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UNRAVELING THE EFFECTS OF SEX AND DISPERSAL: OZARK BIG-EARED BAT (*CORYNORHINUS TOWNSENDII INGENS*) CONSERVATION GENETICS

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The Ozark big-eared bat (*Corynorhinus townsendii ingens*) is federally listed as endangered and is found in only a small number of caves in eastern Oklahoma and northwestern Arkansas. Previous studies suggested site fidelity of females to maternity caves; however, males are solitary most of the year, and thus specific information on their behavior and roosting patterns is lacking. Population genetic variation often provides the necessary data to make inferences about gene flow or mating behavior within that population. We used 2 types of molecular data: DNA sequences from the mitochondrial D loop and alleles at 5 microsatellite loci. Approximately 5% of the population, 24 males and 39 females (63 individuals), were sampled. No significant differentiation between 5 sites was present in nuclear microsatellite variation, but distribution of variation in maternally inherited markers differed among sites. This suggests limited dispersal of female Ozark big-eared bats and natal philopatry. Areas that experience local extinctions are unlikely to be recolonized by species that show strong site fidelity. These results provide a greater understanding of the population dynamics of Ozark big-eared bats and highlight the importance of cave protection relative to maintaining genetic integrity during recovery activities for this listed species.

Key words: *Corynorhinus*, geographic structure, microsatellites, mitochondrial DNA, Oklahoma, philopatry

Townsend's big-eared bats (*Corynorhinus townsendii*) live in a variety of habitats across North America but are much more common in western states (Handley 1959). Of 5 recognized subspecies (*australis*, *ingens*, *pallescens*, *townsendii*, and *virginianus*), the 2 that occur entirely east of the continental divide (*ingens* and *virginianus*) are geographically isolated from one another and other conspecifics (Fig. 1) and are federally listed as endangered (United States Fish and Wildlife Service 1984, 1995). Populations of these 2 eastern subspecies likely always have been limited by availability of necessary habitat, particularly caves for roosting, but they have experienced declines in their ranges over the past few decades (Humphrey and Kunz 1976; United States Fish and Wildlife Service 1984, 1995).

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Historically, Ozark big-eared bats (*C. t. ingens*) occurred in eastern Oklahoma, northern Arkansas, and southern Missouri (United States Fish and Wildlife Service 1995). Only about 100 Ozark big-eared bats could be located during the early 1970s, and they have been extirpated from Missouri (Kunz and Martin 1982; United States Fish and Wildlife Service 1973). With increased survey efforts in the 1980s, numbers of Ozark big-eared bats were estimated variously at <450 to about 1,700 individuals (based on doubling exit counts of primarily females at maternity caves—United States Fish and Wildlife Service 1995). In the 1990s, the total population was estimated at 1,600–2,300 (United States Fish and Wildlife Service 1995). The largest documented colony of Ozark big-eared bats at a single site is 225–325 individuals (Martin et al. 2000). Although the majority of the present range is thought to occur in Arkansas (United States Fish and Wildlife Service 1995; Fig. 1), all caves consistently used by groups of Ozark big-eared bats are located in a single county in eastern Oklahoma (Clark et al. 1996; Wethington et al. 1997), and few bats have been located in Arkansas (Prather and Briggler 2002).

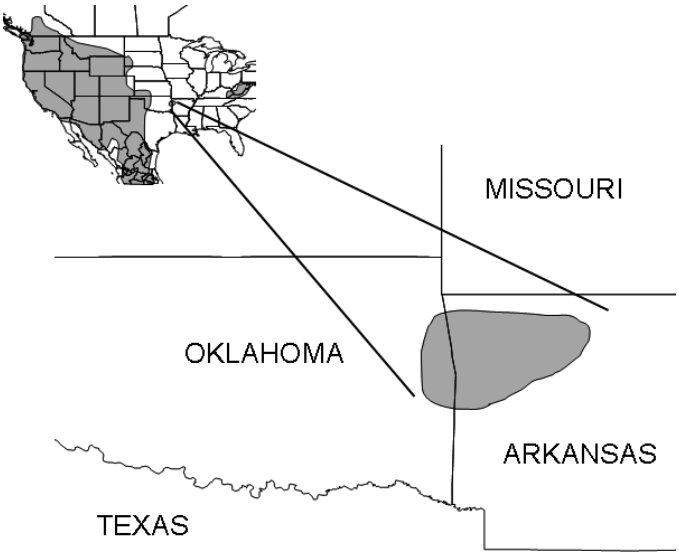

FIG. 1.—Distribution of *Corynorhinus townsendii* in Canada, United States, and north-central Mexico (shaded regions of map; modified from Hall [1981]). The current range of the Ozark big-eared bat (*C. t. ingens*) is shown in enlarged area below.

