42. MOLECULAR SYSTEMATICS AND PHYLOGEOGRAPHIC HISTORY OF *THOMOMYS BOTTAE* IN TEXAS

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Abstract

*Thomomys bottae* is a unique faunal element in southwest Texas and deserves conservation priority at the state level. Conservation efforts should be based on empirical data from ecological, genetic, and morphological studies. This paper provides genetic evidence from the mitochondrial cytochrome *b* gene. Our analysis of Texas populations does not identify unique matrilineal populations that could be used to justify metapopulational status. Existing subspecies (*n* = 7) likely share a recent coalescence and/or presently maintain some level of maternal gene flow. This constrasts with the genetic pattern observed in other areas of the species’ range, most notably California and the Baja California Peninsula. Both genetic and biogeographical data indicate that the species entered Texas from the southwest (Trans-Pecos) and not from the South Plains/Texas Panhandle. However, caution should be exercised not to overextend data from a single, uniparental gene, and further studies are necessary to thoroughly resolve the current subspecific taxonomy in Texas.

Keywords:
Pocket gophers (genus *Thomomys*) have interested mammalian systematists and evolutionary biologists for many years because of the extensive morphological, chromosomal, and genetic variation among local populations (Burt 1954, Durrant 1955, Patton and Smith 1989, Patton and Smith 1990, Smith 1998). Clearly, the level of population subdivision within species and subspecies in this genus results from a unique combination of evolutionary events fostering the apportionment of genetic variation into distinct phylogeographic units (Patton and Smith 1989, Patton and Smith 1990, Smith 1998).

The mitochondrial DNA (mtDNA) cytochrome \( b \) gene has been used extensively to elucidate patterns of genetic structure and evolutionary history among numerous taxa including pocket gophers (Smith 1998). This gene has proven particularly useful in delineating species boundaries (Avise and Walker 1999, Baker et al. 1994, Johns and Avise 1998, Irwin et al. 1991, Wright et al. 1999, Tiemann-Boege et al. 2000, Peppers and Bradley 2000, Bradley and Baker 2001) and in revealing distinct “mtDNA phylogroups” within species which are often associated spatially. Therefore, we analyzed the cytochrome \( b \) gene in samples of *Thomomys* collected from southwest Texas, the Chihuahuan Basin of New Mexico, and high elevations from southeast-central New Mexico. Populations from this region have been somewhat underrepresented in previous comprehensive analyses of *T. bottae* from other parts of the current range (Patton and Smith 1990, Smith 1998). Our goal was to estimate genetic variation in these populations of *Thomomys* to determine if distinct maternal lineages exist in this geographic area. Furthermore, we examined the phylogeographic history of specimens from Texas and New Mexico in light of the available cytochrome \( b \) gene data from *Thomomys* (Smith 1998).

Current issues concerning biodiversity and biocomplexity suggest that some populations may harbor unique combinations of genes, and as such, may serve as critical biological metapopulations. Therefore, we wish to address whether or not there are such distinct metapopulations of *Thomomys* in Texas. We emphasize that this study is limited to investigating maternal relationships among *T. bottae* in Texas and between these same populations and the remainder of *T. bottae*. Therefore, we intend to follow the current taxonomy of *Thomomys* proposed by Patton and Smith (1990) and Smith (1998).

**Materials and methods**

**Samples and Locations**

Twenty-five specimens were examined (Table 1). Subspecific designations follow Hall (1981). Nineteen specimens were collected from southwestern Texas, 1 from the Chihuahuan Basin of New Mexico, and 5 were collected from a montane forest (~ 2000 m elev.) in south-central New Mexico. Forty-six samples representing *T. bottae*, *T. townsendii*, and *T. umbrinus*, analyzed in Smith (1998), were obtained from GenBank (National Center for Biotechnology Information, NCBI). The geographic distributions of these 3 species including the sampling localities are presented in Fig. 1 (adapted from Smith [1998]). All DNA sequences generated for this study have been deposited in GenBank according to the following accession numbers: AF445042-AF445064.

