06-03

INTRODUCTION

In the 20th century, extirpation of carnivores occurred in southwestern United States for the protection of livestock and land development. Through decreased hunting, increased protection, and reintroduction efforts, extirpated mammals are once again filling into their historic ranges. In the time since this extirpation, the landscape these carnivores occupied has changed dramatically. Riparian corridors have disappeared or been greatly reduced, land has been developed into cities and town, and large barriers such as multi-lane highways and canals have been constructed. National parks and wilderness areas have become islands in this sea of development, but connectivity of wildlife between areas is uncertain. As space in the west becomes ever more limited and restricted, two questions are paramount: what is the dispersal ability for carnivores and other mammals between national parks, and how is this connectivity affected by anthropogenic development?

We used a highly mobile and common carnivore (skunks) to help answer important questions of dispersal ability of carnivores within the southwestern states, as well as the linkages that exist between western national parks. The objectives in our study are to determine the population structure of skunks in three national parks to determine relative fragmentation and dispersal abilities among subpopulations. In order to conduct a study over such a large geographic area in a several year time frame, we chose to use genetic analysis techniques. These methods also allowed us to reduce stress on animals, increase our sample size, and determine long-term evolutionary history. We chose to analyze portions of the mitochondrial (mtDNA) genome because multiple mtDNA studies have been done for other mesocarnivore species, allowing for comparison between studied. Additionally, because skunks do not have a sex-biased dispersal ratio (Bjorge et al 1981; Sargeant et al. 1982; Bixler and Gittleman 2000), mtDNA is an appropriate method to use.

This project differs from the original study proposal in the following: we include trap numbers in the three national parks for all four species, however, we only conducted genetic analysis on striped and hooded skunks, due to low trap numbers for spotted and hognose skunks.

METHODS

Study Sites

We collected tissue samples from striped skunks in the following national parks: Saguaro National Park (SAGU) within the Rincon mountain range, Chiricahua National Monument

(CHIR) in the Chiricahua mountain range, and Fort Bowie National Historic Site (FOBO) in the Dos Cabazes mountain range. We trapped both inside these national parks and throughout the mountain ranges in which the parks were located. Additionally, to further understand species distribution, we trapped in the Santa Catalina, Santa Rita, and Pinaleno Mountain ranges. We trapped in a range of elevations from 360-2894 meters in multiple habitats. Additionally, we received samples of urban nuisance skunks from the Arizona Department of Health Services: Tucson Regional Laboratory. Trap locations are illustrated in Figure 1.

Trapping/Sample Collection

All trapping was done in accordance with University of Arizona IACUC protocol #05-058. We trapped 4800 trap nights from September 2005-September 2006. We took a 2mm tissue notch from the ear for genetic analysis. Skunks were non-chemically immobilized (modified from Koprowski 2002) and released at point of capture.

DNA extraction and amplification

We amplified two regions of the mitochondrial DNA (for striped and hooded skunks): a 702 bp region of the D-Loop and a 613 region of the cytotochrome b (concatenated to a 1315 bp sample). We conducted a species verification on all samples collected using reference sequences from GenBank (http://www.ncbi.nlm.nih.gov/). Complete genomic analysis is available upon request from the University of Arizona (Margaret Rheude MS thesis).

Statistical Analysis

We analyzed population structure using ARLEQUIN version 3.0 (Excoffier et al. 2005). We examined Analysis of Molecular Variance (AMOVA) statistics within and among populations, population fixation indices (F_{ST}, a measure of gene flow among populations), multi-dimensional scaling to determine population groupings (Kruskall and Wish 1978) (NTSYSpc 2.02h, 1986-1998, Applied Biostatistics Inc.). To determine movement among mountain ranges we compared genetic to geographic distance using a normalized Mantel statistic (TFPGA 1.3, Miller 1997) using a dissimilarity matrix of F_{ST} values plotted against geographic distances. To determine long-term population history we examined a measure of genetic diversity (Pi) and whether populations were increasing or decreasing (Tajima's D statistics).

