Phylogenetic Relationships, Ecological Correlates, and Molecular Evolution Within the Cavioidea (Mammalia, Rodentia)

Diane L. Rowe and Rodney L. Honeycutt

Department of Wildlife and Fisheries Sciences, Texas A&M University

A molecular phylogeny of the rodent superfamily Cavioidea was derived using two nuclear sequences (exon #10 of the growth hormone receptor gene and intron #1 of the transthyretin gene) and one mitochondrial gene (12S rRNA). A combined analysis produced a highly derived and well-supported phylogenetic hypothesis that differed from traditional taxonomy primarily in the placement of two taxa. *Kerodon*, traditionally included within the subfamily Caviinae with guinea pigs and its relatives, is placed sister to the family Hydrochaeridae and closely aligned with the subfamily Dolichotinae. Inclusion of *Hydrochaeris* within the Caviidae renders the familial classification paraphyletic. Our data further support the taxonomic separation of the families Agoutidae and Dasyproctidae. Both the molecular and traditional morphological interpretations are assessed in testing an ecological constraints hypothesis regarding social behaviors. Whereas traditional taxonomy is consistent with an environmental constraints explanation for social behavior, the molecular data suggest that phylogenetic effects may be a more important factor in the evolution of social behavior in this group. Although lineage-specific rate heterogeneity was identified in all three molecular data sets, no significant support was obtained for the metabolic rate hypothesis. However, both nuclear genes displayed patterns in accordance with the generation time hypothesis.

Introduction

Phylogenetic studies of closely related monophyletic groups are essential for understanding evolutionary processes at both the organismal and molecular levels. The presence of a robust phylogeny provides a framework for the interpretation of evolutionary patterns, allowing inferences to be drawn about either particular character associations (i.e., how evolutionary changes among characters are correlated through evolutionary history) or phylogenetic congruence among diverse biological features. The determination of phylogenetic influences on the phenotypic and ecological characters of organisms has been of particular interest. For instance, understanding the evolution of ecological specialization or generalization remains especially problematic for evolutionary biologists (Futuyma and Moreno 1988; Thompson 1994, pp. 2, 59). To address such issues, statistical relationships (e.g., simple parametric correlations or more complex comparative methods) that consider relationships among phenotype, environment, and phylogeny have been used to connect ecological process with historical patterns (e.g., Harvey and Pagel 1991, p. 1; Faith and Belbin 1994; Crespi 1996; Irwin 1996; Burda et al. 2000). Such methods allow one to determine whether the observed trends among clades reflect variable environmental constraints or whether the character of interest is bounded by phylogenetic history. The same approach can be used to address molecular evolutionary hypotheses. Patterns in rate of nucleotide substitution, for example, stem from variation in the constraints on mutation and fixation. Differences in rates of nucleotide substitution among closely related taxa could be assessed for correlation with differing morphological or physiological constraints, which in turn are hypothesized to dictate mutation rates. How such biological constraints are manifested as mutations may vary within and among genes as well. In other words, a decoupling of patterns may be evident, as genes need not show the same pattern of rate variability across the same set of species (Mindell et al. 1996).

Rodents provide an ideal opportunity to address ecological and evolutionary hypotheses, given the prevalence of well-accepted monophyletic groups of closely related, yet ecologically and morphologically diverse assemblages of species. Among South American hystricognath rodents (i.e., Caviomorpha), the monophyly of the superfamily Cavioidea is well supported (Woods 1993; Nedbal, Honeycutt, and Schlitter 1996; Huchon, Catzeflis, and Douzery 1999). This closely related assemblage, including 33 species (Nowak 1999, p. 1663), retains an extraordinary diversity in behavior, habitat utilization, morphology, and life-history strategies (Cabrera and Yepes 1960, p. 25; Kleiman 1974; Mares and Genoways 1982, pp. 187, 377) (table 1). The rapid and extensive radiation of caviomorph rodents in the early Oligocene or late Eocene (30–40 MYA; Wyss et al. 1993) also complicates interpretation of phylogenetic relationships among these lineages, because of the high degree of parallelism seen in morphological and serological characters (Hartenberger 1985; Nedbal, Honeycutt, and Schlitter 1996). As a result, taxonomic designations, particularly at the familial level, have been inconsistent and widely debated (Cabrera 1961; Anderson and Jones 1984, p. 402; Corbet and Hill 1991, p. 199; Wilson and Reeder 1993, p. 778; McKenna and Bell 1997, p. 191). Currently, little is known about relationships among families and genera of cavioid rodents. A more accurate phylogenetic perspective of these relationships will allow for detailed studies of the evolution of life-history traits within this diverse superfamily. Using an independently derived molecular phylogeny, it may be possible to ascertain whether certain morphological, ecological, or behavioral traits (or all of them) charac-

Key words: environmental constraints, phylogeny, rate heterogeneity, Rodentia, systematics.

Address for correspondence and reprints: Rodney L. Honeycutt, 210 Nagle Hall, 2258 TAMUS, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843-2258. E-mail: rhoneycutt@tamu.edu.

Mol. Biol. Evol. 19(3):263–277. 2002

Table 1
Behavioral, Morphological, Ecological, and Life History Variables Within the Superfamily Cavioidea

Family and Subfamily Genus[a] (Number of species[b])	Common Name	Breeding System[b,c]	Degree of Sociality[d]	Habitat[e]	Adult Body Size (kg)[f]	Gestation Time (days)	Metabolic Rate[g] ml·O_2/(g h)
Caviidae							
Caviinae							
Cavia (8)	Cavies	Prom	Low	G	0.25–0.75	56–74	0.55
Galea (3)	Cuis	Prom	Low	G, A	0.20–0.50	49–60	0.82
Kerodon (1)	Rock cavy	Poly	High	R	0.80	76–77	0.45
Microcavia (3)	Mtn. cavy	Prom/Poly	Low	G, A	0.15–0.34	53–55	0.69
Dolichotinae							
Dolichotis (1)	Mara	Mono	High	AS	8.0–16	87–93	0.45
Pediolagus (1)	Dwarf mara	Mono	High	AS	1.6–2.3	75–77	Unknwon
Agoutidae							
Agouti (1)	Paca	Mono	Moderate	TF	8.0–10	111–118	0.30
Stictomys (1)	Mtn. paca	Mono	Unknown	MF	4.3	Unknown	Unknown
Dasyproctidae							
Dasyprocta (11)	Agoutis	Mono	Moderate	TF	2.0–3.9	80–104	0.54
Myoprocta (2)	Acouchis	Mono	Moderate	TF	0.60–1.4	99	0.55
Hydrochaeridae							
Hydrochaeris (1)	Capybara	Poly	High	AQ	30–65	120–156	0.25

[a] Cabrea (1961).
[b] Nowak (1999).
[c] Breeding system: Mono, monogamy; Poly, polygyny; Prom, promiscuity.
[d] Degree of sociality is based on a review of the literature; low infers nonrecurring social interactions, moderate infers territorial defense and pair formation with recurring social interactions, and high infers long-term recurring social interactions from one season to another.
[e] Habitat: A, arid; AS, arid savanna; AQ, semiaquatic; G, grassland; MF, montane forest; R, rock outcrops; TF, tropical forest.
[f] Silva and Downing (1995).
[g] Lovegrove (2000).

terizing lineages are the result of shared ancestry or which could potentially be the result of independent evolution in response to similar environmental conditions.

Morphological and behavioral adaptations associated with the Cavioidea include differences in overall life-history strategies, habitat utilization ranging from generalists (i.e., evenly distributed resources) to semiaquatic and desert specialists (i.e., clumped and monopolizable resources), and a range of breeding systems from hierarchical promiscuity to polygyny and monogamy (Kleiman, Eisenberg, and Maliniak 1979). In terms of life-history traits, Lacher (1981) proposed an ecological constraints hypothesis to explain apparent convergence in sociality between *Kerodon*, a member of the subfamily Caviinae (Cabrera 1961; Corbet and Hill 1991; McKenna and Bell 1997), and members of the subfamily Dolichotinae. Although most species in the Caviinae display promiscuity and lack recurrent social bonds, *Kerodon* and species of dolichotids display a monogamous mating system, with more complex social interactions. Unfortunately, the lack of a well-supported phylogeny prevents one from testing the alternative hypothesis, which suggests that the similarity in mating system and behavior resulting from shared ancestry is a more probable explanation. Such information is central in debates over the role that habitat plays as a precondition for the development of complex social systems in hystricognath rodents in general (Burda et al. 2000).

At the molecular level, a reliable phylogenetic framework is pertinent to testing different hypotheses accounting for observed heterogeneity in rates of molecular substitution among closely related lineages. Although these findings have been criticized by some (e.g., Slowinski and Arbogast 1999), several recent molecular studies have reported lineage-specific rates of substitution that appear to be correlated with life-history traits, body size, and metabolic rates (Martin and Palumbi 1993; Mooers and Harvey 1994; Martin 1995; Mindell et al. 1996). The original explanation for this rate heterogeneity is that species with shorter generation times have a greater number of DNA replications per year, thus incurring an increased chance of replication error per unit time (Li, Tanimura, and Sharp 1987; Ohta 1993; Mooers and Harvey 1994; Li et al. 1996). An alternative explanation is the metabolic rate hypothesis, which attributes a positive correlation between metabolic rate and rate of nucleotide substitution to the effects of oxidative DNA damage (Shigenaga, Gimeno, and Ames 1989).

The order Rodentia provides an excellent model for detailed studies of molecular rate heterogeneity. Although contested by some (e.g., Easteal 1990), several molecular studies have revealed an overall faster rate of nucleotide substitution in rodents relative to other mammalian lineages (Li, Tanimura, and Sharp 1987; Li et al. 1990; Gissi et al. 2000). However, little is known about lineage-specific rate heterogeneity within the order Rodentia. Although O'hUigin and Li (1992) observed rate homogeneity among muroid rodents, their comparisons were limited to three taxa. Huchon, Catzeflis, and Douzery (1999) detected rate heterogeneity within and among several rodent families and suggested a lack of support for a generation time effect. However, no statistical anal-

Table 2
Primers used for PCR Amplification and Sequencing

Gene	Strand[a]	Name	Sequence (5′–3′)
GHR	F	GHREXON10[b]	GGRAARTTRGAGGAGGTGAACACMATCTT
	F	GHR50F[b]	TTCTAYARYGATGACTCYTGGGT
	R	GHREND[b]	CTACTGCATGATTTTGTTCAGTTGGTCTGTGCTCAC
	F	GHR10[b]	ACCAGCAGGNAGTGTRGTCCTTTC
	R	GHRendC[b]	RTGGCTTACTTGGGCATAAAAGTC
TTH.	F	TTHF2	TGAGGCTGACCCTGTGGTGAGTGTTT
	R	TTHR2	CTGGCACAGAAAGRACAAA
	F	CFx	ATGGGTAATAAAAGTAGTCTGA
	R	CFx	GCCCTCTGAGAACAGTTG
	R	CRy	GACCAAATAATYTTTCCDGAGA
12S rRNA.	F	L651[c]	CATAGACACAGAGGTTTGGTGG
	R	12GH[c]	TTTCATCTTTTCCTTGCGGTAC
	R	H147[c]	TGACTGCAGAGGGTGACGGGCGGTGTGT
	F	L109[c]	AAAAAGCTTCAAACTGGGATTAGATACCCC
	R	12EL[c]	TGGGAAGAAATGGGCTACATT
	F	Ha12S[c]	TATCGATTATAGAACAGGCTCC

[a] F = forward strand; R = reverse strand.
[b] Adkins et al. (2001).
[c] Nedbal, Allard, and Honeycutt (1994).

yses were performed to support or refute this observation. Given the diversity of body size, metabolic rate, generation time, and life-history strategies within rodents, more detailed studies of rates of molecular evolution need to focus on specific monophyletic groups and their associated life-history traits. Species within Cavioidea show 100-fold differences in body size and as much as threefold differences in metabolic rate (Silva and Downing 1995, p. 195; Lovegrove 2000), providing an ideal opportunity for detailed assessments of the influence of body size and its correlates on rates of molecular evolution.

