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Demographic history and incomplete lineage sorting obscure population genetic structure of the Texas mouse (*Peromyscus attwateri*)

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The Texas mouse, *Peromyscus attwateri*, is a relatively habitat-specific species that exhibits a discontinuous distribution across the south-central United States. To examine the evolutionary history and contemporary population genetic structure of *P. attwateri* we sequenced an 1,100-base pair fragment of the mitochondrial cytochrome *b* gene for 210 individuals from 22 localities, resulting in 89 unique haplotypes. Low nucleotide diversity ($\pi = 0.0043$) and a median-joining haplotype network indicated low levels of divergence among haplotypes with little geographic structure. Demographic analyses indicated the presence of 2 significant range expansions: the 1st coinciding with the end of the last glacial maximum of the Pleistocene approximately 14 thousand years ago (kya) and the 2nd more recent expansion during the hypsithermal (9–5 kya) of the Holocene. Coalescent simulations under a model of no gene flow indicated that the lack of divergence among populations most likely is attributable to incomplete lineage sorting, and the observed gene flow statistic ($s = 116$) suggests that populations became isolated approximately 7.5 kya. This finding substantiates the hypothesis that the thermal maximum of the hypsithermal may have had a significant impact on small mammals as well as other organisms in the south-central United States. DOI: 10.1644/09-MAMM-A-242.1.

Key words: coalescent theory, cytochrome *b*, *Peromyscus attwateri*, phylogeography, Pleistocene, Texas mouse

The stochastic nature of coalescent processes can make the determination of phylogenetic and phylogeographic relationships difficult. The process of lineage sorting proceeds randomly, with significant variation in the time between initial divergence and the point at which reciprocal monophyly can be obtained for any 2 lineages (Edwards and Beerli 2000; Knowles and Maddison 2002). At the population level differentiating between gene flow and incomplete lineage sorting can be difficult, especially when isolation of populations has occurred extremely recently (i.e., in the last 10,000 years—Holder et al. 2001). In addition, a dynamic demographic history can lead to further confusion, with rapid population growth or radiation leading to the retention of ancestral character states (Knowles and Maddison 2002; Maddison 1997).

The climatic fluctuations of the Pleistocene played a significant role in dictating patterns of species diversity and divergence (Avise et al. 1998; Hewitt 1996, 1999, 2004). The Pleistocene of the south-central United States was a dynamic period, characterized by repeated glacial advance and recession producing a variable environment that translated into repeated faunal and floral shifts. During climatic cooling many temperate species occurring in the Northern Hemisphere reduced their geographic ranges to warmer southern refugia (Hewitt 1996). Subsequent periods of stable climate created opportunities for these temperate species to disperse northward behind warming trends. Evidence for this trend has been observed in population genetic studies of multiple temperate species on several continents (Avise et al. 1998; Hewitt 1996, 1999, 2004), and a similar scenario has been suggested to explain the contemporary distribution of the Texas mouse, *Peromyscus attwateri* (Kilpatrick 1984; Schnake-Greene et al. 1990). In addition to the glacial advances of the Pleistocene, the more subtle warming of the hypsithermal 9–5 thousand years ago (kya) also could have affected the distribution of *P. attwateri*, as has been suggested for other temperate animals (Kulikova et al. 2005; Masta 2000), by altering the distribution of its preferred habitat.

Allen (1895) originally described *P. attwateri* as a distinct species. However, subsequent revisions by Bailey (1906) and

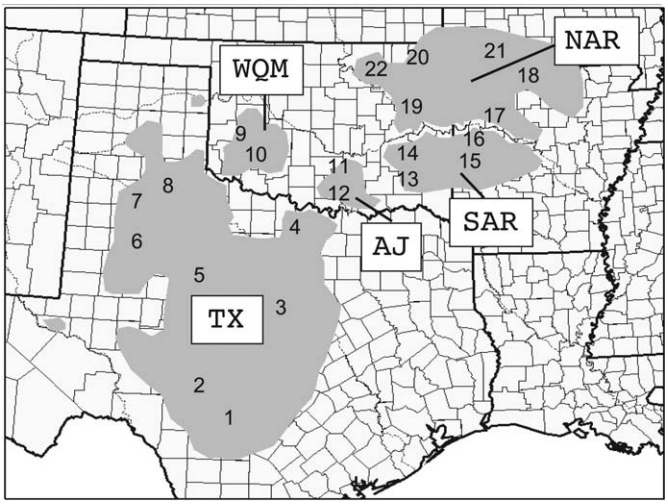

Fig. 1.—Distributional map of the Texas mouse, *Peromyscus attwateri*. Numbers represent counties in Texas (TX), Oklahoma (OK), Arkansas (AR), and Missouri (MO) from which *P. attwateri* were collected, including Kerr Co., TX; Kimble Co., TX; Erath Co., TX; Cooke Co., TX; Coke Co., TX; Garza Co., TX; Floyd Co., TX; Cottle Co., TX; Comanche Co., OK; Greer Co., OK; Murray Co., OK; Johnston Co., OK; Pushmataha Co., OK; Latimer Co., OK; Saline Co., AR; Perry Co., AR; Pope Co., AR; Stone Co., AR; Cherokee Co., OK; Delaware Co., OK; Taney Co., MO, and Osage Co., OK. TX, AJ, WQM, SAR, and NAR indicate regional groups. Sample sizes for each sampling locality are given in Table 1.

Osgood (1909) relegated it to a subspecies of *P. boylii*. Lee et al. (1972) elevated *P. attwateri* to species level on the basis of karyotype, and Schmidly (1973) substantiated this finding with additional chromosomal data and morphology. Additional studies using protein electrophoretic analysis also supported the elevated taxonomic status but indicated that *P. attwateri* was closely allied to *P. boylii* and therefore included it as a member of the *boylii* species group (Avise et al. 1974; Kilpatrick and Zimmerman 1975). However, recent systematic studies have found *P. attwateri* to be allied most closely with *P. difficilis* and *P. nasutus* and therefore a member of the *truei* species group (Bradley et al. 2007; DeWalt et al. 1993; Durish et al. 2004; Janecek 1990; Tiemann-Boege et al. 2000). The repeated shuffling of the taxonomic status of *P. attwateri* warrants a reconsideration of its evolutionary history, as past hypotheses concerning its origin and subsequent expansion have been derived from fossils identified as *boylii*-like and the assumption that *P. attwateri* was most closely allied with the *boylii* species group (Kilpatrick 1984).

