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Author(s): Darin M. Bell, Meredith J. Hamilton, Cody W. Edwards, Lara E. Wiggins, Raul Muñiz Martínez, Richard E. Strauss, Robert D. Bradley and Robert J. Baker

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PATTERNS OF KARYOTYPIC MEGAEVOLUTION IN *REITHRODONTOMYS*: EVIDENCE FROM A CYTOCHROME-*b* PHYLOGENETIC HYPOTHESIS

DARIN M. BELL, MEREDITH J. HAMILTON, CODY W. EDWARDS, LARA E. WIGGINS, RAUL MUÑIZ MARTÍNEZ, RICHARD E. STRAUSS, ROBERT D. BRADLEY,* AND ROBERT J. BAKER

Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409 (DMB, CWE, LEW, RES, RDB, RJB)
Department of Zoology, Oklahoma State University, Stillwater, OK 74078 (MJH)
Centro Interdisciplinario de Investigacion Para el Desarrollo Intregal Regional Unidad Durango, Instituto Politecnico Nacional, Vicente Guerrero, Durango 34980, Mexico (RMM)
Museum of Texas Tech University, Lubbock, TX 79409 (RDB, RJB)

Phylogenetic relationships among 7 species of *Reithrodontomys* were examined using the complete mitochondrial cytochrome-*b* gene. DNA-sequence data were analyzed using parsimony (weighted and unweighted) and genetic distance methods. In all analyses, *R. sumichrasti, R. megalotis,* and *R. zacatecae* formed a monophyletic clade. Likewise, *R. montanus* and *R. raviventris* were depicted as sister taxa in all analyses. Our study supports recognition of *R. zacatecae* as a species distinct from *R. megalotis* and recognition of *R. raviventris* as distinct from *R. montanus.* Patterns of chromosomal evolution were examined using differential staining as resolved by 3 DNA probes and fluorescent in situ hybridization. Using the phylogeny based on DNA sequences, we concluded that there has been extensive chromosomal repatterning (in most cases karyotypic megaevolution) in the evolution of *R. zacatecae, R. megalotis, R. sumichrasti, R. montanus, R. raviventris,* and *R. humulis.*

Key words: chromosomal evolution, DNA sequences, in situ hybridization, karyotypic megaevolution, *Reithrodontomys*

Karyotypic megaevolution was 1st described (Baker and Bickham 1980) based on cladistic analyses of a number of congeneric species that differed drastically in rates and types of chromosomal change. The genus *Reithrodontomys* provides 1 of the best examples of karyotypic megaevolution (Baker and Bickham 1980). For example, *R. fulvescens* has experienced chromosomal stasis, whereas *R. montanus, R. megalotis, R. zacatecae, R. humulis, R. sumichrasti,* and *R. raviventris* have experienced extensive chromosomal repatterning of euchromatic elements as evidenced by G-bands. To better understand this biological phenomenon, we examined the magnitude of mitochondrial DNA evolution, variation in restriction-site definition of heterochromatic repeats, and the chromosomal location of telomeric, ribosomal, and heterochromatic repeats using fluorescent in situ hybridization. Specifically, we examined 7 species of *Reithrodontomys* (*fulvescens, humulis, megalotis, montanus, sumichrasti, raviventris,* and *zacatecae*) representing 4 of the 6 species groups proposed by Hooper (1952). Our goals were to determine phylogenetic relationships, using DNA-sequence variation in the cytochrome-*b* gene, in the context of Hooper's (1952) species groups; to compare relationships generated by molecular data to those

* *Correspondent: rbradley@ttacs.ttu.edu*

developed from morphology, karyology, and allozymes; and to evaluate relationships between DNA-sequence divergence and karyotypic megaevolution.

Materials and Methods

Samples.—Seven species of *Reithrodontomys* were examined in this study: *R. fulvescens* (Oklahoma), *R. humulis* (Oklahoma), *R. megalotis* (Texas), *R. montanus* (Texas), *R. raviventris* (California), *R. sumichrasti* (Mexico), and *R. zacatecae* (Mexico). All species in this study belong to the subgenus *Reithrodontomys.* Two individuals of *R. megalotis, R. raviventris,* and *R. zacatecae* and a single individual for each of the remaining taxa were examined. Specimens were from natural populations or borrowed from the Natural Sciences Research Laboratory, Museum of Texas Tech University (Appendix I). Samples were karyotyped in the laboratory or under field conditions following Baker and Qumsiyeh (1988).