The objectives of this paper are to derive a molecular phylogeny for members of the superfamily Cavioidea. Sequences from two nuclear genes (growth hormone receptor (GHR) exon #10 and transthyretin (TTH) intron #1) and one mitochondrial gene (12S rRNA) are analyzed separately and in combination, and the resultant phylogeny is used to test several hypotheses. First, relationships depicted by the combined tree are compared to those based on previous taxonomic treatments derived from morphology. Second, the molecular phylogeny is used to examine the influence of shared ancestry versus ecological constraints on the origin of complex social systems. Finally, the phylogeny and branch lengths are used to investigate lineage-specific rates of molecular evolution and potential correlates with life-history traits, including body size, gestation time, and metabolic rate.

Materials and Methods

Taxa Examined

Heart, liver, brain, lung, or kidney tissues were acquired from all 11 of the recognized genera within the superfamily Cavioidea, including 14 of the 33 nominal species (Nowak 1999). Taxonomic designations above the species level are consistent with Cabrera (1961). *Capromys piliroides*, representing the superfamily Octodontoidea, served as an outgroup in all analyses. Exon #10 of the nuclear GHR gene, intron #1 of the nuclear prealbumin TTH gene, and the mitochondrial 12S rRNA gene were used to assess phylogenetic relationships among these 14 species. Estimates of metabolic rate, body size, and gestation time were obtained from the literature (Weir 1974; Kleiman, Eisenberg, and Maliniak 1979; Eisenberg 1989, p. 392; Redford and Eisenberg 1992, p. 336; Silva and Downing 1995; Lovegrove 2000). Body size reflects mean male and female adult weights (average of all available population means), avoiding single-sex biases resulting from some taxa having sexual dimorphism or reverse–sexual dimorphism in body size. Metabolic rates are given in units of $ml \cdot O_2/(g\ h)$.

Nucleotide Sequencing

Total genomic DNA was isolated using DNeasy™ Tissue Kits (Qiagen). Double-stranded DNA amplification products were sequenced directly with ABI PRISM™ (Perkin-Elmer) Big Dye Terminator Cycle Sequencing Kits and Applied Biosystems (Perkin-Elmer) 377 automated DNA sequencer. Sequencing primers were chosen to give complete overlap of sequences, reading in both directions.

Primers GHRend, GHREXON10, and GHR50F (table 2) were used to PCR amplify exon #10 of GHR, under the following conditions: (1) hot start of one cycle at 95°C for 5 min; (2) five cycles with denaturation at 95°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min; (3) four sets of five cycles at the same denaturation and extension conditions but with lowering of the annealing temperature each time (59, 57, 55, and 53°C); (4) a single set of 10 cycles with an annealing temperature of 53°C; and (5) a final extension for one cycle at 72°C for 10 min. Two internal primers, GHR10c and GHRendC, were used to obtain completely overlapping sequences. Combinations of primers TTHF2, TTHR2, CFx, CRx, and CRy were used to amplify intron #1 of TTH (table 2). The PCR thermal pro-

file was the same as that used to amplify GHR. The complete 12S rRNA gene, consisting of approximately 1,000 base pairs (bp), was amplified using primers L651 and 12GH. Internal primers from Nedbal, Allard, and Honeycutt (1994) were used to sequence both strands (table 2). PCR conditions were similar to those described in Nedbal, Allard, and Honeycutt (1994) and Nedbal, Honeycutt, and Schlitter (1996). Designation of all heavy (H) and light (L) strand primers refers to positions in the mouse mitochondrial genome (Bibb et al. 1981).

Patterns of Sequence Variation

Sequences were aligned using default settings in ClustalX (Thompson et al. 1997) and optimized visually according to either codon position (GHR) or secondary structure models (12S rRNA; Springer and Douzery 1996). The use of structural models aided in identifying positional homology, especially for loop regions, which contain numerous indels. Areas of ambiguous alignment for 12S rRNA and TTH were excluded from all analyses. Base composition was estimated for all taxa, and homogeneity among taxa was tested using chi-square tests of contingency tables of nucleotide counts, as implemented in PAUP* 4.0b8 (Swofford 1999). Estimates of nucleotide substitution types and nucleotide sequence divergences also were obtained using PAUP*.

Maximum Parsimony Analyses

All maximum parsimony (MP) analyses were conducted using PAUP*4.0b8 (Swofford 1999). Pairwise uncorrected p distances were calculated to assess within and among species differences. Data sets were then reduced to one representative of each species, which was the same individual for all the three gene sequences. Prior to performing MP analyses, all data sets were examined for evidence of saturation by plotting percent change in transitions or transversions between taxa as a function of HKY (Hasegawa, Kishino, and Yano 1995) distances. Because of differential rates at codon positions, saturation plots for GHR were analyzed separately for the first, second, and third codon positions. Likewise, stems and loops were assessed independently for 12S rRNA.

Genes were analyzed separately prior to total evidence analysis; combinability was assessed using the partition homogeneity test (PHT; 1,000 replications, $\alpha = 0.05$; Cunningham 1997). All MP analyses were performed using branch-and-bound search methods. In all cases, both bootstrap replication (1,000 replicates using a full heuristic search; Felsenstein 1985) and Bremer decay indices (Bremer 1994) were used to assess support for individual nodes.

Maximum Likelihood Analyses

All maximum likelihood (ML) analyses were implemented in PAUP*4.0b8 (Swofford 1999) and were conducted on both separate and combined data. The MP tree was used for selection of an appropriate model of evolution for likelihood analyses by first assessing likelihood scores for a nested array of models (Sullivan and Swofford 1997; Posada and Crandall 1998). Substitution models included F81 (Felsenstein 1981), HKY (Hasegawa, Kishino, and Yano 1985), and general time reversible (GTR; Yang 1994*a*). Among-site rate variation models were then tested in a nested manner, under the appropriate substitution model (see Posada and Crandall 1998). Significance in gain of likelihood under increasingly complex models and patterns of rate variation were measured using likelihood ratio tests (LRTs; Yang, Goldman, and Friday 1995), assuming a chi-square distribution of scores (degrees of freedom [df] are equal to the difference in number of parameters estimated under the different models), for all pairwise comparisons and Bonferroni correction (Rice 1989) for multiple testing. With the determined model of choice, heuristic searches were performed using tree-bisection-reconnection (TBR) branch swapping with 10 random addition replicates. Because of computational limitations, 100 bootstrap replicates were implemented using the fast stepwise-addition method (PAUP*4.0b8).

Hypothesis Testing

To address the idea of environmental constraints in the evolution of cavioid rodents, as previously suggested by Lacher (1981), KH-tests (Kishino and Hasegawa 1989) were used to compare the tree generated from sequence data to trees consistent with Lacher's original hypothesis. In addition, patterns of correlated character evolution between habitat characteristics and sociality were assessed with the concentrated changes test (MacClade 3.1; Maddison and Maddison 1992) applied to both previous morphological phylogenies (Quintana 1998; da Silva Neto 2000) and the molecular phylogeny obtained in this paper.

Because methods for assessing lineage-specific substitution rates perform optimally under different situations (Sorhannus and Van Bell 1999; Bickel 2000; Bromham et al. 2000), three different approaches were used to evaluate rate heterogeneity. First, Tajima's relative rate test (RRT; Tajima 1993) was used in pairwise comparisons of taxa to a reference outgroup. This method requires no mathematical model and minimizes the effects of sampling bias. The test does, however, suffer from lack of power, requiring a large number of variable sites and a closely related outgroup (Bromham et al. 2000). In addition, the nonindependent triplet comparisons require Bonferroni correction for multiple testing, thus rendering the test even more conservative. Second, the two cluster test (TCT) and branch length test (BLT; Takezaki, Rzhetsky, and Nei 1995; LINTRE program: http//www.bio.psu.edu/People/Faculty/Nei/Lab/) were used to evaluate several lineages simultaneously, allowing identification of either single or multiple lineages that are evolving significantly fast or slow compared with the average rate for all taxa. This method is sensitive to unbalanced taxonomic sampling (Sorhannus and Van Bell 1999) as well as unbalanced tree topologies (Robinson et al. 1998). Finally, the LRT (Felsen-

stein 1988) was used to compare likelihood scores of a topology derived under the assumption of a molecular clock to one that does not assume a molecular clock. This method suffers from a lack of power when few variable sites are available (Sorhannus and Van Bell 1999). It also fails to identify specific lineages contributing to the heterogeneity.

A nonparametric correlation approach was used to assess the nature of the relationship of substitution rate heterogeneity (i.e., deviation from a molecular clock) to either generation time (or some indicator thereof) or metabolic rate. Tests for serial independence (TFSI; Abouheif 1999) were applied to assess possible associations between body mass, gestation time, or metabolic rate (or all the three) with phylogenetic history. Because this is a parametric test, traits were transformed, when necessary, to obtain normality. Subsequently, Spearman rank correlation analyses, with correction for tied values (Sokal and Rohlf 1998, p. 598), were used to identify significant relationships, on the premise that differences in life-history parameters are expected to yield differences in branch length estimates. If, for example, there is a negative effect of generation time (measured here as gestation time or body size; Eisenberg 1981, p. 241; Calder 1984, pp. 1, 285) on substitution rate, then one might expect lineages with longer generation times (longer gestation times and larger body size) to have shorter branch lengths (i.e., ML branch length estimates) than the average (i.e., clock-constrained branch lengths) for this group of taxa. Likewise, lineages with shorter generation times (shorter gestation times and smaller body size) should have positive branch length values (i.e., individual branch length estimates minus clock-constrained branch length). The same approach was taken for assessing the dependence of rates of evolution on metabolic rate.

