



University of Tennessee, Knoxville
**TRACE: Tennessee Research and Creative
Exchange**

Chancellor's Honors Program Projects

Supervised Undergraduate Student Research
and Creative Work

5-2001

An Analysis of Population Structuring in the Eastern Red Bat (Lasiurus Borealis) Using the Mitochondrial D-loop

Julie Rose Hermann
University of Tennessee-Knoxville

Follow this and additional works at: https://trace.tennessee.edu/utk_chanhonoproj

Recommended Citation

Hermann, Julie Rose, "An Analysis of Population Structuring in the Eastern Red Bat (*Lasiurus Borealis*) Using the Mitochondrial D-loop" (2001). *Chancellor's Honors Program Projects*.
https://trace.tennessee.edu/utk_chanhonoproj/467

This is brought to you for free and open access by the Supervised Undergraduate Student Research and Creative Work at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Chancellor's Honors Program Projects by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.

UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

Name: Julie Hermann

College: Arts & Sciences Department: Ecology & Evolutionary Biology

Faculty Mentor: Gary McCracken

PROJECT TITLE: An Analysis of Population Structuring in the Eastern
Pied Bat (*Lasiurus borealis*) using the Mitochondrial D-loop

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: Gary F. McCracken, Faculty Mentor

Date: May 4, 2001

Comments (Optional):

**AN ANALYSIS OF POPULATION STRUCTURING IN THE
EASTERN RED BAT (*LASIURUS BOREALIS*)
USING THE MITOCHONDRIAL D-LOOP**

Julie Hermann

May 2001

Faculty Mentor: Gary McCracken

ABSTRACT:

Very little is known about the migration patterns of the eastern red bat, *Lasiurus borealis*. Available information about the ecology of the eastern red bat is presented here. This study examined population structuring in the form of female philopatry using a sequence analysis of the d-loop of the mitochondrial DNA. While no significant structuring was found, the results were confounded by an extremely small sample size. Suggestions for further work with mtDNA and the eastern red bat have been outlined.

INTRODUCTION:

A. Ecology

Eastern red bats, *Lasiurus borealis*, vary in color from bright orange to yellowish brown. Their range extends from southern Canada through the eastern half of the United States and south into northern Mexico. Red bats roost in pine and mixed hardwood communities containing sweet gums, water oaks, and loblolly pines and in bottomland hardwood swamps that primarily consist of cotton-gum and bald cypress (Menzel et al. 1998).

Lasiurus borealis feed on moths and other insects (including scarab beetles, planthoppers, flying ants, leafhoppers, ground beetles, and assassin beetles) at or above the forest canopy and also around street lamps (Mammals of Texas - Online Edition 1994). Since these bats are often attracted into areas inhabited by people, they are frequently encountered. During the three-year span from February 1984 to February 1987, 626 eastern red bats were reported to the Texas Department of Health. Of these, 46 animals, or 7%, tested positive for rabies infection (Schmidly 1991). Therefore, *L. borealis* has a relatively low incidence of infection as compared to the hoary

bat (*Lasiurus cinereus*) which was reported to have a 25% infection rate. However, it should be noted that the incidence rate in the hoary bat was based on a sample size of only 40 (Schmidly 1991). The spread of rabies through the eastern red bats might be predicted with more information on its population structure and gene flow.

We know very little about the migration patterns of the eastern red bat and essentially nothing about their migration routes. Our best information indicates that eastern red bats lead solitary lives, coming together only for mating and migration. Some observations have suggested that the males and females migrate independently in the spring and occupy different winter and summer ranges in certain areas (Tuttle 1988). However, as many as 100 bats have been observed migrating together (Tuttle 1988). Eastern red bats have been known to join migrating birds, perhaps even using the same orientation cues (Neuweiler 2000). This may be a source of some of the confusion as to the size of their migration groups. Migration of the eastern red bats seems to be dependent on latitude. While the bats in the northern portions of the range tend to migrate during the colder months, many in the warmer zones, like Texas, appear to be year-round residents. In the summer range, small family groups consisting of an adult female and her offspring have been found (Schmidly 1991). For information about possible ranges for the eastern red bat during their winter and summer ranges, see Whitaker and Hamilton (1998, Figure 1)

During the winter, especially in the northern part of the range, eastern red bats undergo periods of hibernation. Because of their long, thick fur, they are able to spend most of the winter outdoors in the branches of fir trees until the temperature reaches around 13°C (Neuweiler 2000). The eastern red bat does not, however, sleep continuously for the entire winter like other mammals. As with other bats, it rarely spends more than 80 days at one time in hibernation. The eastern red bat is unusual in that it only awakens from hibernation when the ambient temperature has reached 16°C (Neuweiler 2000). This is 6° warmer than the average for bats.