Fluorescent in situ hybridization.—For fluorescent in situ hybridization studies, only *R. megalotis, R. montanus,* and *R. zacatecae* were examined. Probes were labeled by standard nick-translation with biotinylated deoxyuridine triphosphate (dUTP) using the Clontech Biotin-21-dUTP nick translation kit following the manufacturer's instructions (Clontech, Palo Alto, California). Hybridization procedures followed those of Hamilton et al. (1990). Signal detection and amplification utilized avidin-conjugated fluorescein isothiocyanate (Vector Laboratories, Burlingame, California) alternated with biotinylated anti-avidin. Counterstaining was accomplished with propidium iodide and diamidino-2-phenylindole. At least 10 complete spreads/individual were analyzed using an Olympus Epi-fluorescent microscope (Olympus Optical Company Ltd., Tokyo, Japan) with a dual band pass filter allowing the simultaneous visualization of propidium iodide and fluorescein isothiocyanate. Microphotographs were recorded on Kodak Royal Gold 1000 color print film (Eastman Kodak, Rochester, New York) and are archived at Texas Tech University.

DNA sequencing.—Mitochondrial DNA was isolated from about 0.1 g of muscle or liver tissue following methods modified from the Wizard Miniprep DNA Purification System (Promega, Madison, Wisconsin). The mitochondrial cytochrome-*b* gene was amplified by polymerase chain reaction (Saiki et al. 1988). The primers MVZ05 and H15915 (Irwin et al. 1991; Smith and Patton 1991), located in the tRNAs (glutamic acid and threonine), were used to amplify the cytochrome-*b* gene in its entirety. Polymerase chain reaction amplifications were conducted using 0.25 µl *Taq* DNA polymerase (Fisher Scientific, Pittsburgh, Pennsylvania), 2.5 µl 10× polymerase chain reaction buffer, 2 µl $MgCl_2$ (25 mM), and 4.0 µl deoxynucleoside triphosphate (2.5 mM). Reaction conditions included denaturation for 1 min at 95°C, annealing for 1 min at 50°C, and extension for 2 min at 72°C for 27 cycles, followed by 1 extension cycle for 7 min at 72°C.

Most polymerase chain reaction products were ligated and cloned using Bluescript plasmids (Stratagene™, La Jolla, California) modified with a T-nucleotide overhang. Double-stranded DNA samples were sequenced with the dideoxy-chain-termination method (Sanger et al. 1977) and following sequencing primers: MVZ05 and H15915 reported by Smith and Patton (1991) and Irwin et al. (1991), and internal primers (L15162 and H15149—Irwin et al. 1991). At least 2 clones were sequenced for each individual. Other polymerase chain reaction products were sequenced using dye-labeled terminators and automated methods following manufacturer's instructions (PE Applied Biosystems, Foster City, California). Nucleotide sequences of both strands were obtained using 6 primers: MVZ05, 400F (Edwards et al., in press), 400R and 700L (Peppers and Bradley 2000), 752R (GCAGGAGTGTAATTAT CGGGGTCTC), and P3′ (Tiemann-Boege et al. 2000). About 60–80 ng of DNA were used with the following cycle sequencing conditions: 25 cycles of 96°C (30 s) denaturing, 50°C (20 s) annealing, and 60°C (4 min) extension temperature. Polymerase chain reaction products were then ethanol precipitated and analyzed with an ABI Prism 310 Genetic Analyzer (Perkin Elmer, Foster City, California). All sequences are deposited in GenBank (accession numbers: AF176248–AF176252, AF176254–AF176258).

Data analyses.—Nucleotide sequences were aligned with MacVector 5.0.2 (Oxford Molecular Group, La Jolla, California). Sequences were treated as discrete, nonordered characters, and the software program PAUP* (Swofford 1999) was used to reconstruct putative phylogenetic re-

TABLE 1.—Comparison of fluorescent in situ hybridization patterns for *Reithrodontomys megalotis, R. montanus,* and *R. zacatecae* using 3 different probes, pMeg-1 = heterochromatic probe, $(TTAGGG)_7$ = telomere repeat probe, and LINE-1 = transposable element probe.