The geographic distribution of *P. attwateri* ranges from central Texas, north throughout most of Oklahoma, southern Kansas and Missouri, and northwest Arkansas (Caire et al. 1989; Choate et al. 1966; Ethridge et al. 1989; Hall 1981; Sealander and Heidt 1990; Fig. 1). Relative to other species of *Peromyscus*, the Texas mouse is habitat specific and exhibits a discontinuous distribution (Kilpatrick 1984; Schmidly 1974). Throughout its range *P. attwateri* can be found in upland barren or rocky habitat in juniper and oak woodlands, mixed hardwood/pine forests, and cedar glades (Brown 1964a, 1964b; Schnell et al. 1980; Stancampiano and Schnell 2004). These habitats are fragmented by low-lying plains, mixed-grass prairie, and river valleys, appearing to create disjunct populations of *P. attwateri*. In addition, *P. attwateri* appears to be highly submissive to other species of *Peromyscus* (Brown 1964a), most notably the geographically widespread white-footed mouse, *P. leucopus*, indicating that interspecific competition might be limiting its dispersal (Sugg et al. 1990b).

Due to the habitat-specific nature of *P. attwateri* and its fragmented distribution, we hypothesize that relatively little gene flow occurs among populations, and therefore ample opportunity exists for populations to diverge. Our primary objective was to determine the magnitude of gene flow that has occurred among populations since their isolation and use measures of genetic diversity to determine the effect(s) of what may be a recent fragmentation of populations. In addition, we attempted to determine historical population demographics to better understand how the dynamic climatic conditions of the Pleistocene affected *P. attwateri* and shaped its current distribution and population genetic structure. We predicted that the climatic fluctuations that occurred during and following the Pleistocene have contributed to the contemporary population genetic structure of *P. attwateri*.

Materials and Methods

To address our objectives specimens were collected from 22 localities across the geographic distribution of *P. attwateri* (Fig. 1). All animals were handled according to the guidelines of the American Scoiety of Mammalogists (Gannon et al. 2007). Whole genomic DNA was isolated from muscle tissue through phenol extraction as described by Longmire et al. (1997). All extracted DNA, tissues, and voucher specimens used in this study are stored in tissue and museum collections at the University of Central Oklahoma (UCO), Tarleton State University, Oklahoma State University, or The Museum, Texas Tech University (see Appendix I). The entire cytochrome *b* gene was amplified via polymerase chain reaction (PCR) in 50-µl reactions using primers L14724 (Irwin et al. 1991) and H15906 (Zheng et al. 2003). Due to inconsistent success of PCR amplification and low yield using the above primer pair, 2 external primers (L_UCO_CYT*b*_PEAT, 5′-AACACCCACTACTCAAAATCATTAAC-3′; H_UCO_CYT*b*_PEAT, 5′-ATGGGTGAATTTTAGTATGTTGTT-3′) were developed to amplify the remaining samples. Reaction mixes consisted of 50–200 ng of DNA, 5 µl of 10× buffer, 1 mM each deoxynucleotide triphosphate, 0.5 mM each primer, 2.5 mM $MgCl_2$, and 1.25 units of *Taq* DNA polymerase (Promega, Madison, Wisconsin). PCR cycles were as follows: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 45°C for 1 min, and 72°C for 1 min 30 s with a final elongation phase of 72°C for 7 min. PCR products were visualized by agarose gel electrophoresis. Amplicons were purified using the Wizard PCR Prep DNA purification system (Promega, Madison, Wisconsin).

Termination sequencing reactions were modified from the manufacturer's protocol (Big Dye; Perkin Elmer, Foster City, California): 1 μl of PCR product, 2 μl of Big Dye Terminator version 3.1 sequence mix, 1.6 μl of primer (10 μg/μl), and 5.4 μl of water. Two internal primers (PEAT_UCO_Hint, 5′-AGTTAAGGCCTGATGGGTTATTG-3′; PEAT_UCO_Lint, 5′-ATCCTACCATTCATCATTACAGC-3′) were developed to produce overlapping sequence at all base positions. Sequence reaction products were visualized using an Applied Biosystems 310 Genetic Analyzer (Foster City, California). Contigs were assembled using the computer program Geneious v4.6 (Biomatters 2005), and complete sequences were aligned in Clustal W (Thompson et al. 1994). The resulting alignment was imported into the computer program MacClade (Maddison and Maddison 2000) for visual inspection and editing, and the REDUNDANT TAXA option was used to identify unique haplotypes.

Mitochondrial DNA haplotype frequencies, haplotype diversity (h), nucleotide diversity (π), uncorrected genetic distance, and population pairwise ϕ_{ST} values were calculated using the computer program ARLEQUIN v2.001 (Schneider et al. 1997). The analysis of molecular variance (AMOVA—Excoffier et al. 1992) option in ARLEQUIN (Schneider et al. 1997) was used to test for population structure. In addition, we conducted a spatial analysis of molecular variance (SAMOVA) using the program SAMOVA 1.0 (Dupanloup et al. 2002). This approach attempts to identify population groups (K) that maximize the amount of variation among groups. Therefore, the number of groups that produces the maximum ϕ_{CT} is the best depiction of population relatedness. This analysis was run for 10,000 iterations for $K = 1, 2, \ldots, 20$ groups using 100 initial conditions. In the event that SAMOVA fails to identify any significant population groupings, we also grouped localities by what appeared to be continuous regions occurring on opposite sides of potential filters or barriers to gene flow (i.e., sampling localities falling in what appear to be continuous distributional regions on opposite sides of the Arkansas River; Fig. 1). Although these groupings might make intuitive sense, they were not based on any distinct genetic clustering or phylogenetic results and instead were used to examine regional differences in demographic history or partitioning of genetic variation and simplify the presentation of results.

Intraspecific data sets can violate many of the assumptions made by standard phylogenetic tree-building algorithms (Posada and Crandall 2001). Therefore, a phylogenetic network that allows for multiple connections will typically provide a more realistic graphical interpretation of population genetic processes because many intraspecific data sets will have both ancestral (internal) and derived (terminal) haplotypes in extant individuals. We generated a median-joining network (Bandelt et al. 1999) using the computer program Network v4.510 (http://www.fluxus-engineering.com).