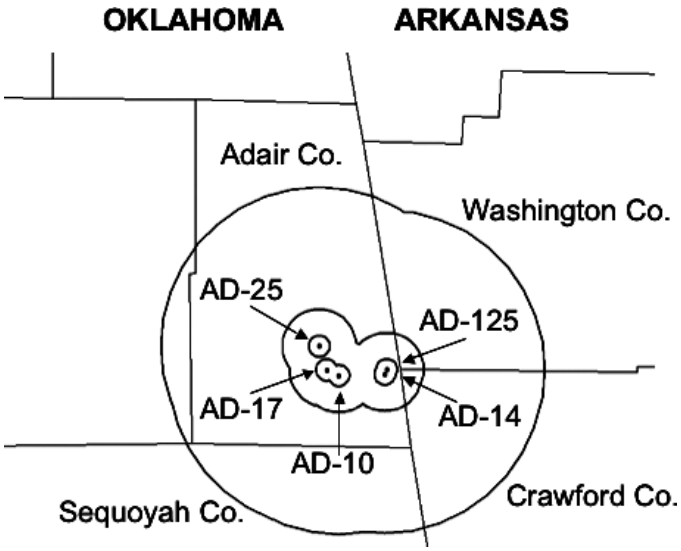

FIG. 2.—Counties in eastern Oklahoma and northwestern Arkansas with sampled caves (AD-25, AD-17, AD-10, AD-14, and AD-125) containing *Corynorhinus townsendii ingens* identified by dots. Caves are surrounded by radii of 2 km, 7 km (i.e., average nightly foraging distance and maximum expected nightly movement, respectively—Clark et al. 1993, 1997; Wethington et al. 1996), and 30 km (i.e., approximate maximum expected dispersal distance revealed by previous banding data—Harvey 1992; United States Fish and Wildlife Service 1995).

Ozark big-eared bats mate during autumn and winter, and sperm is stored in the reproductive tract of females until arousal from hibernation, at which time fertilization occurs (United States Fish and Wildlife Service 1995). Females begin to congregate in late April or May at maternity caves and give birth to young in June (Clark et al. 1996, 2002; United States Fish and Wildlife Service 1995). These maternity colonies typically disband in August (Clark et al. 1996; Wethington et al. 1996). Ozark big-eared bats also form colonies in October and November, where males and females are found together and hibernate through February (Clark et al. 1996, 2002).

In Oklahoma, 12 caves in which Ozark big-eared bats have been found in any number are currently gated; however, most of these are not maternity caves and experience only sporadic use by Ozark big-eared bats (Martin et al. 2000). These may be used as transitory caves between seasons because behavioral studies suggest that Ozark big-eared bats exhibit site fidelity to those caves they use extensively, even returning to the same location within a cave (Clark et al. 1996). Although Ozark big-eared bats do not migrate, they differentially use caves within a region by season (Clark et al. 1997; Humphrey and Kunz 1976). Banding studies showed that the maximum distance traveled by a Ozark big-eared bat was about 30 km (Harvey 1992; United States Fish and Wildlife Service 1995), but examination of radiotelemetry data suggested that typical distances traveled over any 24-h period were generally within 2 km of the roosting site and never exceeded 8 km (Clark et al. 1993, 1997; Wethington et al. 1996; Fig. 2).

