Evolutionary History of the Most Speciose Mammals: Molecular Phylogeny of Muroid Rodents

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Phylogenetic relationships between 32 species of rodents representing 14 subfamilies of Muridae and four subfamilies of Dipodidae were studied using sequences of the nuclear protein-coding genes Lecithin Cholesterol Acyl Transferase (LCAT) and von Willebrand Factor (vWF). An examination of some evolutionary properties of each data matrix indicates that the two genes are rather complementary, with lower rates of nonsynonymous substitutions for LCAT. Both markers exhibit a wide range of GC3 percentages (55%–89%), with several taxa above 70% GC3 for vWF, which indicates that those exonic regions might belong to the richest class of isochores. The primary sequence data apparently harbor few saturations, except for transitions on third codon positions for vWF, as indicated by comparisons of observed and expected pairwise values of substitutions. Phylogenetic trees based on 1,962 nucleotidic sites from the two genes indicate that the 14 Muridae subfamilies are organized into five major lineages. An early isolation leads to the clade uniting the fossorial Spalacinae and semifossorial Rhizomyinae with a strong robustness. The second lineage includes a series of African taxa representing nesomyines, dendromurines, cricetomyines, and the sole living member of mystromyines. The third one comprises only the mouselike hamster *Calomyscus.* The fourth clade represents the cricetines, myospalacines, sigmodontines, and arvicolines, whereas the fifth one comprises four ''traditional'' subfamilies (Gerbillinae, Murinae, Otomyinae, and Acomyinae). Within these groups, we confirm the monophyly of almost all studied subfamilies, namely, Spalacinae, Rhizomyinae, Nesomyinae, Cricetomyinae, Arvicolinae, Sigmodontinae, Cricetinae, Gerbillinae, Acomyinae, and Murinae. Finally, we present evidence that the sister group of Acomyinae is Gerbillinae, and we confirm a nested position of Myospalacinae within Cricetinae and Otomyinae within Murinae. From a biogeographical point of view, the five main lineages spread and radiated from Asia with different degrees of success: the first three groups are now represented by a limited number of species and genera localized in some regions, whereas the last two groups radiated in a large variety of species and genera dispersed all over the world.

Introduction

The rodents of the family Muridae are the most diverse group of mammals, encompassing at least 1,326 species spanning more than 281 genera (Musser and Carleton 1993). The evolutionary systematics of this family have been very difficult, and in spite of many attempts (Miller and Gidley 1918; Simpson 1945; Hooper and Musser 1964; Chaline, Mein, and Petter 1977; Carleton and Musser 1984), several uncertainties, confusions, and conflicting views have persisted for these animals. For this reason, in their recent review, Musser and Carleton (1993) decided to keep a prudent state of uncertainty about the hierarchical pattern of muroid suprageneric groups and to divide the family Muridae into 17 subfamilies considered at the same taxonomic level. These ''major lineages'' within murids are Arvicolinae (26 genera/143 species), Calomyscinae (1/6), Cricetinae (7/18), Cricetomyinae (3/6), Dendromurinae (8/23), Gerbillinae (14/110), Lophiomyinae (1/1), Murinae (122/529), Myospalacinae (1/7), Mystromyinae (1/1), Nesomyinae (7/14), Otomyinae (2/14), Petromyscinae (2/5), Platacanthomyinae (2/3), Rhizomyinae (3/15), Sigmodontinae (79/423), and Spalacinae (2/8).

Key words: muroids, phylogeny, LCAT, vWF.

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However, there remains a strong need to better define the taxonomic boundaries of these subfamilies and especially the relationships existing between them. Many important questions have not yet been adequately answered concerning the evolutionary origins of most of the 17 subfamilies or of their rates of evolution.

In a recent publication based on 40 rodent sequences of the nuclear protein-coding gene Lecithin Cholesterol Acyl Transferase (LCAT) (Michaux and Catzeflis 2000) representing 13 Muridae subfamilies, we proposed a first step toward a better knowledge of this speciose group. The results showed that within Muridae, a first branching leads to the fossorial Spalacinae and the semifossorial Rhyzomyinae. The remaining components of Muridae appeared as a polytomy from which Sigmodontinae, Calomyscinae, Arvicolinae, Cricetinae, Mystromyinae, Nesomyinae and some Dendromurinae (*Steatomys* and *Dendromus*) were issued. This phylogeny was interpreted by Michaux and Catzeflis (2000) as the result of a bushlike radiation at the end of the early Miocene, leading to emergence of most living subfamilies. The separation between three additional taxa, Murinae, Gerbillinae, and ''Acomyinae'' (which comprises the genera *Acomys*, *Deomys*, *Uranomys*, and *Lophuromys*), occurred more recently, from a common ancestor issued from the main basal radiation. As previously shown by other molecular studies, the vlei rats Otomyinae are nested within Old World Murinae. In the same way, the zokors Myospalacinae appear strongly nested within the hamsters Cricetinae. Finally, Michaux and Catzeflis (2000) proposed a sister group relationship be-

Table 1
References for Rodent Tissues Used in the Experiments

Suprafamily	Subfamily	Species	Tissue Sample
Dipodoidea	Sicistinae	*Sicista kazbegica*	T-762
	Allactaginae	*Allactaga elater*	T-1045
	Dipodinae	*Dipus sagitta*	T-869
		Jaculus jaculus	T-552
Muroidea	Calomyscinae	*Calomyscus mystax*	T-1067
	Dendromurinae	*Steatomys* sp.	T-1167
		Deomys ferrugineus	T-778
		Dendromus mystacalis	T-1422
	Gerbillinae	*Tatera gambiana*	T-913
		Gerbillus henleyi	T-1165
	Mystromyinae	*Mystromys albicaudatus*	T-1365
	Nesomyinae	*Macrotarsomys ingens*	T-1150
		Nesomys rufus	T-1125
	Cricetomyinae	*Saccostomus campestris*	T-2088
		Cricetomys gambianus	T-968
	Sigmodontinae	*Neotoma fuscipes*	T-385
		Peromyscus maniculatus	T-142
	Cricetinae	*Phodopus roborovskii*	T-714
		Mescocricetus auratus	T-1162
		Cricetulus migratorius	T-325
	Myospalacinae	*Myospalax* sp.	T-394
	Arvicolinae	*Dicrostonyx torquatus*	T-1337
		Clethrionomys glareolus	T-357
	Murinae	*Lophuromys sikapusi*	T-1179
		Rattus norvegicus	GenBank
		Mus musculus	GenBank
		Micromys minutus	T-1196
		Uranomys ruddi	T-1184
		Acomys cahirinus	T-1670
	Otomyinae	*Otomys angoniensis*	T-718
	Spalacinae	*Nanospalax ehrenbergi*	T-268
	Rhizomyinae	*Rhizomys pruinosus*	T-1284
		Tachyoryctes sp.	T-4991

NOTE.—The taxonomic arrangement follows Wilson and Reeder (1993). The geographic origins of collected/preserved animals, as well as collector's names and individual accession numbers, are available on request from the senior author.

tween Malagasy Nesomyinae and South African Mystromyinae.

In order to verify these hypotheses and to know if the majority of the modern Muridae subfamilies indeed appeared as a bushlike radiation, we here tested the relationships between 14 Muridae subfamilies using another nuclear gene: the von Willebrand Factor (vWF). Then, we combined these new sequences with those of the LCAT gene.