The hypothesized recent origin of the *P. attwateri* lineage (Kilpatrick 1984) and the potential for significant range reduction(s) and/or expansion(s) as recently as ~5 kya might lead to a lack of distinct phylogeographic structure even in the complete absence of gene flow. This is due to insufficient time for complete lineage sorting to occur. To test the hypothesis of incomplete lineage sorting we conducted coalescent simulations in the software package MESQUITE v2.6 (Maddison and Maddison 2004). Population trees were constructed consisting of 22 terminal taxa corresponding to each of the sample populations and with branch lengths corresponding to varying functions of effective population size ($2N_{ef}$, N_{ef}, $0.2N_{ef}$, and $0.02N_{ef}$). Under a model of no migration we simulated 1,000 topologies within each population tree, using a conservative effective population size of 10^5. On the basis of the Bayesian skyline plot (BSP, described below; Fig. 2), current effective population sizes were estimated to fall between 10^5 and 10^6. We chose the lower bound for our simulations because time to complete lineage sorting is directly related to effective population size, and overestimating contemporary effective population sizes will result in artificially high estimates of time to complete lineage sorting (Hein et al. 2005; Neigel and Avise 1986; Pamilo and Nei 1988). Therefore, $N_e = 10^5$ ensured that we would obtain estimates of the minimum time required for current populations of *P. attwateri* to become distinct. For each simulation we calculated the distribution of s, an estimate of the minimum number of migration events consistent with a given phylogeny (Slatkin and Maddison 1989). In addition, we conducted a phylogenetic analysis using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). This analysis consisted of 4 Markov chains of 5×10^6 generations sampling every 100 generations, and burn-in of resulting samples was determined visually, with convergence occurring at approximately 5,000 samples. The observed s value was calculated from the resulting 50% majority consensus tree (not shown) and compared with the distributions generated from simulations to estimate the minimum number of generations required for complete lineage sorting.

The demographic history of *P. attwateri* was investigated using mismatch distributions and BSP. Mismatch distribu-

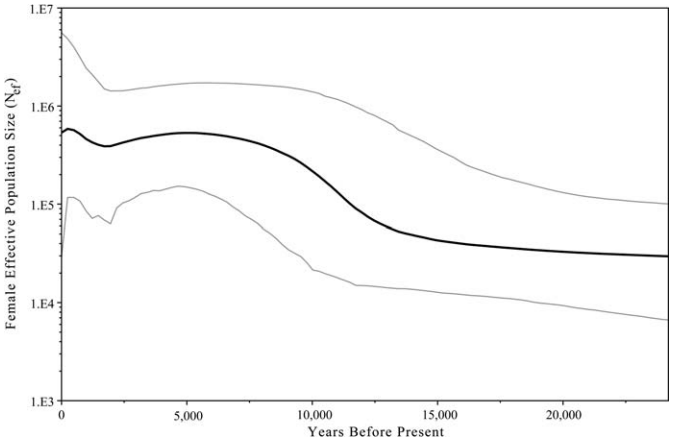

FIG. 2.—Bayesian skyline plot generated with BEAST v1.4.8. x-axis values are thousands of years before present and y-axis values are estimates of female effective population size (N_{ef}). The thick black line is the median estimate and the thin gray lines correspond to the 95% HPD estimate.

tions, which plot the distribution of pairwise genetic differences between pairs of individuals (Rogers and Harpending 1992), were determined using ARLEQUIN and compared with a model of sudden expansion (Rogers and Harpending 1992). This model assumes that an initial population at equilibrium with $\theta = \theta_0$ grows rapidly to a new size with $\theta = \theta_1$, τ units of mutational time before the present, where $\theta = N_e\mu$ and $\tau = 2\mu t$ (N_e = effective population size, μ = mutation rate, and t = time since the expansion in generations—Rogers and Harpending 1992). To date the time of expansion a cytochrome *b* divergence rate of 7.5–12% per million years for rodents was used (Arbogast et al. 2001). Because no studies have clearly revealed the generation time for *P. attwateri*, this parameter was estimated on the basis of the assumption that *P. attwateri* reaches sexual maturity approximately 55 days after birth, as indicated for other species of *Peromyscus* (Clark 1938). This produced a generation time of 0.15 years. Goodness-of-fit tests (Schneider and Excoffier 1999) of the observed and expected distributions were computed. The confidence intervals for τ were obtained from 1,000 bootstrap replicates. Conformation to a model of selective neutrality and population equilibrium by Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) was tested with 5,000 bootstrap replicates.

In addition to the mismatch distribution, we used the coalescent-based approach of the BSP (Drummond et al. 2005) as implemented in the computer program BEAST v1.4.8 (Drummond and Rambaut 2007). The BSP estimates effective population size through time, providing a graphical representation of past population demographics. In addition, this analysis allows for the simultaneous estimation of the time to most recent common ancestor (TMRCA) for all included haplotypes. To produce time estimates in years we used a strict clock and a substitution rate of 7.5–12% divergence per million years. The hierarchical likelihood ratio test (Felsenstein 1981) implemented in the computer program Modeltest (Posada and Crandall 1998) indicated that the HKY + I + Γ model of nucleotide substitution was most appropriate. Our analysis consisted of an initial run of 3×10^7 generations followed by adjustment of operator values to optimize search settings. Two final runs of 3×10^7 generations were conducted with optimized search settings, and the resulting log and tree files were combined to produce final estimates of demographic parameters. All runs were checked for sufficient mixing, stable convergence on a unimodal posterior, and effective sample sizes (Drummond et al. 2002) >100 for all parameters using the computer program TRACER v1.4 (Drummond and Rambaut 2003).

Results

Sequence data for an 1,100-base pair portion of the cytochrome *b* gene was obtained from 210 *P. attwateri* from 22 localities across the species geographic range. All sequences were deposited in GenBank (accession numbers GU251883–GU251971). Nucleotide composition was 30.98% adenine, 27.18% cytosine, 12.58% guanine, and 29.26% thymine. Nucleotide substitutions were present at 74 variable sites with 64 transitions and 10 transversions. Of the 74 variable sites, 13 substitutions occurred at the 1st codon position (17.57%), 6 substitutions occurred at the 2nd codon position (8.11%), and 55 occurred at the 3rd codon position (74.32%). Nucleotide composition and codon bias estimates were consistent with those of cytochrome *b* sequences for other mammals (Irwin et al. 1991; Johns and Avise 1998).

Eighty-nine unique haplotypes were identified, with 18 of these haplotypes occurring in >1 sampling locality. The 2 most common and widely dispersed haplotypes, B and K, occurred 22 and 19 times, respectively. Haplotype B occurred in all Texas localities, 3 Oklahoma localities (Greer Co., Murray Co., and Latimer Co.; sampling localities 10, 11, and 14, respectively; Fig. 1), and 1 locality (Perry Co., sampling locality 16; Fig. 1) in Arkansas south of the Arkansas River. Haplotype K occurred in all localities north of the Red River and south of the Arkansas River except Johnston Co., Oklahoma (sampling locality 12; Fig. 1). Neither of these 2 haplotypes occurred north of the Arkansas River. The only haplotype that occurred on both sides of the Arkansas River was haplotype L, found in Cooke Co., Texas, Cherokee Co., Oklahoma, and Stone Co., Arkansas (sampling localities 4, 19, and 18, respectively; Fig. 1). Mean haplotype diversity was high ($h = 0.8399 \pm 0.0927$), whereas nucleotide diversity was low ($\pi = 0.0043 \pm 0.0043$; Table 1), indicating the presence of a large number of closely related haplotypes. The highest π values were found in Arkansas (SAR and NAR; Fig. 1), whereas the lowest π values were found in the WQM (Fig. 1) populations in southwestern Oklahoma (Table 1). Pairwise genetic distance among haplotypes of all individuals ranged from 0.00% to 1.45%.

