USING GENETIC ANALYSES TO GAIN INSIGHT ON A RARE BAT, *CORYNORHINUS TOWNSENDII PALLESCENS*

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CHAPTER I

USING GENETIC ANALYSES TO GAIN INSIGHT ON A RARE BAT, *CORYNORHINUS TOWNSENDII PALLESCENS*

ABSTRACT

Maternally inherited mitochondrial DNA (mtDNA) and 5 bi-parentally inherited microsatellite DNA markers were used to investigate population and social structure within and among populations and gender, demographic history, and conservation implications of the rare bat, *Corynorhinus townsendii pallescens*, roosting in gypsum caves in western Oklahoma. *C. t. pallescens* was evaluated by grouping individuals into different possible population scenarios. Individuals were grouped 4 different ways and analyzed as: 1 single population; according to caves where captured; according to proximity of caves at distances within known maximum nightly movements of *C. t. pallescens*; and according to continuous distribution of gypsum deposits (named northern and southern population groupings). Results showed that male *C. t. pallescens* had high gene flow and dispersal, but BAPS individual-level clustering analysis revealed 9 genetically divergent admixed clusters. The clustering results suggested possible structure with male dispersal dynamics that were temporally based. Population structure was detected in females, which corresponded to continuous gypsum deposits. Females were hindered in dispersal by a geographic barrier. The barrier was a gap in continuous

gypsum deposits, which consisted of exposed gypsum (and caves) extending from Blaine County northwest into Kansas and another exposed section of gypsum existing in Washita County; 2 separate populations of females were isolated in each. Relatedness was analyzed for social structure with the assumption that *r*-values corresponded to known pedigrees and suggested the northern group was related (< 0.12) and differed from the southern group, which had no average relatedness expressed by *r*-values. Demographic analysis revealed a recent bottleneck in the southern group. Female *C. t. pallescens* in the southern group have been isolated in a small pocket of gypsum deposits and have suffered from low genetic diversity and gene flow. If female numbers significantly decrease or they become extinct, it is unlikely that new recruits will repopulate the area.

INTRODUCTION

Understanding the ecology and behavior of species is essential to their conservation and management. Genetic techniques have opened avenues of research, increasing behavioral and ecological knowledge of wild species, especially those that are difficult to observe and study in the field. Bats (Chiroptera) are often difficult to study, and although genetic studies of bats are increasing, studies of some species are lacking (Burland and Worthington Wilmer 2001). Few studies exist on *Corynorhinus townsendii pallescens* in the southern Great Plains, and none have investigated genetic structuring.

Corynorhinus townsendii occurs in the majority of the western United States, Canada, and northern and central Mexico (Barbour and Davis 1969; Handley 1959). Of the 5 subspecies that occur in North America, *C. t. pallescens* is believed to have the largest distribution, from California eastward to the southern Great Plains, including Kansas, Oklahoma, and Texas. Federal agencies consider *C. townsendii* a species of special concern or a sensitive species (United States Fish and Wildlife Service 1994). In Oklahoma, *C. t. pallescens* is rare and listed as a Tier II species of special concern by the Oklahoma Department of Wildlife Conservation (Oklahoma Department of Wildlife Conservation 2005).

Corynorhinus townsendii pallescens always may have occurred in low numbers in the southern Great Plains, where it settled during the western contraction of their range in the late Pleistocene (Humphrey and Kunz 1976). Humphrey and Kunz (1976) estimated that 14,000 *C. t. pallescens* occurred in the southern Great Plains, but they noted this was likely a 2-factor overestimation. A 2-year survey of gypsum caves in Oklahoma found

only 167 *C. t. pallescens* in 26 of 74 caves surveyed (many caves revisited); only 3 caves had ≥ 30 individuals and 23 had only 1–8 individuals (Caire 1997).

Corynorhinus townsendii has an affinity for caves (particularly maternity colonies) but also will roost in mines, rock crevices, and some human structures (e.g., under bridges—Barbour and Davis 1969; Handley 1959; Humphrey and Kunz 1976; Kunz and Martin 1982; Pierson et al. 1999). In the southern Great Plains, including Oklahoma, *C. t. pallescens* roosts predominately in gypsum caves in what have been described as small, isolated, relictual populations (Barbour and Davis 1969; Caire 1997; Handley 1959; Humphrey and Kunz 1976). Populations of *C. t. pallescens* in Oklahoma may be isolated from each other due to breaks in the gypsum deposits. Hundreds of solution caves have formed in these exposed gypsum beds. Many of the gypsum caves exist as intricate mazes, but many caves are small or occur as sinkholes (Johnson 1972; Twente 1955). In some of the larger gypsum caves, other bat species such as Brazilian free-tailed (*Tadarida braziliensis*) and cave myotis (*Myotis velifer*) roost in larger colonies than *C. t. pallescens* (2–4 times larger —Humphrey and Kunz 1976). *C. t. pallescens* is able to roost in caves that are uninhabitable by other bat species because of their small numbers at roosts. Maternity colonies of *C. t. pallescens* in Oklahoma generally consist of 17–40 individuals (Humphrey and Kunz 1976) and have never been found with >80 individuals as they have in Colorado and New Mexico (Pierson et al. 1999). Male *C. t. pallescens* roost singularly during summer but vary during other times of the year (e.g., singular or ≥ 60 males and females roosting together—Humphrey and Kunz 1976; Twente 1955).

The subspecies is relatively sedentary with migration from summer roosts to hibernacula or transitory stops at gypsum caves within the same area (Barbour and Davis 1969; Humphrey and Kunz 1976; Pierson et al. 1999). Movements of *C. t. pallescens* in Oklahoma and Kansas, from banding recaptures, were generally < 1.6 km with maximum of 8 km; movements from nurseries to hibernacula in autumn averaged 11.6 km with 1 individual moving 39.7 km (Humphrey and Kunz 1976). Telemetry studies of other *C. townsendii* subspecies documented movements ≤ 5 km, with rare movements > 5 km (Adam et al. 1994; Clark et al. 1993; Fellers and Pierson 2002; Wethington et al. 1996).

Mitochondrial DNA (mtDNA; maternally inherited) and nuclear DNA (bi-parentally inherited) markers can be used to investigate ecological patterns, including differences in behavior between genders (Avise 2000, 2004; Burland and Worthington Wilmer 2001; Weyandt et al. 2005). Many mammalian species, including some bats, display female philopatry and male-biased dispersal patterns, but variation in dispersal patterns of different bat species has been found. Populations of female *Macroderma gigas* have extremely high genetic structuring, and males have slightly lower structuring (Worthington Wilmer et al. 1999). Groups of female *Myotis bechsteinii* exhibited genetic structuring comparable to *M. gigas*, but males did not have significant structuring and dispersal was male-biased (Kerth et al. 2000, 2002a).

Defining populations *a priori* can be difficult, especially for volant species, and if incorrectly identified, false information about conservation units and biology of the species can result (Burland and Worthington Wilmer 2001; Manel et al. 2005; Pearse and Crandall 2004). Genetic data can be used to recognize separate populations and answer other important conservation questions (e.g., dispersal patterns, conservation units, and

mating and roosting behavior— Burland et al. 2001; Burland and Worthington Wilmer 2001; Pearse and Crandall 2004). Mating systems vary among bat species, but polygyny or male reproductive skew (disproportionate mating of few males with majority of females) and low colony relatedness are often found, despite female philopatry (Burland et al. 2001; Burland and Worthington Wilmer 2001). The temperate bat *Rhinolophus ferrumequinum* has high relatedness at maternity colonies (suggesting strong philopatry) and some degree of male reproductive skew (Rossiter et al. 2000, 2002). In contrast, *Plecotus auritus* and *Myotis bechsteinii* have low levels of maternity-colony relatedness and low male reproductive skew (multiple males mate with multiple females—Burland et al. 2001; Kerth et al. 2002b).

Genetic data help to understand the history of populations and stochastic events, which can elucidate their current and future dynamics and stability (Burland and Worthington Wilmer 2001; Pearse and Crandall 2004). Due to their high variability, microsatellites are particularly ideal for detecting recent demographic events (e.g., bottleneck—Pearse and Crandall 2004). If a bottleneck occurs, the persistence of a population may be low because of the resulting genetic changes (Luikart et al. 1998).

