Investigating possible hibernaculas for the bat population of the Missouri River in South Dakota

A report for the South Dakota Game, Fish and Parks Small Grants

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INTRODUCTION

From May 2000 to August 2002, a study was conducted to document the distribution, roost sites, and food habits of bats in South Dakota east of the Missouri River. During the summers of 2000, 2001, and 2002, mist netting and acoustic sampling (Anabat system) was conducted at state parks, state recreation areas, and national wildlife refuges. Seven species of bat were recorded from this region: *Myotis septentrionalis*, *Myotis lucifugus*, *Myotis ciliolabrum*, *Eptesicus fuscus*, *Lasionycteris noctivagans*, *Lasiurus borealis*, and *Lasiurus cinereus* (Swier, 2006).

More species were collected along the Missouri River than at any other location in eastern South Dakota during 2001. Bat capture rates (BNN=bats/per net/per night) and species richness were greater within the Missouri River riparian forest than any other habitat in eastern South Dakota. The Anabat system recorded a greater number of bat passes in Missouri River locations than in non-Missouri River locations, indicating greater bat activity along the river.

A radiotracking study was performed in the summer of 2002 to investigate the importance of the Missouri River riparian forest for bats in eastern South Dakota. Four species (*M. septentrionalis*, *M. lucifugus*, *E. fuscus* and *L. noctivagans*) were radiotracked using small radio transmitters in order to follow the bats to their roost sites. Reproductively active *E. fuscus* were captured within a recreation area along the Missouri River in central South Dakota and radiotracked back to a maternity roost in an abandoned building. A postlactating *M. septentrionalis* was also captured, banded, and radiotracked to a dead cottonwood tree in a Missouri River recreation area of central South Dakota. In 2003, this same *M. septentrionalis* was recaptured in the same locality. These bats are actively reproducing and foraging in the riparian forest of the Missouri River during the summer months and are demonstrating site fidelity, but where are they going during the winter and spring months?

Certainly, instead of migrating, some individuals stay in eastern South Dakota. In 2000 and 2001, approximately 620 bats were collected throughout the state for rabies testing (South Dakota Animal Disease Research and Diagnostic Laboratory Report, 2000). A majority (98%) of the submitted bats were *E. fuscus* collected from Sioux Falls in eastern South Dakota. A few of these bats were collected during the hibernation period, April-October, indicating that *E. fuscus* hibernates in eastern South Dakota (Swier, 2003; Swier, 2006).

Another suggestion is that many bats hibernate in the caves and mines of western South Dakota. These bats may follow the tributaries of the Missouri River westward to hibernate in caves or mines of the Black Hills. Western South Dakota is mostly composed of grasslands and rangeland, except for the Black Hills, a remnant area of ponderosa pine forest. Many caves exist in the Black Hills and bats have been found to utilize these caves as maternity colonies or hibernacula roosts. Turner (1974) reported ten species of bats from the Black Hills of South Dakota and Wyoming. At least seven species, including *E. fuscus* are resident throughout the year, forming wintering colonies in caves and mines (Choate and Anderson, 1997). *Eptesicus*

fuscus has been found roosting in Jewel cave of the Black Hills and in a cave in Custer State Park on February 2, 1970 (Martin and Hawks 1972).

A final possibility is that some bats hibernate in Nebraska and follow the Missouri River northward to their summer habitats. Bats may be utilizing the Missouri riparian forest as a corridor to travel through South Dakota, as the trees of riparian zones provide roosting opportunities, an abundance of insect prey, and protection from predators (Downs and Racey, 2000; Carroll et al., 2000). Recent capture records of *Nycticeus humeralis* captured along the Missouri River in southeastern South Dakota (Lane et al., 2003), may indicate a northward extension as this species is commonly found in Nebraska.

Eptesicus is an ideal species for this research question, as it is not known to be a migratory species. *Eptesicus fuscus* has been found to hibernate in temperate regions of North America and to hibernate in buildings but in the coldest months utilizes caves or mines (Barbour and Davis, 1969). Hibernacula have been found in eastern and western South Dakota and we may be able to determine where the Missouri River bats are hibernating as we have voucher specimens from hibernacula in eastern South Dakota and census data from hibernacula in western South Dakota.

I proposed to sequence the mitochondrial DNA (cytochrome b) genome from *E. fuscus* collected along the Missouri River of South Dakota and compare these sequences to *E. fuscus* from the Black Hills region, and eastern South Dakota. Other studies have utilized this powerful molecular approach to determine the relatedness of bats within colonies to bats collected outside of the colonies. Kerth et al. (2000) obtained wing biopsies from *Myotis bechsteinii* to isolate mtDNA sequences and determine the haplotype diversity among colonies in Germany. They were able to match haplotypes from bats captured in solitary roosts to bats captured in these colonies. One of the singularly roosting bats matched a colony located 7 km from the single roost, indicating dispersal of these bats from their natal colonies.