The SAMOVA was unable to identify any significant structure of populations. ϕ_{CT} values increased progressively as K increased, with ϕ_{CT} ranging from 0.289 to 0.366 and all values statistically significant ($P << 0.001$). In addition, as groups were added for each subsequent analysis, the results most often consisted of all but 1 group containing a single population and all other populations lumped into a final group. AMOVA for the total population revealed that 65.24% of variation was found within individual sampling localities, whereas 34.76% of variation was found among sampling localities, indicating significant population structure (Table 2). ϕ-statistics were indicative of significant population structuring ($P < 0.001$; Table 2). When populations were grouped regionally, all ϕ-statistics (ϕ_{CT}, ϕ_{SC}, and ϕ_{ST}) were significant (Table 2). Also, the majority of pairwise ϕ_{ST} values were significant (169/231 = 73.16%; not shown), indicating significant population structuring at the sampling locality level but displaying no geographic pattern.

Despite significant ϕ_{ST} values for both the total population and the majority of pairwise population comparisons, the median-joining network shown in Fig. 3 showed no clear pattern of genetic structure. The 2 most frequent haplotypes (B and K; Fig. 3) do occur internally as coalescent theory

Table 1.—Sample size (*n*), haplotype diversity (*h*) ± *SE*, nucleotide diversity (π) ± *SE*, Tajima's *D*, and Fu's F_S for *P. attwateri* from 21 sampling localities in Texas, Oklahoma, Arkansas, and Missouri. Asterisks indicate statistically significant values ($P < 0.05$).

Locality numbers/County	*n*	*h*	*SE*	π	*SE*	Tajima's *D*	Fu's F_S
1. Kerr	10	0.9111	0.0773	0.0032	0.0020	−0.058	−1.640
2. Kimble	9	0.8333	0.1265	0.0030	0.0019	1.312	−0.915
3. Erath	10	0.9556	0.0594	0.0046	0.0028	0.150	−1.930
4. Cooke	8	0.8571	0.1083	0.0042	0.0025	0.381	−2.220
5. Coke	10	0.9556	0.0594	0.0022	0.0015	−0.551	−1.295
6. Garza	10	0.8889	0.0754	0.0024	0.0016	−0.348	−2.460*
7. Floyd	10	0.9111	0.0773	0.0038	0.0024	0.413	0.410
8. Cottle	10	0.7111	0.1175	0.0034	0.0021	0.760	2.126
9. Comanche	10	0.8444	0.1029	0.0011	0.0009	0.218	0.645
10. Greer	9	0.6667	0.1318	0.0018	0.0013	−0.193	−1.803
11. Murray	10	0.8889	0.0754	0.0018	0.0013	−1.925*	3.989
12. Johnston	10	0.2000	0.1541	0.0038	0.0023	0.333	−0.034
13. Pushmataha	8	0.9643	0.0772	0.0039	0.0025	−1.326	−2.283
14. Latimer	10	0.8444	0.1029	0.0012	0.0009	−1.035	−3.023*
15. Saline	10	0.8222	0.0969	0.0035	0.0022	0.974	0.936
16. Perry	10	0.8222	0.0969	0.0032	0.0020	−0.030	0.691
17. Pope	10	0.8444	0.0796	0.0061	0.0035	1.590	2.362
18. Stone	10	0.9556	0.0594	0.0051	0.0030	1.399	1.849
19. Cherokee	10	0.8000	0.1001	0.0066	0.0038	0.939	−1.051
20. Delaware	10	0.9333	0.0773	0.0027	0.0017	0.229	−3.581*
21. Taney	10	0.9333	0.0620	0.0051	0.0030	0.259	−0.468
22. Osage	6	0.9333	0.1217	0.0026	0.0018	0.375	−3.453*
Total	210	0.8399	0.0927	0.0035	0.0043		

predicts, and many haplotypes do group regionally. The majority of haplotypes found in Texas cluster together, with a few exceptions. However, several haplotypes found in NAR, SAR, and AJ (Fig. 1) form a cluster terminal to the haplotypes found in Texas, with a relatively large number of mutations separating these haplotypes and others from the same sampling locality. When haplotype relationships are considered independent of regions, no clear pattern among individual localities is seen. The lack of geographic structure is further augmented by the presence of a large number of loops, which make any inferences drawn from this network speculative and subject to high degrees of error.

The mismatch distribution analysis of the complete data set revealed a unimodal distribution of the frequency of pairwise differences (Fig. 4a), indicating a historical range expansion. The sum of squares deviation (SSD = 0.002, $P_{SSD} = 0.740$) and raggedness index (0.006, $P = 0.940$) were unable to reject the hypothesis of a recent expansion. The expansion for the total population dated to approximately 13.2–21.1 kya, coinciding with the end of the Pleistocene and the beginning of climatic stabilization. SSD and raggedness index values were unable to reject a recent expansion for any region, but a unimodal distribution of pairwise differences was observed for TX and WQM (Figs. 4b and 4c, respectively). The peaks for these 2 populations do not occur at the same position on the *x*-axis. The TX peak (Fig. 4b) occurs at approximately the same position as the peak for the total population (Fig. 4a), between 9 and 11 pairwise differences, suggesting that both data sets indicate the same expansion event. The WQM peak (Fig. 4c) occurs between 3 and 4 pairwise differences. This same peak occurs in the total population (Fig. 4a), TX (Fig. 4b), SAR (Fig. 4e), and NAR (Fig. 4f) mismatch distributions, suggesting a secondary expansion approximately 6.7–10.7 kya. In addition, the low nucleotide diversity of the WQM region, coupled with the mismatch peak being significantly shifted to the left, suggests that this region was either not founded until the secondary expansion or underwent a significant recent bottleneck. Parameter values and goodness-of-fit results for all

Table 2.—Analysis of molecular variance (AMOVA) for the haplotypes of the Texas mouse, *Peromyscus attwateri*, indicating the degree and significance of population structuring. Regionally grouped sampling localities correspond to groupings defined in Fig. 1.