Genetic analysis was done, using mtDNA and nuclear microsatellite data, to gain a better understanding of the ecology and behavior of *C. t. pallescens*. I investigated inter- and intra-colony structure to assess population dynamics and boundaries, separating genetically divergent populations. Relatedness analysis was used to further understand the mating and social behavior of *C. t. pallescens*. Finally, I tested for bottleneck effects on populations of *C. t. pallescens*.

MATERIALS AND METHODS

Sampling sites and collection.—Mist nets were placed at entrances of 12 gypsum caves in western Oklahoma that had been searched for roosting individuals or colonies of *C. t. pallescens*. Mist nests were supplemented with fish netting that was draped over gaps around mist nets and at other openings of the same cave to decrease escape by bats. Netting occurred from late May to mid-August in 2003 and 2004 and resulted in capture of *C. t. pallescens* at 8 of the 12 cave entrances. The 8 caves occurred in 4 counties in western Oklahoma: Alabaster Caverns in Woodward County; Saloon, Nescatunga, and Cult caves in Major County; Ake's Cave in Blaine County; and Fink 1, Ratzlaff, and 3 Domes caves in Washita County (Fig. 1a). Maximum distance among the 3 caves in Major County was 4.6 km, and 3.8 km among the 3 caves in Washita County. The distance between the 3 caves in Major County and the 3 caves in Washita County was ca. 108 km (Fig. 1a). Distance between Alabaster Caverns and the caves in Washita County was ca. 144 km; all other distances among the 8 caves were > 38 km.

Tissue samples consisted of wing biopsies (1 from each wing) taken from captured *C. t. pallescens* with a sterile 3-mm biopsy punch (Worthington Wilmer and Barratt 1996). Tissue was taken from the distal one-third of the plagiopatagium after sterilizing it with ethyl alcohol. Wing biopsies were stored in lysis buffer until whole genomic DNA was extracted. Isolation of DNA was performed using a phenol extraction and salt precipitation procedure (Longmire et al. 1997; Zeugin and Hartley 1985). Ages of captured individuals (adult or juvenile) were determined by backlighting wings to observe the cartilaginous epiphyseal plates of the bone joints (Anthony 1988).

Because populations of volant species were difficult to delineate *a priori*, individual samples were grouped *a posteriori* for statistical analysis by different geological and ecological factors. Samples were placed into the following groups: (A) all individuals as 1 population; (B) 8 populations based on the 8 cave entrances of capture; (C) 4 populations with caves grouped if closer than the known maximum nightly movements (= 7 km) from concurrent telemetry study and previous studies (Adam et al. 1994; Clark et al. 1993; Fellers and Pierson 2002; Humphrey and Kunz 1976), effectively lumping the 3 caves in Major County and the 3 caves in Washita County as distinct populations; and (D) 2 populations separated by a distinct break in the distribution of continuous gypsum deposits (Fig. 1).

Microsatellite variation and analysis.—Using primers isolated from *Eptesicus fuscus* (Family Vespertilionidae—Vonhof et al. 2002), 5 di-nucleotide microsatellite loci were amplified using polymerase chain reaction (PCR). Reactions consisted of 15-µl containing 50 ng genomic DNA, 10 pmol of each primer, 9-µl ABI Prism True Allele PCR Premix, and 3.8 µl ddH_2O. Cycling conditions were 12 min at 95ºC followed by 35 cycles of 94ºC for 30 s and annealing temperatures of 40° (EF14), 45° (EF1, EF6), 53° (EF21), or 55ºC (EF15) for 45 s, 72ºC for 45 s, and a final 10-min incubation at 72ºC. Then, 1.5 µl of the PCR product was added to 3.5 µl of loading mix, containing an internal size standard (ROX); 1.5 µl of that combination were loaded into a lane in a 6% acrylamide gel. To determine genotypes, PCR products were visualized with an ABI-377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, California) and GeneScan 2.0 and Genotyper 2.0 software (Applied Biosystems, Inc., Foster City,

California). To minimize bias (i.e., from stutter), 2 people scored microsatellite markers independently.

Statistical analysis of average alleles across loci, allelic frequency, deviation from Hardy-Weinberg and linkage equilibrium, and observed (H_O) and unbiased expected (H_E) heterozygosity were performed using Arlequin (Schneider et al. 2000). Arlequin also was used to test population variation with analysis of molecular variance (AMOVA) for the 4 population groupings (Fig. 1b) at 4 hierarchical levels (among groups, among populations within groups, among individuals within populations, and within individuals). Population groupings were further evaluated using subgroupings (delineated in the same manner as population groupings; Fig. 1b) to test for subpopulation structure (i.e., among populations within groups) and to explore optimal structuring of individuals in the study area. Population differentiation and spatial structuring were assessed with Φ-statistics, including pairwise φ_{ST} (with 10,000 steps in the Markov chain). Sequential Bonferroni correction (Rice 1989) was used to correct for multiple tests.

Bayesian Analysis of Population Structure (BAPS v. 3.1—Corander and Marttinen 2005) was used to reveal genetically distinct populations or clusters. The program used stochastic optimization to infer the posterior mode of genetic structure and considered allelic frequencies and number of genetically diverged groups as random variables (Corander and Marttinen 2005). The Bayesian model used a joint probability distribution, where past distribution was considered in conjunction with likelihood after the posterior analysis. Mixture clustering was performed at the group- and individual-level (Corander et al. 2003), wherein mixture results were examined for individuals

containing lineages among the genetically divergent groups (i.e., admixture). When performing the analysis for individual-level mixture, the user designates the suspected number of genetically divergent groups (i.e., K), but for group-level mixture analysis, the suspected populations are already defined in the data set. I designated suspected clustering in the data for group-level mixture analysis using the different population groupings (Fig. 1b). At the individual-level mixture analysis, BAPS was performed numerous times with integers defined for K, from 3 to 25, using different series of numbers, with the same numbers repeated several times within an analysis (the possibility of K = 1 was automatically considered). Designation of K was performed in this manner because each analysis of the same K could produce different results due to stochasticity of the algorithm. To prevent K from fixing at a local mode (Corander and Marttinen 2005), analysis was performed with different maximum K integers (e.g., K = 3–15 for 1 analysis and K = 3–9 for another). The BAPS analysis looks for optimal partitioning with $k \leq K$ (Corander and Marttinen 2005). The mixture analysis compared data from multiple tests, by examining the natural logarithm of the marginal likelihood of the data (*logml*), which was used to determine goodness-of-fit for assigned clustering (Corander and Marttinen 2005). When mixture results were subjected to admixture analysis, I designated 5 as the threshold for the minimum size of a population.

Adults were used to examine relatedness within and between populations and sex using RELATEDNESS 5.0 (Queller and Goodnight 1989). The program produced a correlation index (*r*) that used population allelic frequency to estimate proportion of alleles identical by descent between 2 individuals. Population groupings (Fig. 1b) were used again to examine allelic frequencies among populations and for pairwise *r*-values.

The *r*-value ranged from -1 to 1, with values ≤ 0 indicating no relationship and values > 0 indicating relatedness. All tests were symmetric and weighted equally among individuals. The program GENECAP version 1.1 (Wilberg and Dreher 2004) was used to calculate probability of identity, which determined the probability that 2 randomly selected individuals had identical genotypes at multiple alleles by descent. A more conservative estimate, sibling probability of identity [$P(ID)_{sib}$], was determined and compared with pairwise *r*-values of relatedness. I used $P(ID)_{sib}$ because it quantified genetic diversity levels, including social structure (Waits et al. 2001). Initially, pairwise relatedness was evaluated with the assumption that pedigree relationships follow: 1st order, $r = 0.50$; 2nd order, $r = 0.25$; and 3rd order, $r = 0.125$. Subsequently, 1st order relationships were compared with mtDNA results and $P(ID)_{sib}$ results.

