exotic) was higher in areas without horned lizards than in areas with horned lizards (Table 1). A list of L1 and L2 species can be found in Table 2 and Table 3.

Table 1. Diversity indices of ground vegetation (L1), canopy vegetation (L2), all grass, exotic grass, and native grass species in areas with and without horned lizards.

Treatment	Cat	Uniques Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Shannon Mean	Simpson Inv Mean
HL	L1	71.36	81.36	74.11	107.74	3.19	13.39
No HL	L1	48	100.33	97.11	112.86	3.35	14.7
HL	L2	11.92	15.89	12.58	37.15	1.63	3.98
No HL	L2	10	19.49	17.37	33.95	1.75	4.22
HL	Grass	3.01	24.76	25.22	26.28	2.29	5.34
No HL	Grass	0	31	31	33.36	2.42	5.47
HL	Grass Exotic	11.92	11.92	11.92	12.53	1.54	2.81
No HL	Grass Exotic	4	14	14	14.85	1.62	2.81
HL	Grass Native	11.38	11.38	11.67	12.77	1.83	4.74
No HL	Grass Native	7	15	15	16.75	2.1	6.19

Table 2. Ground vegetation (L1) species in plots with and without horned lizards.

Common Name	Scientific Name	HL	No HL
Alamo vine	<i>Merremia dissecta</i>	12	1
African coral vine	<i>Antigonon leptopus</i>	0	2
anacua	<i>Ehretia anacua</i>	8	0
annual sunflower	<i>Helianthus annuus</i>	2	1
barnyardgrass	<i>Echinochloa crusgalli</i>	3	0
Bermuda grass	<i>Cynodon dactylon</i>	221	287
bindweed	<i>Convolvulus equitans</i>	1	5
brownseed paspalum	<i>Paspalum plicatulum</i>	2	0
buffalo grass	<i>Bouteloua dactyloides</i>	4	58
buffelgrass	<i>Cenchrus ciliaris</i>	62	11
chili petin	<i>Capsicum annuum</i>	1	0
Chinaberry tree	<i>Melia azedarach</i>	0	0
common purslane	<i>Portulaca oleracea</i>	1	0
common ragweed	<i>Ambrosia artemisiifolia</i>	4	0
Drummond's woodsorrel	<i>Oxalis drummondii</i>	13	0
<i>Euphorbia</i> sp	<i>Euphorbia</i> sp.	83	9
exotic <i>Lilium</i> sp.	<i>Lilium</i> sp.	0	1
exotic windmill grass	<i>Chloris</i> sp.	17	29
fall witchgrass	<i>Digitaria cognata</i>	2	0
false ragweed	<i>Parthenium hysterophorus</i>	34	11
four-flowered trichloris	<i>Trichloris pluriflora</i>	7	0
four o'clock	<i>Mirabilis jalapa</i>	0	1
giant ragweed	<i>Ambrosia trifida</i>	11	8
goathead	<i>Tribulus terrestris</i>	11	1
Gregg's tubetongue	<i>Justicia pilosella</i>	8	14
guineagrass	<i>Urochloa maxima</i>	29	6
Halls panicum	<i>Panicum hallii</i>	0	8
honey mesquite	<i>Prosopis glandulosa</i>	15	8
huisache	<i>Acacia farnesiana</i>	1	0
Illinois bundleflower	<i>Desmanthus illinoensis</i>	60	26
Johnson grass	<i>Sorghum halepense</i>	22	12
Kairn's sensitive-briar	<i>Mimosa latidens</i> Small	0	7
Kleberg bluestem	<i>Dichanthium annulatum</i>	8	14
Kleingrass	<i>Panicum coloratum</i>	4	0
<i>Lantana</i> sp.	<i>Lantana</i> sp.	2	0
little mallow	<i>Malva parviflora</i>	3	0
live oak	<i>Quercus virginiana</i>	0	4
lotebush	<i>Ziziphus obtusifolia</i>	1	0
low wild mercury	<i>Argythamnia humilis</i>	2	0

Common Name	Scientific Name	HL	No HL
manzanita	<i>Malpighia glabra</i>	0	14
mealy sage	<i>Salvia farinacea</i>	0	7
Mediterranean love grass	<i>Eragrostis barrelieri</i>	23	10
milkweed vine	<i>Matelia</i> sp.	1	0
morning glory	<i>Evolvulus</i> sp.	0	1
Morus sp.	<i>Morus</i> sp.	2	0
<i>Nama</i> sp.	<i>Nama</i> sp.	0	2
netleaf hackberry	<i>Celtis reticulata</i>	0	2
nutgrass (sedge)	<i>Cyperus rotundus</i>	3	7
old man's beard	<i>Clematis drummondii</i>	7	2
old world bluestem	<i>Bothriochloa</i> sp.	19	23
pigeonberry	<i>Rivina humilis</i>	1	0
pigweed, lamb's-quarters	<i>Chenopodium album</i>	154	70
plains bristle grass	<i>Setaria vulpiseta</i>	63	12
prickly pear	<i>Opuntia ellisiana</i>	2	0
prickly-leaf dogweed	<i>Thymophylla acerosa</i>	2	0
prickly-mallow	<i>Sida spinosa</i>	9	5
proso millet	<i>Panicum miliacem</i>	3	0
purple threeawn	<i>Aristida purpurea</i>	10	22
Purslane	<i>Portulaca umbraticola</i>	2	0
rain lily	<i>Cooperia pedunculata</i>	34	40
retama, Jerusalem thorn	<i>Parkinsonia aculeata</i>	2	0
ruellia	<i>Ruellia nudiflora</i>	30	14
sand dropseed	<i>Sporobolus cryptandrus</i>	9	3
sandbur	<i>Cenchrus incertus</i>	33	29
scarlet sage	<i>Salvia coccinea</i>	1	1
Scutellaria sp.	<i>Scutellaria</i> sp.	1	0
shaggy portulaca	<i>Portulaca pilosa</i>	7	4
<i>Sida</i> sp.	<i>Sida</i> sp.	0	4
Silver bluestem	<i>Bothriochloa laguroides</i>	3	0
silverleaf nightshade	<i>Solanum elaeagnifolium</i>	12	25
slender yellow woodsorrel	<i>Oxalis dillenii</i>	1	1
pigweed sp.	<i>Chenopodium</i> sp.	1	0
Southwestern bristlegrass	<i>Setaria scheelei</i>	1	5
spiny hackberry, granjeno	<i>Celtis pallida</i>	13	1
Spurge sp.	<i>Euphorbiaceae</i> fam.	0	6
St. Augustine	<i>Stenotaphrum secundatum</i>	0	4
sticky florestina	<i>Florestina tripteris</i>	5	0
straggler daisy	<i>Calyptocarpus vialis</i>	167	71
sugar hackberry	<i>Celtis laevigata</i>	9	4

Common Name	Scientific Name	HL	No HL
talinum	<i>Talinum paniculatum</i>	2	5
Texas grama	<i>Bouteloua rigidiseta</i>	2	0
Texas millet/Texas signalgrass	<i>Urochloa texana</i>	28	4
Texas nightshade	<i>Solanum triquetrum</i>	8	3
three-lobed false mallow	<i>Malvastrum coromandelianum</i>	192	163
<i>Tradescantia</i> sp.	<i>Tradescantia</i> sp.	7	6
<i>tridens</i> sp.	<i>Tridens</i> sp.	2	0
tropical amaranth	<i>Amaranthus polygonoides</i>	56	0
tumble windmill grass	<i>Chloris verticillata</i>	47	60
Unknown	N/A	63	11
white tridens	<i>Tridens albescens</i>	22	1
widow's tears	<i>Commelina erecta</i>	4	1
wild onion	<i>Allium canadense</i>	0	1
wild poinsettia	<i>Euphorbia cyathophora</i>	3	2
yellow ground cherry	<i>Physalis viscosa</i>	1	1
yellow indian mallow	<i>Abutilon malacum</i>	1	0

Table 3. Canopy vegetation (L2) species in plots with and without horned lizards.

Common Name	Scientific Name	HL	No HL
anacua	<i>Ehretia anacua</i>	47	26
Chinaberry tree	<i>Melia azedarach</i>	1	2
Euphorbia sp	<i>Euphorbia</i> sp	1	0
honey mesquite	<i>Prosopis glandulosa</i>	112	42
Juniper	<i>Juniperus</i> sp.	0	2
live oak	<i>Quercus virginiana</i>	1	23
manzanita	<i>Malpighia glabra</i>	1	0
Mexican ash	<i>Fraxinus berlandieriana</i>	6	10
<i>Morus</i> sp.	<i>Morus</i> sp.	2	0
netleaf hackberry	<i>Celtis reticulata</i>	4	0
one-seed Croton	<i>Croton monanthogynus</i>	0	1
Palm sp.	<i>Arecaceae</i> fam.	0	3
spiny hackberry, granjeno	<i>Celtis pallida</i>	1	1
sugar hackberry	<i>Celtis laevigata</i>	35	56
three-lobed false mallow	<i>Malvastrum coromandelianum</i>	1	0
Unknown	N/A	26	0
wild poinsettia	<i>Euphorbia cyathophora</i>	0	1

Areas with and without horned lizards had a similar amount of ground vegetation (L1) and canopy vegetation (L2) (Fig. 9) and similar frequencies of bare ground, litter, native grass, and exotic grass (Fig. 10).