In northern latitudes, breeding occurs in August and September. Sperm is then stored in the uterus and oviducts until the spring when fertilization and parturition take place. Up to five offspring, but typically twins, are born in May, June, or July after an 80-90 day gestation period. This is known as delayed fertilization and further complicates the determination of parentage and thus, population structure. Delayed fertilization can be adaptive for the eastern red bats because in the temperate zones, the number of insects increases immediately after the winter when the

bats have just awakened from hibernation. Since fertilization occurs only 1 to 3 days after arousing from hibernation, the females and their offspring are able to take advantage of this abundant food source. Also, having offspring early in the summer allows the females to raise their young and increase their own store of body fat before the next hibernation period begins. In the southern ranges, especially Texas, it is thought that copulation might occur in the spring since these red bats are active throughout the year (Schmidly 1991).

Delayed fertilization and unknown migration patterns and mating systems have complicated the search for population structuring within the eastern red bats. Still, for purposes of predicting the spread of viruses through the populations and for conservation strategies, it is important to document population structuring in the eastern red bats. This study examines population structuring using sequence comparison of the mitochondrial d-loop.

A further application of this study is to facilitate our discrimination among the eastern red bat (*Lasiurus borealis*), the western red bat (*Lasiurus blossevillii*), and the seminole bat (*Lasiurus seminolus*). The ranges of these species overlap, and their appearance is so similar that they are often difficult to distinguish in the field. Indeed, a morphological study by Koopman and McCracken (1998) found no evidence that *L. borealis*, *L. blossevillii*, and *L. seminolus* should be classified as separate species. Seminoles are cited as having a deeper mahogany color than the eastern red bat (Tuttle 1988) and have been shown to roost at an average height (16.3 m) that is 10 m higher than the eastern red bat (Menzel, et al. 1998). However, in practice, it is very difficult to tell individual seminole bats from the closely-related eastern red bats. The eastern red bat, western red bat, and seminole bat were first acknowledged as separate species by Baker, et al. (1988) using allozymes, and through the use of the high-resolution mtDNA, a more precise level of genetic divergence could be determined.

B. Mitochondrial DNA

Mitochondrial DNA is maternally-inherited and therefore inherited only through females and not subject to recombination. When looking only at the mtDNA, the effective population size becomes smaller and more susceptible to genetic drift. Compared to nuclear DNA, mtDNA can discern more subtle structuring between populations because it evolves faster and accumulates more between-population variation in a shorter evolutionary time span. Because it is inherited through the female line, mtDNA can be used to assess structuring that is limited to

females only, or female philopatry. Female philopatry occurs when females stay in their natal territory while males disperse to other populations.

Mitochondrial DNA consists of several genes coding for enzymatic proteins of the respiratory chain, two ribosomal RNAs, 20-28 genes for transfer RNAs, and a noncoding sequence that serves as a control region (called the “d-loop” in vertebrates) in replication and transcription of the mitochondrial genome (Baker 2000). The d-loop is the most variable region in the mitochondrial DNA and evolves faster than the mitochondrial genes for rRNA and tRNA. Part of the d-loop contains areas of tandem repeat units which have been speculated to “provide signal redundancy and a primitive repair mechanism in the event of somatic mutations” (Wilkinson, et al. 1997)

C. Previous Research

1. Research on the genus, *Lasiurus*

Baker, et al. (1988) used allozymes to distinguish between the eastern red bat, *Lasiurus borealis*, and the western red bat, *Lasiurus blossevillii*. This was the first study to recognize the separate species based on genetic differences. Previously, the genus *Lasiurus* had been divided into species solely according to morphological characteristics.

Morales and Bickham (1995) used restriction-site mapping of ribosomal genes of the mitochondrial DNA to clarify the phylogenetic relationships of the species in the genus *Lasiurus*. They found that *L. borealis*, *L. seminolus*, and *L. blossevillii* should be considered separate species and were able to determine relationships among subspecies of *L. blossevillii*. Information from restriction fragment analysis of the ribosomal genes of mtDNA can therefore discern variation to the level of subspecies. Because the control region of the mtDNA evolves faster than the ribosomal genes, the control region (d-loop) can be used to study more shallow phylogenetic relationships, such as population structuring within a species or subspecies.