To assess whether the distribution of rate heterogeneity was similar among genes analyzed, correlation statistics were implemented. Branch length correlation, total evolution correlation, and correlated rates of evolution were estimated using Pearson product moment correlations, Spearman rank correlations, and binomial tests for overall pattern across taxa (Omland 1997). For binomial tests, patterns of rate similarities between the genes were obtained from clade contrasts because of the lack of power in using only terminal sister taxa comparisons.

Results

GHR Exon

All data were retained, as there was no indication of saturation for either transition or transversion substitutions at any of the three codon positions (plots not shown). All taxa were similar in base composition (chi-square test; $P = 1.00$, df = 48), having the same nucleotide frequency biases at each of the three codon positions. Within species differences (uncorrected p distances) were relatively small, most ranging from 0% (*Capromys*, *Galea* species, and *Pediolagus*) to 0.25% (*Hydrochaeris*). Both *Agouti paca* specimens were maintained in subsequent analyses because they differed by 0.49%. This difference is similar to that seen between the designated species *A. paca* (Bolivia) and *Stictomys taczanowskii* (0.74%). Other species differences (within genera) for this group ranged from 0.37% (*Myoprocta* species) to 4.28% (*Cavia* species). Distances among genera ranged from 3.0% (*Pediolagus-Hydrochaeris*), 1.0%, including the *Pediolagus-Dolichotis* comparison, to 9.0% (*Dolichotis-Myoprocta*).

A total of 814 bp from GHR was used in an equally weighted parsimony analysis, which resulted in one most parsimonious tree (fig. 1*a*) of length 300 (consistency index [CI] = 0.85, retention index [RI] = 0.81). The placements of *Hydrochaeris* and *Kerodon* were unstable, and a tree just one step longer (length = 301) was consistent with the ML topology (fig. 1*b*). Using the MP tree topology, LRTs suggested that HKY + γ (Yang 1994*b*) was the most appropriate model of evolution for ML analysis. A heuristic search with TBR branch swapping and 10 random additions was implemented and all appropriate parameters estimated. The $-\ln$ likelihood (L) score of the best tree was 2,759, the transition to transversion (ti/tv) ratio was estimated to be 2.50, and the gamma shape parameter (α) was estimated at 0.70.

Transthyretin Intron

There was no indication of saturation for either transitions or transversions (plots not shown), and all taxa were similar in base composition bias (A + T = 0.62), as indicated by a chi-square test ($P = 1.00$, df = 48). Uncorrected p distance measures within species typically ranged from 0% (*Capromys*) to 0.66% (*Galea musteloides*). However, *Microcavia australis* and *A. paca* had large intraspecific differences (3.32% and 2.50%, respectively), of the order of that seen for species differences within genera (0.11% within *Cavia* to 3.55% within *Galea*). Based on these distance measures, *S. taczanowskii* appears to be more similar to the Bolivian *A. paca* (0.76%) than the two *A. paca* specimens are to one another (2.50%). Among genera, distances varied from about 2.0% (*Myoprocta-Dasyprocta*) to 14.0% (*Dasyprocta-Microcavia*).

The TTH data set consisted of 1,004 bp (after exclusion of 3 bp of questionable positional homology; positions 61–63) plus 22 indels. Because the tree topology was not affected by their presence, indels were excluded from subsequent analyses. MP analysis under equal weights yielded one tree of length 604 (CI = 0.82, RI = 0.80; fig. 2*a*). This tree was subsequently used to estimate ML parameters under three different substitution models. The most appropriate model for the ML analysis was GTR + γ. All applicable parameters were estimated, using the heuristic search option and TBR branch swapping with 10 random additions. The $-\ln L$ score of the best tree was 4,439, with $\alpha = 1.46$. This tree topology (fig. 2*b*) was identical to the MP tree.

Mitochondrial 12S rRNA

Stems and loops, both separately and combined, showed no saturation effects for either transitions or

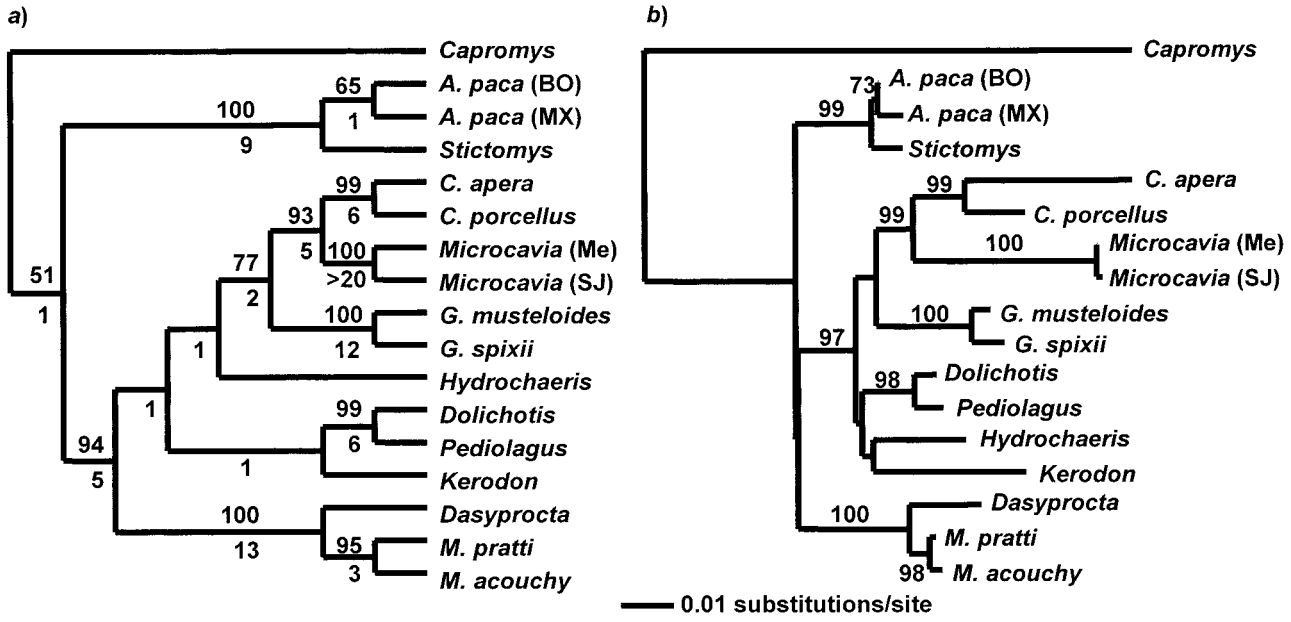

FIG. 1.—(*a*) Single most parsimonious topology resulting from equally weighted MP analysis of exon #10 of the GHR gene. Tree length = 300, CI = 0.85, RI = 0.81. Bootstrap support, based on 1,000 replicates, is given above the nodes (only scores >50% are reported) and Bremer decay indices are given below the nodes. (*b*) ML topology using GHR sequences with an HKY + γ model of evolution ($-\ln L$ = 2,759; ti/tv = 2.5; $\alpha = 0.70$). Branch lengths are scaled to the number of substitutions per site, and bootstrap support (>50%; 100 replicates) for nodes is indicated. This topology is consistent with an equally weighted parsimony tree of length 301.

transversion substitutions. A chi-square test showed no deviation in nucleotide frequencies among taxa (P = 1.00, df = 48), with all taxa having similar patterns for both stems and loops. Uncorrected p distances within species typically ranged from 0% (*Cavia porcellus*, *G. musteloides*, and *Pediolagus salinicola*) to 0.37% (*Hydrochaeris*). *Agouti paca* and *M. australis* had unusually large within-species distances of 1.48% and 3.60%, respectively. Therefore, both specimens of each species were maintained in all subsequent analyses. Species differences within genera ranged from 1.74% (*Cavia* species) to 6.71% (*Galea* species). Among genera, distances were between 6.7% (*Dasyprocta-Myoprocta*) and 14.5% (*Cavia-Agouti*), down to 3.0% if *Stictomys* is included as a distinct genus.

Although the complete 12S rRNA was examined, a total of 161 ambiguous base pairs (57–62, 75–85, 115–121, 161–166, 217–231, 289–293, 299–303, 314–326, 367–382, 477–481, 653–660, 739–746, 751–760, 776–781, 881–915) were removed, leaving 807 bp for phylogenic analysis. The MP analysis, with loops weighted twice as much as stems (accounting for compensatory changes occurring in stem regions), yielded one most parsimonious tree of length 899 (CI = 0.56, RI = 0.59; fig. 3*a*). However, all deep-level nodes were quite unstable, as indicated by bootstrap values. A GTR + γ + Inv model was chosen, using the LRT for nested models of evolution. Tree searches were performed as above, resulting in a best $-\ln L$ score of 3,748, with $\alpha = 0.47$, and the estimated proportion of invariable sites (Inv) 0.39. Because of computational limitations, only 50 bootstrap replicates, using the fast stepwise-addition method, were performed. The ML topology (fig. 3*b*) was inconsistent with all other analyses, with the *Galea* species being located at the base of the Cavioidea clade. However, constraining the 12S rRNA MP topology did not result in a significantly less likely topology ($-\ln L$ = 3,755; KH-test; $P = 0.11$).

Combined Data

The PHT ($P = 0.25$) suggested that the data partitions (12S rRNA, GHR, and TTH) did not have significantly different underlying processes or patterns. Combining the data sets (2,625 bp) yielded a single MP tree of length = 1,490 (CI = 0.72, RI = 0.71). The MP topology was identical to the ML tree generated under a GTR + γ model of evolution, with six rate classes (fig. 4; $-\ln L$ = 11,268; $\alpha = 0.46$). In addition, most nodes were strongly supported (bootstrap support and Bremer decay indices) in the combined MP tree; however, the placement of *Galea* and Dasyproctidae received less support under the ML bootstrap analysis. Forcing Dasyproctidae to be the basal clade was a significantly less likely topology under both MP and ML methods (KH-test; $P < 0.01$ and $P = 0.04$, respectively). The same was true when monophyly of Agoutidae-Dasyproctidae was enforced (KH-test; $P < 0.01$ and $P = 0.03$ for MP and ML, respectively).