To determine if the status of genetic variation was consistent with historical demographics, Bottleneck 1.2.02 (Cornuet and Luikart 1996) was used with the 2-phased model (TPM). The TPM incorporated the infinite alleles model (IAM) and the stepwise mutation model (SMM), with the user specifying percentage of SMM in the analysis. The more conservative mutation model, SMM, experiences stepwise loss or gain (forward or backward) of a single tandem repeat (Lowe et al. 2004), while on the other extreme, in IAM, new mutations can arise in any part of a sequence with alleles of any size or character (Kimuri and Crow 1964; Lowe et al. 2004). The TPM incorporates both SMM and IAM into the analyses by allowing the expected majority of mutations of stepwise additional repeats in either direction and allows the occasional new mutational units to occur more distantly in the sequence (Di Rienzo et al. 1994). I used the Wilcoxon sign-rank test in the Bottleneck program, which compared observed

heterozygosity (gene diversity) with the heterozygosity expected if the population was at mutation-drift equilibrium. Heterozygosity excess (compared to equilibrium) is expected of bottlenecked populations because allelic numbers decrease faster than heterozygosity with decreasing effective population size. The TPM was used in the analysis at 70%, 80%, and 90% SMM and 30%, 20%, and 10% variance in mutation lengths, respectively. All population groups (Fig. 1b) were subjected to the analyses, which were performed with 5,000 iterations for all tests.

Mitochondrial DNA variation and analysis.—Primers that have been successful in ≥ 5 families of chiropterans (Wilkinson and Chapman 1991) were used to amplify about 485 bp of the mtDNA control region (d-loop) using PCR. Amplifications were performed using 50 ng DNA, 50 pmol each primer, 10mM dNTPs, 1.5 mM $MgCl_2$ and 1 unit *Taq* DNA polymerase. Cycling parameters consisted of 2 min at 95ºC, then 35 cycles of 95ºC for 1 min, 55ºC for 1 min, and 72ºC for 1 min, followed by a final 30 min at 72ºC. Purification of the double-stranded amplicons was done using the Wizard PCR Prep DNA Purification System (Promega, Madison, Wisconsin), and they were sequenced in both directions using Big-dye chain terminators and a 377 Automated DNA Sequencer (Applied Biosystems, Inc., Foster City, California). Overlapping sequence fragments were pieced together using AssemblyLIGN 1.0.9 (Oxford Molecular Group PLC 1998), and multiple sequence alignment was performed using CLUSTAL X (Thompson et al. 1997). Aligned sequences were imported into MacClade 4.0 (Maddison and Maddison 2000) for visual inspection and determination of unique haplotypes using the redundant taxa option.

Arlequin ver. 2.00 (Schneider et al. 2000) was used to determine intrapopulation haplotype (h) and nucleotide (π) diversity (analyzed using the same population groupings used for microsatellite analyses). Arlequin also was used for AMOVA at 3 hierarchical levels (among groups, within populations, and among populations within groups) and Φ-statistics (including pairwise φ_{ST}). Sequential Bonferroni correction (Rice 1989) was used to correct for multiple tests.

RESULTS

Sampling sites and collection.—A total of 96 *C. t. pallescens* (adult and juvenile) were sampled from 8 gypsum cave entrances in western Oklahoma. Individuals captured by cave were: 7 from Alabaster Caverns (2 females, 5 males), 18 from Saloon Cave (14 females, 4 males), 5 from Nescatunga Cave (3 females, 2 males), 14 from Cult Cave (9 females, 5 males), 15 from Ake's Cave (10 females, 5 males), 13 from Fink 1 Cave (9 females, 4 males), 6 from Ratzlaff Cave (2 females, 4 males), and 18 from 3 Domes Cave (11 females, 7 males). Colonies of ca. 30–50+ were found in Alabaster Caverns, Cult Cave, Ake's Cave, Fink 1 Cave, and 3 Domes Cave. Despite a relatively large colony of *C. t. pallescens* at Alabaster Caverns, few individuals were captured because the cave was large and had many openings. No colonies were visually seen in Saloon or Nescatuga caves, but they also were large caves and colonies of *C. t. pallescens* could have been in inaccessible areas. Ratzlaff Cave was thoroughly investigated, and no colonies were found; 2 separately roosting *C. t. pallescens* were observed. Of the 8 caves, Saloon, Ake's, and 3 Domes caves were the only confirmed maternity sites, but demography of *C. t. pallescens* in the other caves could not be definitively determined.

Microsatellite variation.—Average number of alleles over all loci when examining populations designated by cave entrances (Fig. 1b) was 5.2 (range = 3.8–6.2; Table 1). The most variable locus was EF15 with 15 alleles. Rare alleles occurred at Cult, Ake's, Fink 1, and 3 Domes caves. With population groupings designated by proximity (Fig. 1b), a rare allele occurred at both Ake's and Major County caves and another rare allele occurred at Alabaster Caverns and Major County caves.

When populations, as designated by cave entrance, were examined (Fig. 1b), only 2 pairs of loci were significantly associated at Cult Cave, after sequential Bonferroni corrections. Linked loci at Cult Cave were EF1 to both EF6 and EF21, but upon combining with Saloon and Nescatunga caves to form the Major County cave grouping (Fig. 1b), linkage disequilibrium did not occur. One locus (EF6) significantly deviated from Hardy-Weinberg expectations. Significant deficiencies of heterozygotes (allele EF6) occurred at Saloon Cave and in combination with other caves in Major County and the northern population. Mean observed heterozygosity (H_O) was lower than expected heterozygosity (H_E) for all sampling units with H_O and H_E ranges of 0.567–0.743 and 0.709–0.791, respectively (Table 1). Hierarchical AMOVA for nuclear DNA (nDNA) data depicted a lack of genetic structuring for all sampling units (φ_{ST} = 0.000 for all units). None of the pairwise φ_{ST} revealed significant differentiation, except between Cult and Fink 1 caves and Nescatunga and Ratzlaff caves (p = 0.000).

Population and social structure.—Mixture clustering at the group-level for all population groupings resulted in optimal partitioning of 1 cluster, with population grouping of all individuals as 1 population (group A, Fig. 1b; *logml*-value = -1638.2). Admixture for group-level clustering also revealed 1 optimal cluster using results from

mixture analysis. Mixture clustering at the individual-level produced *logml*-values that ranged from -1493.8 to -1501.4, with optimal partitioning of 11–13 clusters. Results with *logml* of -1493.8 (with optimal partitioning of 13 clusters) were used for admixture analysis because it produced the best goodness-of-fit of the multiple runs, or the exponential of their absolute differences in *logml*-values [i.e., -1493.8 is exp (8) times better than *logml* of -1501.4, as measured in the posterior probability—Corander and Marttinen 2005]. Admixture clustering at the individual-level resulted in optimal partitioning of 9 clusters. Those 9 clusters from individual-level analysis, or inferred populations from admixture analysis, did not adhere strictly to any predefined population groupings I suspected (Fig. 1a); instead, population assemblages contained individuals from multiple clusters or genetically diverged groups (Fig. 2). Admixture analyses identified individuals that represented > 1 cluster or genetically diverged group, or who showed evidence of admixture; analysis revealed significance of 1 individual for admixed lineage (Fig. 2). Admixture analysis, further discarded 8 individuals as outliers or those belonging to clusters whose population size was < 5 (designated threshold for the analysis).

Although population groupings did not correlate with the BAPS results for individual-level admixture analysis, some patterns were observed. Specifically, when individuals were grouped according to gypsum deposits (Fig. 1b), 7 of the 9 clusters revealed disproportionate occurrence, and 2 clusters were about evenly distributed between the northern and southern populations (Fig. 2). The northern population grouping had the majority of individuals (70–100%) contained in 5 of the 7 clusters, and

2 clusters predominated in the southern population grouping (≥ 80 %). There were no significant contrasts in other population groupings (Fig. 2).

All adults sampled (n = 51) were used to obtain r-values using RELATEDNESS 5.0: 2 from Alabaster Cavern (1 females, 1 males), 8 from Saloon Cave (7 females, 1 males), 2 females from Nescatunga Cave, 8 from Cult Cave (6 females, 2 males), 10 females from Ake's Cave, 10 from Fink 1 Cave (7 females, 3 males), 4 from Ratzlaff Cave (2 females, 2 males), and 7 females from 3 Domes Cave. Average relatedness among adults at all 8 caves approximated 0.00 (Table 5). Adults from each cave entrance, within Major County caves and Washita County caves, and within the northern population (Fig. 1b) had average r-values approximating 0.00 (range = −0.10–0.06), except Alabaster Cavern (r = 0.217; Table 5). Examination of average relatedness among all females and among all males approximated 0.00 but increased within gender at several caves (i.e., Saloon, Cult, Ratzlaff caves for females and Cult and Fink 1 caves for males; Table 5). Examination of northern and southern population groupings revealed no relatedness in the southern population (averaged r-value approximated 0.00) among all adults, females, or males; conversely, relatedness in the northern population was higher (r = 0.10) for all adults and by gender (Table 5).