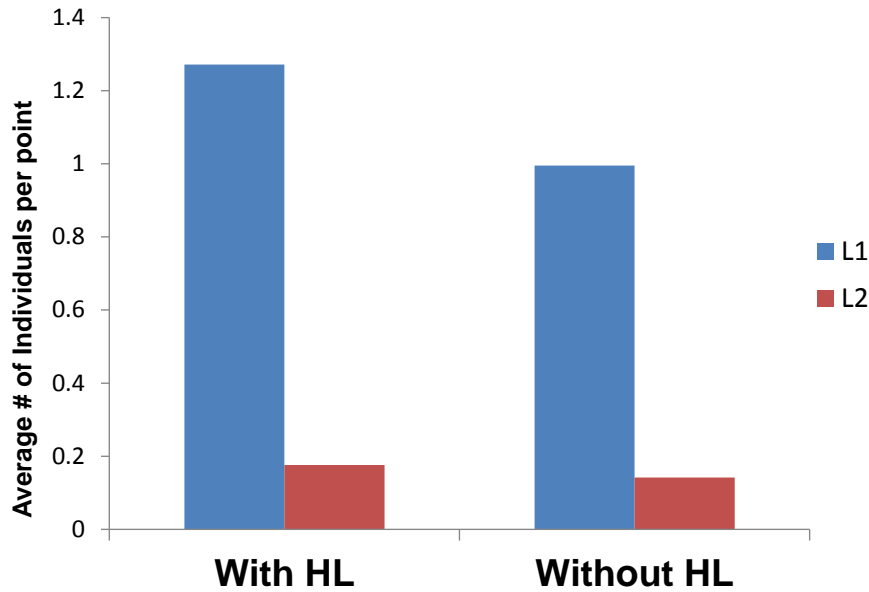


Figure 9. Average number of individuals touching the point at ground level (L1, blue) and canopy level (L2, red) species in areas with and without Texas horned lizards.

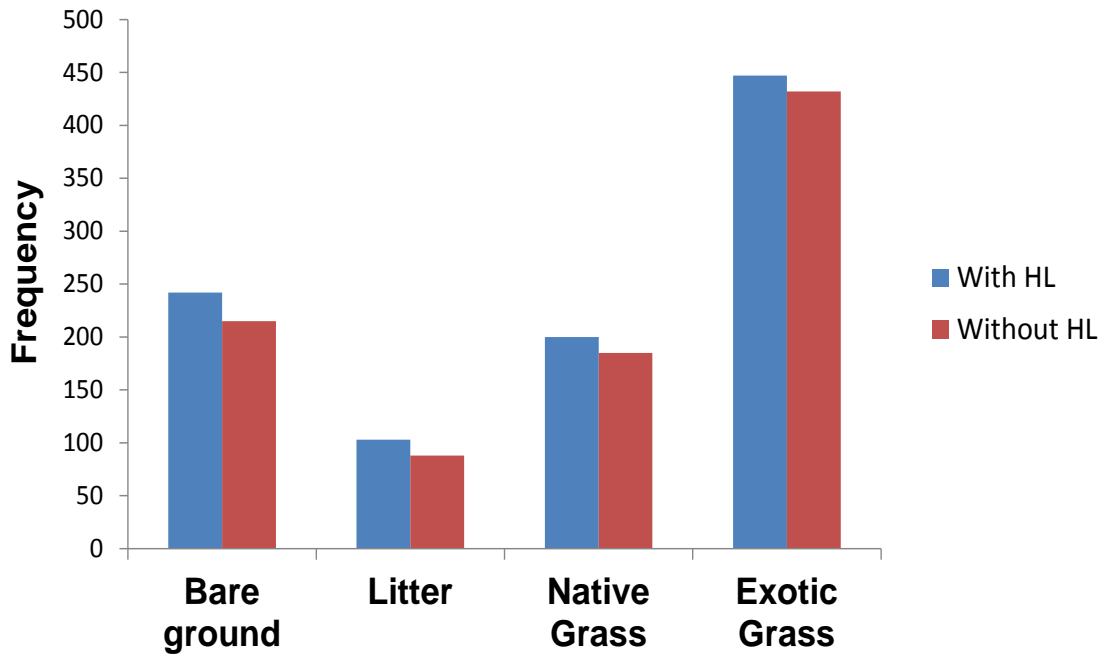


Figure 10. Frequency of bare ground, litter, and native, exotic, and unknown grasses in areas with (blue) and without (red) Texas horned lizards.

Genetics

We found 27 sets of genetic matches between samples, with a maximum of 5 matching samples. Duplicates were removed from all analyses. Out of the 102 extracted scat samples, 55 consensus genotypes with more than 7 loci were obtained. The consensus genotypes had on average 12 loci and 86% had more than 9 loci. On average, the success rate of amplifying a locus was 48%, allele dropout rate was 10%, and rate of false alleles was 2% (Table 4). Without duplicates, we analyzed 76 swab and 17 scat samples from Karnes City (N = 93), 18 swab and 11 scat samples from Kenedy (N = 29), 5 swab and 3 scat samples from the ranch (N = 8).

Table 4. The PCR success rate, rate of allele dropout, and rate of false alleles for replicated amplifications of DNA extracted from feces.

	Success Rate	Allele Dropout	False Alleles
Pc41	0.6	0.079	0.023
Pc49	0.51	0.145	0.05
Pc60	0.49	0.076	0.009
Pc61	0.58	0.115	0.034
PcD01	0.58	0.114	0.015
PcD09	0.55	0.099	0.076
Pc70	0.52	0.129	0.016
PcD14	0.47	0.043	0
PcD20	0.45	0.043	0.022
PcD52	0.54	0.085	0.003
Pc83	0.4	0.065	0.013
PcD26	0.44	0.093	0.026
PcD31	0.27	0.171	0.011
PcD53	0.38	0.148	0.031
Mean	0.48	0.100	0.024

For all 14 loci, the probability of identity (PI) and probability of identity of siblings (PIsibs) was 2.2×10^{-14} and 2.2×10^{-5} for Kenedy and 7.2×10^{-20} and 3.6×10^{-7} for Karnes City. The probability of identity and probability of identity of siblings begins to level off at 7 loci, and so we used genotypes with 8 or more loci (Fig. 11).

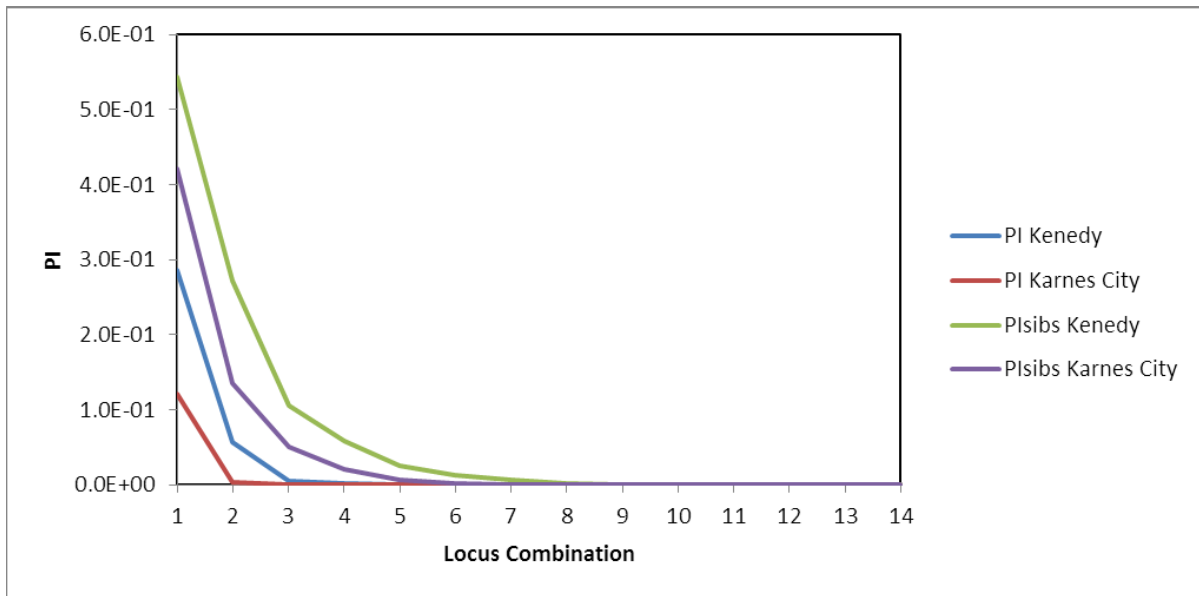


Figure 11. The probability of identity and probability of identity of siblings at varying number of loci.

The Rolling Plains population exhibited significant heterozygote deficits at the Pc49 locus, the Kenedy population at Pc49, Pc70, and PcD31 loci, and the northeast Karnes City population at Pc49, Pc61, PcD20, PcD26, PcD31, and PcD53 loci (Table 5).

Table 5. Number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Fis) of populations in Rolling Plain (N = 56), Tinker AFB (N = 31), Kenedy (N = 29), and Karnes City (N = 93) at each of the 14 microsatellite loci. Asterisks represent loci with heterozygote deficits.