I proposed to use this same genetic marker to explore the genetic relatedness within *E.fuscus* that reside in South Dakota, to better understand the movements and winter roost locations of this species. As these winter roosts may be shared with other species such as *M. septentrionalis*, *M. lucifugus*, *M. ciliolabrum*, *M. thysanodes*, *M. volans* and *Corynorhinus townsendii* which are known to roost in caves of the Black Hills (Turner, 1974; Choate and Anderson, 1997).

METHODS

Sixty *E. fuscus* individuals, collected from the Eastern, Missouri River, and Western regions of South Dakota by the SD Department of Heath, were sent to the South Dakota State Veterinary lab at Brookings, South Dakota for rabies testing. Scott Pedersen and Roxy Larson of South Dakota State University took tissue samples from these individuals as well as skins and skeletons to be deposited in the Texas Tech University Museum, National Science Research Laboratory, Lubbock Texas. Genomic DNA was extracted from these individuals by the Longmire et al. (1997) method with the addition of Proteinase K. The concentration of genomic DNA differed per individual due to the storage age and method of transport to Texas. The control region of the d loop of the cytochrome b gene was amplified by PCR amplification. Primers were supplied by Michael Sorenson of Boston University. The 5' to 3' primer, batTPRO (TCCTACCATCAGCACCCAAAGC) and 3' to 5' primer, EFCR1 (CACGGAGGTAGGTAGATTAATAAACC) were used to amplify 500 base pairs of the control region. As the concentration of each sample differed, and as the annealing temperature was unknown, the PCR procedure was finally optimized at 95 C for 3 minutes, 94 C for 40 seconds, 1

second to 64.8 C, 64.8 C for 40 seconds, 1 second to 72 C, 72 C for 1 minute for 35 cycles. All but two samples (7 and 8) have been amplified with this procedure (Figure 1).

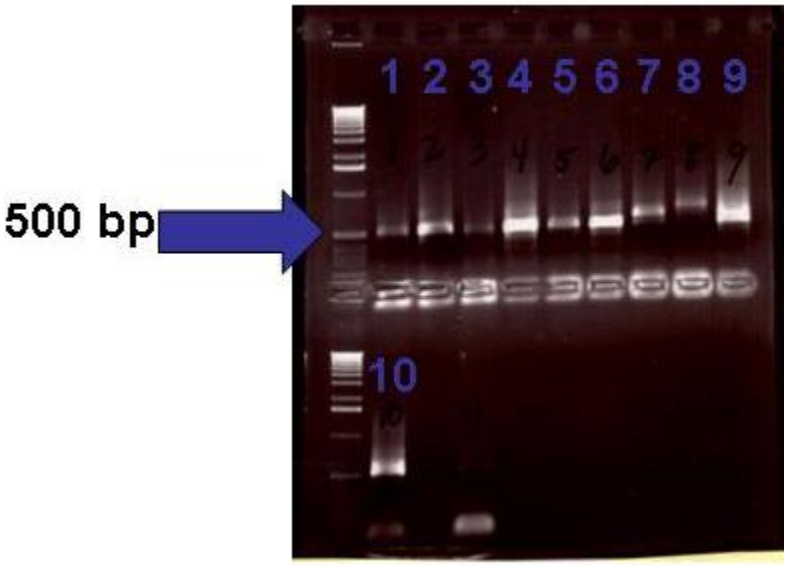

Figure 1: PCR products amplified from the *Eptesicus fuscus* cytochrome b control region. Each product was run through gel electrophoresis to determine the size and number of bands. Note lanes 1-6, 9 and 10 have only one band at a size of 500 base pairs.

After the PCR products were purified using a Qiagen purification kit, the PCR products were labeled with a cycle sequencing reaction, the sequences were precipitated and the product was sequenced using a 3100-Avant automated sequencer from Applied Biosystems. Forward primer batTPRO and reverse primer EFCR1 were used to obtain the sequences. These sequences were aligned and checked for consistency in the Clustal X program. After alignment, the consensus sequences were checked for gaps and suspicious bases using the Maclade program. After the alignment, only 409 base pairs were found to be accurate for further analysis.