RESULTS

From all areas we obtained tissue samples from 280 skunks: 151 striped skunks, 82 hooded skunks, 23 hognose skunks, and 19 spotted skunks (Figure 1). Within the mountain ranges containing our national parks we sampled 57 skunks, 19 of which were trapped in our three national parks (Table 1). Results of trap effort varied widely. We only conducted 30 trap nights in the Dos Cabazes, and captured 6 skunks. Conversely, over 700 trap nights in the Rincon Mountains yielded only eight live individuals, attributable perhaps to a recent rabies epidemic. Many of the skunks sampled from the Rincon Mountains, including Saguaro National Park, were from recovered carcasses. Of the two species we genetically analyzed, we observed a marked difference in distribution of urban and non-urban skunks. Of our 151 striped skunks, 119 were found in non-urban trap sites, 32 were found in urban or urban/wildland interface trap sites. For our 82 hooded skunks, only 15 were captured in non-urban trap sites; 67 were found in urban or urban/wildland interface trap sites (Figure 2). We never captured hooded skunks east of the San Pedro River Valley, although striped skunks were present throughout the entire study area (Figure 1). All figures and tables from this study are available from the University of Arizona (Rheude, 2008)

Determination of population structure and dispersal patterns

Striped Skunks

Striped skunk populations grouped by mountain range (six subpopulations total) showed defined population structure, indicating that the population is not panmictic across southeastern Arizona. There was obvious gene flow observed among populations, with an overall $F_{ST} = 0.053$. The Multi-dimensional scaling grouped populations spatially in the same manner as would be expected geographically (stress value = 0.297). We achieved the best-fit (Mantel test, $r^2 = 0.54$, $p = 0.01$) for subpopulations separated by straight-line distances, rather than any combination of major and minor washes. There is no current evidence to indicate that striped skunks disperse in specific riparian corridors. The multi-dimensional scaling analysis grouped all mountain ranges closer to each other than to urban areas that were geographically closer (stress value = 0.415), indicating that striped skunks in urban areas were not part of the historic populations structure. F_{ST} values were the same for similar geographic distances, regardless of whether these distances were traversed within a river valley or among a river valley (Figure 3). Striped skunks yielded a nucleotide diversity (π) of 0.00445, and a Tajima's D value of -.36813(P>/0.10), indicating a stable (not increasing or decreasing) and genetically diverse population.

Hooded Skunks

Because hooded skunks were not trapped in the same area as striped skunks, we were not able to directly compare the two populations. We analyzed seven sub-populations of hooded skunks. We found these populations to be more structured than the striped skunk populations, with an overall F_{ST} value of 0.10494, indicating less gene flow between the populations. Five of our sites were within the Santa Cruz River Valley; two were within the San Pedro river valley. Our multi-dimensional scaling grouped these river valleys into distinct groups (Stress = 0.00535), and our F_{ST} values indicated hooded skunks were able to move within the river valley fairly easily, but not between the river valleys. We also found no correlation between genetic and geographic distance (Mantel Test, $r^2 = 0.2082$, $p > 0.05$) (Figure 3). The genetic diversity of the hooded skunk population was $\pi = 0.00288$, indicating a less genetically diverse population than striped skunks, with a negative Tajima's D value (-0.84986), indicating an expanding population. Both of these statistics indicate that the majority of the hooded skunks found in southeastern Arizona are fairly recent immigrants from Mexico. All figures and tables from this study are available from the University of Arizona (Rheude, 2008)

DISCUSSION

Overall skunk populations

Striped skunks were the most common skunk observed in our study, as well as the most common skunk observed in our three national parks. Outside of urban areas, we captured the most hooded skunks in Saguaro National Park (which is adjacent to urban Tucson). We did not observe any hooded skunks east of the San Pedro River Valley. Capture numbers for hognose and spotted skunks were very low; further studies should determine if they exhibit more trap-shyness or if they actually exist in lower frequency in southeastern Arizona. The Chiricahua Mountains (including CHIR) yielded the highest capture success of skunks. The Dos Cabezas Mountains (including FOBO) yielded six skunks from minimal capture effort, indicating the skunk population is fairly robust in this mountain range. The Rincon Mountains (including SAGU) yielded very poor capture success with half of the samples from carcass recovery, possibly due to a rabies epidemic the previous year.