Habitat and Behavior

Relationships within Caviinae have been problematic. Therefore, two proposed phylogenies were assessed as templates for Lacher's (1981) environmental constraints hypothesis. The shortest tree consistent with the environmental constraints hypothesis (i.e., a monophyletic Caviinae; fig. 5*a*) is supported by recent morphological work on this group (da Silva Neto 2000). This

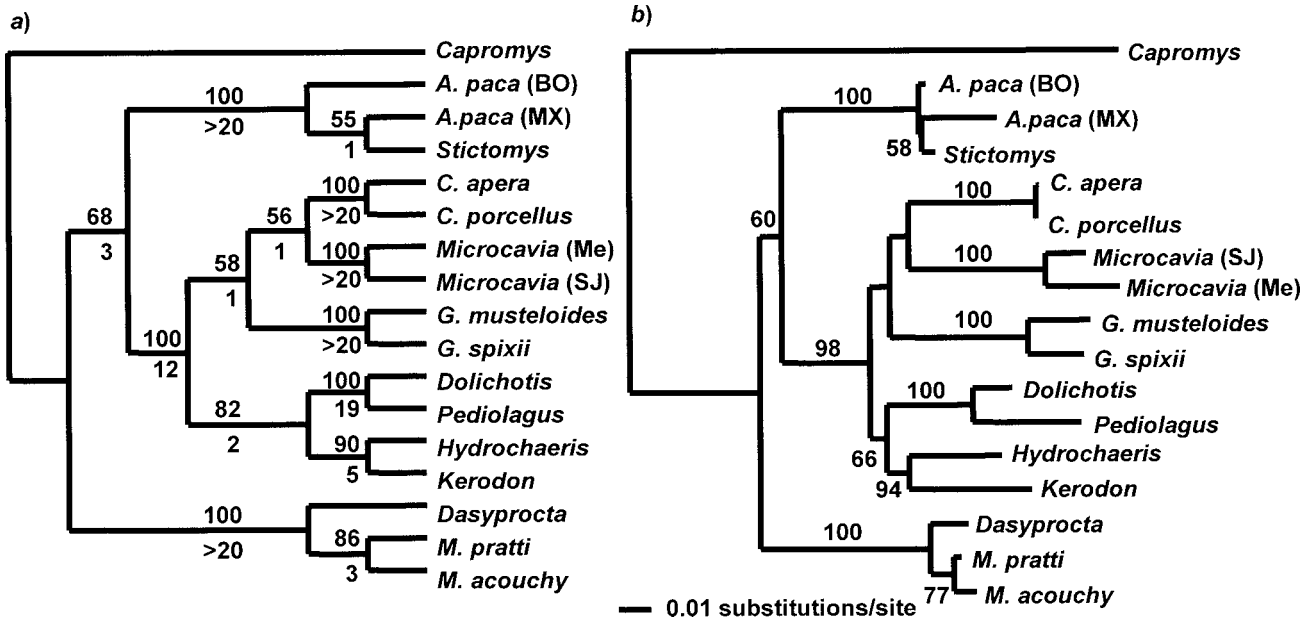

FIG. 2.—(*a*) Single most parsimonious topology resulting from equally weighted MP analysis of intron #1 of the transthyretin gene (TTH). Tree length = 604, CI = 0.82, RI = 0.80. Bootstrap support, based on 1,000 replicates, is given above the nodes (only scores >50% are reported) and Bremer decay indices are given below the nodes. (*b*) ML topology using TTH sequences, with a GTR + γ model of evolution ($-\ln L$ = 4,439; α = 1.46). Branch lengths are scaled to the number of substitutions per site, and bootstrap support (>50%; 100 replicates) for nodes is indicated.

topology was significantly longer and less likely than the molecular phylogeny (fig. 4) for both MP and ML interpretations (KH-tests; $P < 0.01$ and $P = 0.04$, respectively). The same was true for Quintana's (1998) interpretation of caviid relationships (fig. 5*b;* KH-tests; $P < 0.01$ for both MP and ML). Given the results of the concentrated changes test, this morphologically based phylogenetic interpretation supports an environmental constraints explanation for the social behavior of *Kerodon*. Because the concentrated changes test (MacClade 3.1; Maddison and Maddison 1992) is restricted to use of binary characters, habitat (i.e., resource distribution) and behavior (i.e., sociality) were coded accordingly (table 3). Habitat was defined by resource dis-

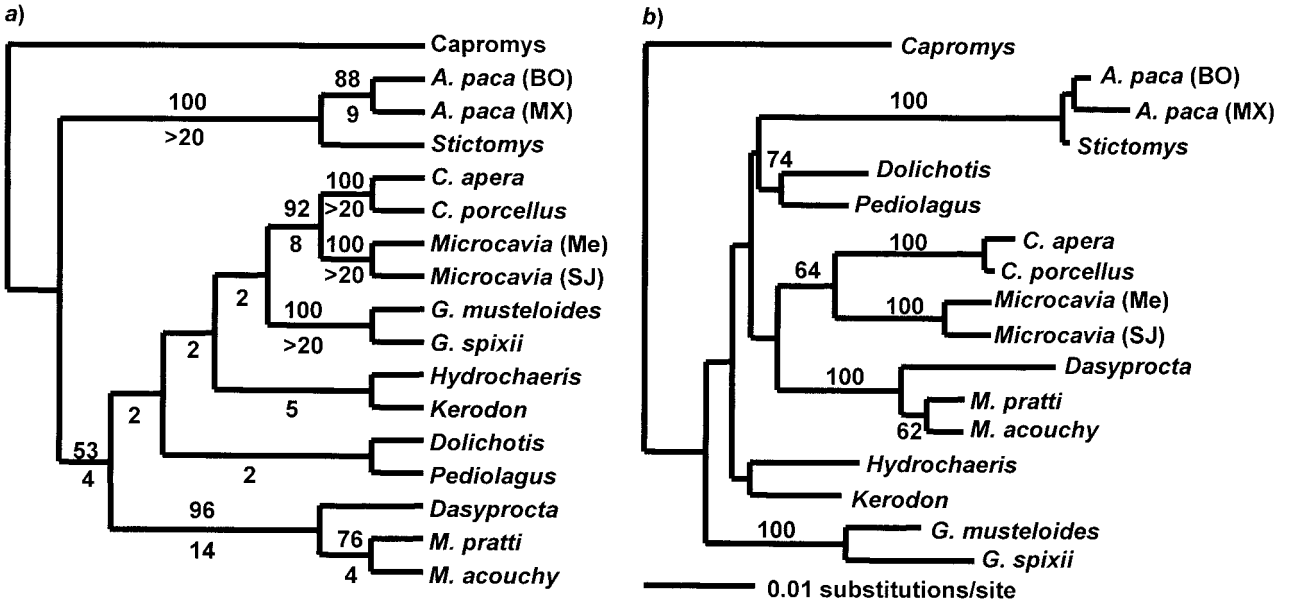

FIG. 3.—(*a*) Single most parsimonious topology resulting from weighted MP analysis of the mitochondrial 12S rRNA gene. Loops were weighted twice as much as stems to counterbalance compensatory changes in stem regions. Tree length = 899, CI = 0.56, RI = 0.59. Bootstrap support, based on 1,000 replicates, is given above the nodes (only scores >50% are reported) and Bremer decay indices are given below the nodes. (*b*) ML topology using 12S rRNA sequences, with a GTR + γ + invariants model of evolution ($-\ln L$ = 3,748; α = 0.47; invariant = 0.39). Branch lengths are scaled to the number of substitutions per site, and bootstrap support (>50%) for nodes is indicated. Because of computational limitations, owing to homoplasy in this data set, only 50 bootstrap replicates were performed.

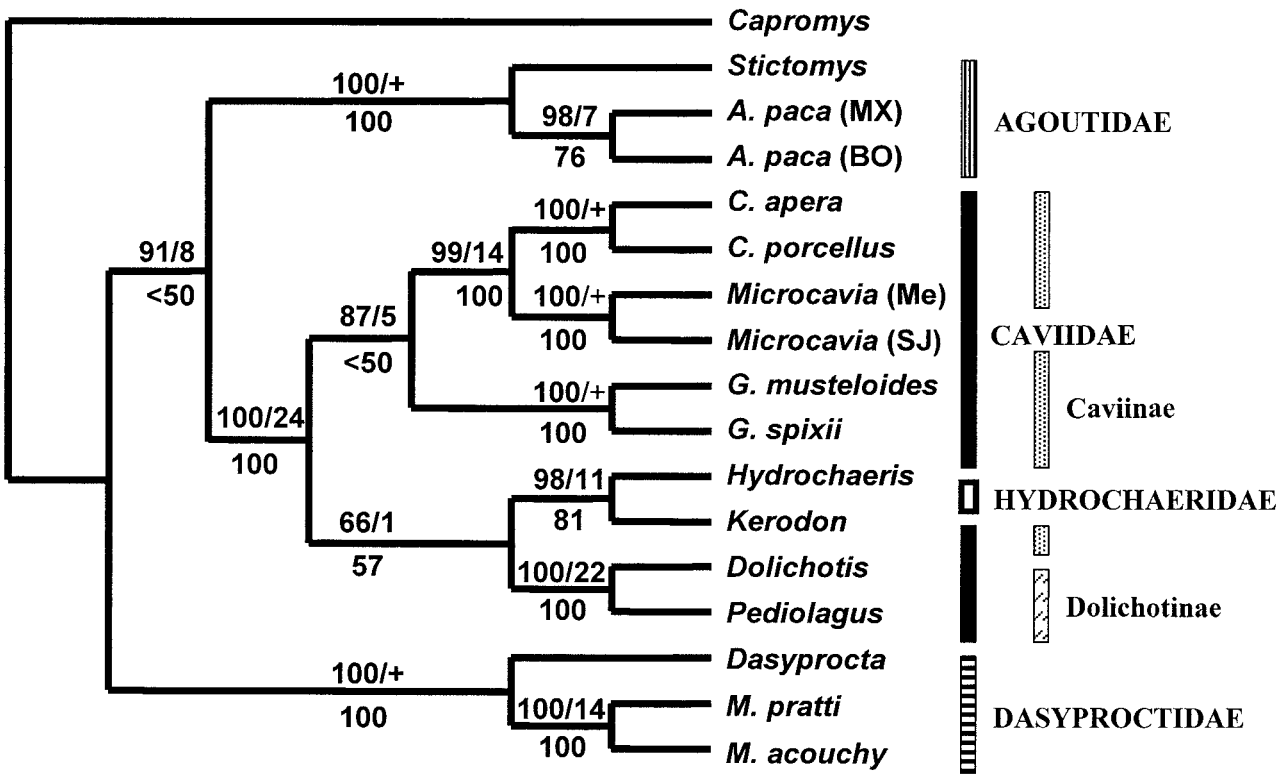

FIG. 4.—Topology for the combined data set of GHR, TTH, and 12S rRNA genes. Both equally weighted MP (length = 1,490; CI = 0.72; RI = 0.71) and ML analysis, using a GTR + γ model of evolution with six rate classes ($-\ln L = 11{,}268$; $\alpha = 0.46$), yielded the same topology. Bootstrap values (based on 100 replicates), followed by Bremer's decay indices, are given above the nodes for the MP analysis. A positive sign (+) indicates Bremer support greater than 20 steps. Bootstrap values below the nodes (based on 100 replicates) correspond to the ML analysis.