Probe	Taxon		
	R. megalotis	*R. montanus*	*R. zacatecae*
pMeg1	Centromeric and heterochromatic short arms	Centromeric and heterochromatic short arms	Centromeric and heterochromatic short arms
$(TTAGGG)_7$	3 interstitial sites	Telomeres only	Telomeres only
LINE-1	Interspersed in euchromatin and some centromeres	Interspersed in euchromatin and some centromeres	Interspersed in euchromatin and some centromeres

lationships among taxa. *Peromyscus leucopus* and *Osgoodomys banderanus* (GenBank AF131926 and AF155383—Tiemann-Boege et al. 2000) were used as outgroup taxa in all analyses.

Two weighting schemes were used to investigate effects of transition versus transversion substitutions. These included equal weighting of transitions and transversions and downweighting (of transitions by a factor of 4.8) the transition: transversion ratio calculated from our data. Nucleotide positions (1st, 2nd, and 3rd) were weighted using the ratio of 3.7:28.7:1 (the empirical ratio). In all analyses, uninformative characters were excluded. The heuristic search option estimated the most parsimonious tree(s). Support for individual clades was calculated using bootstrap (Felsenstein 1985) and Bremer decay indices (Bremer 1994) using PAUP* and Autodecay (Eriksson 1997).

Genetic distances calculated using the Tamura–Nei (Tamura and Nei 1993) model of evolution were used to construct neighbor-joining trees (Saitou and Nei 1987). The gamma version for the Tamura and Nei model (gamma = 0.154) also was used to assess the effect of among-site rate variation on the tree topology.

Maximum-likelihood analyses included the estimation of parameters (transition: transversion ratios and gamma-shape parameters) for the HKY85-Γ model of evolution (Hasegawa et al. 1985). Model parameters estimated for the equally weighted parsimony tree were used for subsequent maximum-likelihood searches following Sullivan et al. (1997). Analyses employed empirical base compositional biases, 10 random input orders, and tree-bisection-reconnection branch swapping.

The Kishino–Hasegawa (Kishino and Hasegawa 1989) test was used to test for significant differences among tree topologies. That test was used to examine topologies generated by equal weighting, downweighting of transitions by a factor of 4.8, positional weighting (3.74:28.7:1), genetic distance, and likelihood analyses.

RESULTS

Fluorescent in situ hybridization.—Fluorescent in situ hybridization was performed with 3 probes to visualize distinct repetitive elements of the genome (Table 1). Those included a centromeric heterochromatin probe (pMeg-1), a transposable element probe (LINE-1), and a telomeric repeat probe $(TTAGGG)_7$. In *R. zacatecae* and *R. megalotis,* the pMeg-1 probe hybridized to centromeric regions and large blocks on the heterochromatic short arms in a pattern similar to that described for *R. megalotis* and *R. montanus* (Hamilton et al. 1990). Hybridization of LINE-1 (probe Man106, GenBank U70930—Casavant et al. 1996) to chromosomes of *R. zacatecae* and *R. megalotis* revealed banding patterns typical of LINE-1 hybridization in peromyscine rodents (Baker and Kass 1994; Casavant et al. 1996) with hybridization along the lengths of chromosomes, alternating regions of greater and lesser intensity, and no hybridization to heterochromatic regions. Patterns generated with those probes (pMeg-1 and LINE-1) revealed no differences that distinguished *R. zacatecae* and *R. megalotis* from each other. Hybridization with $(TTAGGG)_7$ revealed 2 distinct patterns, 1 restricted to the telomeric regions of all chromosomes in *R. zacatecae* and *R.*

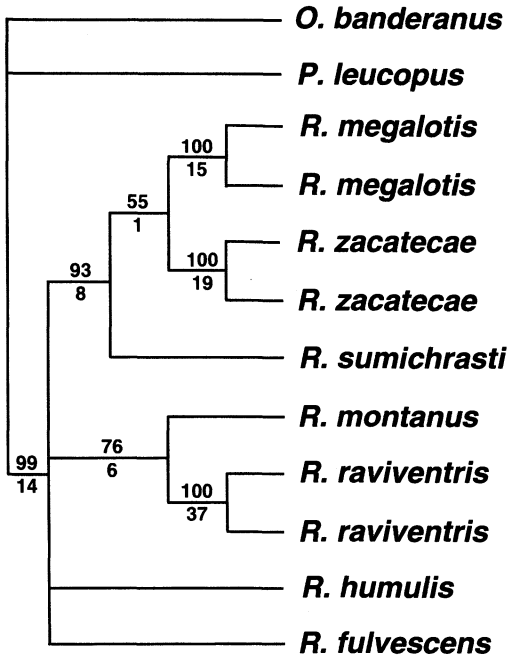

FIG. 1.—Most parsimonious tree for 7 species of *Reithrodontomys* (653 steps, consistency index = 0.5130, retention index = 0.5534) resulting from equal weighting of transitions and transversions.

montanus, and the other that indicated that *R. megalotis* has 3 additional interstitial sites (Meyne et al. 1990).