Materials and Methods

DNA used to sequence exon 28 of the vWF gene was extracted and purified from ethanol-preserved tissues taken from the collection of mammal tissues housed at Montpellier (Catzeflis 1991). Whenever possible, we selected two species for each studied subfamily (see table 1). This biological sampling was aimed at getting an equilibrated representation of each murid lineage diminishing a possible ''long-branch attraction effect.'' These species were also chosen according to the sampling already performed on the nuclear LCAT gene (Michaux and Catzeflis 2000) in order to combine the sequences of both genes.

DNA Sequencing of vWF Gene Exon 28

DNA extraction from the 95% ethanol-preserved tissues was performed according to Sambrook, Fritsch, and Maniatis (1989). The main part of exon 28 from the vWF gene (1,265 bp) was amplified using the PCR primers V1 (5′-TGTCAACCTCACCTGTGAAGCCTG-3′) and W1 (5′-TGCAGGACCAGGTCAGGAGCC TCTC-3′) previously designed by Huchon, Catzeflis, and Douzery (1999). All PCRs used 5 min at 94°C; 33 cycles of 45 s at 94°C, 30 s at 52°C, and 1 min at 72°C; and 10 min at 72°C in an Appligen Crocodile 3 or a Labover PTC100 thermal cycler. The total reaction volume was 50 μl. PCR products were purified using the Ultra-free DNA Amicon kit (Millipore) and directly sequenced. Sequencing on both strands was done using a dye terminator or Big dye terminator (Perkin Elmer) sequencing kit and ABI 373 and ABI 310 (Perkin Elmer) automatic sequencers. The external (V1 and W1) and internal (V2 and W2) primers designed by Huchon, Catzeflis, and Douzery (1999) were used for sequencing.

Technical difficulties prevented us from obtaining a high-quality sequence of *Tachyoryctes* for the LCAT gene, whereas we obtained only a partial sequence (624 nt) for *Rhizomys* exon 28 of the vWF gene. Thus, our representation of the taxon Rhizomyinae is chimeric: complete sequence of vWF for *Tachyoryctes* and of LCAT for *Rhizomys.* We nevertheless checked that *Tachyoryctes* and *Rhizomys* were sister taxa (100% bootstrap support; data not shown) when the only 624 available sites of vWF were compared for 33 Myodonta

taxa. Thus, we feel confident that, as documented by comparative morphology of fossil and living taxa (Flynn 1990), *Rhizomys* and *Tachyoryctes* are sister genera among all other rodents of our study.

Sequence Alignment and Saturation Analysis

Previously known sequences for vWF and LCAT genes were extracted from GenBank and aligned with the new sequences using CLUSTAL W (Thompson, Higgins, and Gibson 1994) and the ED editor (MUST package; Philippe 1993). The program AFAS (MUST package; Philippe 1993) was used to combine the aligned matrices of vWF and LCAT.

We performed a saturation analysis for vWF as described by Philippe and Douzery (1994) and Hassanin, Lecointre, and Tillier (1998). Details for such an analysis are provided by Michaux and Catzeflis (2000).

Phylogenetic Reconstructions

The aligned sequences were treated with different approaches: the stationary Markov model (Saccone et al. 1990) (also called general time reversible [GTR]) in PAUP 4.0b2 (Swofford 1998), the Tamura and Nei (1993) model, and the LogDet (Lockhaert et al. 1994) estimator were used for the calculation of genetic distances. The last estimator was used to take into account the differences in GC composition between species. The GTR analyses were also performed assuming a gamma distribution for substitution rates across sites, where the parameter alpha (Yang 1996) and the proportion of invariant sites (I) were estimated with the maximum-likelihood (ML) method assuming the GTR–Markov evolution (ME) phylogeny using PAUP 4.0b2. Maximum-parsimony (MP; heuristic search, TBR branch swapping option) and ML (GTR model of sequence evolution) analyses were also conducted using PAUP 4.0b2 (Swofford 1998).

The robustness of inferences was assessed through bootstrap resampling (BP) under ML after 100 replicates (with neighbor-joining [NJ] starting trees, NNI branch swapping, and model parameters fixed to values estimated from the original data) and under MP and NJ or ME after 1,000 replicates. Bremer's support index (BSI, Bremer 1988) was also calculated on the most parsimonious tree with enforcement of topological constraints. Alternative topologies were evaluated by the Kishino and Hasegawa (1989) test implemented in PAUP 4.0b2.

The level of incongruence between the two genes was tested using PAUP4 (option Hompart; Swofford 1998). This approach used the incongruence length difference (ILD) test with the parsimony criterion (Farris et al. 1995). One thousand randomizations were performed on variable sites only (Cunningham 1997).

Relative-Rate Test

Relative-rate tests were conducted both with RRTree, version 1.0 (Robinson et al. 1998) (which improves the test of Wu and Li [1985]), by taking into account the taxonomic representativity and its phylogenetic relationships, and with the likelihood approach of Muse and Gaut (1994). In the latter case, statistical significance of the test was assessed by means of the chi-square test ($P < 0.05$). Quantifications of the rate differences were performed either on the proportions of synonymous (K_s) and nonsynonymous (K_a) substitutions for RRTree, or on all three codon positions and GTR model for the likelihood approach. Relative-rate tests were performed among rodents at intrafamilial levels; Dipodidae were chosen as outgroups.

Results

Sequenced Species

The 27 new rodent sequences of vWF gene exon 28 have been deposited in the EMBL gene bank under accession numbers AJ297764, AJ297765, and AJ402693–AJ402717. The newly determined sequences were compared with four rodent sequences determined by Huchon, Catzeflis, and Douzery (1999), as well as with a *Mus musculus* sequence available in GenBank (U27810).

Evolutionary Properties of LCAT and Exon 28 vWF Genes

The data matrix for 32 Myodonta taxa had 1,962 nucleotidic sites, of which 711 (about 36%) were parsimony-informative. Exons of LCAT comprised 804 sites; about half of them were variable, and 239 (30%) were parsimony-informative; exon 28 of the vWF gene included 1,158 positions, of which 710 (61%) were variable and 472 (41%) were parsimony-informative. Thus, there was more absolute and relative variation in vWF than in LCAT. The average ratio of transitions to transversions was 2.00 for LCAT, ranging from 1.1 (*Uranomys ruddi*/*M. musculus* comparison) to 5.00 (*Allactaga elater*/*Dipus sagitta*). For vWF, this ratio was 1.94, ranging from 1.23 (*Saccostomus campestris*/*D. sagitta* and *S. campestris*/*Jaculus jaculus* comparisons) to 3.52 (*Mesocricetus auratus*/*Myospalax* sp.).

From comparing the 496 pairs of sequences, it appears that LCAT exons have evolved slower than exon 28 of vWF in muroids and dipodoids. The LCAT/vWF ratios for nucleotidic and amino acid percentages of differences were 0.78% ± 0.16% and 0.63% ± 0.21%, respectively. Nonsynonymous changes (K_a values) were much less frequent in LCAT (values of up to 0.08), on average 59% ± 20% of the values observed in vWF (values of up to 0.15); synonymous changes were as frequent in LCAT as in vWF (values of up to 0.45 and 0.55, respectively), and this might well be due to saturation of both genes for this kind of change (fig. 1).