Source of variation	Variance components	Percentage of variance	ϕ-statistics	*P*
All populations				
Among populations	1.015	34.76	$\phi_{ST} = 0.348$	$P < 0.0001$
Within populations	1.905	65.24		
Regionally grouped				
Among groups	0.536	17.76	$\phi_{CT} = 0.178$	$P < 0.0001$
Among populations within group	0.576	19.08	$\phi_{SC} = 0.232$	$P < 0.0001$
Within populations	1.906	63.16	$\phi_{ST} = 0.368$	$P < 0.0001$

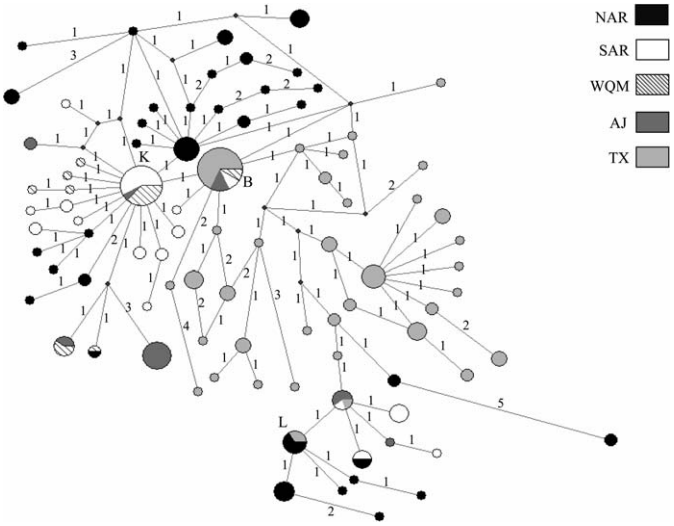

Fig. 3.—Median-joining network of all unique haplotypes. Circle sizes correspond to haplotype frequency. The smallest circles are median vectors joining 2 or more haplotypes. Shading and patterns correspond to regional groupings depicted in Fig. 1. Haplotypes with multiple shades or patterns occurred in multiple regions, and the size of color slice corresponds to the frequency of the haplotype in the corresponding region. Numbers along lines correspond to the number of mutations separating haplotypes or vectors. B, K, and L correspond to haplotypes described in the text.

mismatch analyses are given in Table 3. Further support for the presence of 2 major expansion events is provided by the BSP (Fig. 2). This analysis indicates an initial expansion between 10 and 15 kya and a 2nd expansion occurring in the past few thousand years. The TMRCA for all haplotypes sampled was 48.8 kya (highest posterior density [HPD] 28.1–73.1 kya).

The total population exhibited significantly negative values for Tajima's D ($D = -1.55$, $P = 0.021$) and Fu's F_S ($F_S = -3.4 \times 10^{38}$, $P = 0.014$; Table 3). When populations were analyzed according to region (Fig. 1) all F_S values were significantly negative except for region AJ. With the exception of AJ, all D values were negative, although none were significant (Table 3). When sampling localities were analyzed separately the majority of D and F_S values were negative, but only Floyd Co., Johnston Co., Latimer Co., Delaware Co., and Osage Co. localities deviated significantly from random predictions ($P < 0.05$; Table 1).

The resulting distribution of the test statistic s from coalescent simulations derived from a model of no migration and the assumption of $N_e = 10^5$ are shown in Fig. 5. The observed gene flow statistic (s) derived from the Bayesian phylogeny (not shown) was $s = 116$. The minimum possible migration events to produce the 22 observed populations is $s = 21$, and therefore the distribution of s values that encompasses this minimum provides an indication of the minimum number of generations required for complete lineage sorting to occur. Coalescent simulations indicate that an approximate minimum of 10 million generations is required (Fig. 5), a value translating into approximately 1.5 million years. In terms of population divergence, coalescent simulations indicate that the 22 populations sampled here diverged approximately 50,000 generations ago (Fig. 5) with no subsequent gene flow, a value translating into a minimum of 7.5 kya.

Discussion

The southernmost glacial advance of the Pleistocene reached Kansas and Missouri in the central United States (Coleman 1941). During these advances conditions near the ice front were similar to those of the present-day arctic tundra, with temperatures south of the ice front significantly reduced (Bryant and Holloway 1985; Coleman 1941; Frye and Leonard 1965). Consequently, the plant community composition in unglaciated areas of the south-central United States likely shifted to cooler-adapted species (Delcourt 2002; Frye and Leonard 1965). Approximately 14 kya a climatic shift toward a warmer climate led to the retreat of the last ice sheet (Bryant and Holloway 1985; Nordt et al. 1994; Ogden 1967; Reid et al. 1970), resulting in the re-establishment of many plant species currently occurring throughout the south-central United States (Geis and Boggess 1968). After the end of the Pleistocene and the cold Younger Dryas (11.5 kya—Gibbard and van Kolfschoten 2004), temperatures warmed and reached a warming maximum during the hypsithermal 9–5 kya (Deevey and Flint 1957).

In the south-central United States Hutchinson and Templeton (1999) detected a significant population expansion for collared lizards (*Crotaphytus collaris*) after the Pleistocene. *C. collaris* occurs in the rocky habitats that *P. attwateri* appears to favor, and a similar pattern in *P. attwateri* supports the hypothesis that many temperate species in the south-central United States tracked the southward retreat of their habitats during the glacial advances of the Pleistocene. Both mismatch distributions and BSP indicate that *P. attwateri* underwent a significant population expansion at the end of the Pleistocene, and timing estimates for this expansion are in agreement for both methods. To understand the magnitude of this expansion the BSP indicates at least an order of magnitude increase in female effective population size with an upper HPD limit in excess of 10^6.

The presence of southern refugia in species that occurred in the Northern Hemisphere during the glacial maximum(s) of the Pleistocene has become a widely accepted concept, supported by an abundance of both fossil and genetic data (Bennett 1997; Hewitt 1996, 1999, 2004). Kilpatrick (1984) hypothesized that *P. attwateri* originated when a *boylii*-like ancestor became isolated during the Pleistocene on the Edwards plateau in southern Texas. This was based on fossils recovered from Schulz Cave, on the Edwards Plateau and originally described by Dalquest et al. (1969). However, more recent systematic treatments of the genus *Peromyscus* indicate that *P. attwateri* is most closely related to *P. nasutus* and *P. difficilis* and may belong in the *truei* species group (Bradley et al. 2007; Durish et al. 2004; Tiemann-Boege et al. 2000).