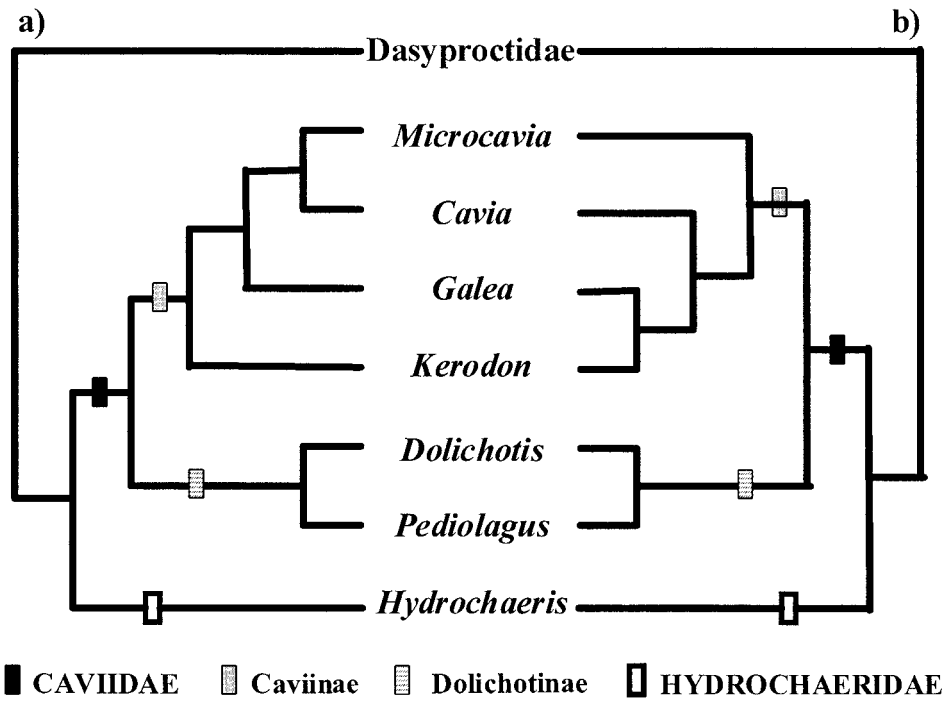

FIG. 5.—(*a*) Proposed phylogenetic relationships for species taxonomically defined as belonging to Caviidae and Hydrochaeridae. This topology is the shortest tree, given the combined molecular data, which is consistent with Cabrera's (1961) taxonomy. This topology is also supported by recent morphological work on the Caviidae (da Silva Neto 2000). (*b*) Phylogenetic relationships for members of the taxonomic designations Caviidae and Hydrochaeridae, based on Cabrera's (1961) taxonomy and Quintana's (1998) morphological evaluation. This is consistent with the relationship patterns assumed in Lacher's (1981) environmental constraints proposal for the evolution of social behavior in the Caviidae.

Table 3
Habitat and Behavioral Traits Coded as Binary Characters

Genus	Habitat[a,c]	Behavior[b,c]	Reference
Cavia	G	N	Sacher (1998)
Dolichotis	S	S	Taber and Macdonald (1992)
Galea	G	N	Lacher (1981)
Hydrochaeris . . .	S	S	Macdonald (1981)
Kerodon	S	S	Lacher (1981)
Microcavia	G	N/S	Rood (1970)
Pediolagus	S	S	Nowak (1999)

[a] Habitat: G = generalist; S = specialist.

[b] Behavior: N = nonsocial; S = social.

[c] Classification of habitats is determined by resource distribution, with clumped and monopolizable resources classified as habitat specialists. Behavior is classified as social or nonsocial based on interactions between males and females. Social species are those with recurring interactions between specific individuals.

tribution, with clumped and defensible resources (i.e., food or denning sites) considered habitat specialization. Although some species display colonial habits, their resources are not necessarily clumped (e.g., *Cavia* and *Galea*). The same is true of social behavior. Colonial species may display amicable relations, but the key factor in sociality, as defined here, is whether actions are recurrent between specific individuals. By the definition used here, interactions must be recurring in a predictable fashion (i.e., within a season or between seasons) in order to be considered a social species. Given these requirements, the evolution of sociality had two equally parsimonious patterns, given the morphological tree (fig. 5*b*) and assuming *Microcavia* to be social. The most parsimonious explanation involved either two independent losses of sociality or one gain and one loss of social behavior. If *Microcavia* was assumed to be nonsocial, the interpretation involved one loss and one gain of sociality. In either case, the probability of habitat specialization (i.e., environmental constraints) influencing the presence of social behavior was relatively high. The probability of the habitat-behavior association occurring by chance on this phylogeny was 0.20 (two independent gains scenario) or 0.24 (one gain and one loss scenario), assuming *Microcavia* to be social. Assuming *Microcavia* is nonsocial, the probability of a one gain-one loss scenario occurring by chance is also low (25%), considering the small sample size. A resource-sociality pattern consistent with da Silva Neto's (2000) (fig. 5*a*) and our molecular phylogeny (fig. 4) were much less likely, given the fact that the probability of association occurring by chance on these phylogenies was 0.56 and 0.58, respectively.

Rate Heterogeneity

Statistical support for rate heterogeneity among lineages was obtained using both ML and relative rate methods. For all genes, the clock-constrained trees had significantly lower likelihood scores than their nonconstrained counterparts (table 4), indicating the presence of some lineages that deviated from rate homogeneity. Tajima's RRT (Tajima 1993) indicated numerous rate differences between pairs of taxa for all three genes assessed (table 5). The genera *Agouti*, *Cavia*, and *Microcavia* appeared to account for most of the rate heterogeneity in both nuclear data sets. The Tajima test detected no significantly heterogeneous lineages for 12S rRNA after Bonferroni correction. This is not surprising given that this test is known to be ineffective at low rates of substitution where few changes in the ingroup have occurred. The Tajima test is unlikely to detect even moderate levels of rate variation for sequences with few-

Table 4
Likelihood Ratio Tests (LRTs) and Assessment of Overall Rate Heterogeneity Among Taxa

Gene	LRT[a] (df = 15)	TCT[b,c] (df = 15)	Clades Contributing to Rate Heterogeneity and Their Rate Relationships[d]	*P*-value
GHR	$P < 0.001$	$Q = 139$	4 > 5	<0.01
		$P < 0.0001$	11 > 10	<0.01
			4-5-12-13 > 8-9	0.04
			4-5-12-13-8-9- > 10-11	<0.01
			4-5-12-13-8-9-10-11 > 7-16	<0.01
			4-5-12-13-8-9-10-11-7-16 > 6-14-15	0.02
			4-5-6-7-8-9-10-11-12-13-14-15-16 > 1-2-3	0.02
TTH	$P < 0.001$	$Q = 69$	2 > 3	0.02
		$P < 0.001$	16 > 7	<0.01
			2-3 > 1	0.02
			12-13 > 4-5	0.01
			4-5-7-8-9-10-11-12-13-16 > 1-2-3	<0.01
			1-2-3-4-5-7-8-9-10-11-12-13-16 > 6-14-15	<0.01
12S rRNA	$P < 0.001$	$Q = 99$	10 > 11	<0.01
		$P < 0.001$	6 > 14-15	<0.01
			6-14-15 > 7-16	<0.01
			4-5-8-9-12-13 > 6-14-15-7-16-10-11	<0.01

[a] Felsenstein (1981).

[b] Two cluster test (TCT) identifies overall rate heterogeneity as well as clades with significant rate differences (Takezaki, Rzhetsky, and Nei 1995).

[c] *Q*-values, for overall rate heterogeneity, *P*-values determined from a chi-square distribution.

[d] Numbers correspond to taxa, as identified in table 5.

Table 5
Tests for Rate Heterogeneity

Taxa Number	Name	Tajima's RRT (df = 15)[a]			BLT (df = 15)[b]; *P*-value, Rate of Evolution		
		GHR	TTH	12S	GHR	TTH	12S
1	*Agouti paca* (Bolivia)	4,12,13	2,16	NS	<0.001, −	0.01, −	NS
2	*Agouti paca* (Mexico)	4	1,3	NS	<0.001, −	NS	NS
3	*Stictomys taczanowskii*	4	2	NS	<0.001, −	0.06, −	NS
4	*Cavia apera*	1,2,3,7,16	NS	NS	<0.001, +	NS	NS
5	*Cavia porcellus*	NS	NS	NS	NS	NS	NS
6	*Dasyprocta punctata*	NS	NS	NS	NS	0.10, −	NS
7	*Dolichotis patagonum*	4	NS	NS	0.04, −	NS	<0.001, −
8	*Galea musteloides*	NS	NS	NS	NS	NS	NS
9	*Galea spixii*	NS	NS	NS	NS	NS	NS
10	*Hydrochaeris hydrochaeris*	NS	NS	NS	NS	NS	NS
11	*Kerodon rupestris*	NS	NS	NS	NS	NS	<0.001, −
12	*Microcavia australis* 1	1	NS	NS	<0.001, +	0.10, +	NS
13	*Microcavia australis* 2	1	NS	NS	<0.001, +	0.01, +	NS
14	*Myoprocta acouchy*	NS	NS	NS	NS	0.02, −	NS
15	*Myoprocta pratti*	NS	NS	NS	NS	0.04, −	NS
16	*Pediolagus salinicola*	4	1	NS	NS	0.01, +	<0.001, −

NOTE.—*P*-values are reported, followed by a sign indicating a rate increase (+) or a rate decrease (−) in comparison to an average rate for all taxa. Nonsignificant results are denoted NS.

[a] Tajima's (1993) relative rate test (RRT); Numbers for the RRT refer to taxonomic designations, identifying lineages with significantly different rates of evolution after Bonferroni correction for multiple testing.

[b] Takezaki, Rzhetsky, and Nei's (1995) branch length test (BLT). The models used for the BLT were; GHR data using HKY + γ (α = 0.70), TTH data using Tamura Nei + γ (α = 1.5), and 12S rRNA data using Tamura Nei + γ (α = 0.47).

er than 400 sites free to vary, as is the case here. Takezaki, Rzhetsky, and Nei's (1995) BLT was used not only to identify lineages that did not show rate homogeneity but also to assess whether rates were slow or fast. When the appropriate model (i.e., the one chosen for ML analysis) was not available, then the most complex model allowable was implemented (see table 5). For both nuclear genes, *Agouti* was identified as a slowly evolving lineage, whereas *Microcavia* was rapidly evolving. The pattern for the 12S rRNA data set was strikingly different, with *Dolichotis*, *Kerodon*, and *Pediolagus* identified as significantly slower than average. Because of the high rate of type-II errors (i.e., failure to detect a difference in rate) associated with RRTs, any lineage identified as deviating from rate constancy should be considered to represent a substantial amount of rate variation. RRTs also are limited by their ability to compare only two taxa. The TCT has the advantage of detecting concerted acceleration or deceleration in rates across multiple lineages. In effect, the TCT was used to identify all lineages deviating from rate homogeneity. In general, Dasyproctidae and Agoutidae were identified as slowly evolving, whereas the Caviidae and Hydrochaeridae demonstrated rate acceleration. For GHR, there was additional rate heterogeneity within the Caviidae (table 4).