Our objectives were to evaluate connectivity among colonies of Ozark big-eared bats resulting from effective movement of each sex by examining genetic variation in the mitochondrial and nuclear genomes. Based on current knowledge and documentation of site fidelity (Clark et al. 1996, 1997), we predicted that genetic differentiation between maternity caves was likely to be detected. However, specific information on the behavior and roosting patterns of male Ozark big-eared bats in spring through autumn months is lacking. Male Ozark big-eared bats typically are solitary during this time and roost in caves or on bluff faces, making observational studies difficult (Clark et al. 1997; United States Fish and Wildlife Service 1995). We predicted that dispersal of males would result in a population that appeared panmictic based on nuclear data. This is likely because of the trend toward male-biased dispersal in mammals and potential for copulation to occur when Ozark big-eared bats are moving between summer and winter roosts and at hibernacula (Avise 2000; Burland et al. 1999). We used distribution of mitochondrial haplotypes to determine if female big-eared bats used caves without preference and compared those data with the distribution of microsatellite alleles to infer if levels of dispersal of males were similar to those of females (Burland et al. 1999; Kerth et al. 2000; Petit et al. 1999; Petit and Mayer 2000; Petri et al. 1997; Rossiter et al. 2000; Worthington Wilmer et al. 1999).

MATERIALS AND METHODS

Mist nets were placed outside entrances of 5 caves (referred to as AD-10, AD-125, AD-14, AD-17, and AD-25; Fig. 2) to capture Ozark big-eared bats as they emerged to forage, usually about 30 min after sunset. Sampling was conducted from July to October in 2002 and April to October 2003. Individuals were sexed and photographed, and each wing membrane was punched with a 3-mm-diameter sterile biopsy instrument (Worthington Wilmer and Barratt 1996). Procedures followed guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998) and after handling, all bats

immediately took flight and appeared normal. Wing biopsy tissues were stored in lysis buffer until whole genomic DNA was isolated through phenol extraction and salt precipitation (Longmire et al. 1997; Zeugin and Hartley 1985).

Five microsatellite loci were amplified via polymerase chain reaction (PCR) by using primers isolated from *Eptesicus fuscus* (Vonhof et al. 2002). Reactions were conducted in a 15-µl volume containing 50 ng of genomic DNA, 10 pmol of each primer, 9 µl of ABI Prism True Allele PCR Premix (Applied Biosystems, Inc., Foster City, California), and 3.8 µl of double-distilled H_2O and cycling conditions of 12 min at 95°C followed by 35 cycles of 94°C for 30 s, 40–55°C for 45 s, and 72°C for 45 s and a final 10-min incubation at 72°C. Locus EF14 required the lowest annealing temperature of 40°C, 45°C was used for loci EF1 and EF6, 53°C for locus EF21, and 55°C for locus EF15. Amplifications were run on an acrylamide gel with an ABI-377 automated DNA sequencer (Applied Biosystems, Inc.), by using an internal size standard (ROX) in each lane. Genotypes were determined by analyzing PCR products with GeneScan 2.02 and Genotyper 2.0 software (Applied Biosystems, Inc.).

Deviations from Hardy–Weinberg equilibrium and estimates of genetic variation were tested by using ARLEQUIN version 2.00 (Schneider et al. 2000). Number of alleles per locus, frequency of alleles, observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), degree of genetic structure by using *F*-statistics (Wright 1965), an exact test of population differentiation (with 10,000 steps in the Markov chain), and analysis of molecular variance (AMOVA) at 3 hierarchical levels (within individuals, within colonies, and among colonies) were calculated. We also tested for recent reductions in effective population size by using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996), which uses sign and Wilcoxon tests to examine heterozygosity in relation to observed number of alleles and compares allele frequencies to the expected L-shaped distribution of mutation–drift equilibrium.

Approximately 480 base pairs (bp) of the mitochondrial genome control region (D loop) were sequenced by using primers known to amplify DNA in ≥5 families of chiropterans (Wilkinson and Chapman 1991). PCR conditions were as follows: 50 ng of DNA, 50 pmol of each primer, 10 mM of deoxynucleoside triphosphates, 1.5 mM of $MgCl_2$, and 1 unit of *Taq* DNA polymerase; 2 min at 95°C, then 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final 30-min incubation at 72°C. Double-stranded amplicons were purified by using the Wizard PCR Prep DNA Purification System (Promega, Madison, Wisconsin) and sequenced in both directions by using BigDye chain terminators and a 377 automated DNA sequencer (Applied Biosystems, Inc.). AssemblyLIGN 1.0.9 (Oxford Molecular Group, PLC 1998) was used to piece together overlapping fragments for each individual, and CLUSTAL X (Thompson et al. 1997) was used to align multiple sequences. Alignments were then imported into MacClade 4.0 (Maddison and Maddison 2000) to identify variable nucleotide positions and determine haplotypes.