The nucleotidic and amino acid compositions were different for each gene ($P < 0.05$; nonparametric Mann-Whitney *t*-tests) at each codon position (nucleotides) and for each amino acid except proline ($P = 0.47$). LCAT had much more T in first codon positions and A and T in third positions, whereas vWF contained much more A in first positions and G in third positions. The most pronounced differences in frequencies of amino

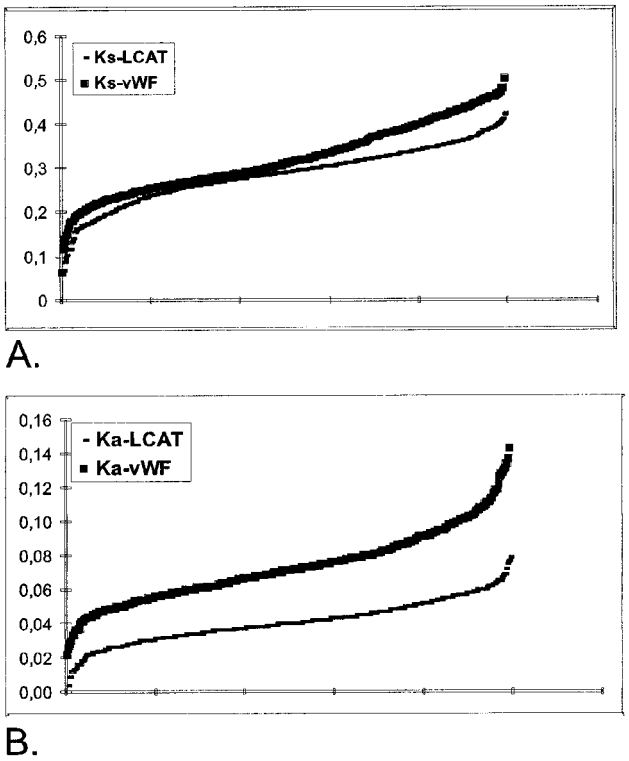

FIG. 1.—Synonymous (K_s) and nonsynonymous (K_a) changes for 496 pairs of rodent sequences in the LCAT gene and exon 28 of the vWF gene. In the two curves, data are organized by increasing values in order to illustrate (*A*) the similar rates of synonymous change in both genes and (*B*) the faster rate of nonsynonymous change in vWF sequences.

acids concerned an excess of C, N, D, and Y as opposed to a deficiency of K, I, S, A, and E for LCAT. Exon 28 of vWF had a very low representation of amino acids C (0.51% ± 0.06%) and W (0.03% ± 0.09%) (data not shown).

GC_3 Content

The percentages of GC at third codon positions (GC_3) spanned a much larger scatter in vWF (64.1–89.1) than in LCAT (54.8–67.6) (data available on request). On average, the four dipodoids had a higher GC_3 (81.7 ± 8.3 for vWF; 64.4 ± 3.1 for LCAT) than the 28 muroids (68.6 ± 2.9 for vWF; 59.7 ± 3.1 for LCAT) (unpaired Mann-Whitney *t*-tests, $P < 0.009$). These differences held also for vWF ($P < 0.005$) in comparisons of dipodoids and several subsets of muroids, such as Gerbillinae + Murinae + Acomyinae, or Arvicolinae + Sigmodontinae + Cricetinae, or Nesomyinae + Cricetomyinae + Dendromurinae. For LCAT, such comparisons also indicated that advanced muroids had a lower GC content, although the differences were marginally significant (*P* values of 0.070, 0.019, and 0.052, respectively). We here confirm, based on a larger data set, that the murids have a lower GC_3 value for LCAT (Robinson, Gautier, and Mouchiroud 1997), and demonstrate that for vWF the average GC_3 difference is still larger for murids and nonmurids.

Saturation Analyses

The levels of saturation for LCAT had already been assessed by Michaux and Catzeflis (2000), showing that the C-T and A-G transitions exhibit a lower slope (average of 0.33 for A-G and 0.35 for C-T) with regard to the transversions, whatever the codon position. Consequently, Michaux and Catzeflis (2000) performed a weighted analysis according to the slopes of each substitution. However, although the slopes were significantly lower than those observed for vWF (see below), the results of the weighted analysis did not show any interesting differences with regard to the unweighted parsimony analysis. Thus, all substitutions were retained for phylogenetic reconstruction performed with the LCAT gene.

The levels of saturation for vWF were moderate on each kind of nucleotide substitution and position (slopes of regression analysis of 0.51 for TS1 and 0.54 for TS2 and of 0.86, 0.87, and 0.87, respectively, for TV1, TV2 and TV3), with the exception of the TS at the third position (fig. 2). However, the graph shows that intra-Muridae pairwise comparisons also appear to be moderately subjected to saturation (slope of the regression analysis $S = 0.50$). Thus, our major interest being in

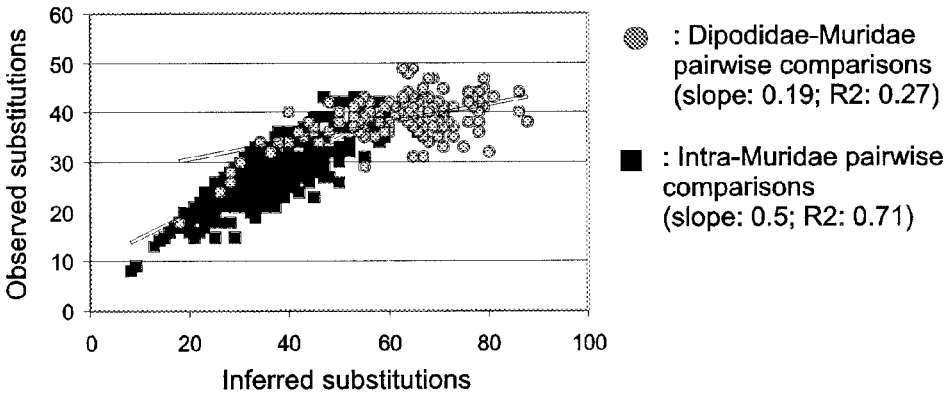

FIG. 2.—Graphic estimation of saturation in vWF for transitions at third codon positions. The inferred number of changes, derived from the patristic distance matrix taken from a most-parsimonious tree, are plotted against the pairwise numbers of observed differences. Equations of the linear regression (straight lines) and correlation coefficients are given for Dipodidae-Muridae and intra-Muridae pairwise comparisons.

intra-Muridae relationships, all substitutions were retained for phylogenetic reconstructions derived from the vWF gene.

Phylogenetic Reconstructions

According to the results obtained with the LCAT nuclear gene (Michaux and Catzeflis 2000) and other nuclear genes (GHR [Adkins et al. 2001], IRBP [DeBry and Sagel 2001], A2AB [D. Huchon, personal communication]) which showed a high robustness for a sister group relationship between Dipodidae and Muridae and for the monophyly of each superfamily, we used four species of Dipodidae as an outgroup for the Muridae.

LCAT Gene

The first set of analyses considered the 32 rodent species sequenced for the LCAT gene. The MP reconstruction yielded one most-parsimonious tree (L = 1,080 steps; consistency index [CI] = 0.41 and retention index [RI] = 0.52). Bootstrapping and BSI values are indicated in table 2 for the ancestral segments labeled 1–30 in figure 3. The majority of the results of this analysis and those of the ML and distance approaches have been presented in Michaux and Catzeflis (2000). To summarize, the first dichotomy isolates the Spalacinae and the Rhizomyinae. The remaining 13 Muridae subfamilies are clustered together in a strongly supported clade. The first branching event of this clade is a large polytomy leading to eight lineages, of which only four encompass more than one subfamily: Mystromyinae and Nesomyinae (node 10), Cricetomyinae and Dendromurinae (node 12), Cricetinae and Myospalacinae (node 19), and Murinae, Otomyinae, Gerbillinae, and Acomyinae (node 21). Thus, with this gene, most of the ''advanced'' murid subfamilies appear to be of a polytomous origin, suggesting the phenomenon of a spectacular bushlike radiation having led to the majority of them.