Analysis of restriction enzyme digestion with *Eco*RI revealed that the 350 base pair monomer repeat in *R. montanus* and *R. megalotis* (Hamilton et al. 1990) also was in *R. zacatecae* and *R. raviventris.* Restriction enzyme digestion of DNA from *R. fulvescens* did not remove the heterochromatic regions (Hamilton et al. 1990).

Data analyses.—The complete cytochrome-*b* gene (1,143 base pairs) was sequenced for 7 species of *Reithrodontomys.* In all analyses, the 2 individuals representing *R. megalotis, R. zacatecae,* and *R. raviventris* formed sister taxa.

The analysis in which transitions and transversions were equally weighted resulted in a single most parsimonious tree (Fig. 1). The topology of that tree depicted 2 clades. In the 1st clade, *R. megalotis* and *R. zacatecae* formed a sister taxon relationship and were then joined to *R. sumichrasti. R. montanus* and *R. raviventris* formed a 2nd clade. Those 2 clades were joined together, and *R. humulis* and *R. fulvescens* were added in a stepwise manner. Bootstrap and Bremer support values were high for each of the respective nodes within those 2 clades; however, little or no support existed for their close association or for the stepwise addition of *R. humulis* and *R. fulvescens.* Those relationships collapsed under bootstrap analyses, resulting in an unresolved polytomy (Fig. 1).

Analyses in which transitions were downweighted produced 1 most parsimonious tree. That tree retained the *R. montanus–R. raviventris* and *R. megalotis–R. zacatecae–R. sumichrasti* clades. However, *R. humulis* and *R. fulvescens* were joined to the *R. megalotis–R. zacatecae–R. sumichrasti* clade in a stepwise manner. Bootstrap and Bremer values showed strong support for the *R. megalotis–R. zacatecae–R. sumichrasti* and for the *R. montanus–R. raviventris* clades; however, no support was found for the placement of *R. humulis* and *R. fulvescens.*

Analyses in which 1st, 2nd, and 3rd nucleotide positions were weighted 3.7:28.67:1, resulted in a single most parsimonious tree. That tree was identical in topology to the equally weighted tree (Fig. 1), except for the placement of *R. humulis* as sister to the *R. montanus–R. raviventris* clade. Bootstrap and Bremer values showed little or no support for the *R. montanus–R. raviventris–R. humulis* clade nor for the relationship of the 2 major clades. As in the equally weighted analysis, an unresolved polytomy was formed among the 2 clades, *R. humulis,* and *R. fulvescens.*

Neighbor-joining trees, based on Tamura and Nei and Tamura and Nei-Γ (gamma = 0.154) models of evolution, produced the same 2 clades as the parsimony analyses except that *R. sumichrasti* was sister to *R. zacatecae* and *R. humulis* was sister to the *R. zacatecae–R. sumichrasti–R. megalotis*

clade. The association of *R. humulis* to the *R. zacatecae–R. sumichrasti–R. megalotis* clade collapsed in the bootstrap analysis, as did the relationship between the 2 clades and *R. fulvescens.*

Genetic distances (Table 2) calculated using the Tamura and Nei model ranged from 0.0798 for *R. sumichrasti* and *R. zacatecae* to 0.1736 for *R. fulvescens* and *R. montanus.* Intraspecific comparisons ranged from 0.0018 for 2 samples of *R. raviventris* to 0.0161 for 2 samples of *R. zacatecae.*

The maximum-likelihood analysis utilized the HKY85-Γ model of evolution with a transition : transversion ratio = 4.8 and gamma-shape parameter = 0.154 (Hasegawa et al. 1985). The topology obtained in that analysis was identical to that obtained when transitions were downweighted. Given lack of support for placement of *R. humulis* and *R. fulvescens* in previous analyses, we constrained all possible associations (6) of *R. humulis* and *R. fulvescens* to the 2 clades (*R. zacatecae–R. sumichrasti–R. megalotis* and *R. montanus–R. raviventris*). None of the topologies differed ($P \leq 0.05$) based on the Kishino-Hasegawa test (Kishino and Hasegawa 1989).