Mitochondrial DNA (mtDNA) haplotype frequencies, haplotype diversity (*h*), nucleotide diversity (π), *F*-statistics, and exact tests of population differentiation (with 10,000 steps in the Markov chain) were calculated in ARLEQUIN (Schneider et al. 2000). Uncorrected percentage sequence divergence among haplotypes was calculated by using PAUP* (Swofford 2000). Nested-clade analysis was used to evaluate phylogeny of haplotypes with the program TCS, version 1.13 (Clement et al. 2000), which was an appropriate alternative to tree-building techniques for intraspecific phylogenies because it incorporated predictions of coalescent theory (e.g., the likelihood of both ancestral and derived haplotypes being found in the sampled population—Crandall and Templeton 1996). Reductions in effective population size based on mitochondrial data were tested by using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) for the entire population.

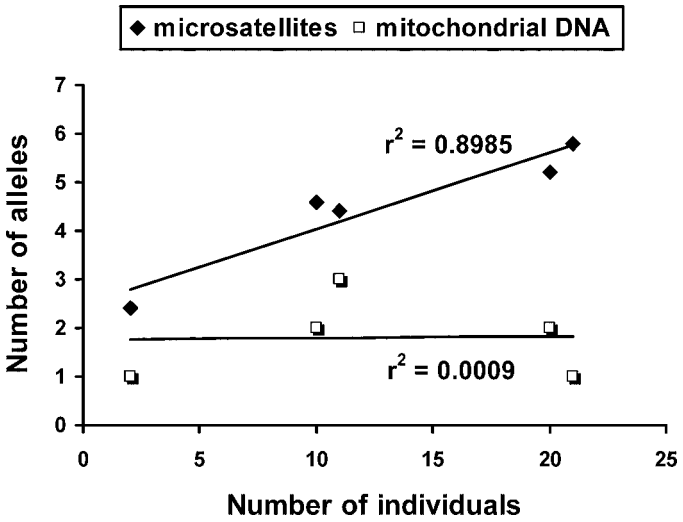

FIG. 3.—Average number of microsatellite alleles and number of mitochondrial haplotypes detected in colonies of *Corynorhinus townsendii ingens* at each cave relative to the number of individuals sampled per cave.

RESULTS

Wing biopsies were collected from 63 Ozark big-eared bats (24 males and 39 females). Although the sample was small, it represented 5% of the known population of this very rare subspecies in Oklahoma (approximately 1,200 bats—United States Fish and Wildlife Service 1995). Twenty individuals were captured at cave AD-10 (14 females and 6 males), 20 individuals at cave AD-125 (10 females and 10 males), 11 individuals at AD-14 (6 females and 5 males), 10 individuals at AD-17 (8 females and 2 males), and 2 individuals at AD-25 (1 female and 1 male). Northern long-eared myotis (*Myotis septentrionalis*) and eastern pipistrelles (*Pipistrellus subflavus*) also were caught at caves AD-10, AD-125, AD-14, and AD-25. Only northern long-eared myotis was found with Ozark big-eared bats at AD-17.

Based on genotyping 63 Ozark big-eared bats for 5 microsatellite loci, no pairs of loci consistently displayed signs of linkage disequilibrium or significant deviations from Hardy–Weinberg expectations. Number of alleles detected at each microsatellite locus ranged from 3 to 17, with expected heterozygosities (H_E) ranging from 0.144 to 0.888. The average number of alleles per locus was 7.4, and both observed (H_O) and expected heterozygosities were >0.500 for all loci except EF14. The average number of microsatellite alleles detected at each site displayed a positive relationship with the number of individuals sampled (Fig. 3). Allelic diversity and heterozygosity values averaged across loci for each cave ranged from 2.4 to 5.8 and 0.364 to 0.700, respectively (Table 1).