Striped vs Hooded Skunks

Our results indicate that striped skunks live in mountain ranges, and not in-between, except occasionally in urban areas. These skunks have a stable, genetically diverse population and have most likely been in southeastern Arizona for a long time. Hooded skunks, on the other hand, were found mostly in urban areas, are not as genetically diverse as striped skunks, and display an increasing population. Hooded skunks have probably always occurred in Arizona in low numbers, but are expanding their population size and range along with expanding urbanization. Their population is highly structured between river valleys, possibly due to the absence of urban areas in-between the Santa Cruz and the San Pedro River Valleys. Whether they are unable to cross large expanses of desert, or they actively prefer urban areas as habitat is not known.

CONCLUSIONS

Skunk populations seem to be variable among different national parks in southeastern Arizona. Striped skunks are the most common skunk, and are long-term residents of Arizona. Hooded skunks have less well established populations and are not frequently found in non-urban places; however, these skunks may become more common in our national parks as urbanization encroaches on the boundaries of our national parks. Striped skunks appear to be able to disperse across desert valleys without using riparian corridors, whereas hooded skunks either cannot disperse easily across the desert or have not been in Arizona long enough to attempt these crossings. Populations of hognose and spotted skunks appear to occur in lower numbers, and should warrant further research on these less common skunks. This study provides insight into conservation of small carnivores in our national parks, as well as helping to determine paths of disease spread in Arizona.

Figure 1 –Study site, southeastern Arizona. Mountain ranges and urban areas sampled and occurrence of *Mephitis mephitis* and *M. macroura*. Mountain ranges sampled are shaded grey, mountains that yielded only yielded striped skunks are outlined in dots, those that only yielded hooded skunks have a dashed outline, and those that yielded skunks of both species have a solid black outline. Urban areas from where striped skunk samples were taken are indicated by stars, stars size is proportional to town size. River valleys are highlighted in black.