Discussion

One tree (Fig. 1) summarizes topologies produced with the various weighting schemes and methods (parsimony, distance, and likelihood) that we used. All analyses except the distance analyses (Tamura and Nei, Tamura and Nei-Γ) depicted *R. megalotis* and *R. zacatecae* as sister taxa. Distance analyses showed *R. zacatecae* and *R. sumichrasti* as sister taxa. Regardless of the arrangement, the common ancestry of these 3 taxa (*R. megalotis, R. zacatecae,* and *R. sumichrasti*) was supported by high bootstrap and Bremer support values. Likewise, all analyses depicted *R. raviventris* and *R. montanus* as sister taxa. Bremer and bootstrap values strongly supported this relationship. Placement of *R. humulis* and *R. fulvescens* was unresolved in parsimony and distance analyses when bootstrap anal-

TABLE 2.—Tamura–Nei genetic distances (Tamura and Nei 1993) for the 7 species of *Reithrodontomys* examined are shown below the diagonal. Multiple individuals per taxon are designated samples 1 and 2 (Appendix I).

Taxon	Taxon										
	R. megalotis 1	*R. megalotis* 2	*R. montanus*	*R. zacatecae* 1	*R. zacatecae* 2	*R. raviventris* 1	*R. raviventris* 2	*R. sumichrasti*	*R. fulvescens*	*R. humulis*
R. megalotis 1	—									
R. megalotis 2	0.0142	—								
R. montanus	0.1568	0.1516	—							
R. zacatecae 1	0.0896	0.0905	0.1612	—						
R. zacatecae 2	0.0894	0.0838	0.1623	0.0161	—					
R. raviventris 1	0.1507	0.1439	0.1348	0.1634	0.1466	—				
R. raviventris 2	0.1511	0.1443	0.1351	0.1639	0.1470	0.0018	—			
R. sumichrasti	0.0985	0.0973	0.1658	0.0798	0.0912	0.1452	0.1456	—		
R. fulvescens	0.1443	0.1446	0.1736	0.1490	0.1452	0.1510	0.1512	0.1379	—	
R. humulis	0.1377	0.1416	0.1719	0.1463	0.1449	0.1519	0.1522	0.1454	0.1615	—

yses were used. Although *R. fulvescens* and *R. humulis* were added in a stepwise fashion to the *R. megalotis–R. zacatecae–R. sumichrasti* clade in the likelihood analysis, that topology was not different (Kishino–Hasegawa test; $P \leq 0.05$) from the 6 independent arrangements of those 2 taxa to either the *R. zacatecae–R. sumichrasti–R. megalotis* clade or the *R. montanus–R. raviventris* clade. This seems to imply that placement of *R. fulvescens* and *R. humulis* was not strongly supported in the likelihood analysis.

Despite several attempts to determine systematic relationships among species of *Reithrodontomys* (Arnold et al. 1983; Carleton and Myers 1979; Engstrom et al. 1981; Hood et al. 1984; Hooper 1952; Howell 1914; Jones and Genoways 1970; Jones and Lawlor 1965; Nelson et al. 1984; Robbins and Baker 1980), complete congruency among data sets has been rare. Hooper's (1952) revision of the genus *Reithrodontomys* represents the most extensive and complete examination of morphologic data. Hooper (1952) recognized 4 species groups (*R. megalotis, R. fulvescens, R. mexicanus,* and *R. tenuirostris*) with the suggestion that 2 additional species groups (*R. humulis* and *R. sumichrasti*) were embedded in the *R. megalotis* group. However, Hooper's (1952) study did not use character polarity as a basis for determining evolutionary relationships.

Karyotypic analyses, including data from studies of nondifferentially stained, G- and C-banded, and in situ hybridized chromosomes have been used (e.g., Carleton and Myers 1979; Engstrom et al. 1981; Hamilton et al. 1990; Hood et al. 1984; Robbins and Baker 1980; Rogers et al. 1983) to examine phylogenetic relationships of taxa (*R. creeper, R. fulvescens, R. humulis, R. megalotis, R. mexicanus, R. montanus, R. raviventris, R. sumichrasti, R. tenuirostris,* and *R. zacatecae*). In general, these studies led to the recognition of a high diploid, mostly acrocentric group (*R. creeper, R. fulvescens, R. mexicanus, R. tenuirostris,* and *R. humulis*) and a low diploid, entirely biarmed group (*R. montanus, R. raviventris, R. megalotis, R. sumichrasti,* and *R. zacatecae*). These studies have documented extensive chromosomal evolution within these 5 species and provided evidence that radical euchromatic rearrangements have occurred (Baker and Bickham 1980; Hood et al. 1984; Robbins and Baker 1980). In general, these data contradict or complicate the proposed species groups and subgenera outlined by Hooper (1952).