Exon 28 of vWF

The second set of analyses considered the 32 rodent species sequenced for vWF. The MP reconstruction yielded one most-parsimonious tree (L = 2,043 steps; CI = 0.42 and RI = 0.51). Bootstrapping and BSI values are indicated in table 2 for the ancestral segments labeled 1–30 in figure 3. As already observed with LCAT, the first dichotomy isolates the Spalacinae and the Rhizomyinae. The remaining Muridae built up a strongly supported clade (node 4: BP = 100%, °BSI = +17). However, in contrast to the LCAT gene results, these ''modern'' subfamilies do not appear to be of a polytomous origin. Several clades appear clearly, i.e., an ''African'' clade (node 6) clustering the Nesomyinae, Mystromyinae, Cricetomyinae, and Dendromurinae (as defined in Michaux and Catzeflis 2000) or a ''Cricetoid'' clade (node 15) uniting the Arvicolinae, the Cricetinae, the Sigmodontinae, and the Myospalacinae.

However, some terminal nodes (i.e., the monophyly of the Cricetomyinae, the position of *Otomys* within the Murinae) appear less robust with the vWF gene than with LCAT. Thus, as these two genes seem complementary, we decided to combine the two data matrices in order to perform a new combined analysis.

Combined Analysis of the LCAT and vWF Genes

The ILD test revealed that there was some incongruence between the two nuclear genes ($P = 0.002 < 0.05$). This result might be explained by the extensive heterogeneity of the two data matrices (large differences in GC contents, some species are evolving very quickly or slowly with regard to the other taxa, etc.) and by the fact that these genes seem to evolve at different rates (see above). According to our results, LCAT yields a better (i.e., more robust) resolution for terminal nodes, whereas vWF seems to perform better for the deeper ancestral segments.

Thus, these genes, although they are suggested to be incongruent by the ILD test, also appear rather complementary. For this reason, the two data sets were concatenated.

The MP analysis yielded one most-parsimonious tree (L = 3,145 steps; CI = 0.41 and RI = 0.51) identical to the ML and NJ and ME trees (fig. 4 and table 2). Once again, the first dichotomy isolates Spalacinae and Rhizomyinae, and the remaining Muridae built up a strongly supported clade (node 4: BP = 100%, °BSI = +24).

The monophyly of almost all subfamilies represented by at least two genera is robust (nodes 7, 9, 11, 16, 17, 19, 23, 24, 28 supported by BP of 97%–100% and by BSI of +10–+40): Nesomyinae (node 7), Dendromurinae (as previously defined in Michaux and Catzeflis 2000) (node 9), Cricetomyinae (node 11), Arvicolinae (node 16), Sigmodontinae (node 17), Cricetinae (node 19), Gerbillinae (node 23), Acomyinae (as previously defined in Michaux and Catzeflis 2000) (node 24), and Murinae (node 28). These different subfamilies are divided into four clades. The first (node 6), highly supported (BP = 97%, BSI = +9), includes the Dendromurinae, *Mystromys*, the Cricetomyinae, and the Nesomyinae. Within it, the first three subfamilies seem related, although with a lower robustness (BP = 75%, BSI = +3) (node 8). This result is probably linked to a conflict between the two genes. Indeed, LCAT associates *Mystromys* with the Nesomyinae (node 10, fig. 3). The second major lineage includes *Calomyscus* only. The third clade associates the Arvicolinae, the Cricetinae, the Sigmodontinae, and the Myospalacinae (node 15: BP = 89%, BSI = +5). Finally, the last group includes the Murinae, the Gerbillinae, and the Acomyinae (BP = 98%, BSI = +13) (node 21). Within it, the ancestral fragment uniting the Gerbillinae with Acomyinae is strongly supported (BP = 96%, BSI = +8) (node 22). A relationship between the third and the fourth lineages is also observed (node 14: BP = 86%, BSI = +5).

The NJ (GTR and LogDet), ML, and ME analyses yielded results similar to those obtained with the parsimony approach. However, some ancestral segments merit further comment, as they appear to be more robustly supported by some analyses—the ''Holarctic''

Table 2
Indices of Robustness for the Nodes (labeled 1–30) of the Phylogenetic Trees Represented in Figures 4 and 5 Using Maximum-Parsimony (MP), Distance (NJ), Maximum-Likelihood (ML), and Markov Evolution Analyses on the Three Codon Positions (ME123) (only for the combined analysis)

	vWF					LCAT					vWF + LCAT + 12S					
	NJ GTR	NJ LogDet	MP	BSI	ML	NJ GTR	NJ LogDet	MP	BSI	ML	NJ GTR	NJ LogDet	MP	BSI	ML	ME123
1 ..	100	100	98	+12	95	100	100	100	+13	100	100	100	93	+38	100	100
2 ..	100	98	100	+47	100	—	—	94	+0	—	100	97	100	+10	100	98
3 ..	100	100	100	+27	100	96	98	100	+11	92	100	100	100	+51	100	100
4 ..	100	100	100	+17	100	90	92	92	+9	92	100	100	100	+24	100	100
5 ..	96	96	95	+7	95	96	96	80	+4	62	100	100	94	+10	91	100
6 ..	99	98	95	+8	98	—	—	—	+0	—	98	99	97	+9	99	100
7 ..	100	100	99	+10	100	81	78	69	+0	54	100	100	99	+12	99	100
8 ..	85	78	91	+4	96	—	—	—	+0	—	—	—	75	+3	62	—
9 ..	85	84	91	+6	93	54	—	—	+0	70	90	93	97	+10	100	89
10 ..	—	—	—	+0	—	96	93	89	+6	93	52	52	—	+0	—	60
11 ..	—	—	—	+0	—	100	99	100	+11	100	99	99	99	+11	99	99
12 ..	—	—	—	+4	—	94	93	86	+0	92	75	78	98	+3	97	66
13 ..	—	—	66	+4	92	—	—	—	+0	—	76	71	64	+2	91	66
14 ..	81	84	63	+2	86	—	—	—	+2	—	55	53	86	+5	97	—
15 ..	90	94	85	+5	96	—	—	—	+0	—	100	97	89	+5	99	90
16 ..	100	100	100	+22	100	99	99	97	+13	99	100	100	100	+38	100	100
17 ..	96	100	98	+10	99	87	94	70	+2	83	100	100	100	+13	100	100
18 ..	—	65	—	—	—	60	67	—	+0	69	74	72	71	+2	77	90
19 ..	100	100	98	+7	98	99	98	95	+6	94	100	100	100	+14	100	100
20 ..	96	98	100	+15	100	60	49	88	+4	71	97	98	100	+21	100	100
21 ..	88	86	95	+6	97	55	63	65	+2	72	99	100	98	+13	100	96
22 ..	98	99	96	+4	97	—	56	49	+1	68	95	96	96	+8	100	98
23 ..	100	100	100	+31	100	100	100	100	+9	100	100	100	100	+40	100	100
24 ..	100	100	99	+14	98	98	99	98	+8	98	100	100	100	+22	100	100
25 ..	—	56	—	+0	—	—	—	—	+0	—	85	87	—	+3	—	88
26 ..	89	91	—	+0	—	—	—	—	+0	—	84	81	—	+0	—	82
27 ..	—	—	—	+0	—	88	91	95	+6	97	—	—	—	+0	—	—
28 ..	100	100	100	+18	100	100	100	100	+9	100	100	100	100	+34	100	100
29 ..	81	88	51	+3	77	—	—	—	+0	54	73	>50	65	+5	79	64
30 ..	60	65	—	+1	59	99	99	98	+8	99	97	99	97	+10	99	98

NOTE.—Bootstrap percentages computed after Neighbor-Joining (NJ) on GTR (Swofford 1998) with gamma rates (alpha, respectively, of 0.99, 0.61, and 0.77) or LogDet (Lockhaert et al. 1994) distances, MP, ML, and ME123 are reported. Finally, Bremer support indices (BSIs) are indicated.