Analysis of nuclear variation revealed no significant genetic structuring among colonies using different caves (overall F_{ST} of 0), and only 1 of 10 pairwise F_{ST} comparisons (AD-17 to

Table 1.—Genetic diversity from colonies of Ozark big-eared bats (*Corynorhinus townsendii ingens*) in 5 caves: number of individuals sampled per site (*n*), number of microsatellite alleles averaged across loci (A), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), number of mitochondrial haplotypes (a), haplotype diversity (*h*), and nucleotide diversity (π).

Cave	*N*	A	H_O	H_E	a	*h*	π
AD-10	21	5.8	0.524	0.580	1	0.000	0.000
AD-125	20	5.2	0.540	0.590	2	0.268	0.006
AD-14	11	4.4	0.364	0.593	3	0.473	0.008
AD-17	10	4.6	0.480	0.744	2	0.356	0.001
AD-25	2	2.4	0.700	0.633	1	0.000	0.000

AD-25) was significant (data not shown). F_{IS} and F_{IT} averaged over all loci were 0.148 and 0.141, respectively. Results of an exact test substantiated the lack of genetic structure among colonies, with none of the pairwise comparisons showing significant differentiation (Table 2). AMOVA identified variation among individuals within caves and variation within individuals as accounting for 100% of the variance. When data were partitioned by cave or locus, or when all individuals were considered as a single population, examination of nuclear data did not suggest significant evidence of a bottleneck (an effect of reduction[s] in population size).

In the 484-bp fragment of the mtDNA control region, alignment of sequences revealed 12 variable nucleotide positions (all due to transition substitutions), resulting in 4 haplotypes (Table 3). Representative sequences of haplotypes A–D were deposited in GenBank (accession numbers AY706337–AY706340). Percentage sequence divergence among haplotypes ranged from 0.21% to 2.48%, with a mean of 1.27%. Haplotype B, which differed from haplotypes A, C, and D at 10–12 nucleotide positions (Table 3), was excluded from the statistical parsimony network created in TCS. Haplotypes C and D were each linked to haplotype A via single mutational steps at positions 265 and 302, respectively (Table 3). Tests for reductions in effective population size did not support a population bottleneck as responsible for the current distribution of haplotypes.

Number of haplotypes per cave ranged from 1 to 3, and only haplotype A was found at all caves (Table 1; Fig. 4). Haplotype C was restricted to cave AD-17, and haplotype D was found only at cave AD-14 (Fig. 4). Number of haplotypes detected at a cave could not be explained by the number of individuals sampled (i.e., $r^2 < 0.001$; Fig. 3). Haplotype diversity (*h*) within colonies ranged from 0 to 0.473, but nucleotide diversity (π) always was low (Table 1). In contrast to the nuclear microsatellite results, analysis of mtDNA variation revealed that 94.74% of the genetic variation was attributable to differences among individuals within a cave, whereas 5.26% of the variation was partitioned among caves ($F_{ST} = 0.0526$). When analyzed in a pairwise fashion, only 1 of 10 comparisons (cave AD-10 to cave AD-14) yielded a statistically significant F_{ST} value, but 4 of 10 comparisons revealed statistically significant values in the exact test of differentiation and the overall test of population differentiation was significant (Table 2).

Table 2.—Pairwise exact tests of differentiation between colonies of Ozark big-eared bats (*Corynorhinus townsendii ingens*) based on nuclear microsatellite data (above the diagonal) and mitochondrial sequence data (below the diagonal). Bolded values are statistically significant at the $P < 0.05$ level.

Cave	AD-10	AD-125	AD-14	AD-17	AD-25
AD-10	—	0.9953	0.0988	0.6687	0.6588
AD-125	**0.0000**	—	0.9230	0.9999	0.9999
AD-14	**0.0075**	0.3152	—	0.2423	0.9384
AD-17	**0.0000**	**0.0410**	0.1592	—	0.7416
AD-25	0.9999	0.2631	0.5822	0.3225	—

Table 3.—Variable nucleotide positions in the alignment of mitochondrial DNA sequences of *Corynorhinus townsendii ingens* (haplotypes A, B, C, and D); dots show positions where the sequence is identical to haplotype A.