**DNA Sequencing**

Genomic DNA was isolated using a cell lysis-protein digestion-organic dissolution procedure sometimes followed by overnight dialysis (Strauss 1994, Longmire et al. 1997). Approximately 200 ng of genomic DNA were used to amplify the mtDNA cytochrome \( b \) gene either completely (primers LGL765 × H15915) or in two segments (LGL765 × H15149 and Tbottt31L [5′-CGT TAG TCT TAC TAC TAC TAC AAA G-3′] × H15915) using the polymerase chain reaction. Sequences for LGL765 are reported in Bickham et al. (1995) and sequences for H15149 and H15915 are reported in Irwin et al. (1991). A final concentration 1.5 U of DNA Taq polymerase (Promega Corp.; Madison WI), 3 mM MgCl\(_2\) (Promega Corp.), 1 X Taq buffer (Promega Corp.), 0.2 mM dNTPs (GeneAmp®, Applied Biosystems [ABI]; Foster City CA), and 0.5 mM each primer (Integrated DNA Technologies; Coralville, IA) were used in each reaction (Q5 with sterilized, distilled H\(_2\)O). The following thermal profile (step cycle format, 32 cycles, PE480 thermal cycler [Perkin-Elmer; Norwalk, CT]) was used for all amplification reactions: 95 °C for 20 seconds, 50 °C for 40
seconds, and 72 °C for 2 minutes. Amplicons were visualized by agarose gel electrophoresis and column purified (QIAGEN Corp.; Valencia CA). Purified amplicons were then cycle-sequenced with the PCR primers and an additional sequencing primer Tbo1664 (5'-YCA YCC ATA YTA CTC AAC YAA AG-3') using the ABI PRISM® dRhodamine or ABI PRISM® BigDye® dye-terminator chemistry following the manufacturer’s recommendations (ABI). DNA sequences were generated using capillary electrophoresis and a fast sequencing protocol on an ABI PRISM® 310 Genetic Analyzer according to the manufacturer’s recommendations (ABI). Chromatograms and sequences were then proofed and aligned using the Sequencher ver. 3.1 software for the Macintosh (Gene Codes Corp.; Ann Arbor, MI).

Analysis

Phylogenetic analyses were performed using the PAUP® ver. 4.0b8 for Windows and PAUP® ver. 4.0b8 for Macintosh software programs (Swofford 1998). Optimality criteria were set to parsimony, maximum likelihood, and distance. The heuristic search algorithm was used in parsimony and maximum likelihood analyses. Dipodomys agilis was designated as the outgroup taxon, and an additional heteromyine (Perognathus longimembris) and two geomyines (Orthogeomys heterodus and Geomys breviceps) were included to polarize the dataset and examine the monophyly of the genus Thomomys (Smith 1998). These 4 DNA sequences were obtained from GenBank and can be found in Smith (1998).

Fig 1. Map (adapted from Smith [1998]) of the southwestern United States and Mexico showing the distributions of Thomomys bottae, T. townsendii, and T. umbrinus. Numbers and dots indicate sampling locales and can be cross-referenced with Smith (1998), Table 1 (this study), and all phylogenetic reconstruction Figs.
Parsimony

Four different character-state weighting schemes were used. The first involved weighting all character-states equally. The second involved weighting transversions 3.4 over transitions. Values used for these two approaches involving character weighting were derived using maximum likelihood parameter estimation of the parsimony reconstruction estimated without any weights. The third involved a codon positional weighting scheme of 2.7:7.7:1. In addition, as the parsimony method is not suited for estimating lineage heterogeneity, estimates assumed homogeneous rates of DNA sequence evolution. The fourth involved the transversion-only parsimony approach used in Smith (1998). All transversions were treated equally and transitions were excluded. In all weighting schemes, the random sequence addition procedure with 10 replicates was applied.

Distance

The Tamura-Nei distance model was used to estimate genetic distances among taxa (Tamura and Nei 1993). This divergence model estimates nucleotide evolution in which there is a biased nucleotide composition, an unequal transition: transversion ratio, and among-site substitution rate heterogeneity. The neighbor-joining method (Saitou and Nei 1987) was used to cluster genetic distances.

Maximum Likelihood

The maximum likelihood analysis involved optimizing model parameter values based on empirical base frequencies and estimates of the transition:transversion ratio (Ts:Tv = 5.12:1) and among-site rate heterogeneity (g shape = 0.237) derived from the unweighted strict consensus parsimony reconstruction. Different evolutionary models were compared statistically using the likelihood ratio test (Huelsenbeck and Crandall 1997). The model that was significantly better (a < 0.05) was used to estimate the likelihood phylogeny. Parsimony and neighbor-joining reconstructions were bootstrapped (1000 replicates) using the fast heuristic and neighbor-joining search algorithms respectively to indicate consistency in clade formation (Felsenstein 1985). All reconstructions were visualized using the program TreeView version 1.6.1 (Page 1996).