At the finer scale, adult females were examined using pairwise relatedness to possibly evaluate philopatric behavior, with the assumption that unknown pedigrees follow known pedigrees of 1st-order relationships (equating to parent-offspring or full siblings), followed by 2nd- and 3rd-order relationships (r = 0.50, 0.25, and 0.125, respectively). Most of the adult females at Saloon, Cult, Ake's, and Fink 1 caves had ≥ 1, 2nd-order relationships or higher (r > 0.24) with another individual from the same cave.

Two individuals at 3 Domes Cave had 2nd-order relationships, while the others only had 3rd-order relationships with ≥ 1 other females in the group. Other population groupings revealed more 1st-order relationships, but when compared with mtDNA, only 7 pairs of 1st-order comparisons had the same haplotypes. Results for probability of identity [$P(ID)_{sib} = 0.01039$] depicted a 1 in 96 probability (i.e., inverse of $P(ID)_{sib}$) of randomly sampling 2 individuals with identical genotypes. Comparison of $P(ID)_{sib}$, mtDNA, and pairwise *r*-values revealed that 1st-order *r*-values for unknown pedigrees of *C. t. pallescens* do not correlate to known pedigrees.

Demographic history.—In analysis of population groupings of bats by cave entrances using BOTTLENECK, Alabaster Caverns, Nescatunga, and Ratzlaff caves (all had $n < 10$) were excluded because the Wilcoxon test has more power with ≥ 10 individuals. When parameters were set at 70% proportion SMM and 30% variance in mutation lengths with population grouping designated by cave entrances, Cult, Fink 1, and 3 Domes caves had excess heterozygosity compared with the average expected value at mutation-drift equilibrium ($p = 0.031$, 0.047, and 0.016, respectively). Marginal heterozygosity excess occurred at the Washita County caves ($p = 0.047$ for both population groupings C and D; Fig. 1b). When parameters were set at 80% SMM and 20% variance in mutation length, only 3 Domes Cave had heterozygosity excess compared with expected heterozygosity at equilibrium ($p = 0.031$); no other population groupings were significant. Heterozygosity excess was not seen for Group A (i.e., all individuals as 1 population; Fig. 1b) with any SMM percentage in the analysis.

mtDNA variation.—Mitochondrial sequence data were obtained for 90 of the 96 individuals sampled from the 8 cave entrances. Variability was noted in 4 nucleotide

positions, resulting in 4 unique haplotypes. Haplotype H occurred only in samples from Alabaster Caverns, Saloon, Cult, and Ake's caves (Table 1; Fig. 3), and haplotype E predominated in samples from Washita County. Haplotype diversity was high for all caves except Alabaster Caverns, Fink 1, and 3 Domes caves (range = 0.000–0.800; Table 1). Haplotypic diversity was high for Nescatunga and Ratzlaff caves, but both had small sample sizes (n = 5 and 6, respectively). However, differences occurred between haplotypic diversity in samples from Washita County (southern grouping: h = 0.203) and Major County (h = 0.713 and northern grouping: h = 0.666). Nucleotide diversity (π) was low for all sampling units.

Regardless of sampling unit or grouping, AMOVA results depicted higher variation within populations than among populations (Table 2; Fig. 4). The population grouping designated by gypsum deposits (Fig. 1b) had the most variation at the among populations hierarchal level at 47.71% between northern and southern populations suggesting optimal geographic grouping (Excoffier et al. 1992). When further subdivision was examined, the hierarchical level among-population-within-groups did not have variation comparable to the other hierarchical levels (range = 7.97–12.26; Table 2). Overall, φ_{ST} was high for all sampling units (range: 0.441–0.549; Table 2).

Pairwise φ_{ST} revealed a general split between caves in Washita County and all other caves (Table 3), with the exception of comparisons of Alabaster Caverns, Nescatunga, and Ratzlaff caves to each other and to other caves (probably due to their small samples). Differentiation was apparent between Washita County caves and Alabaster Caverns, Major County caves and Ake's Cave, and between northern and

southern populations ($p = 0.000$; Table 4; Figs. 4 and 5). Alabaster Caverns, Major County caves, and Ake's Cave were not significantly differentiated.

DISCUSSION

Genetic variability and differentiation within and among populations.—Microsatellite data revealed high male gene flow of *C. t. pallescens*, as indicated by the AMOVA and φ_{ST} results, which has had a homogenizing effect across the study area. However, the mtDNA data depicted low gene flow and resultant high structure of females. Microsatellite data showed all variation was within individuals relative to male influence on gene flow, regardless of population grouping. Microsatellite markers have a tendency toward high heterozygosity (Avise 2004; Lowe et al. 2004), but heterozygosity was not higher than expected. Several factors might attribute to lower than expected heterozygosity (e.g., drift, gene flow, or natural selection—Lowe et al. 2004), including characteristics (e.g., null alleles) of primers and alleles amplified (Weyandt et al. 2005).

High φ_{ST} (> 44 %; Table 2) suggest low female gene flow indicative of a sedentary and philopatric behavioral trait. Maternally inherited mtDNA data suggested high intra- and inter-population variability of female *C. t. pallescens* (Table 2), with most variability seen in the northern population (Group D; Fig. 1b). The southern population (Group D; Fig. 1b) had lower haplotypic diversity and variability than the northern population. By examining multiple population groupings (Fig. 1b), among-population variation of haplotypes was optimized when segregated into individuals contained by continuous gypsum distribution (Table 2; Fig. 5). The structural optimization of 2 populations in the among-population hierarchical level may be attributed to structure

above subpopulations (Excoffier et al. 1992). Regardless of population grouping, φ_{ST} was always higher when the sub-grouping was 2 (Table 2; Fig. 3–5), further suggesting a geographic criterion as the basis for female populations. Further evidence of female division into northern and southern populations was seen with dominance of haplotype H in the northern population and predominance of haplotype E in the southern population; significant pairwise φ_{ST} also resulted in the same distinction. Pairwise φ_{ST} for the other population assemblages (except Nescatunga Cave and Ratzlaff Cave due to small samples) paralleled AMOVA results, showing differentiation between northern and southern populations (Fig. 5). Analyses of mtDNA data are congruent with 2 populations of females in the study area that are isolated from each other due to a geographic barrier to dispersal. Such a geographic barrier occurred between exposed gypsum deposits in Washita County and those that occurred in a thin continuous strip along the Canadian River from Kansas southeast into Blaine County.

Morphometric analysis of *C. t. pallescens* in Oklahoma and Texas, separated by breaks in exposed gypsum, paralleled my results (Smith and Tumlison 2004). Individuals found in caves in gypsum deposits that extended from Kansas southeast to Blaine County differed morphometrically from individuals found from caves in gypsum deposits in Washita County (Smith and Tumlison 2004). Furthermore, *C. t. pallescens* examined southwest of Washita County and in Texas differed from those sampled in my study. Despite the lack of samples of *C. t. pallescens* in caves southwest of Washita County, distances between gaps of suitable habitat are sufficient to hypothesize further genetic differentiation of females from those sampled in this study. Most genetic studies of bats have investigated the macrogeographic scale, but crucial information (e.g., population

structure, dispersal patterns, and social structure) has been found from several microgeographic studies of bat species (Burland et al. 1999, 2001; Kerth et al. 2002a, b; Petri et al. 1997; Rossiter et al. 2000, 2002; Weyandt et al. 2005).

Variation of mtDNA was nearly equal among populations (48%) and within populations (52%), and minimal variation among populations within groups (≤ 12 %) that indicated low probability of further subdivision. Previous genetic studies of bats have shown that the majority of variation occurred either within populations or among populations, but not both, as in my study. When the majority of genetic variability was distributed among populations, bats usually are isolated, may be monophyletic (e.g., 1 haplotype present at a maternity colony), and are highly differentiated, usually attributed to a geographic barrier or isolation by distance (Castella et al. 2000; Ruedi and Castella 2003; Salgueiro et al. 2004). When the majority of variability has occurred within populations, high gene flow occurred between populations with few barriers (Petit and Mayer 2000).