Comparison of the phylogenetic relationships depicted by karyotypic data (Hood et al. 1984) to those generated by DNA-sequence analysis revealed several similarities. First, *R. montanus* and *R. raviventris* are sister taxa in both analyses. Second, *R. megalotis, R. zacatecae,* and *R. sumichrasti* were aligned closely in that they formed a single clade. In most of our analyses, *R. megalotis* and *R. zacatecae* are sister taxa and are then joined by *R. sumichrasti,* whereas these 3 taxa are unresolved in the karyotypic analysis.

Phylogenetic reconstruction based on allozymic data (Arnold et al. 1983; Nelson et al. 1984) has been problematic because many of the derived proteins are autapomorphies or are polymorphic among taxa. However, relationships presented by Nelson et al. (1984) support the general pattern of conclusions depicted in the G-band study of Hood et al. (1984) and our DNA-sequence analyses. In fact, the clade depicted in Nelson et al. (1984) was similar to that generated in our DNA-sequence analysis except for the sister taxon relationship of *R. humulis* and *R. fulvescens.*

Karyotypic megaevolution is described as a radical reorganization of the karyotype in which normally stable G-band patterns are disrupted or rearranged to a point that makes it difficult to observe normal patterns typically shared among closely related species (Baker and Bickham 1980). Karyotypic megaevolution differs from karyotypic orthoselection (White 1975), where a single type of chromosomal rearrangement

occurs repeatedly within a species or species group. However, in karyotypic megaevolution, several different kinds of chromosomal rearrangements are involved. The 1st described example of this phenomenon involved muntjaks, where *Muntiacus reevesi* has 2n = 46 and *M. muntjak* has 2n = 6 for females; 2n = 7 for males (Ellerman and Morrison-Scott 1951). That phenomenon was documented by a cladistic analysis of congeneric species where ≥ 1 contained an unaltered primitive karyotype typical of chromosomal stasis; whereas, a 2nd congener had a radically reorganized karyotype that was difficult to relate back to the basal condition (Baker and Bickham 1980). In this context, it is parsimonious to conclude that time required to radically reorganize the karyotype is no greater than the time since divergence of these 2 congeneric species. The premise that chromosomal evolution does not behave in a clocklike fashion has been hypothesized not only for karyotypic megaevolution but also for karyotypic saltation (Lewis 1966) and the breakage–fusion–bridge cycle (McClintock 1978).

Although many studies question the accuracy of any molecular clock calibrated based on the rate of change in a given gene, it is often assumed that sequence divergence is a time-related phenomenon that may provide valuable information to better understand the mode and tempo of evolution (Hillis et al. 1996; Li 1993). Arnold et al. (1983) and Hood et al. (1984) concluded that *R. zacatecae, R. montanus,* and *R. megalotis* have undergone karyotypic megaevolution, whereas *R. fulvescens* has maintained karyotypic stasis exhibiting the G-band linkage groups typical of peromyscine rodents (Koop et al. 1984). Based on classical morphologic studies, *R. zacatecae* was recognized as a subspecies of *R. megalotis.* When viewed against the wealth of published cytochrome-*b* data for mammals (Fumagalli et al. 1999; Johns and Avise 1998), the level of sequence divergence between *R. zacatecae* and *R. megalotis* is within the range of values typical for biological species rather than that of intraspecific variation. Furthermore, in our other analyses (likelihood and parsimony), the sister relationship of these 2 taxa is apparent.

TABLE 3.—Time-since-divergence values for selected taxa in the genus *Reithrodontomys* were calculated from Tamura–Nei genetic distances (Tamura and Nei 1993) and calibrated assuming 3.5% change/million years sensu Arbogast (1999). Following Hillis et al. (1996), Poisson expectations were used to estimate 95% *CI* limits for time since divergence.