Results

Parsimony Analysis

The entire cytochrome b gene was sequenced for the 25 specimens; all had the “AGA” termination codon resulting in 1,140 bp rather than 1,143 (“TGA” codon) as reported for some subspecies of T. bottae (Smith 1998). When all taxa were examined (2 samples were omitted from all final analyses to eliminate redundant haplotypes within subspecies 43 = 38, 53 = 51; Table 1), 545 character-state changes were apparent, with 129 in the first codon position, 48 in second codon position, and 368 in the third codon position. The heuristic search yielded 176 trees, each 2726 steps in length (CI = 0.31, HI = 0.69, RI = 0.7). The strict consensus of the unweighted heuristic search results in 8 groups (clades I-VIII; Fig. 2). Clade I comprises T. talpoides and T. monticola. Specimens of T. bottae from extreme southern California and the Baja Peninsula (clade II, bootstrap value [bs] = 100) are basal to other samples of T. bottae and T. umbrinus (clades III-VIII). Specimens of T. bottae and T. townsendii from central, southcentral, and northeastern California, Nevada, Utah, and Colorado form a group (clade VII, bs = 51). Specimens from northern California form another group (clade VI, bs = 100). Thomomys b. awahnee from north-central California exhibits no clear affinity with other taxa (clade V). Specimens from the southwestern United States (U.S.) and northern Mexico form a group clade VIII (bs = 66). Thomomys umbrinus formed two distinct groups, clade IV (bs = 92) and clade III (bs = 100).

Within clade VIII, which includes taxa that are the primary focus of this study, there are several subdivisions. Specimens from southwestern Texas and the Chihuahuan Basin of New Mexico form a group and appear sister to T. b. analogus from northern Mexico. Specimens from Cladocroft, NM, group with the previously reported specimens of T. b. ruidosae (Smith 1998) and T. b. connectens. This group then forms a sister group with T. bottae from southeastern California, Arizona, southwestern Texas, and the desert basin of New Mexico. Bootstrap values indicated a high level of support (bs = 90) for the samples from southwestern Texas.
and the Chihuahuan Basin of New Mexico (Fig. 2). Affinity of *T. bottae* from northern and south-central New Mexico was supported by a bootstrap value of 66. Samples from northern and south-central New Mexico had high support (bs = 96). The resolution of sister relationships beyond this was not evident.

Additional Character State and Sequence Characterization

Considering only those taxa restricted to *Thomomys*, there were 489 apparent character-state changes, with 101 in the first codon position, 37 in the second codon position, and 351 in the third codon position. The maximum likelihood estimate of the transition:transversion ratio was 6.0:1. Considering only those taxa restricted to southwestern Texas and the Chihuahuan Basin of New Mexico (see on Fig. 2), there were 75 apparent character-state changes, with 14 in the first codon position, 4 in the second codon position, and 57 in the third codon position. The maximum likelihood estimate of the transition:transversion ratio was 13.1:1.

Neighbor-Joining Analysis

Unlike the parsimony analysis, the neighbor-joining analysis of the Tamura-Nei divergence values indicated that samples of *T. bottae* comprised a single group (Fig. 3). However, the level of sequence divergence between *T. umbinus* and *T. bottae* (and specifically *T. bottae* from southwestern California...
Fig. 2. Parsimony reconstruction represented by a strict consensus of 176 trees each 2,726 steps in length. Numbers in parentheses correspond to locations on Fig. 1. The terminal taxa labeled with “TAG” are those with a cytochrome b gene 1,143 bp in length terminating in “TAG” while the remainder have a cytochrome b gene 1,140 bp in length. *Thomomys bottae* (*T*. b. ssp.), *Thomomys townsendii* (*T*. t. ssp.).

and the Baja Peninsula) is relatively small (0.02%). Relationships within *T. bottae* and *T. umbrinus* were essentially identical to those in the parsimony analysis.