In my study, populations of females were highly differentiated with restricted gene flow but also were highly variable within populations. Intra-population variability was difficult to explain but was consistent with the moderately high haplotypic diversity found in most population groupings (except Alabaster Caverns, Fink 1 and 3 Domes caves, and the southern population), which was expected because subdivision was seen (Excoffier et al. 1992). Haplotypes were variable at only 4 base pairs (all transition substitutions), suggesting minimal genetic divergence between populations, which is why nucleotide diversity remained low despite admixed haplotypes within populations (Petit and Mayer 2000). Ruedi and Castella (2003) found 96% haplotypic variation at nurseries

of *Myotis myotis* and concluded that divergent matrilines within the same populations were either ancestral polymorphism from coalescences or mixing of subpopulations that previously evolved in isolation. Some individuals in the northern population with haplotype E and decreasing haplotypic diversity in the southern population suggested continued isolation of the southern population. This also may explain why Alabaster Cavern, Major County caves, and Ake's Cave were not differentiated from each other, despite distances among those caves greater than this species has been documented to move. *C. t. pallescens* in Oklahoma is believed to have settled in refuges during the Pleistocene (Humphrey and Kunz 1976), suggesting that intra-population variability was due to ancestral polymorphism.

Population structure.—When microsatellite data were analyzed at the group-level for genetically divergent populations, using all population groupings (Fig. 1b), results paralleled summary statistics (φ statistics and AMOVA). The BAPS program differs from summary statistics (e.g., AMOVA), because it also can determine genetically divergent populations by examining the individual-level; BAPS individual-level results did not parallel summary statistics and did not conform to any of the possible population scenarios I anticipated (i.e., population groupings; Fig. 1b). Individual-level analysis revealed 9 genetically divergent populations, from which 7 had disproportionate representation (Fig. 2) when individuals were contained in the northern or southern population grouping (Fig. 1b). Why were individuals not completely isolated into either the northern or southern population (corresponding to mtDNA summary statistics)? The migration-drift relationship theory is a possible explanation, where even 1 individual is enough to keep isolated populations from becoming fixed for a neutral allele at any locus

(Lowe et al. 2004; Slatkin 1987). Why did the individual-level analysis not show a homogenizing effect across the study area (corresponding to nDNA summary statistics)? Natural selection is a possible explanation, where some loci are favored over others despite gene flow, causing sufficient differentiation at those loci; other loci are weakly selected resulting in uniformity among populations (Slatkin 1987).

American puma (*Puma concolor*—McRae et al. 2005) and North American thinhorn sheep (*Ovis dalli*—Worley et al. 2004) showed structuring of genetically divergent clusters that coincided with geological barriers to dispersal (e.g., mountain range or gaps in suitable habitat). Bats may not have genetic structuring of populations as strong as some mammals because they are volant, but average wing span and low aspect ratio of *C. t. pallescens* may inhibit long-distance dispersal (Norberg and Rayner 1987). Individual-level cluster analysis differed from my population groupings and haplotypic differentiation among female suggesting temporal changes in demographics of *C. t. pallescens* in Oklahoma. Temporal dynamics, especially at mating sites, are mostly unknown for *C. t. pallescens*, except that mating mainly occurs when males visit females in winter hibernacula (Pearson et al. 1952). Individual-level clustering may correlate with bachelor colonies that split up and move in different directions to mate with females.

Social structure.—Low mean relatedness of adults in my study area was similar to several other bat species (Burland et al. 2001; Burland and Worthington Wilmer 2001; Kerth et al. 2002b; Rossiter et al. 2002), but *r*-values were higher for the northern population grouping than the southern (Table 4). Relatedness of all adults and adult females in the northern population was higher than that found in other temperate bat species. The southern population was similar to other bat studies; for example, maternity

colonies of *M. bechsteinii* in Germany located within a few kilometers from each other with little exchange of individuals had low mean colony relatedness (r = 0.02—Kerth et al. 2002b). Despite female philopatry, adult *R. ferrumequinum* in a large maternity colony in the United Kingdom had low mean relatedness (r = 0.002—Rossiter et al. 2000, 2002). Low relatedness of adult *C. t. pallescens* in the southern population may be due to the small sample because jack-knifed standard errors were high. The northern population had higher relatedness with lower standard errors, which may indicate stronger social structure or kin-based coloniality. It is unclear why individuals sampled in the northern population are more related than the southern, but caves in Washita County may be suboptimal, and individuals there may be subject to precarious ecological pressures or stochastic events.

Low mean colony relatedness found in other bat species is often attributed to low male reproductive skew (or non-polygynous mating—Burland et al. 2001; Burland and Worthington Wilmer 2001; Kerth et al. 2002b; Petri et al. 1997; Rossiter et al. 2000, 2002). Low mean relatedness in my study also could be attributed to mating behavior. Assuming close relationships among females (seen from pairwise relatedness) and polygynous mating, paternal dyads would raise mean r-values because many offspring would have similar maternal and paternal dyads in a colony. I did not see this in *C. t. pallescens* in western Oklahoma, suggesting a mating strategy other than polygyny. Results for *C. t. pallescens* in western Oklahoma are consistent with what is known about mating behavior of *C. townsendii*, where the majority of mating occurs in winter hibernacula (Pearson et al. 1952). Most females have been inseminated once by October,

but females do not form a vaginal plug and insemination by many different males is probable throughout the winter (Pearson et al. 1952).

In contrast to average *r*-values of *C. t. pallescens* (Table 5), pairwise relatedness indicated philopatry. A similar scenario was found for *M. bechsteinii* in Germany, where mean relatedness approximated 0.00, but 75% of the adult females in the colonies had ≥ 1 close relative ($r \geq 0.25$—Kerth et al. 2002b). The majority of adult female *C. t. pallescens* in the different caves (where > 2 captured) appeared to have an intricate web of relatedness ($r \geq 0.25$). Many 1st-order pairs of relatives were linked by 2nd-order relationships to others captured at the same caves, thus forming tight kin-based coloniality, despite average *r*-values. In contrast to all other maternity caves in my study, only adult females at 3 Domes Cave had low pairwise relationships. However, there was little likelihood that adult females at 3 Domes Cave were not philopatric because they shared only 1 haplotype and mtDNA haplotypes overall did not correlate with relatedness results. When examining probability of identity [$P(ID)_{sib} = 1/0.01039$], *C. t. pallescens* in Oklahoma had *r*-values of relatedness that did not correspond to *r*-values seen within known pedigrees. Alleles that are identical by descent indicate that individuals descended from the same ancestral group, and the low levels of $P(ID)_{sib}$ seen in *C. t. pallescens* in western Oklahoma suggest possible inbreeding (Avise 2004). Because probability of identity was high for *C. t. pallescens*, 1st-order *r*-values would most likely be higher than other known pedigrees on the eastern periphery of 0.50, and so on. This is possible because *C. t. pallescens* in Oklahoma occur on the eastern extent of their range, separated from the main distribution of the subspecies because of a lack in suitable habitat in the Texas panhandle (Handley 1959), and they are rare.

Dispersal.—Short distance movements or dispersal of *C. t. pallescens* documented in previous studies (Barbour and Davis 1969; Clark et al. 1993; Humphrey and Kunz 1976; Handley 1959; Pierson et al. 1999; Wethington et al. 1996) coupled with results from my study suggest stepping-stone dispersal of females, with males having different but unknown dispersal behavior. If female *C. t. pallescens* in western Oklahoma occur in 2 populations associated with continuous gypsum deposits, the northern population has individuals separated by > 38 km between Alabaster Cavern and Saloon Cave and > 66 km between Saloon Cave and Ake's Cave. The longest documented movement of *C. t. pallescens* in the area was 39.7 km from a single individual recaptured in western Oklahoma (Humphrey and Kunz 1976), but several *C. t. virginianus* in Kentucky and West Virginia moved up to ca. 64.4 km (Barbour and Davis 1969).

Stepping-stone dispersal may occur with subpopulations exchanging individuals across a short distance over a specified time scale or new subpopulations developing from migrants from older subpopulations in 1 dimension (Slatkin 1993). Furthermore, lack of differentiation between subpopulations would take many generations with stepping-stone dispersal patterns (Ibrahim et al. 1996), which might explain the lack of differentiation in the northern population in conjunction with little effective dispersal known for *C. townsendii*. *C. townsendii* is known to roost in small numbers in small caves that are uninhabitable by other cavernous species of bats (Humphrey and Kunz 1976). Small caves were numerous in my study area, likely fostering dispersal of *C. t. pallescens*. The unique haplotype in the northern population, AMOVA results, and Φ-

statistics supported stepping-stone dispersal with a geographic barrier of > 45 km separating the 2 populations of females (Fig. 5).