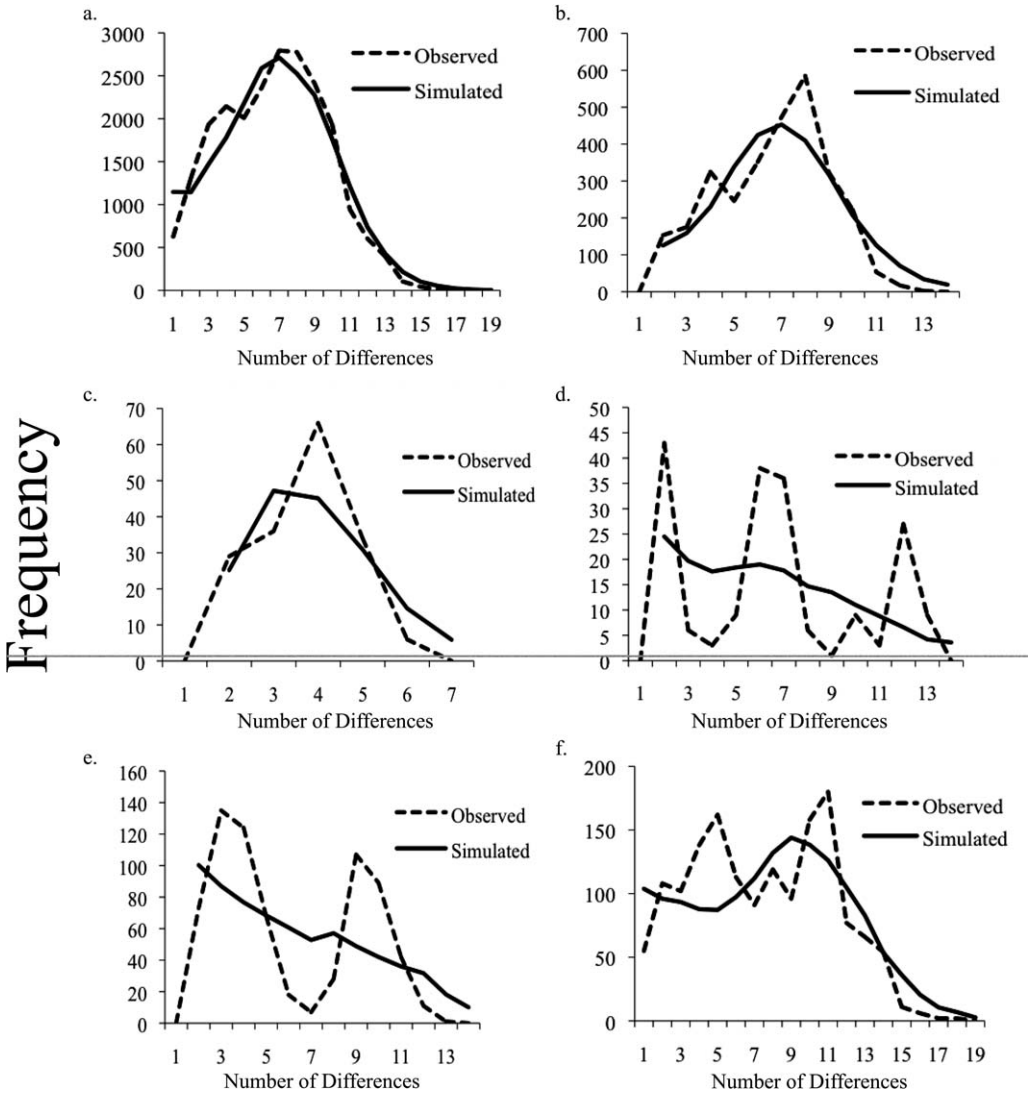

Fig. 4.—Mismatch distributions for Texas mouse, *Peromyscus attwateri*, representing the distribution of pairwise differences for the (a) total population, (b) TX, (c) WQM, (d) AJ, (e) SAR, (f) NAR. Observed distributions are represented by dashed lines and expected distributions under the sudden expansion model are represented by solid lines. See Table 3 for mismatch parameters and goodness-of-fit values.

Table 3.—Mismatch distribution parameters, goodness-of-fit results, and selective neutrality test values for total population and individual regions as defined in Fig. 2. *S*, number of polymorphic sites; θ_0, population size before expansion; θ_1, population size after expansion; τ, the expansion parameter; SSD, sum of squared deviations.

Demographic parameters	Total population	TX	WQM	AJ	SAR	NAR
S	74	31	8	13	21	39
θ_0	0.179	0.003	0	2.766	0.004	0.081
θ_1	18.936	23.338	1122.812	7.509	5.774	13.418
τ	6.728	5.641	2.016	4.832	8.699	9.230
Goodness-of-fit tests						
SSD	0.002	0.008	0.021	0.068	0.033	0.007
PSSD	0.74	0.22	0.05	0.06	0.42	0.75
Ragged.	0.006	0.021	0.096	0.118	0.04	0.011
P	0.94	0.29	0.22	0.15	0.65	0.81
Tests of selective neutrality						
Fu's F_S	-3.4×10^{38}	-3.4×10^{38}	−2.892	1.392	−4.372	−11.867
P	0.014	0.001	0.024	0.761	0.050	0.001
Tajima's *D*	−1.55	−0.837	−0.851	0.968	−0.660	−0.797
P	0.021	0.229	0.194	0.847	0.298	0.227

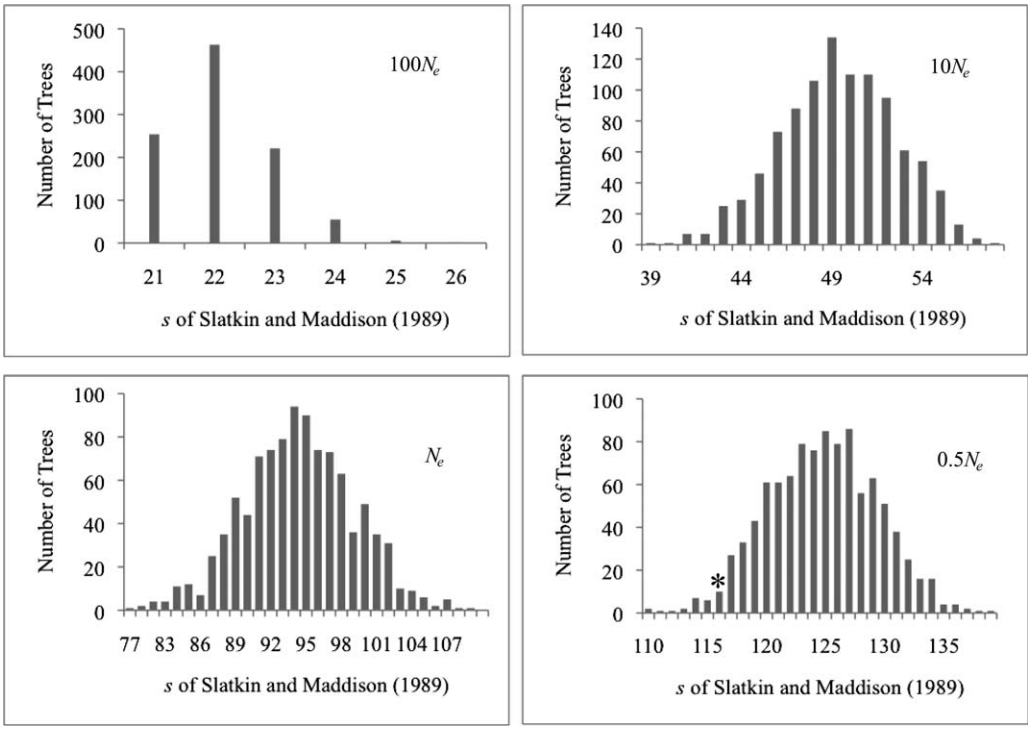

Fig. 5.—Distributions of the gene flow statistic *s* (Slatkin and Maddison 1998) for 1,000 simulated gene trees within 4 population trees. Each distribution corresponds to population trees of varying branch lengths expressed as a function of the effective population size (N_{ef}; shown in each frame). Observed $s = 116$ is indicated by an asterisk and corresponds to the minimum number of gene flow events among the 22 populations.