Pop	Locus	N	Na	Ho	He	F
Rolling Plains	Pc41	56	11	0.875	0.845	-0.045
	Pc49	53	25	*0.887	0.926	0.033
	Pc60	54	10	0.778	0.808	0.028
	Pc61	56	15	0.875	0.885	0.002
	PcD01	56	15	0.875	0.887	0.005
	PcD09	54	11	0.833	0.876	0.040
	Pc70	56	9	0.804	0.824	0.016
	PcD14	53	14	0.906	0.861	-0.062
	PcD20	53	17	0.830	0.910	0.079
	PcD52	55	7	0.764	0.770	-0.001
	Pc83	55	16	0.745	0.901	0.165
	PcD26	56	9	0.857	0.870	0.006
	PcD31	52	11	0.788	0.829	0.040
	PcD53	56	14	0.661	0.657	-0.015
Tinker AFB	Pc41	36	7	0.889	0.794	-0.135
	Pc49	36	15	0.833	0.897	0.058
	Pc60	34	7	0.765	0.723	-0.073
	Pc61	36	14	0.833	0.842	-0.003
	PcD01	33	13	0.909	0.876	-0.054
	PcD09	33	6	0.848	0.814	-0.058
	Pc70	35	8	0.800	0.784	-0.035
	PcD14	34	9	0.853	0.788	-0.099
	PcD20	35	12	0.943	0.895	-0.069
	PcD52	35	5	0.571	0.674	0.140
	Pc83	36	12	0.806	0.820	0.004
	PcD26	35	6	0.771	0.816	0.041
	PcD31	34	7	0.765	0.775	-0.001
	PcD53	32	11	0.781	0.877	0.096
Kenedy	Pc41	28	6	0.679	0.619	-0.115
	Pc49	27	12	*0.370	0.715	0.472
	Pc60	24	6	0.625	0.731	0.127
	Pc61	29	9	0.517	0.636	0.172
	PcD01	29	12	0.724	0.696	-0.059
	PcD09	29	6	0.586	0.532	-0.120
	Pc70	29	6	*0.448	0.678	0.327
	PcD14	26	9	0.808	0.858	0.040
	PcD20	25	11	0.880	0.739	-0.215
	PcD52	29	8	0.759	0.757	-0.019

Pop	Locus	N	Na	Ho	He	F
NE Karnes City	Pc83	25	7	0.400	0.545	0.251
	PcD26	26	8	0.231	0.376	0.375
	PcD31	19	6	*0.316	0.612	0.470
	PcD53	25	11	0.760	0.884	0.123
	Pc41	61	7	0.705	0.757	0.061
	Pc49	62	14	*0.581	0.782	0.251
	Pc60	60	5	0.750	0.678	-0.116
	Pc61	63	8	*0.397	0.596	0.329
	PcD01	61	9	0.738	0.792	0.061
	PcD09	61	9	0.770	0.784	0.009
	Pc70	62	9	0.823	0.791	-0.048
	PcD14	60	9	0.733	0.779	0.050
	PcD20	59	13	*0.729	0.890	0.175
	PcD52	62	10	0.710	0.765	0.065
NW Karnes City	Pc83	63	13	0.794	0.833	0.040
	PcD26	60	10	*0.750	0.830	0.089
	PcD31	55	11	*0.636	0.807	0.204
	PcD53	61	12	*0.770	0.853	0.089
	Pc41	13	6	0.615	0.822	0.221
	Pc49	13	9	0.692	0.775	0.071
	Pc60	13	4	0.615	0.655	0.023
	Pc61	13	5	0.385	0.729	0.451
	PcD01	13	8	0.692	0.785	0.082
	PcD09	13	4	0.923	0.711	-0.351
	Pc70	12	5	0.667	0.768	0.094
	PcD14	13	5	0.692	0.794	0.093
	PcD20	13	8	0.923	0.818	-0.173
	PcD52	13	5	0.769	0.742	-0.079
S Karnes City	Pc83	12	11	0.667	0.819	0.150
	PcD26	13	6	0.769	0.822	0.026
	PcD31	10	7	0.400	0.742	0.433
	PcD53	13	7	0.692	0.785	0.082
	Pc41	17	3	0.353	0.551	0.340
	Pc49	16	7	0.875	0.792	-0.140
	Pc60	16	3	0.563	0.542	-0.071
	Pc61	17	4	0.706	0.699	-0.041
	PcD01	16	6	0.875	0.837	-0.080
	PcD09	17	5	0.765	0.709	-0.111
	Pc70	17	4	0.412	0.357	-0.190
	PcD14	16	4	0.563	0.627	0.074
	PcD20	16	8	0.875	0.853	-0.059
	PcD52	17	4	0.647	0.642	-0.039

Pop	Locus	N	Na	Ho	He	F
	Pc83	16	8	0.813	0.804	-0.043
	PcD26	17	4	0.706	0.763	0.047
	PcD31	16	6	0.500	0.764	0.325
	PcD53	17	5	0.471	0.766	0.367

All loci were in genotypic linkage equilibrium. Observed gene diversity was higher than expected under equilibrium conditions only for Tinker AFB for the TPM (one-tailed Wilcoxon test for H_E excess, $P=0.008$). None of the sites exhibited a mode shift in allele frequency categories. Allelic richness averaged 7.71 (range 4.00 – 10.67) and 7.43 (range 5.04 – 10.39) for Karnes City ($N = 93$) and Kenedy ($N = 29$), respectively, 7.25 (range 4.50 – 9.37) for Tinker AFB ($N = 31$), and 10.38 (range 6.30 – 16.59) for Rolling Plains ($N = 56$). Allelic richness was significantly lower in Kenedy ($t = 3.23$; $p = 0.004$), Karnes City ($t = 2.91$; $p = 0.008$), and Tinker AFB ($t = 3.46$; $p = 0.002$) than in the natural Rolling Plains site (Fig. 12).

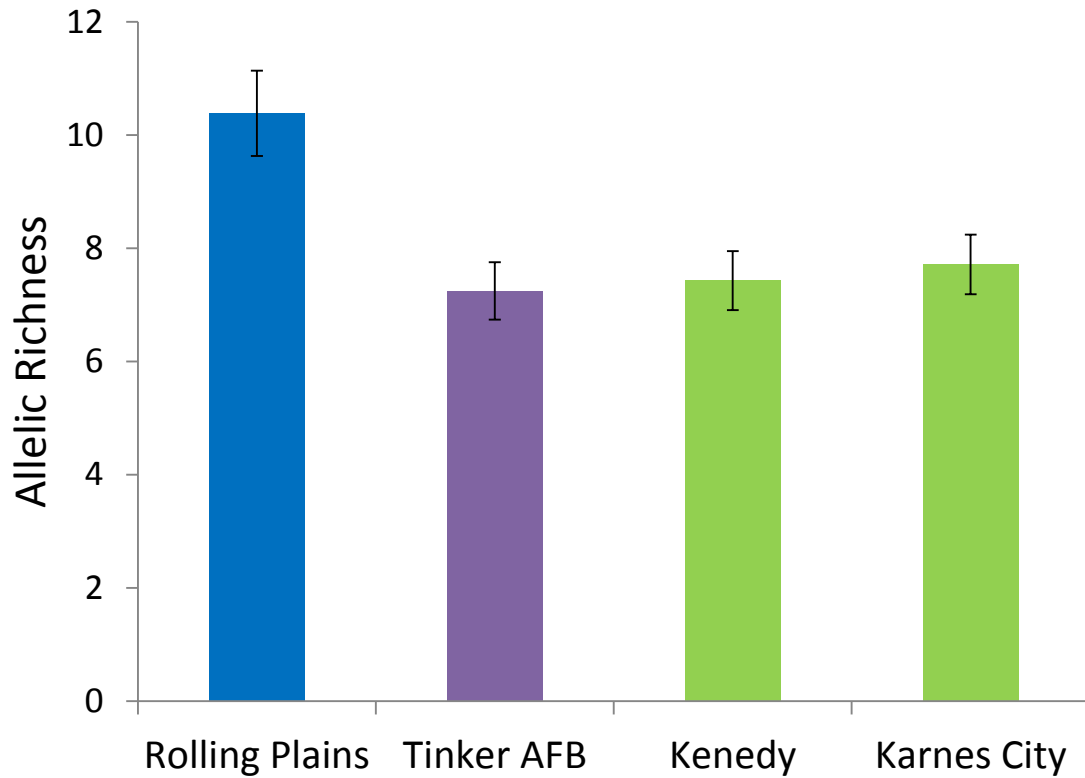


Figure 12. Allelic richness in Karnes City (N = 93) and Kenedy (N = 29) compared to Rolling Plains (N = 56) and Tinker AFB (N = 31). Allelic richness was significantly lower in Kenedy, Karnes City, and Tinker AFB compared to Rolling Plains.

Observed heterozygosity averaged 0.69 (range 0.45 – 0.79) and 0.58 (range 0.23 – 0.88) in Karnes City (N = 93) and Kenedy (N = 29), respectively, 0.84 (range 0.63 – 0.93) in Tinker AFB (N = 31), and 0.82 (range 0.66 – 0.91) in Rolling Plains (N = 56). This was significantly lower in Kenedy ($t = 4.74$; $p < 0.001$ and $t = 4.42$; $p < 0.001$) and Karnes City ($t = 4.78$; $p < 0.001$ and $t = 4.44$; $p < 0.001$) than in Tinker AFB or Rolling Plains (Fig. 13).

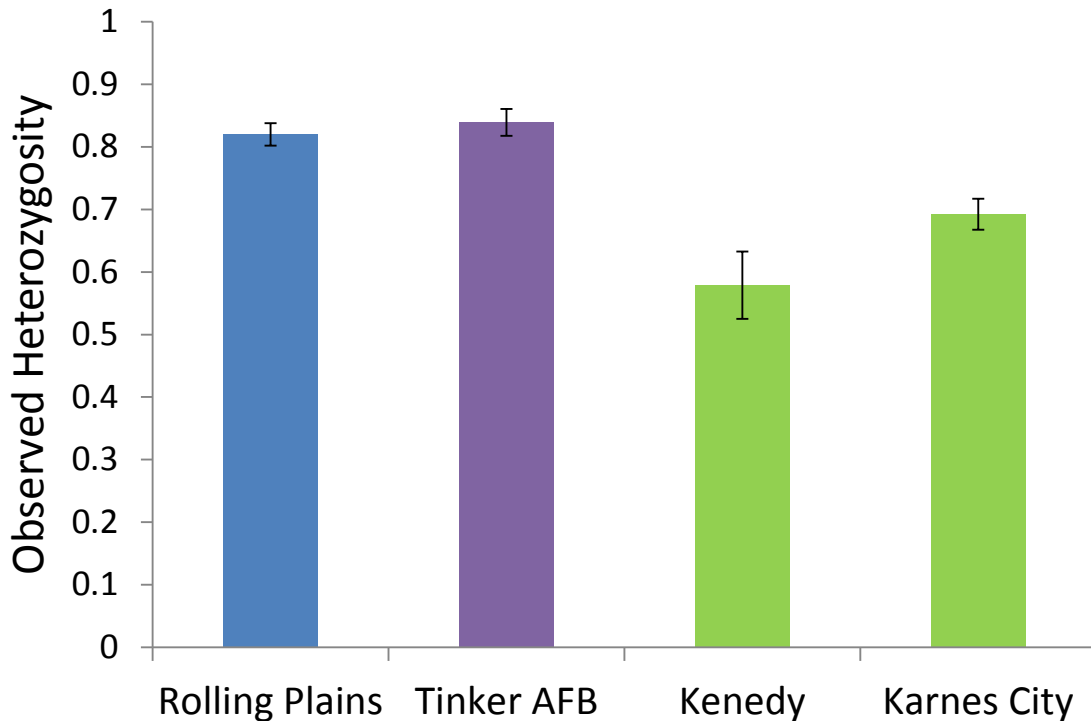


Figure 13. Observed heterozygosity in Karnes City (N = 93) and Kenedy (N = 29) compared to Rolling Plains (N = 56) and Tinker AFB (N = 31). Observed heterozygosity was significantly lower in Kenedy and Karnes City compared to Tinker AFB and Rolling Plains ($p < 0.001$).