2. Mitochondrial DNA Research

Mitochondrial DNA has been used to resolve issues concerning conservation, evolutionary history of a species, the adaptive value of female philopatry, and migration. The range of the

ghost bat, *Macroderma gigas*, has undergone a major and unexplained contraction during the past 100 years. Worthington Wilmer, et al. (1994) analyzed sequences of the hypervariable region of mtDNA in order to determine if female-mediated gene flow is restricted among the remaining populations and if isolation of the populations has increased with the contraction of the range. Using mtDNA sequence data and restriction fragment length polymorphisms, they were able to determine that this species exhibited extremely strong female philopatry. The structuring was so deep that the researchers were forced to conclude that the isolation of the current populations began before the contraction of the range. In terms of conservation, Worthington Wilmer et al. (1994) concluded that the extant populations should be managed as separate units because the low incidence of female dispersal would be ineffective in replacing local population extinctions. Thus, not only does mtDNA give information about the current gene flow, but in some cases it can discern historical population structure as well.

In Bechstein's bat (*Myotis bechsteinii*), Kerth, et al. (2000) used three variable sites within the control region of the mtDNA – one of which was an 81-bp repeat used by Wilkinson and Chapman (1991) – to discriminate between colonies as close together as 3 km. Bechstein's bat is not migratory and from field studies, groups of females have been shown to roost together in the summer repeatedly over many years. A Mantel-test revealed a weak, but significant correlation between the linear geographic distance and the haplotype overlap between colonies. Dispersal barriers were ruled out as a cause for this structuring among females because of the observed male dispersal. Therefore, it was concluded that the female philopatry must have some adaptive value, such as allo-mothering or information transfer.

Wilkinson and Chapman (1991) explored the length and sequence variation of the mitochondrial d-loop in the Evening bat, *Nycticeius humeralis*. They used three pairs of primers, including the P and CSB-F primers used in this study, to amplify and sequence a segment of 81-bp direct repeats containing from 5 to 8 repeat units. The goal of this study was to determine how the observed sequence and repeat variation arose between and within species. Two nonexclusive hypotheses were tested – 1) nuclear-mitochondrial genome coevolution, or that the mitochondrial d-loop sequences have been under selection for their ability to bind a nuclear enzyme and 2) concerted evolution, or that sequence variation is due to the creation of tandemly repeated sequences through replication slippage or unequal crossing over. They found that the variation of the first repeat unit nearest the proline tRNA gene was consistent with the hypothesis for

concerted evolution, while the other repeat units were acted on by selection, or through nuclear-mitochondrial genome coevolution.

Using the same primers, P and CSB-F, Wilkinson and Fleming (1996) resolved two possible migration corridors in the lesser long-nosed bat, *Leptonycteris curasoae*. They took the unique track of first ruling out alternatives to limits to gene flow as causes of sequence variation in the mitochondria; such as diversifying selection and unequal mutation rates. Using a Mantel test that correlated F_{ST} values with geographic distance, they found that these bats move more within these two migration corridors than between them.

In a study of male dispersal in the noctule bat, *Nyctalus noctula*, Petit and Mayer (1999, 2000) compared the information obtained from the use of microsatellites versus mitochondrial DNA. Due to the very different dispersal rates of males and females in the noctule bat, there was a large discrepancy in the amount of genetic variability that could be discerned using the two genetic markers. In mtDNA, 22.6% of the genetic variability was found between the colonies (77% of the variation was within the colonies) while only 0.5% of the microsatellite variation was found between colonies. This is clearly a result of the smaller effective population size of mtDNA and its higher susceptibility to genetic drift. In this case, the noctule bat was found to have very high male dispersal as compared to the females. Still, male dispersal was not panmictic, and Mantel tests were used to determine if this was due to the influence of migration routes or geographic distance on the dispersal of the male bats. Using mtDNA to compare the genetic structure of hibernating winter colonies with the structure of summer nursery colonies, Petit and Mayer (2000) determined that individuals from several nursery colonies gather together in winter roosts.