RESULTS

Six haplotypes were found but with only a few polymorphisms. All polymorphisms were transversions or also known as nonsynonymous substitutions, which have a greater potential of changing a translated protein product than synonymous substitutions. Seven individuals all had the same sequence, haplotype A. In haplotype B, there was 1 substitution of an A to a G in TK 128024 and 128058 at the same site. Both individuals were collected in eastern South Dakota. Haplotype C, had two substitutions of a T to a C in TK 128059. Haplotype D had one substitution of a T to a C in TK 128002 and the other substitution of a C to a T was shared at the same site with TK 128003 (Haplotype E). TK 128002 was collected from Sioux Falls, South Dakota and TK 152003 from Minneapolis, Minnesota. Haplotype F, TK 128053 from Beresford SD, had one substitution of a C to a T (Table 1).

Table 1: The TK number, location, haplotype, and type of substitution for each sequenced *Eptesicus fuscus* individual.

TK number	Location	Haplotype	Substitution
128001	Luverne, Minnesota	A	None
128002	Sioux Falls, SD	D	Two (T to C and C to T)
128003	Minneapolis, Minnesota	E	One (C to T)
128005	Belle Fourche, SD	A	None
128007	Sioux Falls, SD	A	None
128023	Sioux Falls, SD	A	None
128024	Sioux Falls, SD	B	None
128031	Yankton, SD	A	None
128043	Rapid City, SD	A	None
128052	Vermillion, SD	A	None
128053	Beresford, SD	F	One (C to T)
128058	Menno, SD	B	One (A to G)
128059	Tripp, SD	C	Two (T to C)

In the eastern region of South Dakota, five haplotypes (A, B, C, D, F) have been found. Interestingly, three of these haplotypes (A, B, and D) are in Sioux Falls. Within the Missouri River region, haplotype A and F are found, whereas only one haplotype (A) was documented in the Black Hills region (Figure 2). Haplotype A is the only haplotype that was found in all three regions and was also found in Luverne, Minnesota. Unique haplotypes that were only found in one location were haplotype C (Tripp, SD), haplotype D (Sioux Falls, SD), haplotype E (Minneapolis, MN) and haplotype F (Beresford, SD). Yet two of these unique haplotypes (C and D) had the most polymorphic sites.