Overall, dispersal dynamics of male *C. t. pallescens* are unknown, but their dispersal does not seem to be impeded based on my results. The homogenization of a nonmigratory species could be explained by stepping-stone dispersal where homogenization occurs over a much farther distance than movement of individuals and direct exchange of genes (Burland and Worthington Wilmer 2001). Individual-level cluster analysis would correlate with this theory, where clusters showed individuals distributed disproportionately between the northern and southern population (Group D; Fig. 1b). On the other had, Wright's island model also could define dispersal of males in my study, where individuals move or disperse among populations unimpeded by distance (Lowe et al. 2004). Few studies exist on *C. t. pallescens* in Oklahoma, and males may not be as sedentary as seen in other *C. townsendii*. Analysis of isolation-by-distance was not used because numbers of populations in the north or south were < 5, which did not allow enough permutations for matrices in the analysis (Burland and Worthington Wilmer 2001).

Demographic history.—My analysis suggests that a recent bottleneck occurred in the southern population, particularly among females and maternity colonies. Low diversity of bi-parentally inherited DNA was congruent with low diversity of maternally inherited mtDNA in the southern population. That in conjunction with heterozygosity excess relative to the average expected value at mutation-drift equilibrium indicated that a severe bottleneck occurred in the maternity colony at 3 Domes Cave. Although 3 Domes Cave was not significantly differentiated from Ratzlaff or Fink 1 cave samples, a

group of females from the southern population appeared to segregate during the maternity season at 3 Domes Cave. Bats from Fink 1 Cave also were marginal for heterozygosity excess in relation to equilibrium, and when the Washita County caves were combined, analysis still revealed a marginally significant bottleneck. I was not able to differentiate females between caves throughout summer or between summers, so it is possible that females from 3 Domes maternity colony were captured later at Fink 1 or Ratzlaff caves. Nevertheless, females in the southern population appear to have low gene diversity and heterozygosity excess related to equilibrium because allelic numbers decrease faster when a population experiences a bottleneck.

The analysis that I used can only detect a recent bottleneck (0.2−4.0 N_e—Luikart and Cornuet 1998), but little historical information exists for *C. t. pallescens* in western Oklahoma. The Oklahoma Grotto Club documented *C. t. pallescens* in only 22 of 200 caves discussed in their publication in northwestern Oklahoma (Caire 1997). *C. t. pallescens* is sensitive to human disturbance, particularly maternity colonies, and has a propensity to roost near cave entrances (Humphrey and Kunz 1976; Clark et al. 1993). Thus, human disturbance could effectively contribute to declines in *C. t. pallescens* and to a bottleneck, particularly isolated populations such as the one in Washita County. The Oklahoma Grotto Club documented human visitation of caves in Washita County, and most nearby residents were familiar with the caves in their county (Central Oklahoma Grotto 1984). Examination of caves revealed more human graffiti, trash, and destruction in Washita caves than other caves examined in western Oklahoma.

Low haplotypic and nucleotide diversity provided support for the occurrence of a bottleneck in the southern population. Low diversity levels at 3 Domes Cave and Fink 1

Cave and in the southern population (Table 1) also suggests a recent bottleneck or a founder event by 1 or a few mtDNA lineages (Grant and Bowen 1998). Historical demography is needed to verify a bottleneck because many factors could contribute to low diversity and could mask a historical bottleneck (e.g., founding event, disease, predation, or stochastic events—Pimm et al. 1989; Ramey et al. 2000). Other ecological or behavioral metapopulation dynamics also could have negatively affected the southern population (e.g., mating behavior, frequent extinction and recolonization, fragmentation—Pimm et al. 1989; Slatkin 1987). My analyses were a crucial first step, which highlights the need for ongoing monitoring of the southern population. Only long-term analysis with ≥ 10 polymorphic loci on the southern population could provide insight to their historical demography and causes of a genetic bottleneck (Cornuet and Luikart 1996; Luikart and Cornuet 1998; and Luikart et al. 1998).

Conservation implications.—Low dispersal and gene flow of female *C. t. pallescens*, regardless of high dispersal and gene flow of the males, could produce autonomous populations or subpopulations (Avise 2000). Maternity colonies of *C. t. pallescens* are not expected to be recolonized in the event of severe decline or loss, because recruitment of young is highly contingent on reproductive success of females (Avise 2000). Although mtDNA results showed no differentiation across a large section in northwestern Oklahoma, containing the northern population (Group D; Fig. 1b), subpopulations usually do not recolonize areas where extinction occurred unless high density causes dispersal into those areas (Pimm et al. 1989; Slatkin 1987). The status of *C. t. pallescens* in the Washita County area of gypsum deposits is precarious, as consistently suggested by my analyses. Gypsum deposits and associated caves occur in a

relatively small disjunctive pocket in Washita County, resulting in minimal habitat for *C. t. pallescens* and a small isolated population of *C. t. pallescens* with restricted gene flow and dispersal of females. Although the population has low genetic diversity, it is not clear if this resulted from a bottleneck, low fecundity, or high mortality of juveniles (particularly 3 Domes Cave's maternity colony). Any of those could result in inbreeding depression, fixation of deleterious alleles, and negative metapopulation effects (Cornuet and Luikart 1996; Lande 1994; Luikart et al. 1998; Mills and Smouse 1994) resulting in a precarious future for this population. Furthermore, stochastic events could lead to rapid extinction before the population began to suffer from negative genetic related scenarios (Mills and Smouse 1994).

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Table 1.—Genetic diversity of *Corynorhinus townsendii pallescens* from 8 caves in western Oklahoma using mitochondrial sequence data (mtDNA) and microsatellite data (nDNA). Individuals were analyzed according to the 8 cave entrances where they were captured, or grouped (combining caves) based on known maximum nightly movement of *C. t. pallescens* (Major County and Washita County caves) and continuous gypsum distribution (northern and southern populations; Fig. 1b).

	mtDNA[a]							nDNA[b]			
		Haplotypes									
Cave/Group	n_{mt}	E	F	G	H	h	π	n_{ms}	A	H_O	H_E
Alabaster	7			1	6	0.286	0.002	7	4.2	0.714	0.736
Saloon	18	1	2	3	12	0.543	0.003	18	5.4	0.600	0.709
Nescatunga	5	2	1		2	0.800	0.004	5	3.8	0.600	0.773
Cult	13	3	2	6	2	0.744	0.003	14	5.6	0.743	0.763
Ake's	10			7	3	0.467	0.003	15	6.0	0.693	0.712
Fink 1	13	11		2		0.282	0.001	13	5.8	0.615	0.767
Ratzlaff	6	4	1	1		0.600	0.002	6	4.2	0.567	0.791
3 Domes	18	18				0.000	0.000	18	6.2	0.733	0.766
Major County caves	36	6	5	9	16	0.713	0.003	37	7.2	0.654	0.732
All northern caves	53	6	5	17	25	0.666	0.003	59	7.8	0.671	0.719
Washita County caves (= southern population)	37	33	1	3		0.203	0.001	37	7.6	0.665	0.764

[a]Number of individuals sampled (n_{mt}), haplotype frequencies, and haplotype (h) and nucleotide (π) diversities.

[b]Number of individuals sampled (n_{ms}), alleles per locus averaged across loci (A), and mean observed (H_O) and mean expected (H_E) heterozygosity

Table 2.—Analysis of molecular variance (AMOVA) and overall φ_{ST} from *Corynorhinus townsendii pallescens* colonies at 8 caves in western Oklahoma using mitochondrial sequence data. Analysis was done at 3 hierarchical levels: among populations, within populations, and among population within groups. Populations were analyzed to investigate structure within and among population groupings based on cave entrances, maximum nightly movement, and continuous gypsum deposits (Fig. 1b).

Group[a]	Sub-grouping	φ_{ST}	Among populations or groups	Within populations	Among populations within groups
B	1	0.441	44.06	55.94	
	2	0.549	44.79	45.11	10.10
	4	0.495	41.54	50.49	7.97
C	1	0.456	45.58	54.42	
	2	0.510	38.76	48.98	12.26
D	1	0.477	47.71	52.29	

[a]Fig. 1b.