3. Research on *Lasiurus borealis*

Previous studies dealing with *L. borealis* have concentrated on the distinction between species and subspecies. More subtle population structuring within the species *L. borealis* had not been examined. David Wills (2000, unpublished) using two microsatellite loci to look for population structuring among nine populations of *L. borealis*, found no structuring, which is indicative of a high rate of male dispersal (and the absence of male philopatry). Therefore, the next logical step was to search for female philopatry using a high-resolution genetic marker like

mtDNA. In this study, I used the mitochondrial d-loop in order to assess the possibility of female philopatry.

MATERIALS & METHODS:

Sample collection and DNA extraction:

3mm wing punches were taken from 16 eastern red bats from 10 sites: Ohio, Michigan, Indiana, Pennsylvania, South Carolina, West Virginia, East Tennessee, Middle Tennessee, Kentucky, and West North Carolina. These bats were all sampled in their summer ranges. Wing punches were stored at 0°C in DMSO until the DNA was to be extracted. The wing punches were digested using 30µl of 20mg/ml protein kinase and were kept in a 55°C water bath until the tissue was dissolved. DNA was extracted using standard phenol-chloroform extraction and ethanol precipitation (Hillis, et al. 1996).

DNA amplification and Sequencing:

A pair of 22-bp primers were used to amplify a portion of the mitochondrial d-loop containing 81-bp repeats. These primers, P (tRNA^{pro}): 5'-TCCTACCATCAGCACCCAAAGC-3' (light strand) and F (conserved sequence block F): 5'-GTTGCTGGTTTCACGGAGGTAG-3' (heavy strand), had been found to amplify the d-loop in at least five families of bats (Wilkinson and Chapman 1991, Figure 2)

Double-stranded amplifications of the DNA were done using 12µl reactions consisting of: 1.43µl Thermophilic DNA Polymerase 10x buffer (Mg-free), 0.64µl MgCl₂ (at 25mM), 0.28µl dNTPs (at 5µM), 1.19µl of each primer (P and F at 100ng/µl), 1µl template (at 10ng/µl), 0.24µl Taq polymerase (at 5 u/µl), and 6.03µl dH₂O. The PCR protocol consisted of one preliminary denaturation at 95°C (2 min), followed by denaturation at 95°C (30 sec), annealing at 50°C (30 sec), and primer extension at 72°C (30 sec), repeated for 30 cycles.

2% agarose gel electrophoresis and ethidium bromide staining were used to select homoplasmic individuals for sequencing. PCR products were cleaned prior to the sequencing reaction using the QIAquick PCR Purification Kit with an extra step of washing with 750µl GuHCl to remove primer dimer. Primer P was used as a sequencing primer with the ABI Big Dye Chemistry kit.

Sequence Analysis:

Sequences containing 4 repeat units were aligned using Clustal X. Allele phylogeny was estimated using PAUP and parsimony and neighbor-joining trees were created using a *Myotis sodalis* sequence as an outgroup. A *Lasiurus blossevillii* sequence was also included for comparison with the *L. borealis* samples, but the tree was not rooted with the *L. blossevillii*. The stability of branches was evaluated using 5000 bootstrap replications. Tree manipulation was done using TreeView. A Mantel test was conducted using Arlequin to find possible correlation between F_{ST} values and geographic distance. Clustal W was used to determine the percentage of sequence similarity.

RESULTS:

A consensus parsimony tree was made from 24 possible trees. The parsimony tree indicates the minimum number of genetic events needed to create the observed sequence differences. Bootstrap values were found for each of the branches. (Figure 3) A neighbor-joining tree was constructed to show the relative genetic difference between sequences. (Figure 4)

Table 1: Population Pairwise F_{ST} s

	WV	KY	MI	OH	IN	WNC	SC	PA	MTN	ETN
WV	0									
KY	0.04193	0								
MI	-0.20727	0.42308	0							
OH	-0.03403	0.43662	0.05263	0						
IN	-0.32635	0.27835	-0.1831	-0.03297	0					
WNC	-0.54651	0.2	0.21429	0	-0.24	0				
SC	-0.62195	0.75	-0.1	-0.03226	-0.72222	1	0			
PA	-0.56471	0.80952	0.15385	0.21951	-0.55	1	1	0		
MTN	-0.02308	0.91398	0.68571	0.62791	0.10145	1	1	1	0	
ETN	0.39269	0.94444	0.85135	0.81395	0.5894	1	1	1	1	0

**Note: None of the F_{ST} values are significant!

Mantel Test (F_{ST} and geographic distance) $p = 0.247 \Rightarrow$ not significant