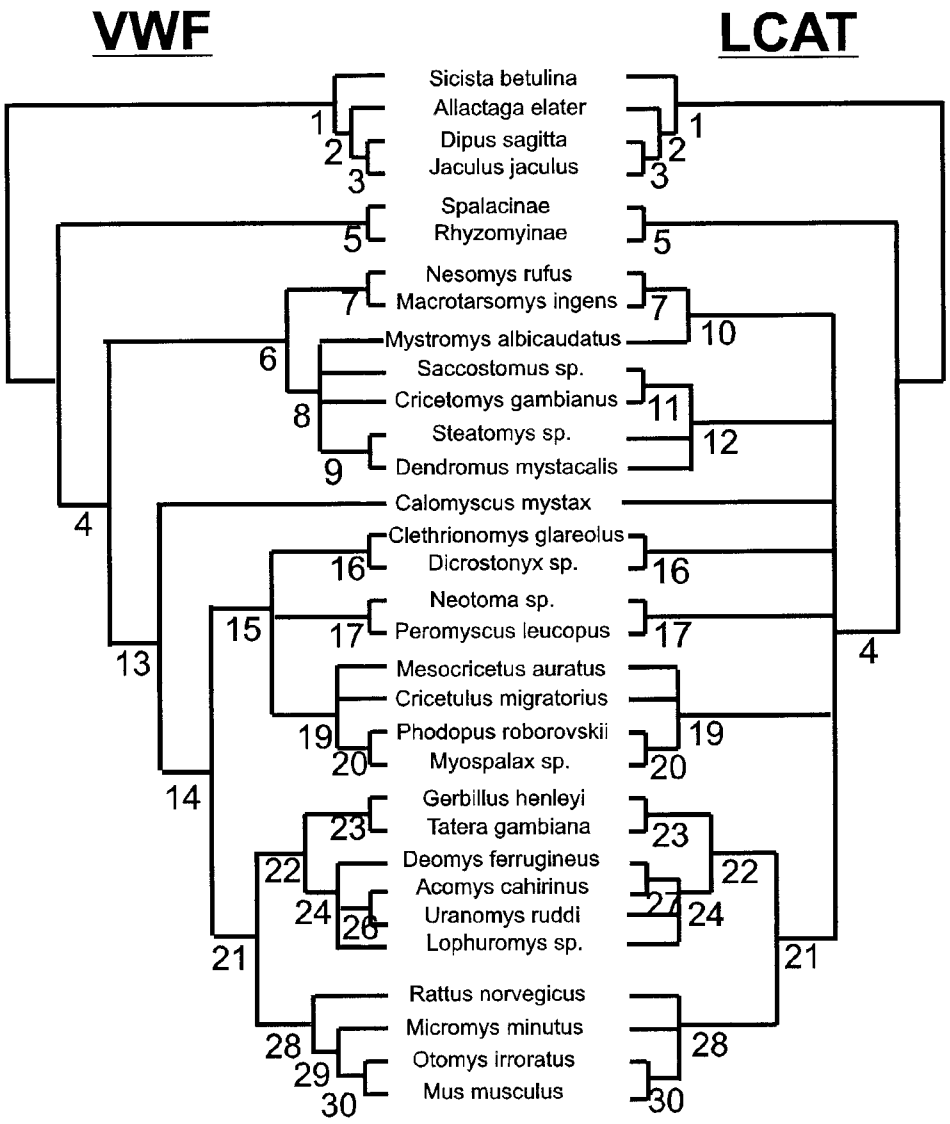

FIG. 3.—Synthetic trees summarizing the results derived from four approaches on 32 mammalian DNA sequences of the LCAT and vWF genes. The robustness of each node (labeled 1–30) is described in table 2 for maximum-parsimony (bootstrap percentage [BP] and Bremer's support index), distance (BP), maximum-likelihood (BP), and Markov evolution analyses (BP). The trees were rooted by the four Dipodidae sequences.

group uniting the third and fourth clades (node 14) (BP for ML = 97%); sister group relationships between the ''Holarctic'' group and *Calomyscus* (node 13) (BP for ML = 91%) and between Sigmodontinae and Cricetinae (node 18) (BP for ME = 94%); within Acomyinae, an external position of *Lophuromys* (node 25) (BP for NJ and ME = 87% and 88%, respectively); and the node 26, uniting *Uranomys* and *Acomys* (BP for NJ and ME = 80% and 86%, respectively).

Likelihood Alternatives to the Best Tree

The highest-likelihood tree (ln $L = -18{,}177.01$) for 32 Myodonta species was identified with PUZZLE (Strimmer and von Haeseler 1996) among 945 alternative trees constructed using MOLPHY 2.3b3 (Adachi and Hasegawa 1996). This tree has the same topology previously obtained by the parsimony and distance criteria and was used as the reference topology to apply Kishino-Hasegawa tests (Kishino and Hasegawa 1989) for assessing the following clades: the existence of the five Muridae lineages (Spalacinae + Rhizomyinae and the four modern groups); the existence of a ''Holarctic'' group; an isolated position of *Calomyscus*; a closer relationship between *Calomyscus* and the ''Holarctic'' subfamilies than with the ''African'' clade (Nesomyinae, Dendromurinae, Cricetomyinae, Mystromyinae); the sister group relationships between (a) *Mystromys*, Cricetomyinae, and Dendromurinae, (b) Gerbillinae + Acomyinae, and (c) Sigmodontinae and Cricetinae; within the Acomyinae, (a) an early offshoot of *Lophuromys* and (b) a closer relationship between *Uranomys* and *Acomys*; the monophyly of (a) the Sigmodontinae, (b) the Nesomyinae, and (c) the Acomyinae; and the nested position of (a) Otomyinae within Murinae and (b) Myospalacinae within Cricetinae.