Because the hypothesis put forth by Kilpatrick (1984) was based on the assumption that *P. attwateri* was a member of the *boylii* species group, the conclusions derived from these fossils warrant reconsideration. The primary character used in distinguishing *P. attwateri* and *P. boylii* fossils is the presence of a mesolophid on m1 occurring at a frequency of 0.99 in *P. attwateri* and 0.02 in *P. boylii* (Schmidly 1973). Kilpatrick (1984) noted that the Schultz cave fossils had a frequency of 0.77 for the m1 mesolophid, aligning them more closely with *P. attwateri*. In addition, Dalquest and Stangl (1983) used this and other characters to identify *Peromyscus* species from lower jaw morphology and found a frequency of 0.68 for the lower m1 mesolophid in *P. difficilis*, a frequency very similar to that of the Schultz Cave *Peromyscus*. Therefore, the exclusion of *P. attwateri* from the *boylii* species group does not preclude the hypothesis set forth by Kilpatrick (1984) that the Edwards Plateau was a likely site of a Pleistocene refugium for an ancestral *P. attwateri* during the Pleistocene. The dental morphology of these fossils is compatible with a *P. attwateri–difficilis–nasutus* ancestor becoming isolated and diverging during the Pleistocene climatic fluctuations. Furthermore, if we apply the range of divergence rates used in this study (7.5–12% per million years) to the mean cytochrome *b* percent divergence Durish et al. (2004) recovered for *P. attwateri* and the *P. difficilis–nasutus* assemblage (8.76%), this places the divergence at approximately 1.17–0.73 mya, during the glacial advances of the middle Pleistocene.

In addition to the initial expansion, mismatch distributions and the BSP indicate a more recent secondary expansion. This is further supported by haplotypes sampled from the NAR, SAR, and AJ regions being most closely related to haplotypes occurring only in TX (Fig. 3). Also, these haplotypes occur at the tips of the haplotype network, indicating that they are recently derived. The hypsithermal might have presented an opportunity for *P. attwateri* to undergo further range expansion and population mixing. Evidence exists for floral shifts during the hypsithermal that significantly affected historical population demographics and contemporary genetic structure of other small mammals (*Glaucomys volans*—Peterson and Stewart 2006; *Sorex cinereus* and *Sorex fumeus*—Sipe and Brown 2004) in the region.

Whereas our results support this prediction, some conflict between mismatch distributions and the BSP remains on the timing of this secondary expansion. Estimations on the basis of mismatch distribution indicate that the secondary expansion likely occurred during the hypsithermal (9–5 kya) of the Holocene. The BSP estimate indicates that the secondary expansion was much more recent, perhaps occurring fewer than 5 kya. One potential explanation for this conflict may be rate variation not accounted for by our strict clock BSP analysis. If evolutionary rates decrease in terminal lineages, a strict clock will underestimate the time required for observed variation to accrue. Although relaxed clock analyses have become much more reliable and straightforward (Drummond et al. 2006) and might have been more appropriate here, a lack of a reliable fossil calibration for *P. attwateri* would make any results obtained from such analyses unreliable. Fortunately, coalescent simulations are not dependent on a molecular clock

or divergence rate and can provide insight into the timing of the secondary expansion. The observed value of s ($s = 116$) occurs in the distribution consistent with 50,000 generations ($0.5N_e$) since populations diverged, with no subsequent gene flow (Fig. 5d). Using the generation time of 0.15, this places the secondary expansion in the middle of the hypsithermal (7.5 kya). The position of the observed s ($s = 116$) in the left half of this frequency distribution suggests that this value also would occur in distributions consistent with more generations since populations diverged. However, even with an upper bound of 70,000 generations ($0.7N_e$), the timing of the second expansion is estimated to be 10.5 kya, a value consistent with the estimate returned by mismatch distributions (6.7–10.7 kya). The agreement between coalescent- and mismatch-based estimates indicates that a model of no gene flow provides a good fit to our data and suggests that changes in climate and habitat during the hypsithermal have limited gene flow among populations of *P. attwateri*.

The low sequence diversity we observed for *P. attwateri* is a pattern congruent with findings reported in previous studies based on protein allozymes (Avise et al. 1974; Kilpatrick 1984; Kilpatrick and Zimmerman 1975; Schnake-Greene et al. 1990). These findings suggest that, although individual populations may be isolated from each other, this isolation has developed relatively recently without sufficient time for divergence to occur. A similar situation was found for *P. polionotus* in Florida, which Avise et al. (1979, 1983) attributed to a relatively recent divergence from ancestral stock, subsequent population expansion, and restricted contemporary gene flow. For *P. attwateri* this pattern is especially apparent in the haplotype network (Fig. 3), where no distinct geographic relationship among haplotypes is revealed. On the basis of morphology, Sugg et al. (1990a) found that populations of *P. attwateri* in Arkansas failed to group geographically, explaining this pattern by population-specific physiologic and ecologic factors molding morphology. Allozymes also indicated an overall lack of divergence among populations, with the majority of diversity occurring within populations and little indication that potential geographic obstacles (i.e., Arkansas River) were limiting gene flow (Sugg et al. 1990b). We suggest the reason for a lack of structure in our study and previous studies is due to insufficient time to allow for distinct population lineages to develop.

Incomplete lineage sorting has been an issue in resolving phylogeographic relationships in a number of species for which recent population divergences are likely (Kulikova et al. 2005; Masta 2000). For *P. attwateri* the maximum number of mutations separating any 2 cytochrome *b* haplotypes is 5, and the 2 most frequent and widespread haplotypes (B and K) are separated by only 1 mutational step. However, the large proportion of significant pairwise ϕ_{ST} (~73%) values suggests that populations are significantly structured. Significant doubts have been raised about the utility of *F*-based statistics in quantifying gene flow (Neigel 2002), so ruling out migration to explain a lack of divergence on the basis of ϕ_{ST} alone may be inappropriate. Our simulations indicate that a minimum of 10^7 generations, or 1.5 million years, would be needed for complete lineage sorting to occur in the absence of gene flow. If even minimal amounts of gene flow were occurring since the establishment of these populations, complete sorting would require even more time. Our conservative estimate of 1.5 million years indicates it is unlikely that populations have had sufficient time for complete lineage sorting to occur. The BEAST analysis estimates the TMRCA for all of *P. attwateri* is approximately 50 kya, during the late Pleistocene. Even if we assume an order of magnitude of error in our estimate (max. = 500 kya), it would fall drastically short of the required time for complete lineage sorting to occur.