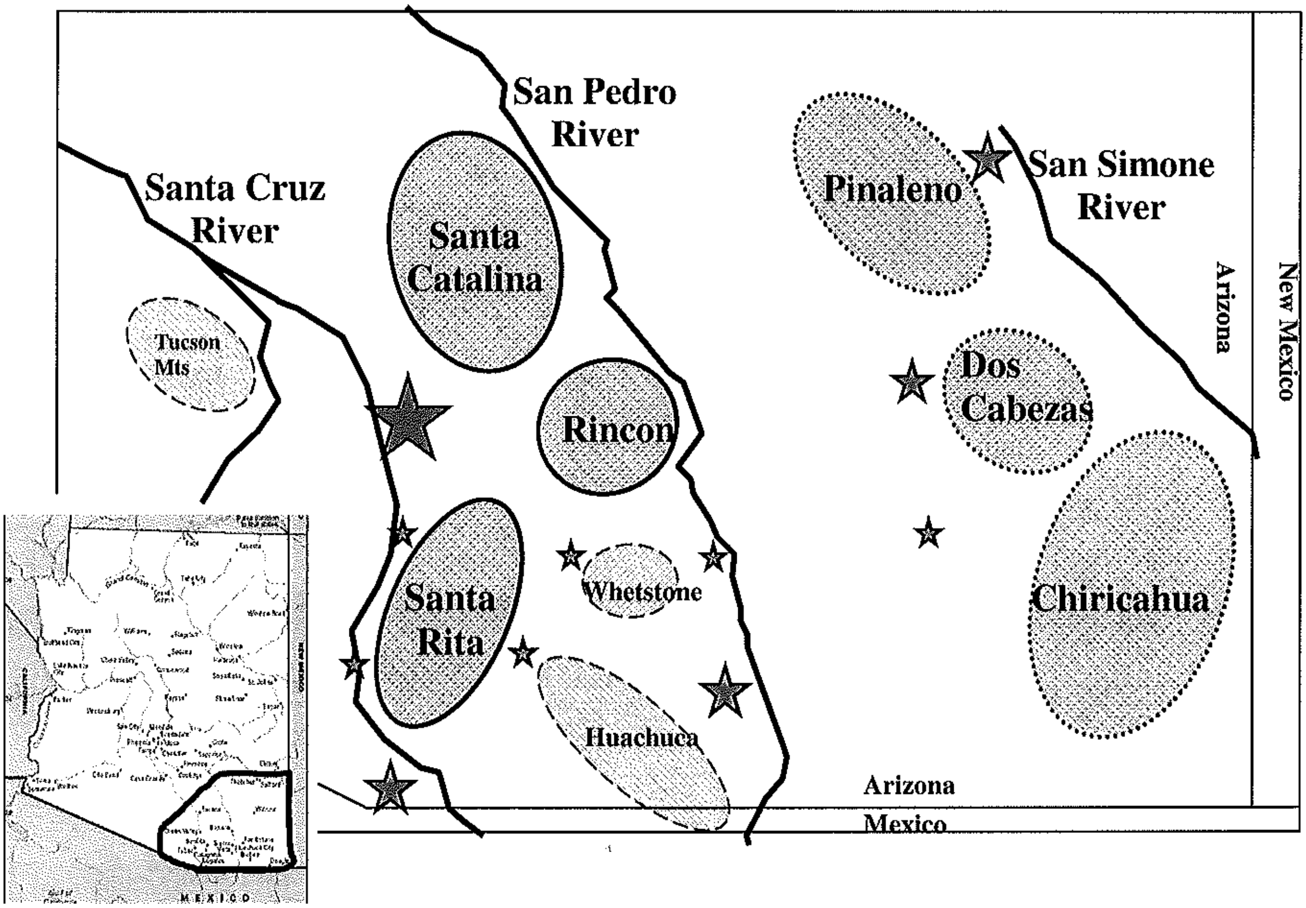

Figure 2: distribution of two skunk species by urban vs non urban areas. Although scales are for different sample sizes, there is a mirror-image in capture trends between the two species.

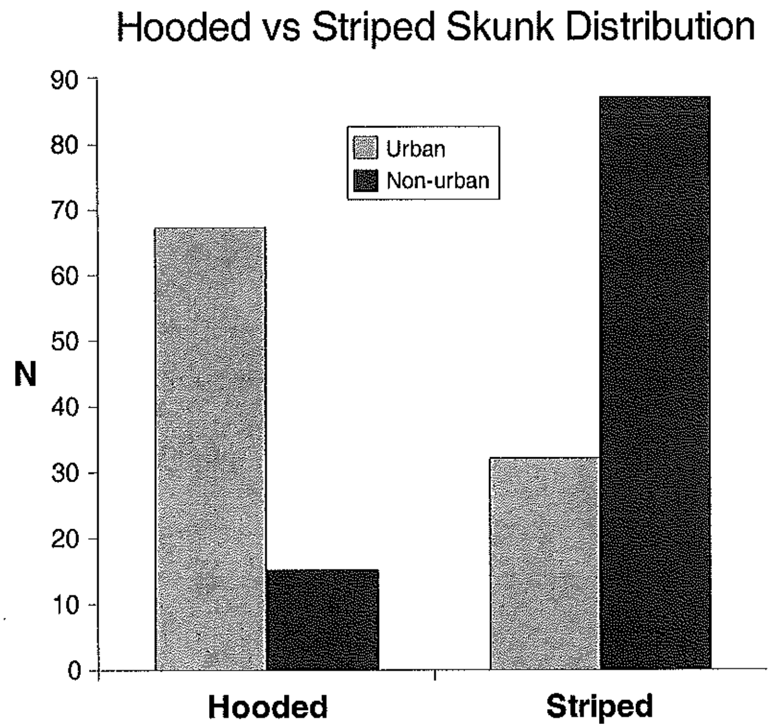

Figure 3 – Comparison of striped (*Mephitis mephitis,* triangles) and hooded (*M. macroura,* diamonds) mantel test to determine relationship between geographic (Km) and genetic (Fst) distance. A. Striped skunks have a strong significant positive linear relationship. B. Hooded skunks have a non-significant, poor relationship between the two variables.