	89	170	198	208	223	230	245	263	265	302	342	451
A	C	C	G	G	T	A	T	C	G	A	T	A
B	T	T	A	A	C	G	C	T	A	·	C	G
C	·	·	·	·	·	·	·	·	A	·	·	·
D	·	·	·	·	·	·	·	·	·	G	·	·

Discussion

Species that are in decline or threatened, as exemplified by Ozark big-eared bats, face numerous potential dangers associated with small population size and fragmentation, making them especially prone to genetic drift and inbreeding (Avise 1994; Daniels et al. 2000; Whitehouse and Harley 2001). Effective monitoring of population trends is vital to the success of conservation efforts, but gathering this information often proves difficult for nocturnal volant mammals (Burland et al. 1999; Clark et al. 1997; United States Fish and Wildlife Service 1995). Understanding genetic variation contributes to

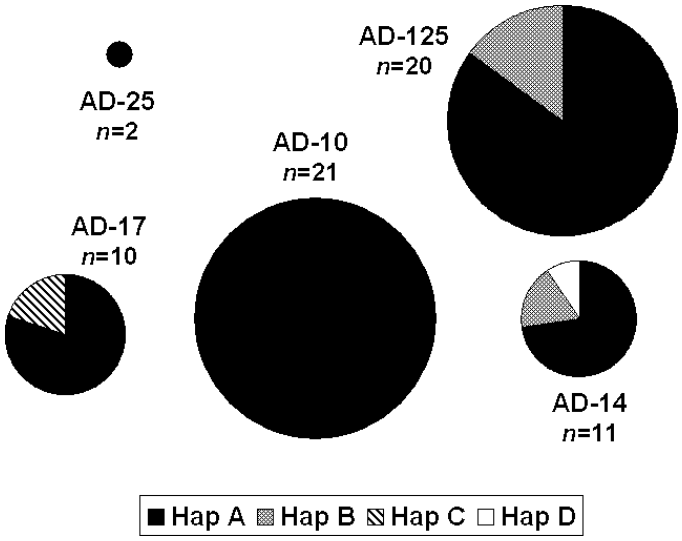

Fig. 4.—Frequency distribution of mitochondrial haplotypes detected in colonies of *Corynorhinus townsendii ingens* at caves AD-25, AD-17, AD-10, AD-125, and AD-14. The size of each chart is proportional to the number of individuals sampled at each site.

effective management strategies by providing information on levels of gene flow among populations or subpopulations (Rossiter et al. 2000; Worthington Wilmer et al. 1994).

Despite the low population estimates of Ozark big-eared bats, particularly in the early 1970s (United States Fish and Wildlife Service 1973), analysis of genetic variation revealed diversity in their mitochondrial and nuclear genomes. A greater average number of alleles was found at nuclear loci than in mtDNA, which is not unexpected because of differences in effective population sizes resulting from ploidy level and mode of inheritance (Birky et al. 1989). Moreover, the genetic effect of a population bottleneck was not detected, indicating that a recent reduction in effective population size has not significantly changed genetic characteristics of the extant population in Oklahoma (Cornuet and Luikart 1996). Therefore, ecological and anthropogenic events much earlier than the 1970s could have shaped current genetic characteristics.

Observed heterozygosity within colonies was less than expected at all caves except AD-25, but this may be due to the use of heterologous primers (isolated in another vespertilionid genus) and further complicated by the presence of null alleles at some loci (e.g., EF21 in *Eptesicus*—Vonhof et al. 2002). Reduced heterozygosity is seen in the moderately positive values of F_{IS} and F_{IT}, but this also can be explained by population subdivision not accounted for in the sampling scheme (Ralls et al. 2001; Wahlund 1928). The inclusion of male Ozark big-eared bats could produce this effect because their status with regard to subpopulations is unclear. Because of concern for the subspecies, we were not permitted to sample enough individuals to allow for comparison of genetic characteristics of male and female Ozark big-eared bats separately, which would have enabled us to address whether a more accurate assignment of males to colonies or subpopulations exists.