Average pairwise genetic divergence values (Tamura-Nei) suggest extensive genetic variation within *Thomomys* (mean=13.9%, standard error(SE) = 0.1%) and the species *T. bottae* (11.9%, SE=0.1%) and *T. umbrinus* (12.5%, SE=1.4%). Clearly, a substantial amount of the genetic variation can be attributed to within species comparisons. Conversely, a relatively small amount of sequence divergence (1.3%, SE = 0.04%) was observed in average pairwise comparisons of those taxa from
southwestern Texas and the Chihuahuan Basin of New Mexico. Fig. 4 represents subdivisions in the cytochrome \( b \) gene exhibiting \( \geq 12\% \) sequence divergence across the range of \textit{Thomomys}. Johns and Avis (1998) and Bradley and Baker (2001) suggested this level of sequence divergence in the cytochrome \( b \) gene reflects a level observed between congeneric species of mammals.

**Maximum Likelihood Analysis**

The maximum likelihood (ML) analysis (Fig. 5) recovered a topology (-lnL = 13116) similar to that of the maximum parsimony analysis (Fig. 2). The HKY85 + g model of molecular evolution was used because statistical comparisons with other models indicated this variant was significantly better \( p < \)
Fig. 4. Genetic divergence isoclines based on the cytochrome *b* gene. Each cluster represents a genetic divergence of $\geq 12.0\%$ (Tamura-Nei) such that independent clusters are at least 12.0% divergent from one another.

Table 2. Results of tests of maximum likelihood models using the data from the unweighted strict consensus maximum parsimony phylogeny of *Thomomys*. Presented are the maximum likelihood models, $-\ln$ likelihood values, and parameters estimated in each model. As noted by the asterisk, the HKY85+g model yielded a statistically significantly better value maximizing the likelihood function, therefore this model was used to estimate the likelihood phylogeny.

<table>
<thead>
<tr>
<th>Model</th>
<th>$-\lnL$</th>
<th>Base Frequencies</th>
<th>TS/TV*</th>
<th>Among-Site Variation</th>
<th>Rate</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC69</td>
<td>16332</td>
<td>Equal</td>
<td>N/A</td>
<td>Equal</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>F81</td>
<td>16106</td>
<td>Equal</td>
<td>N/A</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>JC69+g</td>
<td>14701</td>
<td>Equal</td>
<td>N/A</td>
<td>Heterogenous</td>
<td>0.269</td>
<td>0.266</td>
</tr>
<tr>
<td>F81+g</td>
<td>14404</td>
<td>Equal</td>
<td>N/A</td>
<td>Heterogenous</td>
<td>0.266</td>
<td>0.266</td>
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<tr>
<td>K80</td>
<td>15277</td>
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<td>3.32</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>HKY85</td>
<td>15003</td>
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<td>N/A</td>
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<tr>
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</tr>
<tr>
<td>HKY85+g</td>
<td>13177*</td>
<td>Empirical</td>
<td>5.12</td>
<td>Heterogenous</td>
<td>0.237</td>
<td>0.237</td>
</tr>
</tbody>
</table>

*TS = transition substitution, TV = transversion substitution

empirical base frequencies ($A = 0.297$ $G = 0.248$ $G = 0.133$ $T = 0.321$)

significantly better model of molecular evolution than all others ($p < 0.05$)
0.05) at maximizing the likelihood function explaining the phylogenetic affinities of geomyoid taxa (Table 2). This model allows for unequal numbers of transition and transversion substitutions and among-site rate variation based on empirical base frequencies. Clades 1-4 denote distinct lineages and correspond to clades II, VI, VII, and VIII, respectively, on the parsimony phylogeny (Fig. 2). Clade 1, comprising samples of *T. bottae* from southwestern California and the Baja peninsula, are basal to the remainder of *T. umbrinus* and *T. bottae*. Clade 2 comprises *T. bottae* from northern California and clade 3 comprises *T. bottae* and *T. townsendii* from central California, Nevada, Utah, and Idaho. Clade 4 can be subdivided into 2 distinct groups, “a” comprised of samples from northern and southeastern New Mexico and “b” comprised of samples from southwestern Texas, the Chihuahuan Basin of New Mexico, northern Coahuila, Mexico, Arizona, and southeastern California.
Discussion

Origin of Genetic Variation in Thomomys bottae from Southwestern Texas and the Chihuahuan Basin of New Mexico