Key

ERB = Eastern Red Bat, *Lasiurus borealis*

WRB = Western Red Bat, *Lasiurus blossevillii*

ms = Indiana Bat, *Myotis sodalis*

OH = Ohio

IN = Indiana
 SC = South Carolina
 MI = Michigan
 WV = West Virginia
 PA = Pennsylvania
 ETN = East Tennessee
 MTN = Middle Tennessee
 KY = Kentucky
 WNC = Western North Carolina
 CA = California

Table 2: Percent Similarity Between Sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	100																	
2	95	100																
3	94	93	100															
4	95	95	94	100														
5	91	91	91	93	100													
6	99	94	94	95	91	100												
7	96	97	94	97	94	96	100											
8	96	92	92	91	91	96	95	100										
9	94	90	89	91	90	93	91	92	100									
10	58	76	61	75	70	58	75	60	74	100								
11	81	78	82	80	80	81	80	81	78	49	100							
12	86	82	85	83	83	86	84	86	82	52	82	100						
13	97	93	94	93	91	97	95	96	92	59	82	86	100					
14	96	91	92	92	90	96	94	95	92	59	81	86	96	100				
15	95	91	92	92	90	95	94	95	91	58	83	87	97	97	100			
16	83	83	81	81	78	82	82	81	81	56	68	70	81	80	79	100		
17	77	78	77	78	77	77	79	77	73	55	73	73	77	80	80	68	100	
18	83	79	81	80	79	83	81	83	79	52	78	79	84	82	83	69	75	100

Sequence 1: ERB4OH
 Sequence 2: ERB62_575_KY
 Sequence 3: ERB81_625_MI
 Sequence 4: ERB96_611_WNC
 Sequence 5: ERB107_7052_WV
 Sequence 6: ERB9IN
 Sequence 7: ERB147KY
 Sequence 8: ERB84_660_MI
 Sequence 9: ERB3OH
 Sequence 10: 27msky
 Sequence 11: ERB108_7053_WV
 Sequence 12: ERB11IN
 Sequence 13: ERB34_360_SC
 Sequence 14: ERB77_715_WV
 Sequence 15: ERB115_990_PA
 Sequence 16: WRB183_263_CA
 Sequence 17: ERB179_8_ETN
 Sequence 18: ERB185_1148_MTN

DISCUSSION:

This study suffers from the small sample size -- with only 16 sequences examined, none of the p-values were significant. The Mantel test showed no correlation between the F_{ST} values and geographic distance. Therefore, bats sampled from populations that are farther apart were not genetically more different than bats from populations that are closer together. Considering that the geographic distance between sampling sites ranged from 69 to 775 miles, these data suggest panmixia due to the high vagility of the eastern red bat. From a conservation perspective, these data suggest that there is a high probability of repopulation after a local extinction of populations of eastern red bats. However, these results tell us little about the migratory patterns of *Lasiurus borealis*.

The bootstrap analysis (Figure 3) provide further evidence for high levels of dispersal of eastern red bats, since none of the branches were consistently present in more than 88% of the 5000 trees searched. However, the branch with the highest strength (88%) separated one bat from Indiana, one from West Virginia, and the bats from East Tennessee and Middle Tennessee from the rest of the samples. This is surprising since the branch separating *Myotis sodalis* and *Lasiurus blossevillii* was only present in 78% of the trees. Because my sample size was small, I decided to compare the sequences individually for pairwise comparisons of percent sequence similarity (Table 2). These four bats (mentioned above) were consistently less similar compared to the other samples; especially the two bats from Tennessee, which had sequence similarities comparable to that of the western red bat. However, the Tennessee bat sequences were also only 75% similar to each other, and these two bats together do not clearly represent a single population. These sequence differences were also observed in the neighbor-joining tree (Figure 4).

The results showing that the Tennessee eastern red bats and the western red bat were comparable in percent sequence similarity appear to contradict the species distinction that has been made in the past (Baker, et al. 1988, Morales and Bickham 1995). Also, in several of the parsimony trees, the western red bat sequence showed no separation from the eastern red bat samples. Analysis of the d-loop needs to be done using more sequences of western red bats in order to elucidate the status of the western red bat.