Table 3.—Pairwise φ-statistics using sequential Bonferroni correction (Rice 1989) of *Corynorhinus townsendii pallescens* from 8 caves in western Oklahoma based on mitochondrial sequence data. Bold numbers designate significant differentiation ($p = 0.000$); lower case letters in parenthesis correspond to sub-grouping in AMOVA (individuals grouped by: 1 = all individuals combined; 2 = grouped into northern and southern populations; and 4 = grouped according to maximum nightly movement; Table 2).

Caves	Alabaster	Saloon	Nescatunga	Cult	Ake's	Fink 1	Ratzlaff	3 Domes
Alabaster	——							
Saloon	0.000	——						
Nescatunga	0.210	0.059	——					
Cult	**0.315**$_{(1,2)}$	0.193	0.059	——				
Ake's	0.400	0.255	0.317	0.034	——			
Fink 1	**0.710**$_{(1,2,4)}$	**0.541**$_{(1,2,4)}$	0.273	**0.301**$_{(2,4)}$	**0.590**$_{(1,2,4)}$	——		
Ratzlaff	**0.556**$_{(1,4)}$	**0.386**$_{(1,2,4)}$	0.005	0.085	**0.408**$_{(4)}$	0.000	——	
3 Domes	**0.921**$_{(1,2,4)}$	**0.713**$_{(1,2,4)}$	**0.631**$_{(1,2)}$	**0.569**$_{(1,2,4)}$	**0.828**$_{(1,2,4)}$	0.120	0.333	——

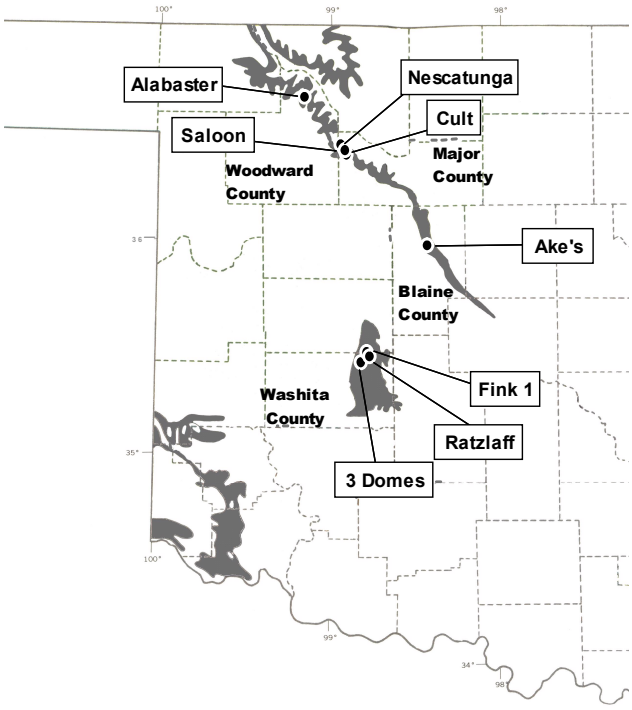
Table 4.— Pairwise φ-statistics using sequential Bonferroni correction (Rice 1989) of *Corynorhinus townsendii pallescens* in western Oklahoma based on mitochondrial sequence data. *C. t. pallescens* was examined as population groupings based on maximum nightly movement (Table 2; Fig. 1b). Bold numbers designate significant differentiation ($p = 0.000$); lower case letters in parenthesis correspond to sub-grouping in AMOVA (individuals grouped by: 1 = all individuals combined and 2 = grouped into northern and southern populations; Table 2).

Population Grouping	Alabaster	Major County caves	Ake's	Washita County caves
Alabaster	——			
Major County caves	0.083	——		
Ake's	0.400	0.123	——	
Washita County caves	**0.777**$_{(1,2)}$	**0.449**$_{(1,2)}$	**0.708**$_{(1,2)}$	——

Table 5.—Relatedness of adult *Corynorhinus townsendii pallescens* sampled in western Oklahoma. Population groups were based on cave entrance of capture, maximum nightly movement, and continuous gypsum deposits (Fig. 1b).

Population grouping	$n_{♀}$	$n_{♂}$	*r*-values		
			All	Females	Males
All cave entrances	42	9	0.004	-0.032	-0.007
Alabaster Cavern	1	1	0.217	---	---
Saloon Cave	7	1	0.056	0.081	---
Nescatunga Cave	2	0	-0.096	-0.096	---
Cult Cave	6	2	-0.004	0.078	0.417
Ake's Cave	10	0	0.056	0.056	---
Fink 1 Cave	7	3	0.054	0.104	0.083
Ratzlaff Cave	2	2	-0.077	0.147	-0.124
3 Domes Cave	7	0	-0.018	-0.018	---
Major County caves	15	3	0.015	0.017	0.158
Northern population	26	4	0.087	0.059	0.083
Washita County caves (southern population)	16	5	0.017	0.028	-0.028

a.



b.

Group	Basis for grouping	Grouped population names	Caves included
A	Individual	*C. t. pallescens*	All caves combined
B	Cave of capture	Individual cave names	All caves individually
C	Maximum nightly movement	Alabaster	Alabaster
		Major County caves	Saloon + Nescatunga + Cult
		Ake's	Ake's
		Washita County caves	Fink 1 + Ratzlaff + 3 Domes
D	Gypsum deposits	Northern	Alabaster + Saloon + Nescatunga + Cult + Ake's
		Southern	Fink 1 + Ratzlaff + 3 Domes

Fig. 1.—a) Locations of caves where *Corynorhinus townsendii pallescens* was sampled; exposed gypsum deposits are dark gray; b) samples were analyzed in population groupings based on behavioral or geographic factors.

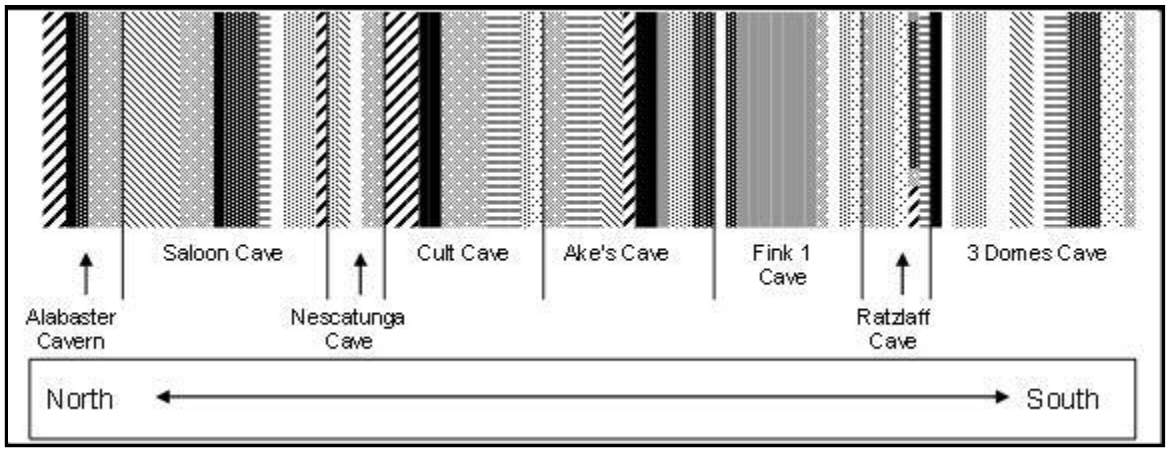


Fig. 2.—Nine clusters from individual clustering analysis using Bayesian Analysis of Population Structure (BAPS). Each individual *Corynorhinus townsendii pallescens* sampled is represented by a vertical bar, and individuals with the same shaded bars belong to the same genetically divergent population or cluster. Caves (acronym below) are separated by vertical black lines and represented in a north-south continuum. Vertical bars where multiple shadings are seen, correspond to ancestral source from admixture clustering (Corander and Marttinen 2005).