These data indicate the populations of Thomomys investigated in this region to date belong to T. bottae and do not ally with T. umbrinus. Both distance and maximum likelihood analyses indicate relatively little genetic divergence or molecular evolution among these taxa. We conclude that this geographic region harbors a natural assemblage of T. bottae (Figs. 3 and 4) whose closest relatives appear to be subspecies of T. bottae from Coahuila, Mexico, (3.7% ± 0.07% divergent) followed by T. bottae from southwestern New Mexico and southeastern Arizona (4.6% ± 0.05% divergent), and northern and southwestern Arizona and southeastern California (6.8% ± 0.06% divergent). A level of 1.3% ± 0.04% sequence divergence was found within the specimens from southwestern Texas and the Chihuahuan Basin of New Mexico. The low level of intersubspecific genetic divergence seen within these subspecies, which comprise 7 subspecies, contrasts with the relatively high levels of sequence divergence that distinguish most subspecies from the remaining range of T. bottae (Smith 1998). The neighbor-joining phylogram (Fig. 3) presents the levels of genetic divergence between subspecies of T. bottae. There are apparent cytochrome b haplotypes that are shared among some of the subspecies from Texas (T. b. limitaris, T. b. confinalis, and T. b. pervarus) sampled in this study. If the phylogenetic relationships depicted in this study are accurate, one explanation for these observations is that there is maternal gene flow between these subspecies. Alternatively, these polyphyletic relationships may be due to the retention of ancestral polymorphisms and/or lineage sorting.

The genetic data indicate that the samples of Thomomys from Texas are closely related. These populations were assigned to 7 subspecies based on morphological variation (Hall 1981). The most morphologically distinct subspecies (T. b. baileyi) probably is extinct. Two authors on this paper (Baker and Schmidly) have independently searched the documented range (Sierra Blanca) for specimens of T. b. baileyi. The only geomyid observed during these expeditions have been specimens of the genus Cratogeomys. Because no cytochrome b gene data exists for T. b. baileyi, our analyses cannot contribute to an understanding of this taxon’s genetic affinity. As for the remaining subspecific taxonomy of the populations sampled in southwestern Texas, our genetic data can be used to better understand the relationships between populations and subspecies. We think that subspecific names should be applied to evolutionary units and that these evolutionary units should have a genetic basis (Durrant 1955, Patton and Smith 1990). There are both advantages and disadvantages to drawing phylogenetic inferences from the mitochondrial genome. The advantages include maternal capture/reticulation and/or lineage sorting that may complicate any simplistic subspecific interpretation (Avise 1994). These problems, though difficult to fully substantiate, are often evident when assessing the relationships among taxa which coalesce in a recent history. However, assuming these disadvantageous properties are negligible (the null hypothesis), mtDNA can provide one measure of time since divergence for assessing evolutionary relationships. Concerning the subspecies of Thomomys recognized in Texas, the cytochrome b data indicate that these subspecies are relatively young as compared to many of the subspecies recognized in the remainder of the distribution of the T. bottae complex. However, care should be taken not to overextend these data. Additional genetic markers and more refined morphological analyses may not support the conclusions drawn from the cytochrome b gene. Ideally, subspecific taxonomy should be based on a synthesis of multiple, independent datasets (Thaeler 1968, Patton and Smith 1990). We interpret our data from this gene as indicating a need for a review of the recognized subspecies from southwestern Texas that we have sampled (Wilson and Brown 1952, Durrant 1955). However, the lack of any substantial matrilineal clusters in Texas suggests no particularly unique evolutionary units exist within T. bottae. However, T. bottae is a unique faunal element in southwestern Texas and the species’ presence in the region should be monitored.

Divergence and radiation of Thomomys bottae in southwestern Texas and the Chihuahuan Basin of New Mexico