Genetic differentiation of microsatellite genotypes between Karnes City and Kenedy was moderately high and significant ($F_{st} = 0.12$; $p < 0.001$). Comparisons between these small towns with populations in the Rolling Plains and Oklahoma revealed genetic differentiation of similar magnitudes (Table 6 and 7).

Table 6. AMOVA table of microsatellite allele frequencies from horned lizards in Kenedy (N = 29), Karnes City (N = 93), Rolling Plains (N = 56), and Tinker AFB (N = 31).

Source	df	SS	MS	Est. Var.	%	P-value
Among Pops	3	173.448	57.816	0.661	11%	0.001
Among Indiv	164	991.388	6.045	0.456	7%	0.001
Within Indiv	168	862.500	5.134	5.134	82%	0.001
Total	335	2027.336		6.250	100%	

Table 7. Pairwise F_{st} of allele frequencies calculated for lizards between Kenedy, Karnes City, Rolling Plains, and Tinker AFB.

	Rolling Plains	Tinker AFB	Kenedy
Tinker AFB	0.07		
Kenedy	0.12	0.17	
Karnes City	0.08	0.11	0.12

We also found significant genetic differentiation within Karnes City between individuals northwest, northeast, and south of two intersecting roads Calvert Ave State Highway 181 (west to east) and Panna Maria Ave (north to south) (Fig. 14). Individuals were significantly different northwest and northeast ($F_{st} = 0.03$; $p < 0.001$), northeast and south ($F_{st} = 0.07$; $p < 0.001$), and northwest and south ($F_{st} = 0.10$; $p < 0.001$) of these intersecting roads.

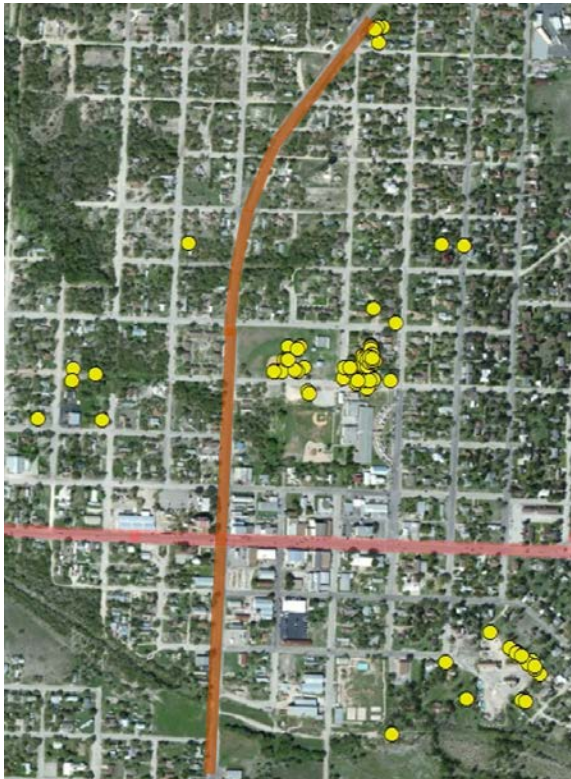


Figure 14. Yellow dots indicated Texas horned lizards sampled in Karnes City. Calvert Ave State Highway 181 runs east to west and Panna Maria Ave runs north to south.

Both the asymptote of $L(K)$ and the method of Evanno et al. 2005, indicated that $K = 5$ was the most likely number of clusters (Fig. 15). Four of these clusters separate individuals from the Rolling Plains, Tinker AFB, Kenedy, and Karnes City. The fifth cluster included individuals from a ranch near Kenedy and seven individuals from Karnes City. Several individuals from Kenedy also had relatively high ancestry in this cluster. One individual from Kenedy was strongly assigned to the Karnes City cluster (Fig. 16).

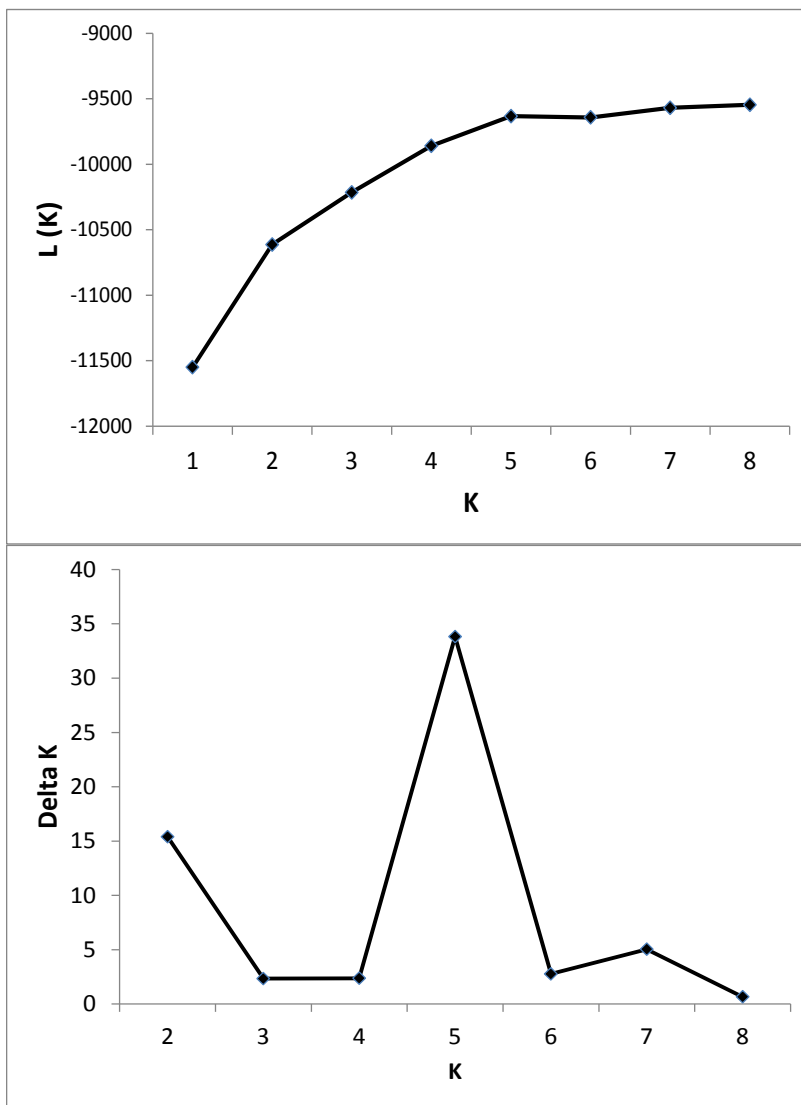


Figure 15. Log probability of data from STRUCTURE output (top graph) and delta K (bottom graph). Both indicate that $K = 5$ is the most likely number of clusters.

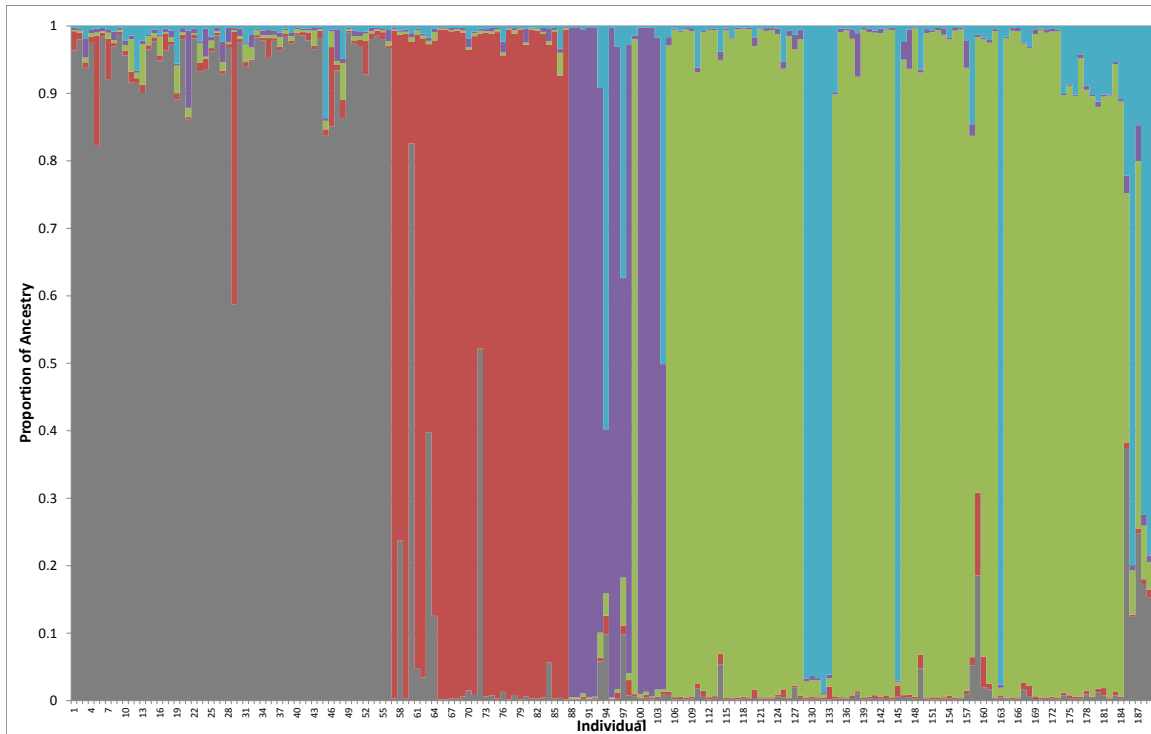


Figure 16. A Bayesian clustering analysis of Texas horned lizards from Rolling Plains (grey; N = 56), Tinker AFB (red; N = 31), Kenedy (purple; N = 17) and Karnes City (green; N = 69), and a ranch near Kenedy (blue; N = 4, plus N = 7 in Karnes City). Each color represents cluster and each vertical line depicts a single individual's proportion of ancestry in a cluster.