Taxon	Time since divergence ($\times 10^6$ years ago)
R. megalotis versus *R. fulvescens*	4.13 ± 0.711
R. zacatecae versus *R. fulvescens*	4.20 ± 0.710
R. montanus versus *R. fulvescens*	4.96 ± 0.787
R. raviventris versus *R. fulvescens*	4.32 ± 0.715
R. sumichrasti versus *R. fulvescens*	3.94 ± 0.681
R. humulis versus *R. fulvescens*	4.62 ± 0.745
R. megalotis versus *R. zacatecae*	2.52 ± 0.547
R. sumichrasti versus *R. zacatecae*	2.44 ± 0.541
R. humulis versus *R. zacatecae*	4.16 ± 0.703
R. montanus versus *R. raviventris*	3.86 ± 0.676
R. humulis versus *R. raviventris*	4.35 ± 0.734
R. megalotis versus *R. sumichrasti*	2.80 ± 0.573
R. humulis versus *R. sumichrasti*	4.15 ± 0.707
R. humulis versus *R. montanus*	4.91 ± 0.784
R. humulis versus *R. megalotis*	3.99 ± 0.699

If one assumes that the average sequence divergence of cytochrome-*b* is about 3.5%/million years (Arbogast 1999; DeWalt et al. 1993; Li et al. 1987, 1990), then the estimated time-since-divergence values (Table

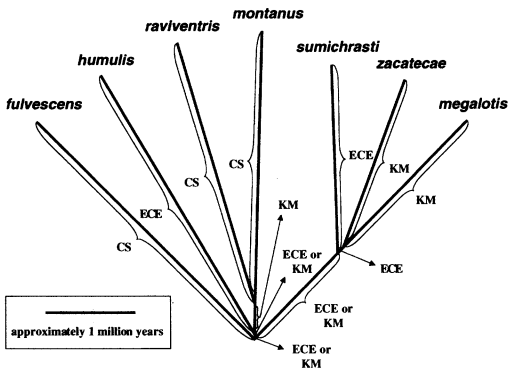

FIG. 2.—Diagramatical interpretation of times-since-divergence values relative to patterns of chromosomal evolution for 7 species of *Reithrodontomys*. Tree topology (Fig. 1) with branch lengths corresponding to time-since-divergence values calculated from Tamura–Nei genetic distances. Patterns of chromosomal evolution are abbreviated as follows: chromosomal stasis (CS), extensive chromosomal evolution (ECE), and karyotypic megaevolution (KM).

3) for species comparisons ranged from 2.44×10^6 (*R. sumichrasti–R. zacatecae*) to 4.96×10^6 (*R. montanus–R. fulvescens*) years ago. Likelihood scores obtained from an equal rate versus a gamma-distribution model were tested using the Tamura–Nei model. Rate variation among DNA-sequence sites did not differ (Kishino–Hasegawa test, $P = 1.000$) and consequently among-site variation was assumed to have little or no effect on estimates of time since divergence. Also, we recognize that there are several methods of estimating time since divergence from sequence data (Rambaut and Bromham 1998); however, the relative values included herein should be comparable.

Relationships of these hypothesized times since divergence and chromosomal evolution are demonstrated in Fig. 2. Several conclusions can be drawn from this relationship. First, within the genus *Reithrodontomys,* some species (i.e., *R. fulvescens*) have experienced continual chromosomal stasis throughout their history; whereas other species (i.e., *R. megalotis, R. montanus, R. raviventris, R. humulis, R. sumichrasti,* and *R. zacatecae*) have experienced substantial chromosomal evolution involving different chromosomal rearrangements (Hood et al. 1984; Koop et al. 1984). Clearly, chromosomal evolution does not behave in a clocklike fashion. Second, some species (*R. raviventris* and *R. montanus*) that have undergone karyotypic megaevolution have returned to a mode of chromosomal stasis. The exact mechanism or event (Baker et al. 1988; Bradley and Wichman 1994; Chesser and Baker 1986; Wichman et al. 1991) that is functioning in a cause and effect relationship in chromosomal change is not understood, but resolution of these processes will best be addressed in cladistical analyses of evolutionary patterns across closely related species.

It is well-documented that chromosomal evolution is not clocklike in its occurrence (Baker and Bickham 1980; Koop et al. 1984), but 1 explanation for extensive chromosome repatterning in supposedly closely related species is that the 2 species actually are not closely related. Our data document that the magnitude of cytochrome-*b* sequence divergence is not atypical for peromyscine genera (Koop et al. 1984). When karyotypic megaevolution was described (Baker and Bickham 1980), these authors assumed that congeneric species that exhibited both chromosomal stasis and extensive chromosomal repatterning were closely related. For the genus *Reithrodontomys,* our cytochrome-*b* data support this conclusion.