A neighbor-joining tree derived from the restriction-site analysis of the ribosomal genes by Morales and Bickham (1995) indicated the ability to discriminate between *L. borealis* found in Texas versus West Virginia. However, their bootstrap analysis showed that one of the branches had a replication percentage of 62% in favor of grouping one of the Texas bats with the West Virginia bats rather than with the other Texas bat. This seems to indicate that the distinction between these two populations also cannot be clearly defined using ribosomal genes of the mtDNA.

From the data collected here, mtDNA did not detect female philopatry in the eastern red bats, as none of the F_{ST} values were significant. Male philopatry also was not detected in microsatellite data (Wills 2000, unpublished). This appears to be a result of the wide ranges covered in the migrations of *L. borealis*.

Looking at this as a pilot study, we see that further work with the d-loop would require a larger sample size and especially the use of bats from Tennessee and farther south. Texas would be a good site to sample since eastern red bats in Texas are reported to be non-migratory. To search for possible migration corridors, it would be useful to get a large sample of bats from both sides of the Appalachians and to look possible limits in dispersal due to geography. Furthermore, all of the bat samples in this study were collected while the bats were in their summer ranges. Structuring might be revealed in their fall and winter roosts where mating actually occurs. An example of the utility of mtDNA in extracting this type of information comes from Petit and Mayer (2000) who were able to show that the hibernating colonies were aggregations formed from individuals from several genetically distinct nursery colonies. It remains possible that the winter (hibernating) colonies of *L. borealis* are structured whereas the summer (natal) aggregations are not. To test this hypothesis, samples are needed from populations in the fall or winter.

In non-migratory and migratory bats where limits to gene flow and structuring exist, information gained from the mitochondrial d-loop would be useful in predicting the spread of rabies through bat populations. Rabies is spread from one bat to another through direct contact, which is most likely to occur during mating. Therefore, when a bat from one geographic location is found to have rabies, the probable direction of the spread of the virus can be determined by analyzing the probability of gene flow in one geographic direction versus another. In migrating, panmictic species, the probable direction of the spread of rabies cannot be ascertained because

gene flow is equally probable in any geographic direction within the species' range. For these species, the utility of mitochondrial DNA lies in its ability to determine whether or not a species is panmictic and whether or not we can predict the spread of rabies through populations.

CONCLUSIONS:

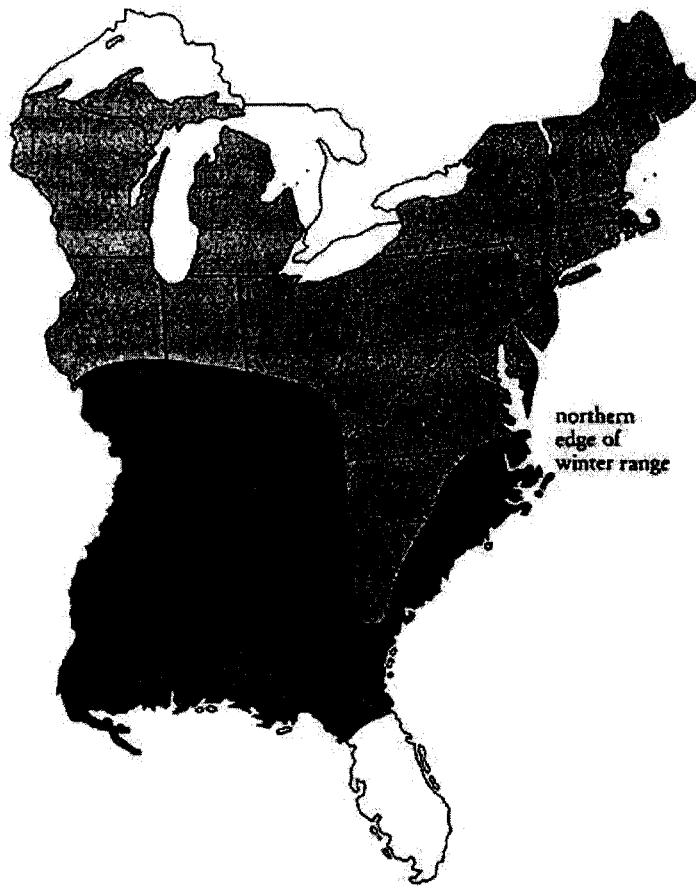
The small sample size of this study does not allow definitive conclusions about the migration or population structure eastern red bat. From this preliminary study, structuring does not appear to exist in the summer range of eastern red bats. However, further research needs to be done using eastern red bats from their winter range and a larger sample size in order to determine if the species is entirely without structure. Also, a discrepancy was seen between the results from this study and the classification of the western red bats in Morales and Bickham (1995) and Baker, et al. (1988). More d-loop sequences of the western red bat need to be compared to the eastern red bat sequences before any conclusions can be drawn.