The mitochondrial data indicated the presence of one haplotype (Haplotype #1) in Karnes City for a haplotype diversity of $h = 0$ and three haplotypes (Haplotype #1, #2, and #3) within Kenedy for a haplotype diversity of $h = 0.42$. Three different haplotypes (Haplotype #4, #5, and #6) were present in the ranch outside of town for a haplotype diversity of $h = 0.61$. Differentiation was very high between Karnes and Kenedy ($\Phi_{iPT} = 0.90$; $p < 0.001$). Differentiation was also high between the towns and the outside ranch ($\Phi_{iPT} = 0.95$; $p < 0.001$ with Karnes and $\Phi_{iPT} = 0.51$, $p < 0.001$ with Kenedy). The single individual from Kenedy with haplotype #1 was the same individual that was assigned to the Karnes City population in STRUCTURE (Fig. 17).

Discussion

We evaluated movement capabilities of Texas horned lizards within two small towns using two lines of evidence. First, radio-telemetry methods revealed movement patterns in the short-term, showing moderate to high road avoidance and small home range sizes. Second, genetic analyses allowed insight into movement over a longer time scale, revealing a lack of gene flow between and within towns and a significant reduction in genetic diversity compared to other areas.

Shedding events sometimes caused transmitters to detach and fall off of the lizard, despite the collar, which heavily influenced the number of locations gathered. We therefore had a small sample size of home range sizes (N = 11) to compare to other studies. Sample size in the Bomer WMA was also small (N = 9). MCP estimates revealed that average home range size of lizards in Karnes County were smaller than those in Tinker AFB, but not the Bomer or Chaparral WMAs. The two towns possessed a significantly smaller variance in home range size than those in Tinker AFB, revealing that home ranges within these towns are more similar in size. Most movement also occurred within a block and very little occurred across roads.

Roads have been shown to impede movement of mammals (Epps et al. 2005, Riley et al. 2006, Golingay et al. 2013), birds (Fernandez-Juricic and Jokimaki 2001, Soule 2007), and reptiles (Brehme et al. 2012). Even small roads (less than 6 m wide with small scale traffic) can act as barriers to movement, as seen in small mammals and reptiles (MacPherson et al. 2011, Brehme et al. 2012).

In an evaluation of scrubland lizard (*Sceloporus occidentalis* and *Aspidoscelis hyperythra*) response to roads, Brehme et al. (2012) found that dirt and secondary paved roads were penetrated by both species while rural highways were actively avoided, suggesting that lizards may avoid the noise, vibration, or visual disturbance produced by roads with steady traffic. Therefore, the lower variance seen in Karnes County home ranges may result from individuals being constrained within a one block area in town. In this study, three lizards crossed a road once, one lizard crossed a road twice, and one lizard crossed at least five times during the duration of the study. One of these roads, in which one lizard crossed once and another lizard crossed twice, had small scale traffic compared to surrounding roads. On either side of this particular road was a vacant lot covered in vegetation with a large brush pile on one side. The other roads were located in residential areas with buildings and houses on either side.

The genetic data is consistent with reduced movement within town and reduced connection to surrounding areas. By restricting gene flow, barriers impede connectivity and create fine-scale genetic differentiation between individuals on either side of the barrier (Golingay et al. 2013). Within Karnes City, we found significant genetic differentiation between individuals north and south of Calvert Ave State Highway 181 and west and east of Panna Maria Ave. Significant genetic differentiation existed between Karnes City and Kenedy at both nuclear and mitochondrial DNA. These patterns have been shown previously for species in fragmented landscapes.

Davies and Melbourne (2007) measured dispersal and calculated degree of isolation of two gecko species (the declining *Oedura reticulata* and the persistent *Gehra variegata*) in fragmented and unfragmented landscapes. There were large genetic differences between the fragments while individuals in nature preserves belonged to one well-mixed population; the declining *O. reticulata* suffered from a higher degree of isolation and showed stronger genetic structuring between fragments than *G. variegata* (Davies and Melbourne 2007). Moore et al. (2008) found significant genetic differentiation across small distances in tuatara (*Sphenodon punctatus*) on a recently fragmented island and hypothesized that reptiles exhibiting limited dispersal may be even more susceptible to the effects of fragmentation.

Mitochondrial DNA revealed that individuals within Karnes City possessed a single haplotype and those within Kenedy possessed three haplotypes, with one individual possessing the haplotype found in Karnes City. That same individual was assigned to the Karnes City population using Bayesian clustering (Fig. 15). Together, this individual's haplotype and proportion of ancestry indicate that this individual was moved from Karnes City into Kenedy. The small home range sizes and avoidance of road crossing indicates that this individual probably did not travel 6 miles across many roads from Karnes City to Kenedy and was likely moved by people either inadvertently in cargo or purposely as an introduction. With the exception of this individual, the mitochondrial data suggests that 1) no female gene flow exists between the two towns and 2) the Karnes City population was founded by a single female lineage, indicating an absence of female gene flow into Karnes City.

In large, connected areas, the number of haplotypes can range from 7 to 12 (Dean Williams unpub data) and in fact the ranch near Kenedy had relatively high haplotype diversity which was not shared with either town.

There was higher genetic differentiation for mitochondrial haplotypes than for microsatellite loci. This suggests that females are relatively philopatric and males disperse further distances than females. Sex-biased dispersal may decrease the chance of inbreeding, as has been suggested for prickly forest skinks (*Gnypetoscincus queenlandiae*) (Sumner 2005).

Genetic diversity was lower within the two towns than to other studied areas, indicating that these populations are smaller in size. Observed heterozygosity was even significantly lower in these towns than in the other fragmented site, Tinker AFB. Because heterozygosity takes a relatively long period of time to decline compared to allelic richness, this finding suggests that Kenedy and Karnes City have been isolated for a fairly long period of time. We did not find statistical evidence of a bottleneck using common tests (mode shift, heterozygosity excess tests). The absence of common bottleneck signals in populations with demographic reductions likely results from gene flow into the population (Busch et al. 2007; Depaulis et al. 2003; Peery et al. 2012; Rodríguez-Zárate et al. 2013). Populations in fragmented areas are often characterized by lower levels of genetic diversity. For example, reduced dispersal of Cunningham's skink (*Egernia cunninghami*) in fragmented areas produced significantly increased relatedness and lower allelic richness compared to nearby natural areas (Stow and Sunnucks 2004; Stow and Briscoe 2005).

Anderson et al. (2004) detected a reduction in genetic variation and a significant correlation between larval survival, mean F_{IS} value, and mean individual inbreeding coefficient in the European tree frog (*Hyla arborea*) due to severe habitat fragmentation.

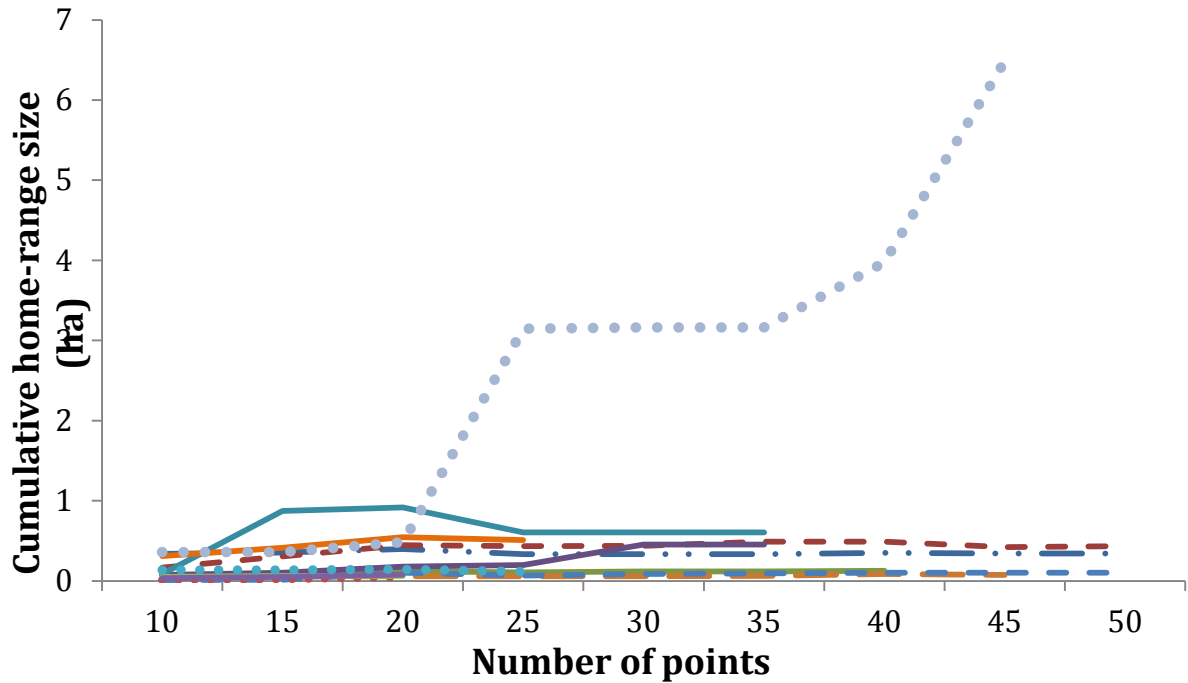
A meta-analysis of previous literature confirmed the significant correlation between genetic diversity and fitness (Reed and Frankham 2003). Low genetic diversity limits adaptability of populations to environmental changes and can result in inbreeding depression (ex. Madsen et al. 1996; Frankham et al. 2007; Keller and Waller 2002). Keller and Waller (2002) urge the necessity of gene flow amongst fragmented habitat patches to prevent the deleterious effects of inbreeding on resistance to disease, predation, environmental stress, and ultimately survival. Also, as population size declines, genetic drift occurs at a faster rate and populations lose diversity more quickly. Due to their small size, populations within Karnes City and Kenedy will be more vulnerable to perturbations like drought, habitat changes, and inbreeding depression.