What is the time frame during which the karyotype has been extensively repatterned? DNA-sequence divergence values for these species of *Reithrodontomys* produced minimal time estimates of about 2.5–4.1×10^6 years ago for this karyotypic megaevolution (Table 3; Fig. 2).

Described as a species by Dixon (1908), *R. raviventris* has a highly restricted geographic range (Hall 1981) and perhaps originated as a result of geographic isolation due to the formation of salt marshes in the San Francisco Bay region (Fisler 1965). Be-

fore 1984 the specific status of *R. raviventris* was based on the assumption that its closest living relative was *R. megalotis* and that it was sympatric with *R. megalotis* (Fisler 1965; Hooper 1952; Shellhammer 1967). With the development of the hypothesis that *R. raviventris* was sister to *R. montanus,* the question became whether *R. raviventris* was an isolated subspecies of *R. montanus,* or was it specifically distinct from *R. montanus.* Analyses of karyotypic and allozymic data presented by Hood et al. (1984) and Nelson et al. (1984) suggested that *R. raviventris* was a species distinct from *R. montanus* and was related more closely to this taxon than to *R. megalotis.* This premise is supported in the cytochrome-*b* sequence data where genetic distances separating *R. raviventris* from *R. montanus* and *R. megalotis* are 13.50% and 14.75%, respectively. These values are greater than those for other currently recognized biological species such as *R. sumichrasti* and *R. megalotis* (9.79%) and *R. sumichrasti* and *R. zacatecae* (8.55%). Given the restricted distribution of this taxon to the highly populated San Francisco Bay region and the ever-increasing threat of loss of habitat, our data indicate that this taxon is unique and contributes to the biodiversity of the genus. Conservation of this taxon is paramount.

Originally described as a subspecies of *R. megalotis* (Merriam 1901), Hooper (1952) suggested that *R. zacatecae* may represent a species distinct from *R. megalotis.* Hood et al. (1984) compared the chromosomal relationships of *R. zacatecae* to several other species of *Reithrodontomys* and concurred with Hooper (1952) that *R. zacatecae* indeed represented a valid species. This position is supported by the cytochrome-*b* sequences that show *R. zacatecae* and *R. megalotis* to be sister taxa, differing by a genetic distance of 8.83%. These data, in conjunction with the sympatric occurrence of these 2 taxa at 2 localities in Durango, Mexico (12 km E Ojitos; 6.1 km W Coyotes), argue for the recognition of *R. zacatecae* as a distinct species. This taxon may be declining and may represent a threatened species (Ramírez-Pulido et al. 1996) and further research should be conducted on the conservation and protection of this taxon.

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APPENDIX I

Specimens examined.—Liver and muscle tissue samples were obtained from the Vital Tissue Collection, Museum of Texas Tech University. Specimen identification numbers (TK) and GenBank accession numbers are listed in parentheses after the taxon name. All localities are in the United States unless otherwise denoted.

Reithrodontomys fulvescens.—Oklahoma: McIntosh County, 3.1 mi E Dustin (TK 23469; AF176257).

Reithrodontomys humulis.—Oklahoma: Pottawatomie County, 3 mi E Tecumseh (TK 26505; AF176258).

Reithrodontomys megalotis.—Texas: Lubbock County, Lubbock Lake Landmark State Historical Park (sample 1; TK 22460; AF176248); Castro County, 5.5 mi S, 2.5 mi W Dimmitt (sample 2; TK 32283; AF176249).

Reithrodontomys montanus.—Texas: Castro County, 5.5 mi S, 2.5 mi W Dimmitt (TK 32314; AF176250).

Reithrodontomys raviventris.—California: Sonoma County, Mouth of Tolay Creek (sample 1; TK 24662; AF176254); Alameda County, 2.5 mi W Newark, Newark Slough (sample 2; TK 13714; AF176255).

Reithrodontomys sumichrasti.—MEXICO: Oaxaca, 3 mi N Suchixtepex (TK 20994; AF176256).

Reithrodontomys zacatecae.—MEXICO: Durango, 3.8 mi W Coyotes, UTM 2634281-13-465908 (sample 1; TK 72369; AF176251); 12 km E Ojitos, UTM 13-2775718-385011 (sample 2; TK 70989; AF176252).