REFERENCES:

- Baker, Allan J., ed. 2000. *Molecular Methods in Ecology*. Blackwell Science Ltd.: MA.
- Baker, R. J., J. C. Patton, H. H. Genoways, and J. W. Bickham. 1988. Genic Studies of *Lasiurus* (Chiroptera: Vespertilionidae). *Occasional Papers, The Museum, Texas Tech University*, 117, 1-15.
- Hillis, David M., Craig Moritz, and Barbara K. Mable, eds. 1996. *Molecular Systematics*. Sinauer Associates, Inc.: MA.
- Kerth, Gerald, Frieder Mayer, and Barbara Konig. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. *Molecular Ecology* 9, 793-800.
- Koopman, Karl F. and Gary F. McCracken. 1998. The Taxonomic Status of *Lasiurus* (Chiroptera: Vespertilionidae) in the Galapagos Islands. *American Museum Novitates* 3243, 1-6.
- Mammals of Texas* – Online Edition. 1994. "Eastern Red Bat." Texas Parks and Wildlife, Nongame and Urban Program.
- Menzel, Michael A., Timothy C. Carter, Brian R. Chapman, Joshua Laerm. 1998. Quantitative comparison of tree roosts used by red bats (*Lasiurus borealis*) and Seminole bats (*L. seminolus*). *Canadian Journal of Zoology* 76(4), 630-634.

- Morales, Juan Carlos and John W. Bickham. 1995. Molecular systematics of the genus *Lasiurus* (Chiroptera: Vespertilionidae) based on restriction-site maps of the mitochondrial ribosomal genes. *Journal of Mammalogy* 76(3), 730-749.
- Neuweiler, Gerhard. 2000. *The Biology of Bats*. Trans. Ellen Covey. Oxford University Press: New York.
- Nowak, Ronald M. 1994. *Walker's Bats of the World*. Johns Hopkins University Press: Baltimore.
- Petit, Eric and Frieder Mayer. 1999. Male dispersal in the noctule bat (*Nyctalus noctula*): Where are the Limits? *Proc. R. Soc. Lond. B* 266, 1717-1722.
- Petit, Eric, and Frieder Mayer. 2000. A Population Genetic Analysis of Migration: The Case of the Noctule Bat (*Nyctalus noctula*). *Molecular Ecology* 9, 683-690.
- Schmidly, David J. 1990. *The Bats of Texas*. Texas A & M University Press: USA.
- Shump, Karl A. and Ann U. Shump. 1982. *Lasiurus borealis*. *Mammalian Species* 183, 1-6.
- Tuttle, Merlin D. 1997. *America's Neighborhood Bats*. University of Texas Press: Austin.
- Whitaker, John O., Jr. and William J. Hamilton, Jr. 1998. *Mammals of the Eastern United States*. Cornell University Press: New York.
- Wilkinson, Gerald S. and Alyson M. Chapman. 1991. Length and Sequence Variation in Evening Bat D-loop mtDNA. *Genetics* 128, 607-617.
- Wilkinson, G. S. and T. H. Fleming. 1996. Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA. *Molecular Ecology* 5, 329-339.
- Wilkinson G. S., F. Mayer, G. Kerth and B. Petri. 1997. Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. *Genetics* 146, 1035-1048.
- Wills, David and Gary McCracken. 2000, unpublished. Population Genetics of the Eastern Red Bats, *Lasiurus borealis*.
- Wilmer, J. Worthington, C. Moritz, L. Hall, and J. Toop. 1994. Extreme population structuring in the threatened ghost bat, *Macroderma gigas*: evidence from mitochondrial DNA. *Proc. R. Soc. Lond. B*, 193-198.