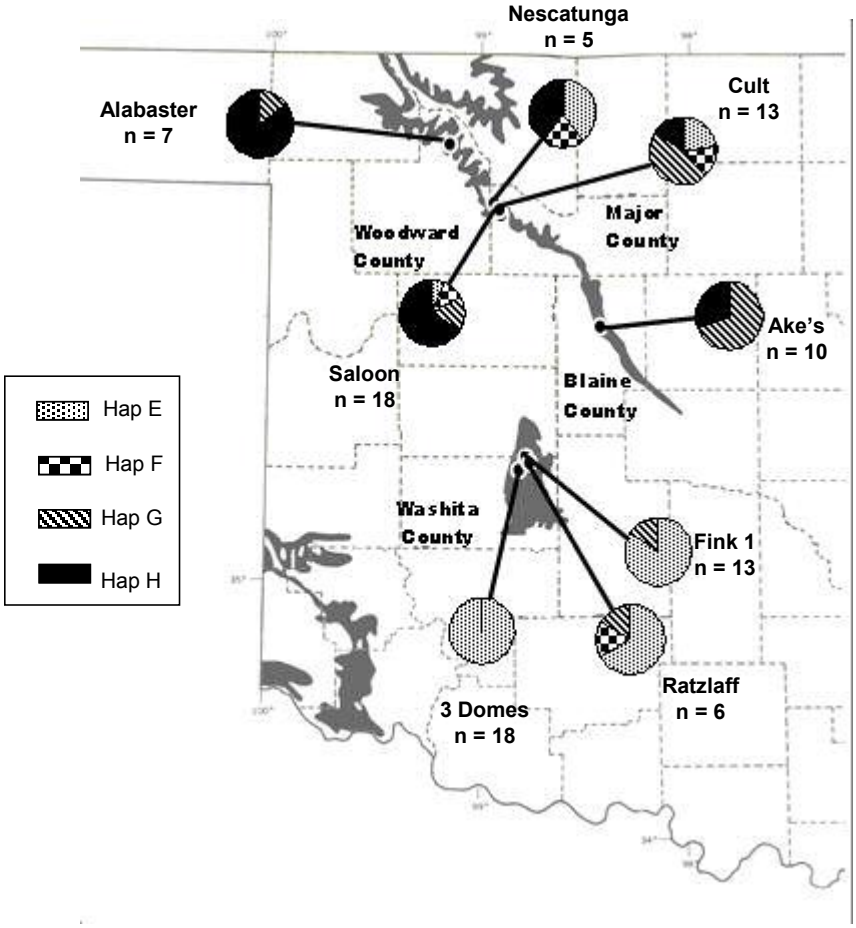


Fig. 3.—Haplotypic associations of *Corynorhinus townsendii pallescens* in western Oklahoma; pie charts display variability in frequencies of haplotypes associated at the 8 caves entrances where *C. t. pallescens* were captured. Distribution of exposed gypsum deposits are dark gray.

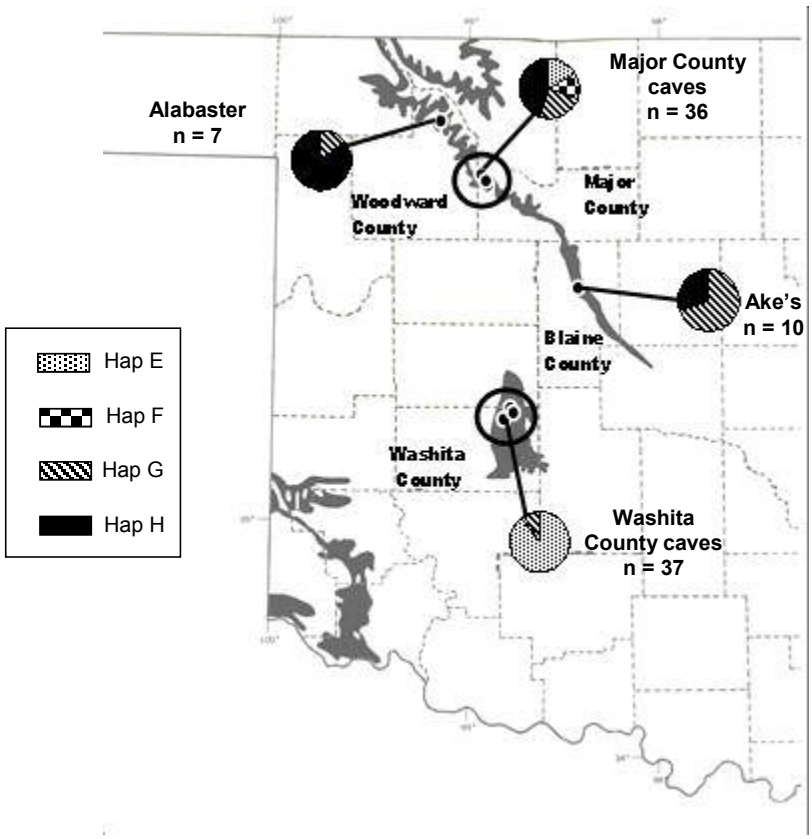


Fig. 4.—Haplotypic associations of *Corynorhinus townsendii pallescens* in western Oklahoma, with caves grouped according to their proximity of known maximum nightly movements of *C. t. pallescens*; pie charts show haplotypic variation in frequencies.

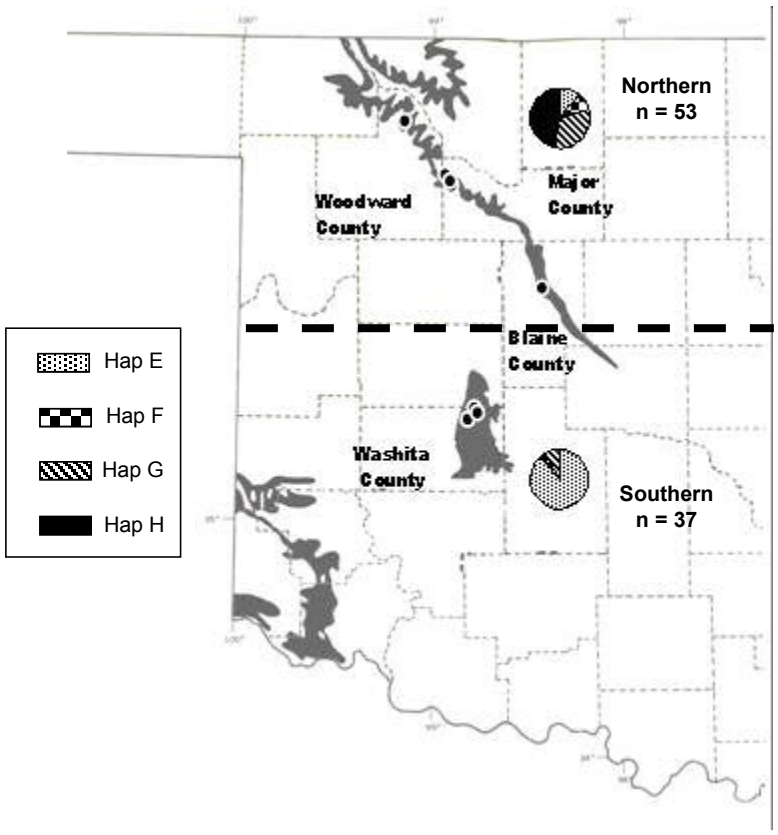


Fig. 5—Haplotypic associations of *Corynorhinus townsendii pallescens* in western Oklahoma, with grouping of individuals by cave, delimited by the distribution of exposed gypsum deposits (dark gray; Fig. 1b); significant pairwise φ_{ST} results separated the northern and southern populations (bold dashed line).

VITA

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Master of Science

Thesis: USING GENETIC ANALYSES TO GAIN INSIGHT ON A RARE BAT, *CORYNORHINUS TOWNSENDII PALLESCENS*

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Maternally inherited mitochondrial DNA (mtDNA) and 5 bi-parentally inherited microsatellite DNA markers were used to investigate population and social structure within and among populations and gender and demographic history of *Corynorhinus townsendii pallescens*, roosting in gypsum caves in western Oklahoma. Results showed that males had high gene flow and dispersal, but BAPS individual-level clustering analysis revealed 9 admixed clusters. Females were hindered in dispersal and had high population structure, with populations separated by a geographic barrier. One population of females used gypsum caves extending from Kansas southeast to Blaine County (northern grouping) and another population used gypsum caves in Washita County (southern grouping). Relatedness was analyzed for social structure with the assumption that *r*-values corresponded to known pedigrees and suggested the northern group was more related than the southern group. Demographic analysis revealed a recent bottleneck in the southern group.

Advisor's Approval: David M. Leslie, Jr.