Vegetation composition was similar between areas with and without horned lizards. Interestingly, some diversity estimates of vegetation were higher in areas without lizards. Overall, the availability of harvester ants in town and the similarity of vegetation between areas with and without horned lizards suggest that many areas with suitable habitat are not being utilized by horned lizards. According to Texas Parks and Wildlife surveys and citizen observations, horned lizards once inhabited many of these now-absent patches. This could suggest that once horned lizards disappear from a patch, recolonization is unlikely due to constrained movement.

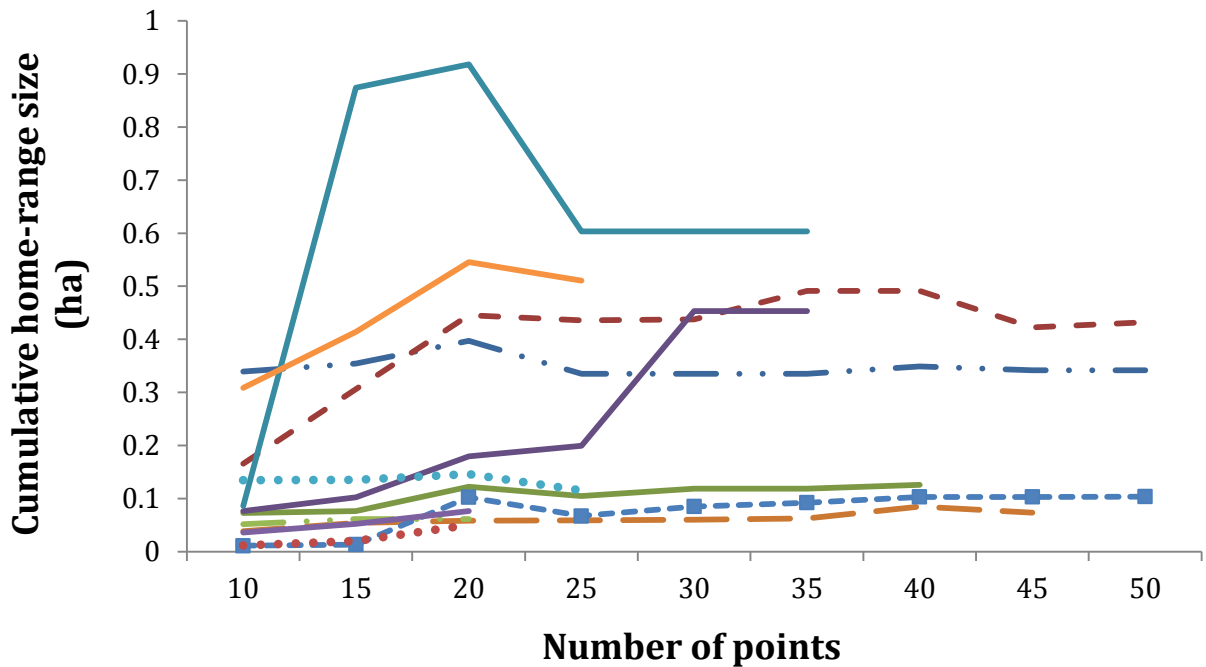
The small home range sizes and low genetic diversity of Texas horned lizards within these towns suggest curtailed movement and minimal gene flow, especially in comparison to individuals in more connected areas.

Conservation efforts should focus on monitoring these populations and regularly searching areas without horned lizards to determine if suitable areas will be recolonized. If recolonization does not occur and populations remain isolated, movement may need to be facilitated. Future research should determine if relocations in town are feasible and if movement could be encouraged through the construction of corridors.

Appendices

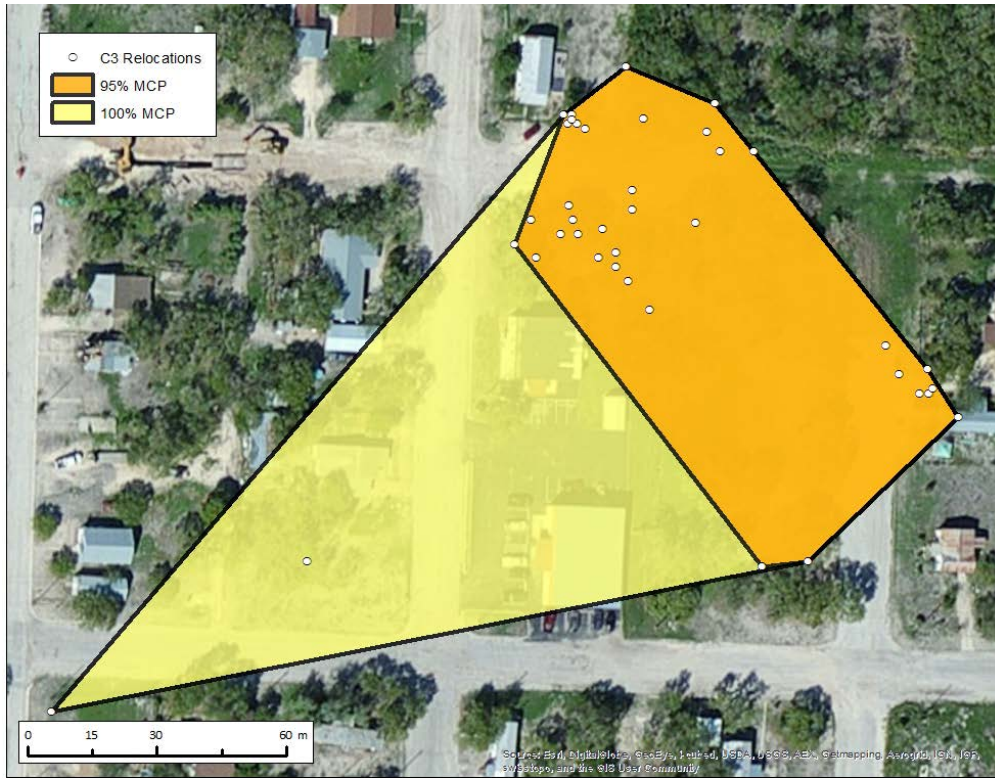


Appendix 1. Cumulative home-range size for radiolocation points collected for all lizards in Kenedy and Karnes City using the 95% Minimum Convex Polygon method.

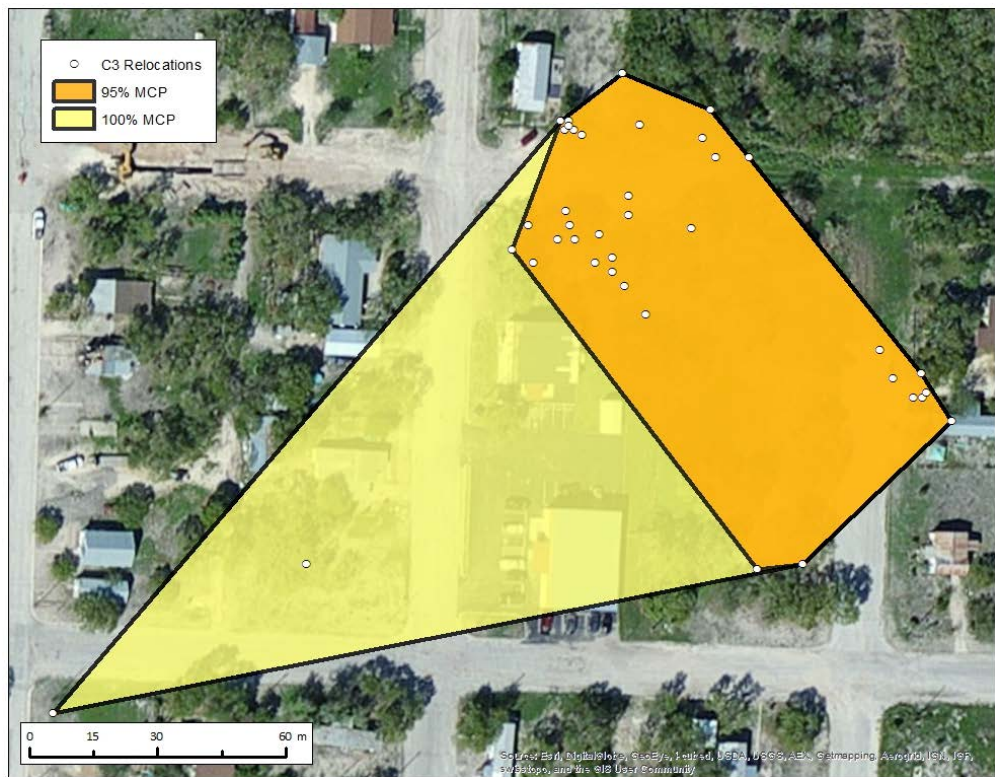


Appendix 2. Cumulative home-range size for radiolocation points for all lizards in Kenedy and Karnes City excluding one outlier lizard using the 95% Minimum Convex Polygon method.

A.



B.



C.



D.



E.