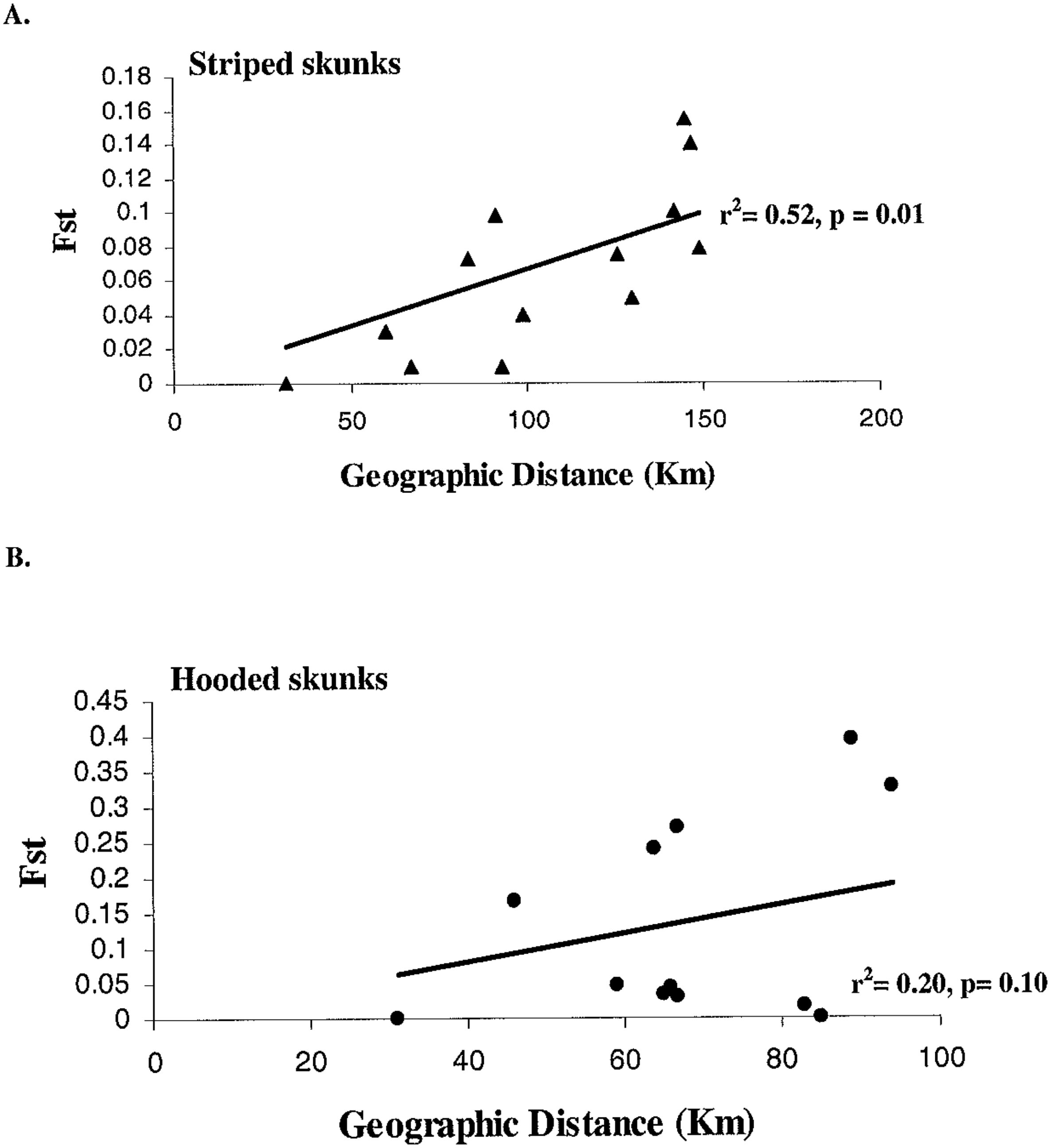

Table 1: Comparison of four species of skunks captured in three national parks, as well as total captures for the mountain ranges in which these national parks are contained. Live capture and carcass collection as well as total samples from the mountain range are listed.

	Striped	Hooded	Spotted	Hognose	Live Capture	Carcass collection	Total
National Park							
CHIR	3	0	1	0	4	0	4
FOBO	6	0	0	0	6	0	6
SAGU	3	3	0	5	4	7	11
Total	**12**	**3**	**1**	**5**			
Entire Mountain							
Chiricahua	32	0	3	0	35	0	35
Dos Cabazes	6	0	0	0	6	0	6
Rincon	6	6	0	4	8	8	16
Total	**44**	**6**	**3**	**5**			

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