When considering the nuclear microsatellite data alone, a lack of structure among colonies of Ozark big-eared bats was found (Table 2). Our ability to detect unique alleles increased linearly with sampling effort (Fig. 3), because the microsatellite variation was evenly distributed among caves. This lack of differentiation among colonies in the nuclear genome is consistent with high rates of dispersal and corresponding gene flow in $\geq$1 sex (Avise 1995) and fits our prediction of male-biased dispersal.

In contrast, examination of maternally inherited mtDNA data showed low but significant partitioning of genetic variation among caves of Ozark big-eared bats (Table 2), and frequency distributions of mtDNA haplotypes among caves support low levels of gene flow through females (Fig. 4). Mitochondrial diversity was not randomly distributed throughout the population; some haplotypes (i.e., C and D) were restricted to single caves, and more intense sampling efforts at those caves relative to the other caves did not result in documentation of additional genetic diversity (Figs. 3 and 4). Not all pairwise comparisons of F_{ST} or the exact test of differentiation were significant, which was likely due to limited sample sizes rather than nonexistent population structure. Our sample of 63 individuals represented approximately 5% of Ozark big-eared bats in Oklahoma (United States Fish and Wildlife Service 1995), which provided substantial documentation of genetic characteristics in this subspecies. An important caveat in interpreting mtDNA results is that selection acting on any one locus can affect linked neutral variation. Such departures from strict neutrality within the mitochondrial genome cannot be ruled out and have the potential to produce patterns of variation similar to that found in this study (Charlesworth et al. 1993). However, matrilineal structure is common in mammals (Avise 2000) and has been previously documented in bats, even when nuclear loci show little structuring (Castella et al. 2001; Petit and Mayer 1999; Petri et al. 1997; Worthington Wilmer et al. 1999). In combination, we suggest that these data are better explained as providing a contrast of population structure resulting from gene flow in males and females, a condition with ''intriguing connection between population demography and matrilineal structure'' (Avise 2000:105).

We predicted that gene flow in females among caves would be limited because past demographic studies suggested that these bats exhibit site fidelity (Clark et al. 1996). Thus, despite close proximity of caves examined in this study (Fig. 2), connectivity of colonies is likely limited by philopatry of females. Radiotelemetry data, which support nightly movements not more than a few kilometers from roost sites (Clark et al. 1993, 1997; Wethington et al. 1996), may therefore be an accurate representation of the apparent lack of connectivity of colonies because of limited movement of females (Fig. 2). When using the 2-km radius as the limit for dispersal of females, interaction would only occur among individuals of cave AD-125 with cave AD-14 and cave AD-17 with cave AD-10 (Fig. 2). With documented movement as high as 7 km from a site, this radius may be a more realistic predictor of dispersal of females, and although it groups cave AD-25 with caves AD-17 and AD-10, it does not suggest interaction between individuals from any of these 3 western caves with individuals from the 2 eastern caves, AD-125 and AD-14 (Fig. 2). The broad geographic distribution of haplotype A and its internal placement in the haplotype network suggest that it is an ancestral sequence (Fig. 4), whereas all other sequences are restricted to either the western or eastern group of caves (Fig. 2). Only when assuming the maximum recorded distance of approximately 30 km (United States Fish and Wildlife Service 1995) as a normative distance for individual movement is there overlap between the areas surrounding all 5 caves (Fig. 2), and this level of connectivity is not supported by the genetic data (Figs. 3 and 4).

Evidence for small-scale genetic structure of temperate bat species among maternity colonies is growing (Burland et al. 1999; Kerth et al. 2000; Wilkinson and Fleming 1996). Microgeographic structuring was 1st described in a bat species by Burland et al. (1999), who documented a pattern of isolation by distance among colonies of brown long-eared bats (*Plecotus auritus*) in Scotland that were 0.1–100 km apart. This conclusion was supported by observational data of restricted movements and the distinct wing morphology of plecotine bats, which is characterized by low wing loading that allows for maneuverable flight but is not economical for long-distance movements

(Entwistle et al. 1996). Ozark big-eared bats share these features of only short-distance recorded movements and low wing loading (Farney and Fleharty 1969; Wethington et al. 1996).