Prior to testing for a correlation between life-history variables and substitution rate, each variable was first assessed for independence of phylogeny in an effort to minimize statistical error and to compensate for the small number of taxa examined (Gittleman and Luh 1994; Hansen and Martins 1996). TFSI (1,000 randomizations) indicated no statistically significant relationship between life-history variables and the given phylogeny. Because the TFSI has been shown to be prone to type-II error (Martins 1996), the a priori probability was set to $P = 0.10$ instead of $P = 0.05$. Although body mass tends to be strongly correlated with its phylogenetic history (Gittleman et al. 1996; Abouheif and Fairbairn 1997), such a pattern was not detected in this study. Although TFSI is not dependent on a particular model of evolutionary change and does not require branch length information, it does assume that the phylogeny is known. Given the results of the TFSI (log body size, $P > 0.38$; gestation time, $P > 0.11$; metabolic rate, $P > 0.10$), straightforward statistical methods were deemed appropriate for assessing the relationship of rate heterogeneity with these life-history variables. Spearman rank correlations (Sokal and Rohlf 1998) revealed significant relationships ($P \leq 0.02$; table 6) between

Table 6
Spearman Rank Correlations and Assessment of Rate Heterogeneity Relative to Life History Traits

Gene	log Body Size	Gestation Time	Metabolic Rate
GHR			
P-value	0.020*	0.020*	0.437
Tied *P*-value.....	0.018*	0.018*	0.463
Association.....	Negative	Negative	NR
TTH			
P-value	0.016*	0.002*	0.925
Tied *P*-value.....	0.016*	0.002*	0.943
Association.....	Negative	Negative	NR
12S rRNA			
P-value	0.527	0.928	0.437
Tied *P*-value.....	0.523	0.932	0.447
Association.....	NR	NR	NR

NOTE.—Statistically significant results are indicated by an *, and values having a $P > 0.05$ indicate that the association of the relationship is not relevant (NR).

rates of molecular evolution and both log body size (GHR and TTH) and gestation time (GHR and TTH) but no significant relationship with metabolic rate. In all cases, the relationships were negative, suggesting an association between shorter branch lengths and both larger body sizes and longer gestation times. Because there is a significant functional relationship between body size and gestation time ($R^2 = 0.75$, $P < 0.01$), a partial correlation analysis was performed to remove the effects of body size. The correlation remained significant for TTH ($P < 0.01$) but not for GHR ($P = 0.30$). Body size also was a potentially confounding variable in the analysis of metabolic rate. Body size appears to be a reliable predictor of metabolic rate ($R^2 = 0.78$, $P < 0.01$), and its affects were therefore removed using a partial correlation. The correlation of metabolic rate with branch lengths, however, remained nonsignificant.

To assess whether the patterns of rate heterogeneity were similar for the different genes analyzed, correlation statistics were implemented. Rates of molecular evolution in GHR and TTH appeared to be coupled. The BLT (see Omland 1997) revealed a positive correlation ($n = 30$, $P < 0.05$; Pearson's $r = 0.54$; Spearman's $r = 0.55$) between the two data sets. A positive correlation in total evolution also existed ($n = 16$, $P < 0.01$; Pearson's $r = 0.71$; Spearman's $r = 0.80$), and the binomial test of clade contrasts was significant at $P < 0.10$ (sign-test; $X \geq 10$, $n = 15$; $P = 0.09$).

Discussion

Taxonomic Relationships

The diagnosis of phylogenetic relationships among caviomorph rodent lineages is complicated by convergent and parallel evolution associated with morphological and serological features, as well as an abundance of autapomorphic characters (Patterson and Wood 1982; Hartenberger 1985; Jaeger 1988), owing to their rapid and extensive radiation during the late Eocene (Wyss et al. 1993). The superfamily Cavioidea has traditionally been divided into three or four families: Caviidae, Hydrochaeridae, Dasyproctidae (Corbet and Hill 1991; Wilson and Reeder 1993), and sometimes Agoutidae (McKenna and Bell 1997). Conflicts over the number of recognized families relate to the status of *Agouti* and *Stictomys*. These genera are sometimes assigned to the family Agoutidae (e.g., Anderson and Jones 1984) and at other times placed with *Dasyprocta* and *Myoprocta* in the Dasyproctidae. Familial and generic designations as well as relationships among these families and their respective genera have been unresolved (Hartenberger 1985; Nedbal, Allard, and Honeycutt 1994).

This is the first detailed phylogenetic analysis of the entire superfamily Cavioidea. Previous studies were either broader in scope, with sparse taxonomic sampling within Cavioidea (e.g., Woods 1982; Wallau, Schmitz, and Perry 2000; Huchon and Douzery 2001), or focused primarily on relationships within the family Caviidae with a priori designation of Hydrochaeridae and Dasyproctidae as outgroups (e.g., Quintana 1998; da Silva Neto 2000). In this study, all 11 genera (Cabrera 1961), six of which are monotypic, are represented. Although sometimes included within the superfamily Cavioidea (Patterson and Wood 1982; McKenna and Bell 1997), based on morphological evidence, the monotypic Dinomyidae (*Dinomys branickii*) has been excluded from this analysis. Because of its taxonomic inconsistency and uncertain phylogenetic affiliation as a member of the ingroup, Cavioidea, or possibly as a member of any closely related outgroup (see White and Alberico 1992), it is inappropriate to include this taxon in our analysis (see Swofford et al. 1996). Our exclusion of *Dinomys* is further supported by recent molecular phylogenetic analyses suggesting that this taxon is not a member of a monophyletic Cavioidea (D. L. Rowe and R. L. Honeycutt, unpublished data; Adkins et al. 2001; Huchon and Douzery 2001). Preliminary analyses (D. L. Rowe and R. L. Honeycutt, unpublished data) suggest Octodontoidea as the sister group to Cavioidea. However, in only one case (12S rRNA) did outgroup selection (Erethizontoidea vs. Chinchilloidea) influence ingroup relationships. *C. piliroides* (an octodontid) thus served as a single outgroup taxon.

The general congruence among the independent molecular data sets, lack of multiple most parsimonious topologies, overall consistency under different methods of analysis, and strong support for all nodes (bootstrap and Bremer decay indices) in the combined analysis imply a robust phylogeny. Both separate and combined analyses provide strong support for the nontraditional placement of the family Hydrochaeridae within the Caviidae, rendering the family Caviidae paraphyletic. Furthermore, our data suggest a paraphyletic Caviinae because of the placement of *Kerodon*, a member of the Caviinae, with the subfamily Dolichotinae. Interestingly, *Hydrochaeris* and *Kerodon* consistently group as sister taxa, a relationship previously suggested by Woods (1984) and dos Reis (1994). This is, however, inconsistent with the majority of morphological interpretations and is somewhat surprising, given the readily apparent differences in morphology and life-history strategies. *Kerodon* is a small-bodied rodent, adapted to rocky outcrops in arid environments, and has small litter sizes (1–3, average = 1.5). On the contrary, *Hydrochaeris* is a very large-bodied aquatic specialist with adaptations for a semiaquatic lifestyle, and has large litter sizes (1–8, average = 5) in relation to other caviomorph rodents (Mares and Genoways 1982).

The data also strongly support designation of two separate families, Agoutidae and Dasyproctidae. Enforcing monophyly, using the combined data set, results in significant changes in tree scores; KH-tests are highly significant for both MP and ML. Although the position of the two families within the Cavioidea clade differs with the three independent data sets, the combined data lends relatively strong support to the position of Agoutidae at the base of the cavioid clade. Enforcing a topology with Dasyproctidae as the basal clade resulted in significant changes in tree scores.

Our findings with regard to the genera *Agouti* and *Stictomys* warrants further phylogeographic investigation. Although five subspecies of *Agouti* have been rec-

ognized (Cabrera 1961), the preliminary data here suggest that the *A. paca* specimens from Mexico and Bolivia may be as genetically distinct from one another as either is from *Stictomys*.

Habitat and Behavior

The evolution of morphological and behavioral differences in the family Caviidae has been proposed to be strongly linked to variable habitat requirements with species occupying habitats characterized by restricted resources (i.e., patchy distribution) or increased susceptibility to predation demonstrating a tendency toward increased sociality (Lacher 1981). For instance, most members of the subfamily Caviinae (e.g., *Galea*, *Microcavia*, and *Cavia*) share numerous morphological similarities and lack recurring pairbonds. For the most part, these three genera occupy productive and diverse habitats with abundant food and shelter. Individuals are unable to monopolize resources, tend to disperse, and demonstrate low social tolerance (i.e., social interactions do not occur repeatedly between specific males and females). *Kerodon*, a member of the Caviinae, occupies areas where resources are distributed among habitat patches (e.g., rock piles). Unlike other members of the Caviinae, *Kerodon* demonstrates a harem-based mating system and high social tolerance. Members of the subfamily Dolichotinae occur in high plains, deserts, and open grasslands of southern South America. They are highly adapted for cursorial life, their offspring are vulnerable to predation, and adequate den sites are limited (i.e., patchily distributed). Presumably, as a consequence of this increased risk to predation and resource limitations in terms of den sites, several mated pairs use a single den site for raising pups, and these pairs demonstrate high social tolerance (Taber and Macdonald 1992). Based on the assumption that *Kerodon* is a member of the Caviinae, Lacher (1981) suggested that the social structure shared between *Kerodon* and members of the Dolichotinae were the consequence of habitat constraints resulting from increased risk of predation and the distribution of resources. If one accepts the traditional taxonomy (fig. 5*b*), whereby *Kerodon* is phylogenetically part of the Caviinae, the concentrated changes test supports Lacher's (1981) contention that environmental constraints have contributed to the evolution of social behavior in this assemblage of rodents.

In contrast, the molecular phylogeny (fig. 4) and the shortest tree consistent with a monophyletic Caviinae (see da Silva Neto 2000) (fig. 5*a*) suggest that sociality did not have two independent origins in response to similar environmental constraints. The molecular data suggest that *Kerodon* is a member of a behaviorally social, monophyletic clade (*Dolichotis*, *Pediolagus*, *Kerodon*, and *Hydrochaeris*). Interestingly, *Kerodon* and *Hydrochaeris* are sister taxa and both have harem-based polygynous breeding systems (Lacher 1981; Macdonald 1981). Like *Kerodon*, *Hydrochaeris* is a habitat specialist (i.e., open water is a patchily distributed resource, especially during the dry season). Although all of the highly social taxa within this rodent assemblage are habitat specialists, they are also all members of a monophyletic clade. The concentrated changes test suggests that the probability of sociality mirroring habitat specialization is quite likely by chance alone in this phylogeny, suggesting that the ancestor to this clade could have been highly social, allowing occupation of harsh environmental niches with patchily distributed resources or high predation pressure (or both). To better ascertain the potential importance of environmental constraints on social behavior, a broader taxonomic sampling must be encompassed (e.g., the entire caviomorph radiation), in which social behavior has potentially arisen multiple times.