The low level of genetic differentiation among specimens from southwestern Texas and the Chihuahuan Basin of New Mexico, suggests a
relatively recent and widespread radiation of this assemblage. Assuming a relatively uniform rate of molecular evolution, which is plausible for such low divergence estimates, the recent divergence and temporal duration of the radiation can be estimated using the value of 2.5% sequence divergence per million years (my) for *Thomomys* (Smith 1998). Based on average sequence divergence values, we estimate that *T. b. analogous* (Coahuila, Mexico) diverged from a common ancestor of specimens within Texas and southcentral New Mexico during the Pleistocene, approximately 1.5 million years ago (mya). Our data further suggest the temporal duration of the putative radiation of the assemblage from southwestern Texas and southcentral New Mexico has spanned about 0.5 my. If one uses an evolutionary rate of 3.5%/my for cytochrome b as suggested by Arbogast and Slowinski (1998) and Arbogast (1999), the divergence of the specimens from southwestern Texas and the Chihuahuan Basin of New Mexico and a common ancestor with *T. b. analogous* has been even more recent (1.1 mya) and the radiation even more rapid (0.4 my). Considering the extent of the geographic range encompassed by *Thomomys* in southwestern Texas and southcentral New Mexico and the low level of sequence divergence among these specimens, this radiation event has been relatively widespread and rapid.

Russell’s (1968) review of the Geomyinae remains the most concise and comprehensive assessment to date on the origin and early history of the modern genera of gophers. Russell logically deduced that the two modern lineages of geomyid rodents—the Thomomynini (*Thomomys*), and Geomyini (*Geomys, Zygogeomys, Orthogeomys, Pappogeomys, Cratogeomys*)—must have diverged from one another at some point during the mid- to early Pleistocene and that the Thomomynini did not experience the Blancan (late Pliocene/early Pleistocene) adaptive radiation undergone by the Geomyini (see Russell [1968]: Fig. 3, p. 544). The ancestral (early Pleistocene) home ranges of geomyid genera appear to have been allopatric or parapatric and the range of *Thomomys* occupied most of the mountainous western half of the present day United States (see Russell [1968]: Fig. 2, p. 491). This historical distribution is very similar to that occupied by *Thomomys* at present.

There is a notable paucity of chronological records since the first appearance of *Thomomys* in the fossil record of the earliest Pleistocene ca. 1.5-1.7 mya (Russell, 1968; Dalquest and Schultz, 1992; Dalquest and Grimes, 1992) and of records from the subsequent gap leading to the well documented period of the latest Pleistocene (Wisconsinan, about 40,000 years ago [ya]; the limits of carbon-dating techniques). Further contributing to the scarcity of records from intervening Pleistocene times is the fact that the approximately 20 successive glacial advances were capable to varying degrees of both displacing *Thomomys* to the south and west and of obliterating much of the fossil evidence from earlier glacial/interglacial events. A notable exception is the 610,000 ya record for *T. bottae* immediately under the layer of potassium/argon-dated Lava Creek B (Type O Pearlette) volcanic ash from Southern Plains sites in southern Kansas and the Texas Panhandle. This area was well beyond the destructive reach of the maximum Wisconsinan glacial advance (Dalquest and Schultz, 1992). Other notable extralimital South Plains records for the species are from the latest Pleistocene (Dalquest and Schultz, 1992).

*Thomomys bottae* remains reported from the late Quaternary (latest Pleistocene through Holocene) faunas are common in the southwestern states of Texas, Oklahoma, and New Mexico (Graham 1987, Dalquest et al. 1969, Dalquest et al. 1990, Dalquest and Schultz, 1992, Stangl et al. 1994 and references cited therein). These and the extralimital Southern Plains records of north Texas from near the terminus of the Pleistocene (Lubbock Lake, 11,000 ya; and Hardeman County, 16,000 ya) led Dalquest and Schultz (1992:120) to postulate that Pleistocene populations of *T. bottae* “probably extended from the populations in southeastern Colorado and northwestern New Mexico southeastward through the Texas Panhandle to the Edwards Plateau.”