Figure 1:



*The summer and winter ranges
of the eastern red bat, Lasiurus
borealis, in the eastern United States*

(Whitaker and Hamilton 1998)

Figure 2:

Location of Primers in mtDNA

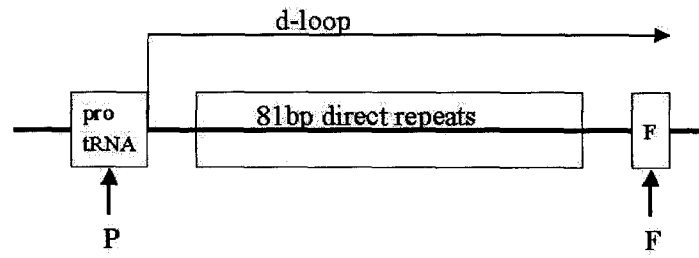


Figure 3: Bootstrap Analysis

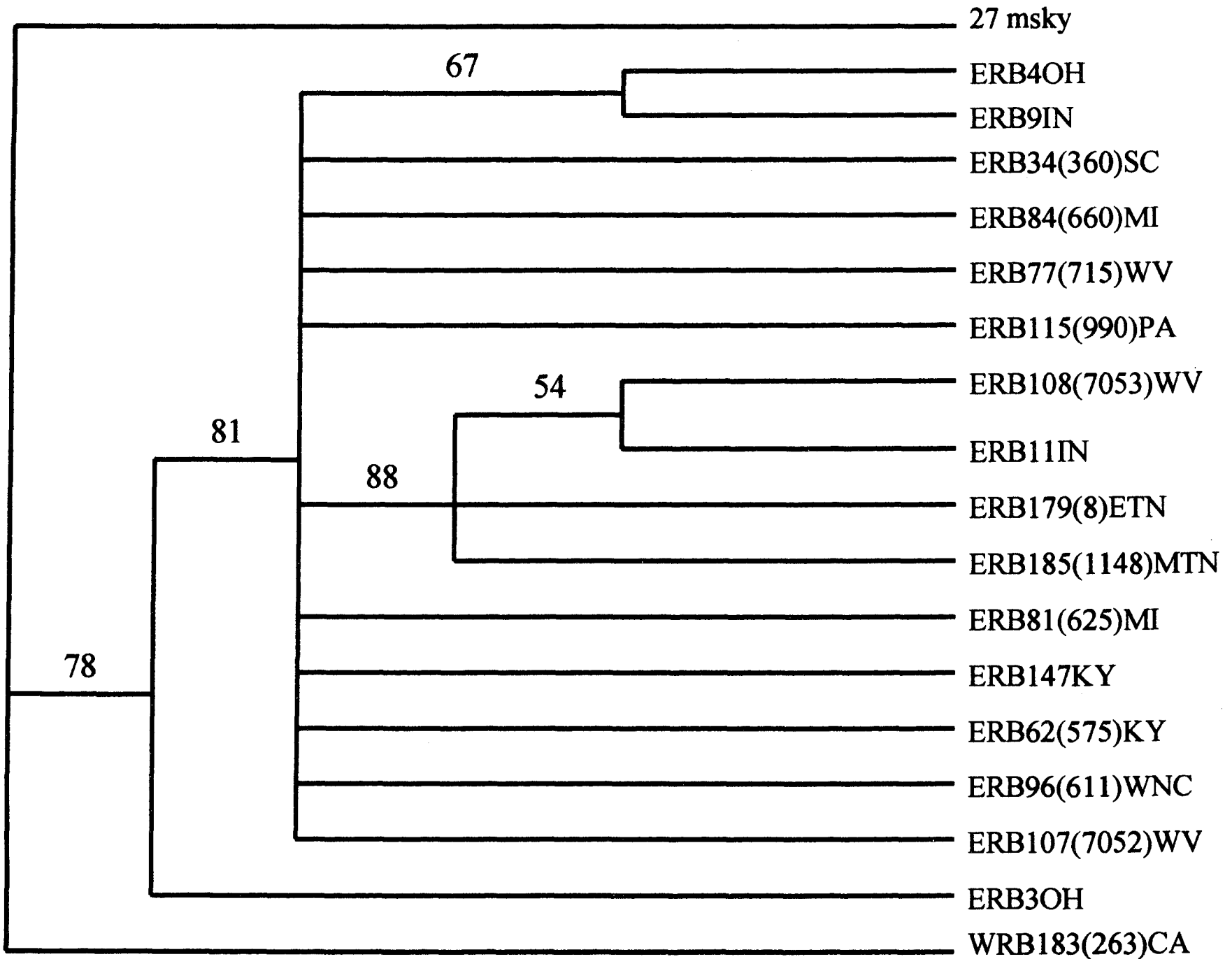


Figure 4: Neighbor-Joining Tree