In conclusion, contemporary phylogeographic patterns of *P. attwateri* have been affected significantly by recent climatic fluctuations that occurred during and following the Pleistocene. The large number of locally restricted haplotypes, low nucleotide diversity, and an absence of suitable habitats in the intervening landscape suggest that contemporary gene flow among populations of the Texas mouse is infrequent. In spite of this, populations show very little genetic divergence, with most haplotypes differing by only 1 mutational event and an overall lack of geographic structure in the haplotype network. Standard phylogeographic analyses, although adequate in detecting genetic patterns among divergent populations, are incapable of differentiating between gene flow and incomplete lineage sorting to explain genetic patterns among closely related populations. However, the flexible nature of coalescent-based analyses and simulations, although somewhat restricted by their model-based approach, possess the sensitivity to detect and differentiate genealogical processes that have occurred as recently as a few thousand years ago.

Our analyses indicate that the *P. attwateri* ancestral stock was isolated and probably locally restricted during the middle Pleistocene, potentially on the Edwards Plateau of south-central Texas. The implication that glacial advances and climatic fluctuations that occurred during and following the Pleistocene affected the historic distribution and population genetic structure of temperate organisms is certainly not a new concept. Our data indicate that the warm hypsithermal played a significant role in shaping diversity for *P. attwateri*. This suggests that the hypsithermal might have had a significant impact on the historic demographics and distributions of other small mammals that occur in the south-central United States.

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Appendix I

Specimens examined.—The 210 specimens examined are listed below. GMW specimen numbers indicate tissues housed in the University of Central Oklahoma mammal collection. SA specimen numbers indicate tissues housed in the Tarleton State University mammal collection. TK, KMD, and RSP specimen numbers indicate tissues housed in The Museum at Texas Tech University.

ARKANSAS. Perry Co.: Ouachita National Forest; 34°51.48′N, 92°54.54′W; 10 (GMW2686–GMW2694, GMW2696). Pope Co.: Piney Creeks Wildlife Management Area; 35°32.19′N, 92°57.09′W; 10 (GMW2667–GMW2671, GMW2673, GMW2679, GMW2680, GMW2682, GMW2684). Saline Co.: Ouachita National Forest; 34°51.48′N, 92°51.48′W; 10 (GMW2700–GMW2707, GMW2721, GMW2724). Stone Co.: Gunner's Pool, Ozark National Forest; 35°59.69′N, 92°12.79′W; 10 (GMW2830, GMW2833, GMW2834, GMW2836, GMW2838, GMW2839, GMW2841, GMW2843, GMW2847, GMW2848).

OKLAHOMA. Cherokee Co.: 1/4 mi. S, 4 1/4 mi. E Cookson, T14N, R23E, Sec. 11; 35°46.38′N, 94°56.01′W; 10 (GMW2155, GMW2157, GMW2162, GMW2166, GMW2170, GMW2175, GMW2182, GMW2188, GMW2190, GMW2194). Comanche Co.: Fort Sill Military Reservation, T3N, R13W; Sec. 29; 34°41.17′N, 98°51.18′W; 10 (TK116787, TK116789, TK116793, TK116795, TK116801, TK116804, TK116805, TK116807–TK116809). Delaware Co.: Spavinaw Hills Wildlife Management Area; 36°22.24′N, 94°59.61′W; 10 (GMW2629, GMW2630, GMW2634–GMW2636, GMW2639, GMW2640, GMW2644, GMW2647, GMW2648). Greer Co.: Quartz Mountain State Park, T5N, R20W, Sec 22; 34°53.59′N, 99°17.92′W; 9 (GMW2854–GMW2862). Johnston Co.: Tishomingo; 34°18.30′N, 96°38.45′W; 10 (GMW2731, GMW2732, GMW2741, GMW2742, GMW2751, GMW2756, GMW2762, GMW2764, GMW2766, GMW2767). Latimer Co.: Robber's Cave State Park, T6N, R19E, Sec. 7; 34°52.10′N, 95°15.51′W; 10 (GMW2311, GMW2314, GMW2317, GMW2321, GMW2340–GMW2342, GMW2344, GMW2345, GMW2348). Murray Co. Arbuckle Mtns.; 34°28.39′N, 97°00.39′W; 10 (TK116758, TK116761, TK116765, TK116768, TK116769 TK116775–TK116778, TK116780). Osage Co.: Tall Grass Prairie Preserve, 36°49.15′N, 96°25.04′W; 6 (OK6023, OK6026, OK6032, OK6048, OK6053, OK6055). Pushmataha Co.: 6.5 mi. S, 1 mi. W Talihina, T2N, R21E, Sec. 11; 34°24.51′N, 95°21.19′W; 8 (GMW2140, GMW2142, GMW2146–GMW2151).

TEXAS. Coke Co.: 31°41.38′N, 100°35.41′W; 10 (SA1, SA2, SA5, SA7–SA13). Cooke Co.: 33°44.59′N, 97°18.10′W; 8 (TK116786, TK116886, TK116951, TK116967–TK116971). Cottle Co.: 34°04.20′N, 100°16.07′W; 10 (TK116987–TK116996). Erath Co.: 32°16.03′N, 98°13.29′W; 10 (KMD2, KMD13, KMD14, KMD16, KMD19, RSP376, RSP422, RSP432, RSP436, RSP440). Floyd Co.: 34°12.40′N, 101°12.35′W; 10 (TK48240, TK51001, TK51005, TK51006, TK51047–TK51050, TK51092, TK51093). Garza Co.: 33°04.11′N, 101°24.20′W; 10 (TK13633, TK24198, TK24200–TK24202, TK24206, TK24207, TK32204, TK32206, TK32264). Kerr Co.: 30°01.47′N, 99°11.43′W; 10 (TK111584, TK111587, TK111590–TK111594, TK111596, TK111598, TK111599). Kimble Co.: 30°29.31′N, 99°39.32′W; 10 (TK111501–TK111505, TK111512, TK111515, TK111517, TK111518).

MISSOURI. Taney Co.: 36°38.14′N, 93°00.56′W; 10 (TK116865–TK116868, TK116871–TK116875, TK116878).