Notable changes of modern generic distributions from Russell’s (1968) proposed generic home ranges of geomyids from the early Pleistocene are the southern retreat of *Zygogeomys* into central Mexico and the filling of the void in present-day New Mexico and Texas by both a southwesterly expansion by *Thomomys* and a northerly expansion by *Cratogeomys* (= *Pappogeomys* of Wilson and Reeder 1993) from Mexico. Regional geology dictates that Texas populations of *Thomomys* must have originated from the West by one or both of two possible routes: 1) a northerly route via Kansas and
adjacent Oklahoma Panhandle (and perhaps also through the Texas Panhandle, via the Canadian River valley); and 2) a more southerly route, from the Trans-Pecos, eastward onto the Edwards Plateau. The first scenario is not supported by the fossil data, for Dalquest and Schultz (1992) provide no more southerly Pleistocene localities of Thomomys in Texas than Lubbock and Hardeman counties, suggesting this gopher never extended much farther south in north Texas than along the Caprock or along eroded river badlands that characterize those sites. The second scenario seems more plausible despite the fact that the Pecos River, a tributary of the Rio Grande, may have presented a historical barrier to dispersal (Jones and Parish 2001). Periods of glacial meltwaters from montane glaciers in New Mexico and Colorado undoubtedly contributed to a greater effectiveness of the Pecos as a partial barrier to the dispersal of small mammals, such as gophers. Hollander et al. (1990) detailed the varying degree of effectiveness of the upper Pecos River Valley in Texas as compared to the more deeply entrenched lower valley confluent with the Rio Grande. Thomomys likely took advantage of an interglacial opportunity to cross the Pecos river, eastward into southcentral Texas. Both molecular and cytogenetic data support this historical pattern of dispersal and the present distribution of Thomomys (Patton and Smith 1990).

In spite of the lack of information from the greatest portion of the Pleistocene, there is no reason to suspect that past distributions of T. bottae have not been subjected to the ebbs and flows that characterize its well documented post-Wisconsinan past. Best (1973) detailed the habitat segregation/competitive exclusion of Thomomys, Cratogeomys, and Geomys in northeastern New Mexico. Past and present records indicate that Thomomys first expanded its range into at least part of the void vacated by Zygogeomys, and that the more recent invader, Cratogeomys, has supplanted (and is continuing to supplant) Thomomys in parts of its southwestern range in Texas, Oklahoma, and New Mexico. The fossil record indicates that Thomomys was widely distributed in the Trans-Pecos desert mountain ranges during the Pleistocene followed by a late Pleistocene-to-present decline. This decline also appears coincident with immigrating Cratogeomys castanops. For example, Williams and Baker (1976) documented the displacement of Thomomys by Cratogeomys in the Davis Mountains. Thomomys was the common gopher in the Pleistocene sediments of Fowlkes Cave, but Cratogeomys dominated the Holocene sediments. Presently, Thomomys is rarely taken in the vicinity of this Apache Mountains cave (Stangl et al. 1994). Those authors also reported the apparent extirpation of Thomomys in Guadalupe Pass within a few years following their first capture of Cratogeomys. Pleistocene and Holocene sediments of northeastern New Mexico and the Oklahoma Panhandle contain only Thomomys, but Cratogeomys is the only gopher occurring in those regions today (Dalquest et al. 1990).

**Implications of Mitochondrial and Diparental Genes to Speciation in Thomomys bottae**

Speciation in pocket gophers is complex and difficult to quantify (Patton and Smith 1990). Implementation of a specific species concept is difficult due to population dynamics, consequences of the fossorial lifestyle, and discordance among datasets (morphology, mtDNA, karyology, allozymes; Thaeler 1968, Patton and Smith 1990, Smith 1998). Typically, 3 species concepts are considered in resolving the number and boundaries within a given complex. First, the Biological Species Concept (BSC; Mayr 1963), in which there is isolation and integrity of respective gene pools, can only be accurately evaluated in situations involving sympatry and is rarely if ever applicable to pocket gophers. Second, the Phylogenetic Species Concept (Cracraft 1983) employs monophyly to delineate species. Third, the Genetic Species Concept, as reevaluated by Bradley and Baker (2001), suggests that levels of nucleotide divergence (likely > 8% and assuredly > 13%) in the cytochrome b gene are concordant with species boundaries in mammals. Below, we employ a combination of the PSC and the GSC to interpret our data and classify the taxa new to this study.

**Mitochondrial (cytochrome b) relationships**

An overview of all of the phylogenetic reconstructions and divergence values indicates several major subdivisions in T. bottae as currently recognized (Smith 1998). In Fig. 5, clades 1, 2, and 3 represent discrete subdivisions within T. bottae. Clade 4 represents another major subdivision relative to our
Facilitate comparison of the mtDNA cytochrome b genetically similar population samples over the geographic range of the species. This figure is included in this paper to facilitate comparison of the mtDNA cytochrome b genetic patterns with those estimated from the nuclear genome. This legend and figure were reprinted with permission from the University of California Press, and we wish to acknowledge Patton and Smith (1990) and the Regents of the University of California for their assistance.