Among colonies of Bechstein's bat (*Myotis bechsteini*) in Germany that were separated by $\leq$60 km, Kerth et al. (2000) concluded that females were philopatric based on the restricted distribution of mitochondrial haplotypes among colonies. They suggested that reproductive success in this species was increased through familiarity with roost sites and surrounding habitat, familiarity and cooperation of individuals, or a combination of these resulting in increased stability of colonies. Female Ozark big-eared bats also may benefit from such effects of philopatry.

Conservation implications.—Genetic differentiation of maternally inherited haplotypes among colonies separated by $<$20 km may be evidence of the most fine-scale geographic structure of any bat studied to date, which has serious implications for the future management of Ozark big-eared bats. Most critically, maternity caves should be managed as demographically autonomous units because if lost, they are unlikely to be recolonized by females from other caves (Avise 1995; Kerth et al. 2000). This could account for the finding of a number of caves and surrounding habitat in eastern Oklahoma that appear suitable for Ozark big-eared bats, but are unused (Clark et al. 1996; Wethington et al. 1997).

Failure to protect each maternity site independently could result in a loss of genetic variation that may not be found in any other colony of Ozark big-eared bats (Kerth et al. 2000; Worthington Wilmer et al. 1994). Gating efforts have been effective in reducing human disturbance inside caves (Martin et al. 2004); however, many maternity caves lack gates (Martin et al. 2000; United States Fish and Wildlife Service 1984, 1995). The philopatry of females to their natal site suggested in this study implies that temporary disturbance associated with construction of gates during months the bats are not present will not permanently deter bats from using those sites, and caves that have been gated are still used by Ozark big-eared bats (Martin et al. 2000; United States Fish and Wildlife Service 1995).

Additional studies are warranted in the arena of metapopulation dynamics relative to the successful recovery of the Ozark big-eared bat. Reproductive success of males in different colonies of Ozark big-eared bats remains an unknown. Studies of genetic structure of endangered greater horseshoe bats (*Rhinolophus ferrumequinum*—Rossiter et al. 2000) and larger mouse-eared bats (*Myotis myotis*—Petri et al. 1997) have emphasized expansion of areas of protected habitat specifically to provide roosting sites for males, ensuring their presence near each colony of females and thus improving connectivity among colonies. Concentrated sampling efforts that target alternative roosts used by males, sampling of maternity colonies strictly between late April and early July, and sampling of hibernacula also will confirm the population structure of Ozark big-eared bats. Although maternity caves should be managed and protected independently, Ozark big-eared bats are primarily sustained over winter by 2 large hibernacula (United States Fish and Wildlife Service 1995), suggesting that population structure may exist in hierarchical levels. Such was identified in populations of the noctule bat (*Nyctalus noctula*), where composition of hibernacula by individuals from multiple maternity colonies revealed higher haplotype diversity (Petit and Mayer 2000).

Current efforts toward identifying additional maternity and hibernating colonies of Ozark big-eared bats should focus on areas near sites of high mitochondrial diversity (e.g., cave AD-14). Priority in gating efforts also might be given to caves that hold disproportionately high genetic diversity. Rare alleles and haplotypes are more likely to be lost because of genetic drift or localized extirpation than those found at higher frequencies, and the maintenance of genetic diversity implies larger, stable populations (Avise 1994; Srikwan and Woodruff 2000). Therefore, nearby unidentified colonies may have supported persistence of high-diversity sites over evolutionary timescales.

At the population level, the relationship between reduced heterozygosity and probability of extinction has been noted in natural populations of the Granville fritillary butterfly (*Melitaea cinxia*—Saccheri et al. 1998) and the greater prairie-chicken (*Tympanuchus cupido*—Bouzat et al. 1998). Diversity in the gene pool is the raw material that evolutionary pressures modify and has innumerable ramifications for a species in the future (Sherwin and Moritz 2000). Although we did not detect a reduction in genetic diversity specifically due to a population bottleneck, future genetic studies of Ozark big-eared bats should attempt to increase the number of individuals sampled and include individuals from other subspecies to ascertain if the patterns of genetic diversity we found are typical of the species, or if its endangered status has had genome-wide consequences. Because the disjunct distribution of the Ozark big-eared bat lies between the endangered Virginia big-eared bat (*C. t. virginianus*) and the western big-eared bat (*C. t. pallescens*), genetic comparisons among these subspecies are particularly important.

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