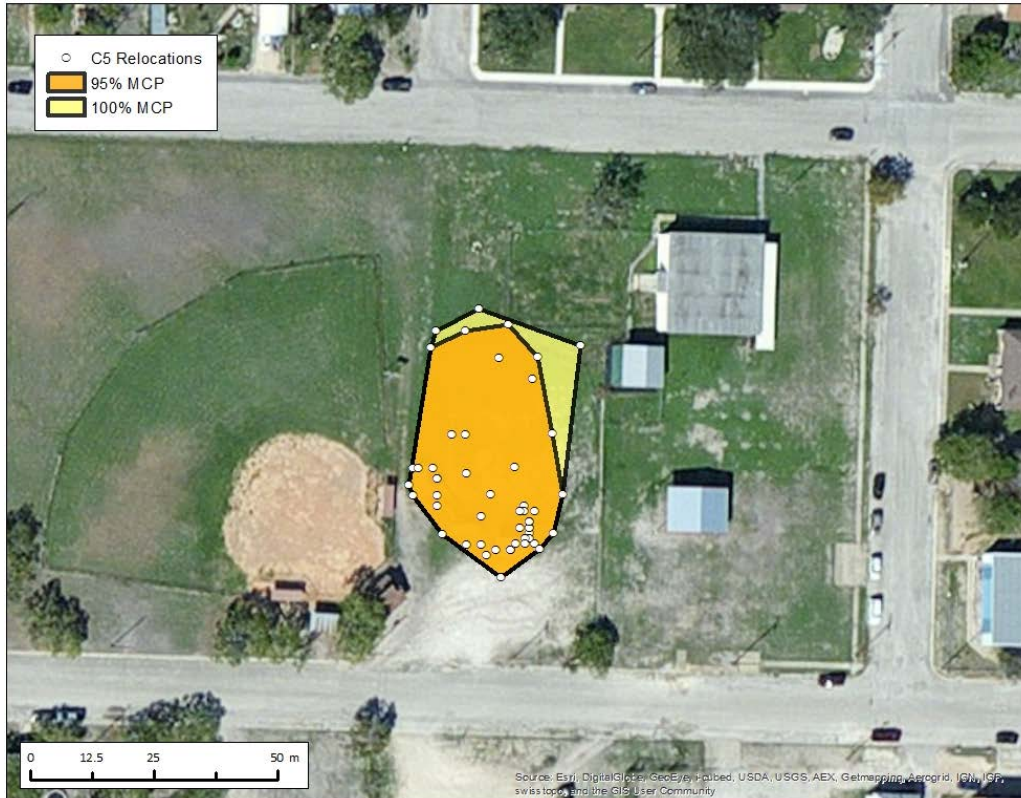
F.



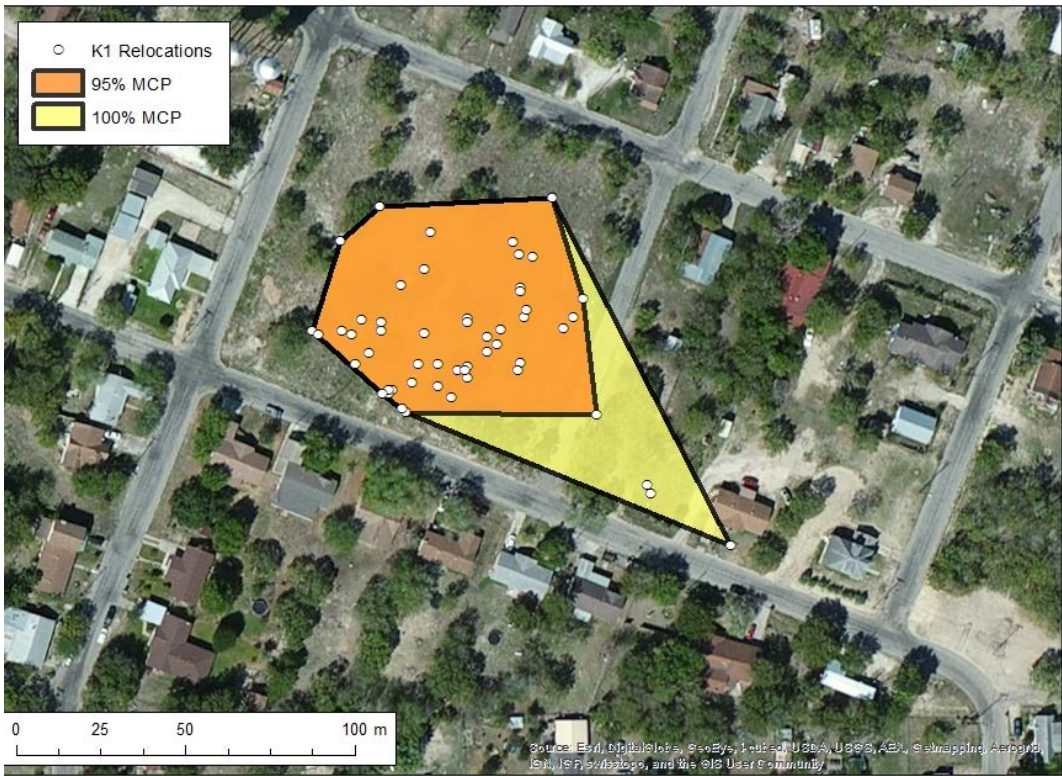
G.



H.



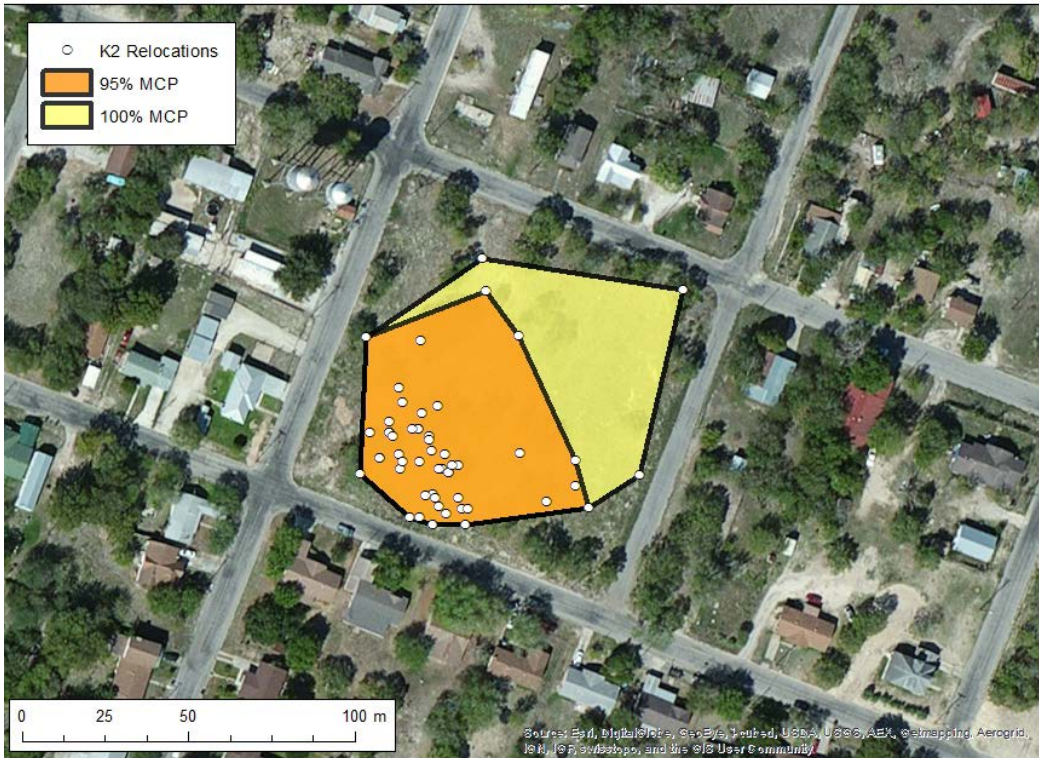
I.



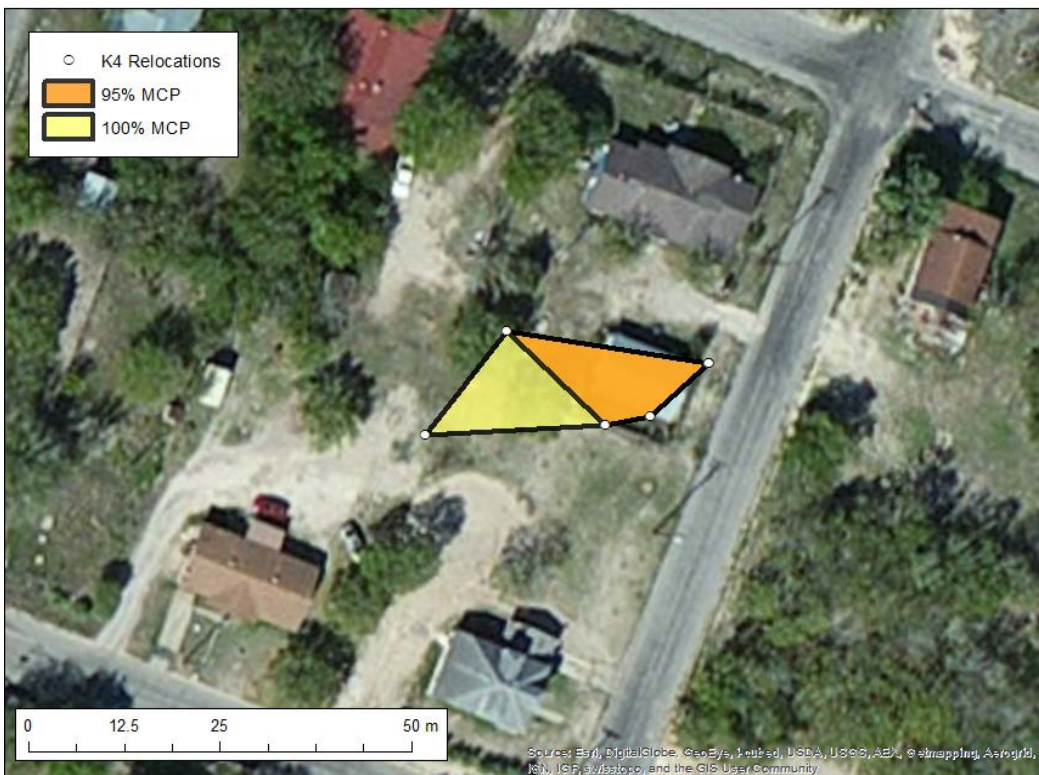
J.