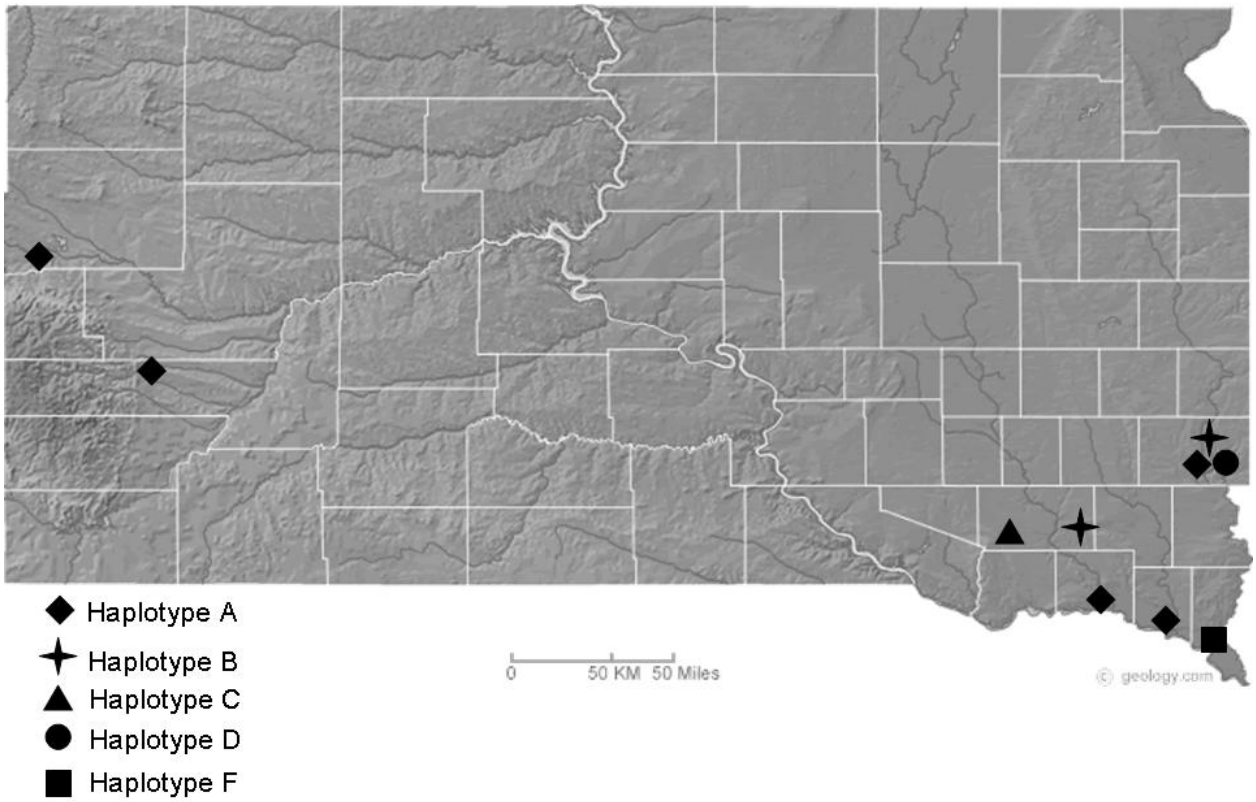

Figure 2: The South Dakota distribution of each *Eptesicus fuscus* cytochrome b control region haplotype. Modified from http://geology.com/state-map/south-dakota.shtml.

DISCUSSION

I expected that *E. fuscus* that inhabit the riparian forests of the Missouri River in South Dakota during the summer months utilize the caves and mines of the Black Hills in greater numbers than the human habitations of eastern South Dakota. If a greater number of bats from the Missouri River had similar haplotypes with the Black Hills populations than the eastern South Dakota population, I may have able to test this hypothesis. Yet, the data showed that this was not the case.

The population of *E. fuscus* in South Dakota are a panmictic population of interbreeding individuals, considering the spread of haplotype A across the three populations (eastern, Missouri River, and western). A sample size of 13 does not make a great estimate of phylogenetic diversity, but it did provide an estimate of gene flow. At this point in time in the genetic history of *E.fuscus* in South Dakota, gene flow is occurring across South Dakota as seen by the prevalence of haplotype A. If gene flow does become restricted between the bats, possibly due to lack of dispersal habitat, enough genetic change could occur between eastern and western populations to cause genetic divergence. Currently, *E. fuscus* individuals are finding means to transverse the state and interbreed with each other. This may be attributed to natural

habitat such as cave hibernaculas and riparian corridors but also to the expansion of anthropogenic structures since increased colonization of South Dakota in the late 1800s.

The occurrence of three different haplotypes in Sioux Falls is interesting but may be attributed to a natural mutation rate as only one or two bases are polymorphic in these haplotypes. As genetic time passes, more or less haplotypes may be generated at this location but further investigation into the nuclear genome and/or morphological traits will give a clearer understanding if these genetic differences are a the result of a true population subdivision.

The cytochrome b data did provide a good starting point towards the analysis of the genetic relatedness of South Dakota *E. fuscus* populations. As this is a maternally inherited mitochondrial marker, gene flow can be documented and large-scale variation between species and within species at the population level can be visualized. Further exploration of individual relatedness within each population subdivision can be found using nuclear markers that are biparentally inherited. Whereas the mitochondrial marker showed that the *E. fuscus* population in South Dakota and eastern Minnesota is one big panmictic population, a nuclear marker may show if there is any population subdivision in the Black hills, Missouri River, or Eastern South Dakota. Mitochondrial data only provides one part (maternal) of the picture but comparing more data sets (mitochondrial, nuclear, and morphological) will give a better picture and may also document greater variation in the population. A greater sample size will also provide a greater resolution to the genetic variation within these bat populations.

Most of the lab work was spent optimizing the PCR amplification, as this proved difficult due to different DNA concentrations and quality of DNA in each individual. The bands were not of equal intensity (Figure 1) and further optimization is required to produce bands of higher intensity, to improve the quality of the sequencing product. In hindsight, the degradation process could have been impeded by placing the tissues directly into lysis buffer after they were extracted from each individual. I was hesitant to ask this because the TTU Museum handles frozen tissues much more readily than tissues in lysis buffer due to the size difference in the tubes. Frozen or cryo tubes are much smaller (2 mL) and a place can be secured for them in the -70 C freezers whereas lysis tubes are bigger (5 mL) and bulkier causing increased postal costs and the ratio of tissue to lysis must be exact or the tissue will not be preserved.

Usually when frozen tissues are shipped, dry ice is placed into the package to insure the tissues will remain frozen. Scott Pedersen shipped all the tissues in dry ice but when the tissues reached Texas Tech, miscommunications and shipping delays prevented frozen tissues from reaching the museum. Of the three packages sent, only one reached the Texas Tech Museum in a frozen state. This caused further degradation of the tissue and more difficulty amplifying and sequencing all 500 base pairs of the cytochrome b control region. In an ideal situation, all animals/tissues used for DNA studies should be stored in -70 C, as -20 C will not prevent DNA degradation. Of course, I had no control over the shipping errors by Fed Ex and any miscommunication errors. However, in the future, I would feel safer using lysis buffer to store tissues to prevent such mishaps.

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