Fig. 6. Geographic relationships of the genetic groups of *Thomomys bottae* defined by the phenogram in Fig. 3.1 (Patton and Smith 1990, p. 15). Contours of genetic distance at the $D_r = 0.15$ and $0.25$ levels serve to link hierarchically genetically similar population samples over the geographic range of the species. This figure is included in this paper to facilitate comparison of the mtDNA cytochrome b genetic patterns with those estimated from the nuclear genome. This legend and figure were reprinted with permission from the University of California Press, and we wish to acknowledge Patton and Smith (1990) and the Regents of the University of California for their assistance.

The lineage most likely to represent specific distinctness from the remainder of *T. bottae* is clade 1 (Fig. 5). This clade is basal to a clade comprising samples of *T. umbrinus* and the remainder of *T. bottae*. To make *T. bottae* a monophyletic assemblage would require the inclusion of *T. umbrinus* within *T. bottae*. Sufficient data exists documenting the isolation of the gene pools of *T. umbrinus* and *T. bottae* (Patton et al. 1972, Patton 1973). Therefore, to retain the monophyly of *T. bottae* and the specific status of *T. umbrinus*, *T. bottae* would need to be divided into 2 species. There are reasons to scrutinize the observed phylogenies in Figs. 2 and 5. One reason is potential homoplasy caused by saturation of 3rd position codon changes. Another reason, which may not be independent of the first, is nucleotide transition saturation. Although it was not specifically stated in Smith (1998), we suspect that this is the reason tranversion-only and weighted transversion analyses were performed in that study. However, the monophyly of *T. bottae* based on mtDNA was only supported in the maximum likelihood analysis of thomomyine taxa in Smith (1998). As-
suming *T. bottae* is monophyletic, the magnitude of molecular divergence suggested by the cytochrome b gene (Fig. 4) reflects a level often observed between congeneric species (Johns and Avise, 1998; Bradley and Baker, 2001). For that reason, the continued evaluation of specific distinction of these populations of *T. bottae* is warranted.

Clade 4a comprises samples collected from relatively high elevations north of Ruidoso, NM, to just south of Cloudcroft, NM. Clade 4b includes samples representing both lowland and mountain top forms from southwestern Texas and the Chihuahuan Basin of New Mexico. These two clades represent systematic hypotheses that should receive further study. For example, possible contact zones should be characterized to determine potential genetic isolation (Thaler 1968, Smith and Patton 1980, Patton and Smith 1981). The relationships suggested by clade 4b indicate that *T. bottae* from southwestern Texas and the Chihuahuan Basin of New Mexico share a common ancestor with subspecies from Coahuila, Mexico, Arizona, and southeastern California.

**Mitochondrial and Nuclear Genetic Relationships**

Figs. 4 and 6 represent maternal and diparental genetic relationships respectively. A comparison of these 2 figures indicates congruence between both patterns. For example, the pattern of genetic relatedness among these two independent datasets indicates that *T. bottae* in southwestern Texas and the remaining desert southwest region of the United States extending to southwestern California, form a cohesive unit exclusive of the remainder of the species. Furthermore, concordant genetic relationships also can be observed in central and northern California. This suggests that mtDNA has a high probability of providing a yardstick for measuring the tempo and duration of the evolutionary processes governing the taxa in the desert southwest U. S. and the taxa in central and northern California. In addition, this congruence suggests that these groups represent distinct evolutionary units.

A notable incongruence is observed between the patterns shown in Figs. 4 and 6. MtDNA data suggest *T. bottae* from southwestern California and the Baja Peninsula share a common maternal history relative to the remainder of *T. bottae*. Protein data suggest that *T. bottae* from southern California are more closely related to those populations from the desert Southwest and that *T. bottae* from the Baja Peninsula is distinct.

Processes which account for both congruencies and incongruencies in pocket gophers have been outlined and discussed (Patton and Smith 1996). These processes are complex and provide stimulating possibilities for testing hypotheses concerning evolutionary relationships and processes particularly in *T. bottae* and mammals in general. Specifically, we do not see evidence to designate any population/subspecies among *T. bottae* in Texas to metapopulation status. At this time, we propose no changes to the taxonomy of *T. bottae* in Texas. We do suggest that additional research be conducted on this species to fully characterize the genetic affiliations among subspecies and to monitor the dynamics of the populations currently documented in southwestern Texas.

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**Literature cited**