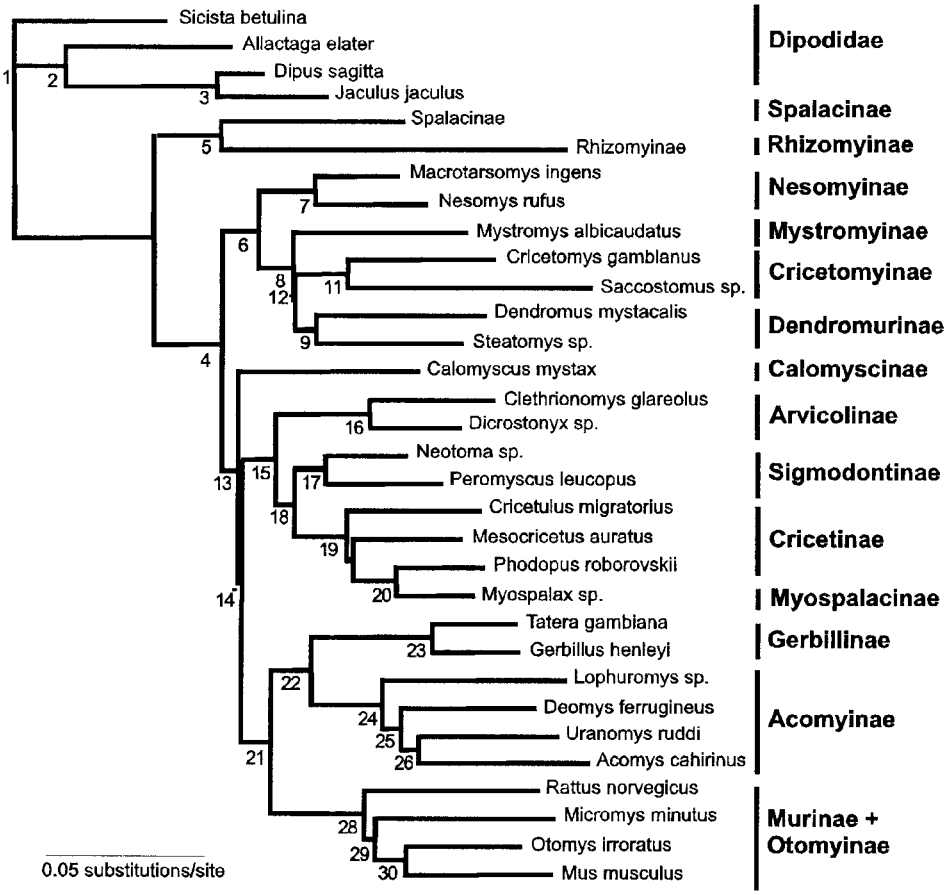

FIG. 4.—Synthetic tree summarizing the results from the combined analysis using LCAT and vWF genes. The robustness of each node (labeled 1–30) is described in table 2 for maximum-parsimony (bootstrap percentage [BP] and Bremer's support index), distance (BP), maximum-likelihood (reliability percentages), and Markov evolution analyses (BP). The tree was rooted by four Dipodidae sequences.

For doing so, we tested 19 different alternative topologies derived from previous morphological or molecular studies (Thomas 1896; Miller and Gidley 1918; Misonne 1971; Chaline, Mein, and Petter 1977; de Graaff 1981; Ameur 1984; Carleton 1984; Flynn, Jacobs, and Lindsay 1985; Dickerman 1992; Musser and Carleton 1993; Dubois, Catzeflis, and Beintema 1999; Michaux and Catzeflis 2000; Chevret, Catzeflis, and Michaux 2001).

Almost all the alternative topologies exhibited significantly worse log likelihoods at 5% probability. However, the alternative hypotheses against a Holarctic group and the sister group relationships between Sigmodontinae and Cricetinae were only significantly worse at 8% probability. Moreover, the topologies uniting *Mystromys* to the Nesomyinae and *Acomys* to *Deomys* and proposing *Uranomys* as an early offshoot of the Acomyinae were not significantly worse than the best tree.

Relative-Rate Tests

To determine if differences of rates of vWF change existed between the different Muridae subfamilies, relative-rate tests were conducted with each of these subfamilies against the remaining lineages. The Dipodidae were used as the outgroup. As previously, synonymous (K_s) changes did not show significant differences between the different Muridae subfamilies. However, K_a comparisons showed that Spalacinae, Nesomyinae, Calomyscinae (*Calomyscus*), and Sigmodontinae were slowly evolving ($P < 0.00001$, 0.01, 0.04, and 0.002, respectively) and that Cricetomyinae and Rhizomyinae were quickly evolving ($P < 0.0001$ and 0.00001). Within Malagasy rodents and the New World Sigmodontinae, further analyses showed that only *Macrotarsomys* and *Neotoma,* respectively, had a lower rate of evolution ($P < 0.02$). Within the African Cricetomyinae, only *Saccostomus* was significantly more quickly evolving ($P < 0.0001$).

As the RRTree test is rather sensitive to the rate of the species used as outgroups (Robinson et al. 1998), we also applied the likelihood approach of Muse and Gaut (1994). This confirmed the results derived from the above RRTree tests based on K_a values.

The different behaviors obtained between K_s and K_a can be explained by the fact that, for the muroids, synonymous substitutions saturate when the phylogenetic level is higher than within the subfamily, as already suggested in Michaux and Catzeflis (2000).

Consequently, in order to apply a molecular clock and date the separation between the murid genera and

Table 3
Estimates of the Separation Times of Different Events Within Muridae on the Basis of the Molecular Data

Separation Events	Separation *Mus*/*Rattus*	SE	Separation *Tatera*/*Gerbillus*	SE	Paleontological Estimates
Mus/*Rattus*	**12**	—	12	1	12 Myr (Jaeger, Tong, and Buffetaut 1986; Jacobs and Downs 1994)
Gerbillus/*Tatera*	6.7	0.6	**8**	—	8–10 Myr (Tong 1989)
Gerbillinae/Murinae/Acomyinae	17.9	0.3	20.8	0	16 Myr (Tong and Jaeger 1993)
Dendromurinae/Cricetomyinae/Mystromyinae/Nesomyinae	16.1	0.5	18.8	1	14–15 Myr (Conroy *et al.* 1992)
Cricetinae/Arvicolinae/Sigmodontinae/Myospalacinae	15.5	0.6	18.2	1	?
Acomyinae/Gerbillinae	16.1	0.5	18.8	1	?
Myospalax/*Phodopus*	5.7	0.5	6.5	1	2 Myr (Chaline, Mein, and Petter 1977; Carleton and Musser, 1984)
Steatomys/*Dendromus*	11.4	0.6	13.3	1	8–11 Myr (McKenna and Bell 1997)
Dicrostonys/*Clethrionomys*	8	0.6	9.3	1	3–4 Myr Chaline and Graf 1988)

Note.—The numbers in bold correspond to the two calibration points used for this analysis: 12 Myr for the separation between *Mus* and *Rattus* (Jaeger, Tong, and Buffetaut 1986; Jacobs and Downs 1994) and 8 Myr for the *Tatera*/*Gerbillus* dichotomy (Tong 1989). SE = standard error values provided by the maximum-likelihood analysis of Puzzle 4.0.

subfamilies, we performed another ML analysis with Dipodidae as the outgroup and all the Muridae except the slowest- and fastest-evolving species (*Spalax, Calomyscus, Rhizomys, Macrotarsomys, Neotoma,* and *Saccostomus*). The inferred ML distances were the basis for estimating separation times. Two calibration points derived from paleontological data were chosen: (1) the *Mus*/*Rattus* dichotomy, set at 12 MYA (Jaeger, Tong, and Buffetaut 1986; Jacobs, Winkler, and Murry 1989; Jacobs et al. 1990; Jacobs and Downs 1994; Muse and Gaut 1994) and (2) The *Gerbillus*/*Tatera* separation, set at 8 MYA (Tong 1989). The estimated time of divergence (41 MYA) between *Mus* and *Rattus* obtained from molecular data (Kumar and Hedges 1998) was not used in this study because recent publications (Adkins et al. 2001; Murphy et al. 2001) have confirmed that it is an overestimate. The ML distance between *Mus* and *Rattus* is 0.049, whereas that between *Tatera* and *Gerbillus* is 0.028. These values give a rate of 0.0041 (*Mus*/*Rattus*) or 0.0035 ML distance per million years. When these rates are applied to the different lineages of Muridae, molecular datings can be suggested for several dichotomies of interest (table 3), such as 17.9–20.8 MYA for the separation between Murinae, Gerbillinae, and Acomyinae or 11.4–13.3 MYA between the two dendromurines *Steatomys* and *Dendromus.*