K.



L.



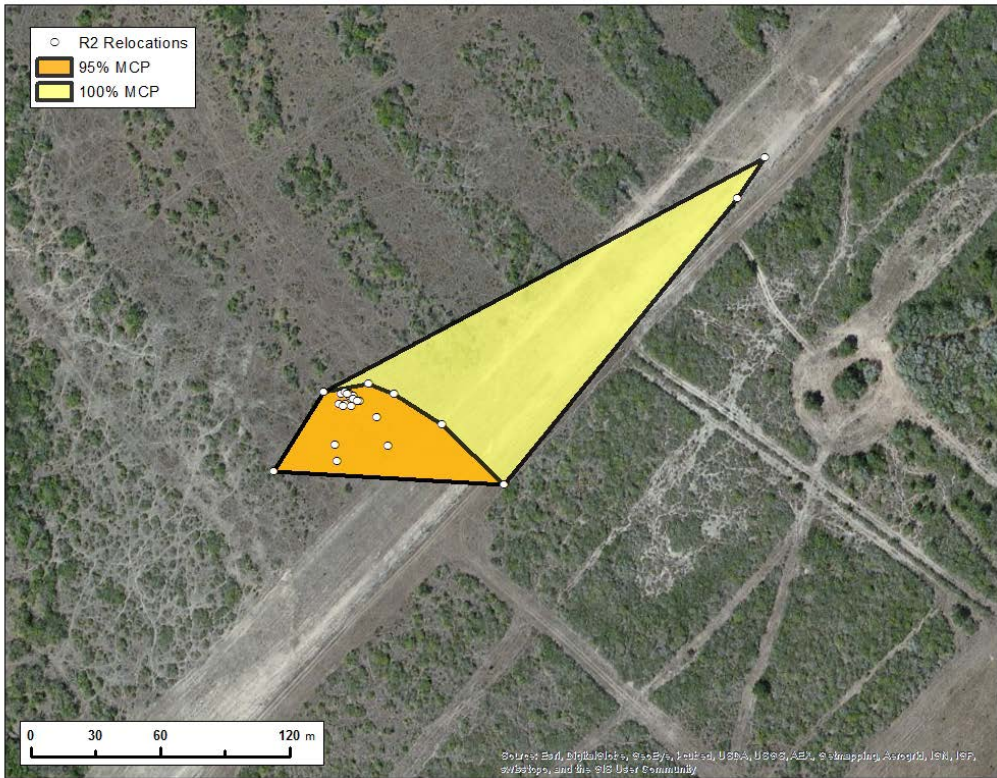
M.



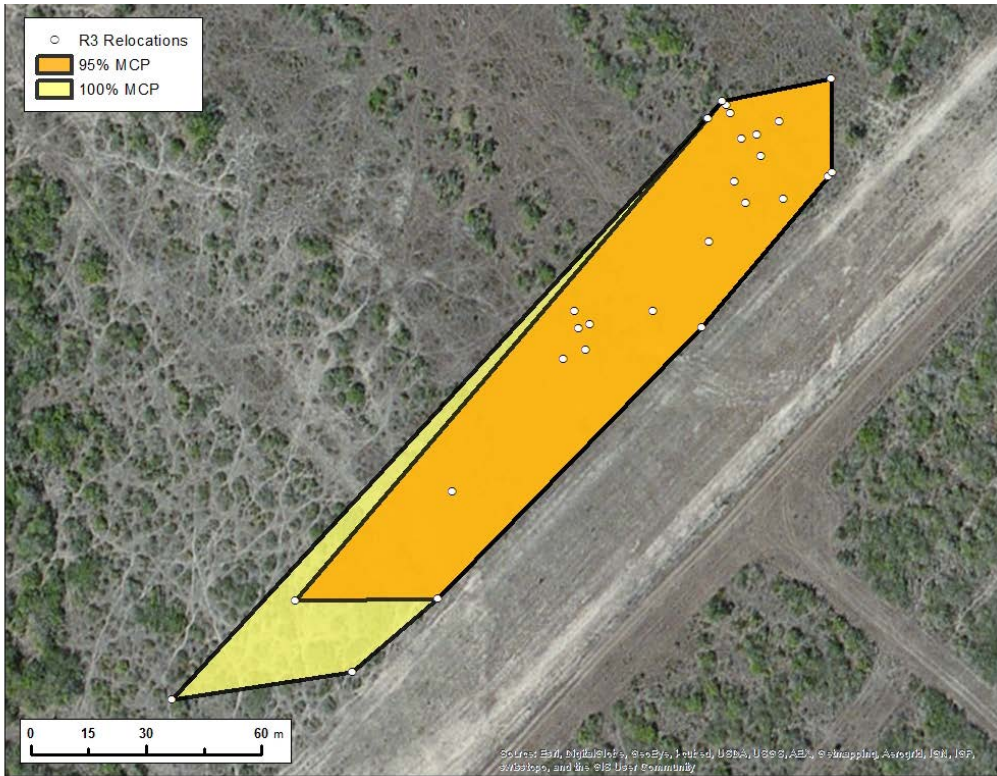
N.



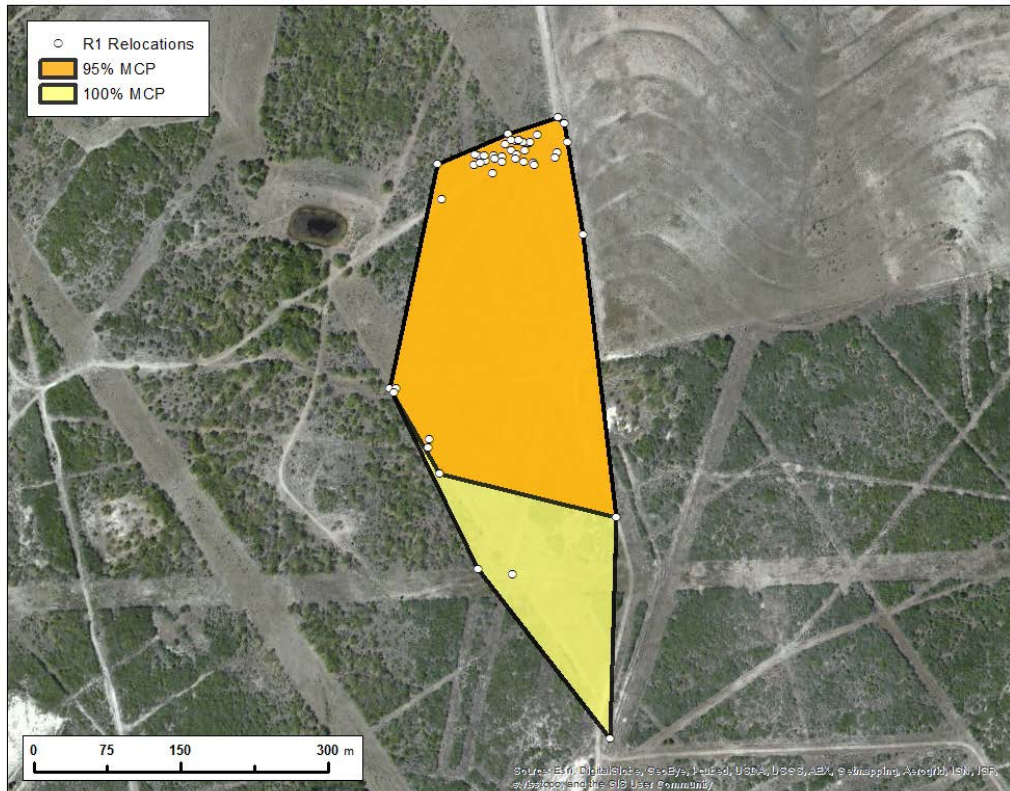
O.



P.



Q.



Appendix 3 (A-Q). Home range constructed for horned lizards in Karnes City (A-H), Kenedy (I-N), and an outside ranch (O-Q) using 95% (smaller polygon) and 100% (larger polygon) MCP.

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VITA

Ashley E. Wall received her Bachelor of Arts with a degree in biology and minor in Environmental Science from Southwestern University, Georgetown, Texas in 2012. There, she studied the ecology and movement of the Georgetown salamander (*Eurycea naufragia*). She graduated with her Master of Science in biology in 2014 from Texas Christian University, where she studied the genetic isolation and movement capabilities of Texas horned lizards (*Phrynosoma cornutum*) in small, urban towns. Ashley enjoys field work, hands-on conservation management, and public outreach.

ABSTRACT

HOME RANGE AND GENETICS OF TEXAS HORNED LIZARDS (*PHRYNOSOMA CORNUTUM*) IN TWO SMALL TOWNS IN SOUTH TEXAS

by

Ashley Elizabeth Wall

Bachelor of Arts, 2012 Southwestern University, Georgetown, Texas

Thesis Advisor: Dr. Dean Williams, Assistant Professor of Biology

Characteristics of urbanization such as roads, buildings, exotic species and vegetative homogenization can create patches of habitat surrounded by poor-quality, unfavorable areas. This inhibits movement, which in turn restricts gene flow. We used radio-telemetry and genetic markers to study the movement patterns in Texas horned lizards (*Phrynosoma cornutum*) in the neighboring towns of Kenedy and Karnes City, Texas. Individuals had small home ranges and rarely moved across roads. Nuclear and mitochondrial DNA markers revealed low genetic diversity in these towns and another urban site at Tinker AFB in Oklahoma compared to a larger natural area in Texas. There was significant genetic structure within town on either side of large roads as well as significant genetic structure between towns. These data suggest that movement is curtailed within and between these towns which may have long-term negative impacts such as inbreeding depression and a lowered ability to adapt to changing conditions.