Rate Heterogeneity

RRTs and LRTs detected significant rate heterogeneity among taxa in all three independent data sets. TFSI, measuring the degree of nonrandomness in continuous variables, suggested that the life-history traits assessed (log body size, gestation time, and metabolic rate) were not strongly correlated with their phylogenetic histories. Correlation analyses provided no support for the metabolic rate hypothesis, with even the mitochondrial gene failing to show a relationship between metabolic rate and variation in rates of molecular evolution. The 12S rRNA branch lengths also showed no significant correlation with body mass or gestation time. However, our inability to detect a clear pattern may be the result of the complex substitution pattern observed for 12S rRNA. Stems and loops demonstrate very different patterns, owing to compensatory changes occurring in stem regions, indels in the loop regions, rapid saturation of transitions in loops or heterogeneity in ti/tv bias (or all) between the two regions (Nedbal, Honeycutt, and Schlitter 1996).

Both the GHR (nuclear exon) and TTH (intron) rates were negatively correlated with gestation time and average adult body mass. This is consistent with the generation time hypothesis, with longer generation times (measured here as gestation time) being associated with slower rates of evolution. However, the significant functional relationship between body size and gestation time makes it difficult to assess exactly what factors are contributing to the rate heterogeneity. If body size effects are controlled, then a significant correlation with gestation time is seen for TTH but not for GHR. The difference between GHR and TTH could arguably be attributed to selective constraints imposed on the coding gene. Third positions are theoretically more likely to be independent of selective constraints and may be expected to show a more similar pattern to the TTH intron. However, most changes in GHR were at third positions, and using only those changes did not significantly alter branch length estimations (i.e., clock minus no-clock values remain negative or positive; data not shown). This suggests that generation time effects may be more pronounced in the patterns seen in the TTH intron, whereas body size effects may be primarily responsible for the rate patterns seen in GHR (see Bromham, Rambaut, and Harvey 1996). Evidence for a body-mainte-

nance effect has been proposed, whereby maintenance of a large body (more cells and cell generations than a small body) necessitates a higher degree of DNA copy fidelity and repair (Promislow 1994; Bromham, Rambaut, and Harvey 1996). Others also have suggested that the cost of change in replication fidelity may vary with life history or genome size, with the total energetic cost likely to be greater in species with larger genome sizes (Drake et al. 1998).

Overall, there appears to be a single underlying mechanism or multiple mechanisms acting simultaneously in a concerted manner. The significant branch length correlation and total evolution correlation (Omland 1997) suggest that rates are variable within but correlated between the data sets. More simply, there is rate heterogeneity among lineages in both GHR and TTH data sets, and this variability is occurring in a similar pattern for both genes. The total evolution contrasts provide further support for the observed similarity in patterns of rate heterogeneity.

Our data support the hypothesized (Li, Tanimura, and Sharp 1987; Ohta 1993; Mooers and Harvey 1994; Li et al. 1996) trend toward slower rates of molecular change in taxa with longer generation times. Consistent with the generation time hypothesis, the nuclear data sets showed a pronounced gestation time effect, and possible body size effect, on molecular evolution but no effect of metabolic rate. This is consistent with the nature of nuclear DNA replication; nuclear replication is linked to cell division, which is often correlated with body size and generation time (Bromham, Rambaut, and Harvey 1996), whereas mitochondrial DNA can replicate independently of cell division (often many times during the lifetime of a cell). The majority of the rate heterogeneity detected here was attributable to silent changes in that they involved an intron for TTH and primarily changes at the third codon position for GHR. These observations suggest that differences in mutation rate are contributing to molecular rate variation.

Supplementary Material

All sequences used in this paper have been deposited in GenBank and assigned the following accession numbers: (1) GHR: AF433927–AF433949; (2) TTH: AF433880–AF433903; (3) 12S rRNA: AF433905–AF433926. Alignments for GHR exon #10 (ALIGN_000234), TTH intron #1 (ALIGN_000235), and 12S rRNA (ALIGN_000236) have been submitted to the EMBL-Align database. Information about specimens examined, including specific localities, museum catalogue numbers, and sources of materials, is provided on the Molecular Biology and Evolution website.

Acknowledgments

We thank several individuals and institutions for generously providing tissue samples: R. J. Baker, Texas Tech University Museum; L. P. Costa, Museum of Vertebrate Zoology, University of California, Berkeley; R. C. Dowler, Angelo State Natural History Collections, Angelo State University; A. Harlin, Texas A&M University; C. W. Kilpatrick, University of Vermont; E. Louis, Henry Doorley Zoo, NE; Michael Mares and Janet Braun, Sam Noble Oklahoma Museum of Natural History; K. Parr, Cleveland Metroparks Zoo; N. Spangler, Department of Pathology, Smithsonian National Zoological Park; and T. L. Yates, Museum of Southwest Biology, University of New Mexico. We would also like to thank C. von Dohlen, P. Wolf, and associated students for their comments on the manuscript. J. Bielawski, two anonymous reviewers, and D. Irwin also provided helpful comments. This research was supported by the National Science Foundation Grant DEB 9615163 to R.L.H. This paper represents contribution # 96 of the Center for Biosystematics and Biodiversity at Texas A&M University.

LITERATURE CITED

ABOUHEIF, E. 1999. A method for testing the assumption of phylogenetic independence in comparative data. Evol. Ecol. Res. **1**:895–909.

ABOUHEIF, E., and D. J. FAIRBAIRN. 1997. A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. Am. Nat. **149**:540–562.

ADKINS, R. M., E. L. GELKE, D. ROWE, and R. L. HONEYCUTT. 2001. Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. Mol. Biol. Evol. **18**:777–791.

ANDERSON, S., and J. K. JONES JR. 1984. Orders and families of recent mammals of the world. John Wiley & Sons, New York.

BIBB, M. J., R. A. VANETTEN, C. T. WRIGHT, M. W. WALBERG, and D. A. CLAYTON. 1981. Sequence and gene organization of mouse mitochondrial DNA. Cell **26**:167–180.

BICKEL, D. R. 2000. Implications of fluctuations in substitution rates: impact on the uncertainty of branch lengths and on relative-rate tests. J. Mol. Evol. **50**:381–390.

BREMER, K. 1994. Branch support and tree stability. Cladistics **10**:295–304.

BROMHAM, L., D. PENNY, A. RAMBAUT, and M. D. HENDY. 2000. The power of relative rates tests depends on the data. J. Mol. Evol. **50**:296–301.

BROMHAM, L., A. RAMBAUT, and P. H. HARVEY. 1996. Determinants of rate variation in mammalian DNA sequence evolution. J. Mol. Evol. **43**:610–621.

BURDA, H., R. L. HONEYCUTT, S. BEGALL, O. LOCKER-GRÜTJEN, and A. SCHARFF. 2000. Are naked and common mole-rats eusocial and if so, why? Behav. Ecol. Sociobiol. **47**:293–303.

CABRERA, A. 1961. Catálogode los mamíferos de America del Sur. II. Rev. Mus. Argentino Cien. Nat. 'Bernardino Rivadavia' **4**:1–732.

CABRERA, A., and J. YEPES. 1960. Mamíferos Sud-Americanos. Historia Natural Ediar, Buenos Aires, Argentina.

CALDER, W. A. III. 1984. Size, function, and life history. Harvard University Press, Cambridge, Mass.

CORBET, G. B., and J. E. HILL. 1991. A world list of mammalian species. 3rd edition. Oxford University Press, Oxford, U.K.

CRESPI, B. J. 1996. Comparative analysis of the origins and losses of eusociality: causal mosaics and historical uniqueness. Pp. 253–287 *in* E. P. Martins, ed. Phylogenies and the comparative method in animal behavior. Oxford University Press, Oxford, U.K.

CUNNINGHAM, C. W. 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. **14**: 733–740.

DA SILVA NETO, E. J. 2000. Morphology of the regiones ethmoidalis and orbitotemporalis in *Galea musteloides* Meyen 1832 and *Kerodon rupestris* (Weid-Neuweid 1820) (Rodentia Caviidae) with comments on the phylogenetic systematics of the Caviidae. J. Zool. Syst. Evol. Res. **38**:219–229.

DOS REIS, S. F. 1994. Allometry, convergence of ontogenetic trajectories and cranial evolution in caviine rodents (Rodentia: Caviinae). J. Morphol. **220**:342.

DRAKE, J. W., B. CHARLESWORTH, D. CHARLESWORTH, and J. F. CROW. 1998. Rates of spontaneous mutation. Genetics **148**:1667–1686.

EASTEAL, S. 1990. The pattern of mammalian evolution and the relative rate of molecular evolution. Genetics **124**:165–173.

EISENBERG, J. F. 1981. The mammalian radiations. The University of Chicago Press, Chicago.

———. 1989. Mammals of the neotropics. The northern neotropics, Vol. 1. The University of Chicago Press, Chicago.

FAITH, D. P., and L. BELBIN. 1994. Distinguishing phylogenetic effects in multivariate models relating Eucalyptus convergent morphology to environment. Pp. 169–188 *in* P. Eggleton and D. Vane-Wright, eds. Phylogenetics and ecology. Academic Press, London.

FELSENSTEIN, J. 1981. Evolutionary trees and DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17**:368–376.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783–791.

———. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. **22**:521–565.

FUTUYMA, D. J., and G. MORENO. 1988. The evolution of ecological specialization. Annu. Rev. Ecol. Syst. **19**:207–233.

GISSI, C., A. REYES, G. PESOLE, and C. SACCONE. 2000. Lineage-specific evolutionary rate in mammalian mtDNA. Mol. Biol. Evol. **17**:1022–1031.

GITTLEMAN, J. L., C. G. ANDERSON, M. KOT, and H.-K. LUH. 1996. Phylogenetic lability and rates of evolution: a comparison of behavioral, morphological and life history traits. Pp. 166–205 *in* E. P. Martins, ed. Phylogenies and the comparative method in animal behavior. Oxford University Press, Oxford, U.K.

GITTLEMAN, J. L., and H.-K. LUH. 1994. Phylogeny, evolutionary models and comparative methods: a simulation study. Pp. 103–122 *in* P. Eggleton and D. Vane-Wright, eds. Phylogenetics and ecology. Academic Press, London.

HANSEN, T. F., and E. P. MARTINS. 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. Evolution **50**: 1404–1417.