Discussion

Base Composition

The GC_3 compositions of vWF and LCAT (64.1%–89.1% and 54.8%–67.6%, respectively) indicate that these genes belong to the richest class of isochores, as already observed in Huchon, Catzeflis, and Douzery (1999) and Robinson, Gautier, and Mouchiroud (1997). Also, other nuclear genes sequenced in different rodent species show similar GC_3 contents, such as the nuclear ribonuclease (67.7%–82.3%; Dubois, Catzeflis, and Bientema 1999) or the p53 gene (54.3%–61.3%; D'Erchia et al. 1999). Accordingly, most of the genes would be distributed in the GC-rich fractions of DNA, which seems to be a general trend in mammals. Indeed, for humans, it has been shown that about 90% of genes are distributed in the two most GC-rich isochores, H2 and H3 (Zoubak, Clay, and Bernardi 1996).

Nevertheless, large differences between Muridae (vWF: 68.6% ± 2.9%; LCAT: 59.7% ± 3.1%), Dipodidae (vWF: 81.7% ± 8.3%; LCAT: 64.4% ± 3.1%), and human (vWF: 82%; LCAT: 77%) base compositions have been observed, being more pronounced for GC_3. These results are in agreement with previous surveys of both LCAT (Robinson, Gautier, and Mouchiroud 1997) and vWF (Huchon, Catzeflis, and Douzery 1999). The observed pattern for murid LCAT and vWF has proved to follow a general trend described for the whole genome of murids and known as the murid pattern (Sabeur et al. 1993; Robinson, Gautier, and Mouchiroud 1997). Accordingly, murids would be characterized by a shift in base composition toward GC-poorer DNA when compared with the pattern observed in nonmurid rodents and all other mammals (general pattern). For Dipodidae, we found that GC_3 values were between human and Muridae values. Similar results were obtained from CsCl profiles, in which Dipodidae showed a skewness value higher than those of murids but slightly lower than those of nonmurid rodents (Douady et al. 2000).

Even though these two genes are characterized by large GC_3 values, the LCAT gene shows lower values than vWF, regardless of the species, Muridae, Dipodidae, or humans. For humans, this difference may be due to the fact that these two genes belong to different GC-rich isochores, namely, H2 and H3 (Zoubak, Clay, and Bernardi 1996). The same thing can be postulated for Muridae and Diopodidae, but taking into account the general shift toward GC-poorer DNA.

From the taxonomic point of view, no apparent relationship between taxonomy (or systematics) and GC_3

content is readily observable for either LCAT or vWF genes within Muridae (data available on request). Average GC_3 percentages for selected taxa are as follows for vWF and LCAT, respectively: Acomyinae (69.3% and 60.0%), Arvicolinae (68.7% and 62.7%), Cricetinae (66.0% and 56.0%), Cricetomyinae (69.5% and 62.5%), Dendromurinae (69.2% and 60.5%), Gerbillinae (71.0% and 61.6%), Murinae (69.1% and 60.2%), Nesomyinae (69.9% and 60.5%), and Sigmodontinae (66.2% and 59.8%).

Rate of Evolution

As we have stressed for base composition, LCAT and vWF show different properties that can be also detected at the level of rate of evolution and, subsequently, at the level of saturation. Indeed, LCAT is more conserved at both the amino acid and the nucleotide levels than is vWF and presents a lower asynonymous substitution rate value than the latter (fig. 1). This variation is thought to reflect differences in functional constraints in both genes, mainly in the proportion of the sequences that are critical to the function of the proteins. Nucleotide substitution rate, and thus the degree of sequence conservation, may be influenced by different factors affecting either mutation or fixation rates:

- Chromosomal position of the gene and even the position within the chromosome (Matassi, Sharp, and Gautier 1999; Perry and Ashworth 1999). The LCAT gene has been mapped on chromosomes 16 and 8 in humans and mice, respectively, while vWF has been mapped on chromosomes 12 and 6, respectively. We can assume that also in most or all other rodent species, these two genes are placed on different chromosomes and thus in different ''evolutionary rate units,'' which could explain the different substitution rates.
- The tissue in which proteins are expressed (Kuma, Iwabe, and Miyata 1995; Hughes 1997; Hurst and Smith 1999). Even though both are plasmatic proteins, vWF is expressed in megakaryocytes and endothelial cells, while LCAT is synthesized by the liver and brain. The existence of differences in asynonymous rates in different tissue-specific proteins may reflect differences in selective pressures rather than differences in mutation rates, as demonstrated by Duret and Mouchiroud (2000).

Regarding synonymous rates of substitution, LCAT and vWF genes show similar substitution rates, as shown in figure 1. Since it seems there is no significant variation of synonymous rates between tissue-specific genes (Duret and Mouchiroud 2000), the observed difference between both genes may be due to differences in mutation rates. Nevertheless, the observed difference is most likely an underestimation of the real values because of a certain level of saturation in the transitions of both the vWF (fig. 2) and the LCAT genes (Michaux and Catzeflis 2000).

Because of the different evolutionary properties of vWF and LCAT, their combination for resolving phylogenetic relationships among muroid rodents has proven to give results with more resolution than those obtained using each single gene (Huchon, Catzeflis, and Douzery 1999; Michaux and Catzeflis 2000)

Fourteen Subfamilies of Muroids Are Organized into Five Major Lineages

An examination of figure 4, with the associated robustness values indicated in table 2, indicates that the combined LCAT and vWF genes suggest a muroid branching pattern with five clades comprising one or more traditional subfamilies.

The most ancient speciation event leads to ancestral segments 4 and 5, a dichotomy separating spalacines and rhizomyines from the rest of the muroids. Both clades are robust (bootstrap values from 91 to 100, Bremer's decay indices of 24 and 10). Moreover, the alternative topologies against this first isolation were always significantly worse. Following other authors (Thomas 1896; Sen 1977), we are tempted to consider spalacines and rhizomyines as the two living components of a muroid family Spalacidae, keeping the taxon name Muridae (Illiger 1811) for all the remaining muroid taxa.

Thus, the ancestral segment 5 would define Muridae, for which our data set includes representatives of 12 subfamilies. The reliability of our Muridae/Spalacidae split will be settled after the inclusion of the three missing subfamilies listed by Musser and Carleton (1993): Lophiomyinae (one genus), Petromyscinae (two genera), and Platacanthomyinae (two genera).

Among the four major lineages of murids, a first clade (ancestral segment 6, bootstrap values from 95% to 99%) unites African taxa representing nesomyines, dendromurines, cricetomyines, and the sole living member of mystromyines. This cluster of genera is reminiscent of Lavocat's (1973, 1978) concept of Nesomyidae, a taxon comprising archaic African ''cricetids'' which could all be derived from the fossil Afrocricetodontinae subfamily. Until additional taxa are examined for their molecular relationships (Malagasy *Eliurus* and *Gymnuromys*, African *Petromyscus* and *Beamys*, additional dendromurines), we refrain from suggesting a name for this clade.