HARTENBERGER, J.-L. 1985. The order Rodentia: major questions on their evolutionary origin, relationships and suprafamilial systematics. NATO ASI Ser. A Life Sci. **92**:1–33.

HARVEY, P. H., and M. D. PAGEL. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, U.K.

HASEGAWA, M., J. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. **21**:160–174.

HUCHON, D., F. M. CATZEFLIS, and E. J. P. DOUZERY. 1999. Molecular evolution of the nuclear von Willebrand factor gene in mammals and the phylogeny of rodents. Mol. Biol. Evol. **16**:577–589.

HUCHON, D., and E. J. P. DOUZERY. 2001. From the Old World to the New World: a molecular chronicle of the phylogeny and biogeography of hystricognath rodents. Mol. Phylogenet. Evol. **20**:238–251.

IRWIN, R. E. 1996. The phylogenetic content of avian courtship display and song evolution. Pp. 234–252 *in* E. P. Martins, ed. Phylogenies and the comparative method in animal behavior. Oxford University Press, Oxford, U.K.

JAEGER, J. J. 1988. Rodent phylogeny: new data and old problems. Pp. 177–199 *in* M. J. Benton, ed. The phylogeny and classification of the tetrapods, Vol. 2. Mammals. Clarendon Press, New York.

KISHINO, H., and M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. **29**:170–179.

KLEIMAN, D. G. 1974. Patterns of behavior in hystricomorph rodents. Symp. Zool. Soc. Lond. **34**:171–209.

KLEIMAN, D. G., J. F. EISENBERG, and E. MALINIAK. 1979. Reproductive parameters and productivity in caviomorph rodents. Pp. 173–183 *in* J. F. Eisenberg, ed. Vertebrate ecology in the northern neotropics. Smithsonian Institution Press, Washington, D.C.

LACHER, T. E. JR. 1981. The comparative social behavior of *Kerodon rupestris* and *Galea spixii* and the evolution of behavior in the Caviidae. Pp. 1–71 *in* H. H. Genoways, D. A. Schlitter, and S. L. Williams, eds. Bull. Carnegie Mus. Nat. Hist. No. 17. Trustees of Carnegie Institute, Pittsburgh, Penn.

LI, W.-H., D. L. ELLSWORTH, J. KRUSHKAL, B. H.-J. CHANG, and D. HEWETT-EMMETT. 1996. Rates of nucleotide substitution in primates and rodents and the generation-time effect hypothesis. Mol. Phylogenet. Evol. **5**:182–187.

LI, W.-H., M. GOUY, P. M. SHARP, C. O'HUIGIN, and Y.-W. YANG. 1990. Molecular phylogeny of Rodentia, Lagomorpha, Primates, Artiodactyla, and Carnivora and molecular clocks. Proc. Natl. Acad. Sci. USA **87**:6703–6707.

LI, W.-H., M. TANIMURA, and P. M. SHARP. 1987. An evaluation of the molecular clock hypothesis using mammalian DNA sequences. J. Mol. Evol. **25**:330–342.

LOVEGROVE, B. G. 2000. The zoogeography of mammalian BMR. Am. Nat. **156**:201–219.

MACDONALD, D. W. 1981. Dwindling resources and the social behavior of capybaras (*Hydrochoerus hydrochaeris*) (Mammalia). J. Zool. Lond. **194**:371–391.

MADDISON, W. P., and D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, Mass.

MARES, M. A., and H. H. GENOWAYS. 1982. Mammalian biology in South America, Vol. 6. Special Publication Series. Pymatuning Laboratory of Ecology, University of Pittsburgh Press, Pittsburgh, Penn.

MARTIN, A. P. 1995. Mitochondrial DNA sequence evolution in sharks: rates, patterns, and phylogenetic inferences. Mol. Biol. Evol. **12**:1114–1123.

MARTIN, A. P., and S. R. PALUMBI. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. USA **90**:4087–4091.

MARTINS, E. P. 1996. Phylogenies, spatial autoregression, and the comparative method: a computer simulation test. Evolution **50**:1750–1765.

MCKENNA, M. C., and S. K. BELL. 1997. Classification of mammals above the species level. Columbia University Press, New York.

MINDELL, D. P., A. KNIGHT, C. BAER, and C. J. HUDDLESTON. 1996. Slow rates of molecular evolution in birds and the metabolic rate and body temperature hypotheses. Mol. Biol. Evol. **13**:422–426.

MOOERS, A. Ø., and P. H. HARVEY. 1994. Metabolic rate, generation time, and the rate of molecular evolution in birds. Mol. Phylogenet. Evol. **3**:344–350.

NEDBAL, M. A., M. W. ALLARD, and R. L. HONEYCUTT. 1994. Molecular systematics of hystricognath rodents: evidence from the mitochondrial 12S rRNA gene. Mol. Phylogenet. Evol. **3**:206–220.

NEDBAL, M. A., R. L. HONEYCUTT, and D. A. SCHLITTER. 1996. Higher-level systematics of rodents (Mammalia, Rodentia): evidence from the mitochondrial 12S rRNA gene. J. Mamm. Evol. **3**:201–237.

NOWAK, R. M. 1999. Walker's mammals of the world. 6th edition, Vol. II. The Johns Hopkins University Press, Baltimore, Md.

O'HUIGIN, C., and W.-H. LI. 1992. The molecular clock ticks regularly in muroid rodents and hamsters. J. Mol. Evol. **35**: 377–384.

OHTA, T. 1993. An examination of the generation-time effect on molecular evolution. Proc. Natl. Acad. Sci. USA **90**: 10676–10680.

OMLAND, K. E. 1997. Correlated rates of molecular and morphological evolution. Evolution **51**:1381–1393.

PATTERSON, B., and A. E. WOOD. 1982. Rodents from the Deseadan Oligocene of Bolivia and the relationships of the Caviomorpha. Bull. Mus. Comp. Zool. **149**:371–543.

POSADA, D., and K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics **14**:817–818.

PROMISLOW, D. E. L. 1994. DNA repair and the evolution of longevity: a critical analysis. J. Theor. Biol. **170**:291–300.

QUINTANA, C. A. 1998. Relaciones filogenéticas de roedores Caviinae (Caviomorpha, Caviidae), de América Sur. Bol. R. Soc. Esp. Hist. Nat. (Sec. Biol.) **94**:125–134.

REDFORD, K. H., and J. F. EISENBERG. 1992. Mammals of the neotropics. The southern cone, Vol. 2. The University of Chicago Press, Chicago.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223–225.

ROBINSON, M., M. GUOY, C. GAUTIER, and D. MOUCHIROUD. 1998. Sensitivity of the relative rate test to taxonomic sampling. Mol. Biol. Evol. **15**:1091–1098.

ROOD, J. P. 1970. Ecology and social behavior of the desert cavy (*Microcavia australis*). Am. Midl. Nat. **83**:415–454.

SACHER, N. 1998. Of domestic and wild guinea pigs: studies in sociophysiology, domestication and social evolution. Naturwissenschaften **85**:307–317.

SHIGENAGA, M. K., C. J. GIMENO, and B. N. AMES. 1989. Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc. Natl. Acad. Sci. USA **86**:9697–9701.

SILVA, M., and J. A. DOWNING. 1995. CRC handbook of mammalian body masses. CRC Press, Boca Raton, Fla.

SLOWINSKI, J. B., and B. S. ARBOGAST. 1999. Is the rate of molecular evolution inversely related to body size? Syst. Biol. **48**:396–399.

SOKAL, R. R., and F. J. ROHLF. 1998. Biometry. 3rd edition. W. H. Freeman and Company, New York.

SORHANNUS, U., and C. VAN BELL. 1999. Testing for equality of molecular evolutionary rates: a comparison between a relative-rate test and a likelihood ratio test. Mol. Biol. Evol. **16**:849–855.

SPRINGER, M. S., and E. DOUZERY. 1996. Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. J. Mol. Evol. **43**:357–373.

SULLIVAN, J., and D. L. SWOFFORD. 1997. Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. J. Mamm. Evol. **4**:77–86.

SWOFFORD, D. L. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, Mass.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, and D. M. HILLIS. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. 2nd edition. Sinauer, Sunderland, Mass.

TABER, A. B., and D. W. MACDONALD. 1992. Spatial organization and monogamy in the mara *Dolichotis patagonum*. J. Zool. Lond. **227**:417–438.

TAJIMA, F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis. Genetics **135**:599–607.

TAKEZAKI, N., A. RZHETSKY, and M. NEI. 1995. Phylogenetic test of the molecular clock and linearized tree. Mol. Biol. Evol. **12**:823–833.

THOMPSON, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, and D. G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24**:4676–4680.

WALLAU, B. R., A. SCHMITZ, and S. F. PERRY. 2000. Lung morphology in rodents (Mammalia, Rodentia) and its implications for systematics. J. Morphol. **246**:228–248.

WEIR, B. J. 1974. Reproductive characteristics of hystricomorph rodents. Pp. 265–301 *in* I. W. Rowlands and B. J. Weir, eds. The biology of hystricomorph rodents. Academic Press, London.

WHITE, T. G., and M. S. ALBERICO. 1992. *Dinomys branickii*. Mamm. Species, No. 410.

WILSON, D. E., and D. M. REEDER. 1993. Mammal species of the world. A taxonomic and geographic reference. Smithsonian Institution Press, Washington, D.C.

WOODS, C. A. 1982. The history and classification of South American hystricognath rodents: reflections on the far away and long ago. Pp. 377–392 *in* M. A. Mares and H. H. Genoways, eds. Mammalian biology in South America, Vol. 6. Special Publ. Ser., Pymatuning Laboratory of Ecology, University of Pittsburgh Press, Pittsburgh, Penn.

———. 1984. Hystricognath rodents. Pp. 389–446 *in* S. Anderson and J. K. Jones Jr., eds. Orders and families of Recent mammals of the world. John Wiley & Sons, New York.

———. 1993. Suborder Hystricognathi. Pp. 771–806 *in* D. E. Wilson and D. M. Reeder, eds. Mammal species of the world. 2nd edition. Smithsonian Institution Press, Washington, D.C.

WYSS, A. R., J. J. FLYNN, M. A. NORELL, C. C. SWISHER III, R. CHARRIER, M. J. NOVACEK, and M. C. MCKENNA. 1993. South America's earliest rodent and recognition of a new interval of mammalian evolution. Nature **365**:434–437.

YANG, Z. 1994*a*. Estimating the pattern of nucleotide substitution. J. Mol. Evol. **39**:105–111.

———. 1994*b*. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. **39**:306–314.

YANG, Z., N. GOLDMAN, and A. FRIDAY. 1995. Maximum likelihood from DNA sequences: a peculiar statistical estimation problem. Syst. Biol. **44**:384–399.

DAVID IRWIN, reviewing editor

Accepted November 1, 2001