The second lineage in murids includes *Calomyscus* only (see below). The third (ancestral segment 15) and fourth (ancestral segment 21) major murid clades are related, although not convincingly so (bootstrap values from $<$50% to 97%; a Bremer's decay index of 5; alternative topologies against this relationship were only significantly worse at 8% probability). All taxa representing the cricetines, myospalacines, sigmodontines, and arvicolines are united by a strong ancestral segment (15) (BP 89%–99%).

Finally, four ''traditional'' subfamilies (Gerbillinae, Murinae, Otomyinae, and Acomyines) cluster in a robust clade (ancestral segment 21: bootstrap values from 94% to 100%; a Bremer's decay index of 13).

An Isolated and Ancient Muroid: Calomyscus

The mouselike hamster *Calomyscus* (six living species) was only recently given a subfamilial rank (Calomyscinae) (Vorontsov and Potapova 1979) with regard

to sharp differences in several morphological characters. Although previous studies had suggested affinities of *Calomyscus* with different muroid taxa, sometimes with compelling evidence (similarities in the auditory ossicles led Pavlinov [1980] to relate mouselike hamsters with sigmodontine *Reithrodontomys*), its relationships have remained obscure. Even chromosomal data were ambiguous (Matthey 1961), with autosomal chromosomes similar to those of Eurasian cricetines, and sexual chromosomes reminiscent of North American peromyscines. Following Fahlbusch (1969) and other paleontologists, Carleton (1984) and Carleton and Musser (1984) concluded that ''*Calomyscus* could be justifiably classified among the cricetodontines, a group hitherto supposed extinct.''

Both LCAT and exon 28 of the vWF gene concur for an isolated position of *Calomyscus*, which might be more related (node 13: bootstrap values from 64% to 91%) to Holarctic cricetids (arvicolines, sigmodontines, cricetines) and advanced murids (gerbillines, murines, acomyines) than to the African muroids (nesomyines, cricetomyines, dendromurines). Moreover, this hypothesis was confirmed with the Kishino-Hazegawa test, which showed that alternative topologies against these relationships always exhibit significantly worse log likelihood values. In any case, *Calomyscus* belongs to the same radiation (nodes 5, 13, and 14 combined) which has led to the three major clades (ancestral segments 6, 15, and 21) of murids.

Acomyines: A Clade Sister to the Gerbils

Much progress with regard to the systematics of *Acomys* has been made since Sarich's (1985) immunological study, which was the first to suggest that spiny mice were not related to true murines (rats and mice). Different morphological and molecular studies have since shown evidence of a cluster of related genera, comprising *Acomys*, *Uranomys*, *Lophuromys*, and *Deomys*, which are neither murines nor dendromurines (see references cited in Denys et al. 1992; Chevret et al. 1994; Hänni et al. 1995; Dubois, Catzeflis, and Beintema 1999). Hänni et al. (1995) named ''acomyines'' the clade containing *Acomys*, *Uranomys*, and *Lophuromys*, to which *Deomys* was subsequently added (Denys et al. 1995; Verheyen, Colyn, and Verheyen 1996; Chevret, Catzeflis, and Michaux 2001).

Although convincing biochemical and molecular data exist for sustaining this cluster, the relationships of acomyines with regard to other living muroids have remained obscure or controversial. Whereas the DNA-DNA hybridization of Chevret et al. (1993) suggested an acomyine–gerbilline relationship, more recent experiments with additional taxa have been equivocal (Chevret, Catzeflis, and Michaux 2001). Sequence data have provided contradictory and weakly supported results (Hänni et al. 1995; Dubois, Catzeflis, and Beintema 1999; Chevret, Catzeflis, and Michaux 2001), with acomyines being either external to a murine + gerbilline clade or belonging to a polytomy also comprising murines and cricetines.

The combined use of two nuclear genes (LCAT and exon 28 of vWF) provides a clear and strong picture: acomyines cluster with gerbillines (ancestral segment 22; bootstrap values 96%–100%; BSI = 8, alternative topologies always significantly worse), and these two lineages are sister to murines (segment 21: BP = 94%–100%; BSI = 13). That these relationships are not biased by saturation is indicated by the robustness of deeper ancestral segments such as 14, 13, and 5 of figure 4. Although our taxonomic sampling is rather limited, each of the clades acomyines, gerbillines, and murines is defined by high values of robustness (BP = 100%; BSI = 22, 40, and 34, respectively). According to the molecular-clock analysis, the separation between the three subfamilies appeared 17.9–20.8 MYA. This approximation is slightly older than those obtained with the previous paleontological (16 MYA; Tong and Jaeger 1993) and molecular (15.5–17 MYA; Michaux and Catzeflis 2000) data. Thus, on the basis of these results, the first radiation event within the modern muroids should have appeared earlier than previously supposed. Moreover, a second event of separation seems to have occurred 16.5–18.5 MYA. This led to the separation between Acomyinae and Gerbillinae, but also to the radiation of the ''Cricetid'' group (Arvicolinae, Sigmodontinae, Cricetinae, Myospalacinae) and the ''African'' clade (Nesomyinae, Mystromyinae, Cricetomyinae, Dendromurinae) (see table 3). According to Aguilar et al. (1996) and Aguilar, Escarguel, and Michaux (1999), this period (end of early Miocene) was characterized by changes in climate which favored the spread in Europe, Africa, and America of allochtonous rodent groups probably coming from Asia.

The South African Mystromys *Clusters with African Murids*

Carleton (1984) and Carleton and Musser (1984) summarize perfectly the uncertain classification of the African white-tailed hamster *Mystromys*, which has been either allied to Holarctic cricetines or placed in its own subfamily (Mystromyinae) (Vorontsov 1966). Lavocat (1973) suggested that Miocene Cricetodontidae found in African (Ethiopia) deposits were ancestral to fossil and recent *Mystromys* and proposed a classification of African muroids into two families: the family Nesomyidae, including not only the Malagasy Nesomyinae and the Mystromyinae, but also Cricetomyinae, Lophiomyinae, Tachyoryctinae, and Otomyinae; and the family Muridae, comprising Murinae, Rhizomyinae, and Dendromurinae. Gerbillinae were not allocated by Lavocat (1973, p. 237), and were left incertae sedis.

Our present knowledge allows us to exclude Otomyinae and Tachyoryctinae from the Nesomyidae of Lavocat (1973), whose emended taxon thus corresponds to the clade defined by ancestral segment 6 in figure 4. Thus, *Mystromys* belongs to a group of archaic African muroids, together with nesomyines, cricetomyines, and some dendromurines. Its relationships among this cluster are not resolved in this study, although exon 28 of the vWF gene suggests the tritomy *Mystromys*-criceto-

myines-dendromurines. Additional taxa (such as *Petromyscus* or *Beamys, Lophiomys*) and gene sequences will be necessary to confirm these observations.

A Nesting Position of Myospalacinae Within Cricetinae and of Otomyinae Within Murinae

The analyses performed on the basis of the vWF sequences confirm those obtained with the LCAT gene (Michaux and Catzeflis 2000) about a nesting position of Myospalacinae within Cricetinae, particularly with the Asiatic species *Phodopus.* Thus, following a previous hypothesis based on morphological characters (Simpson 1945), we propose invalidation of the Myospalacinae subfamily and consideration of the genus *Myospalax* as defining a tribe among the subfamily Cricetinae. In the same way, the Otomyinae must be definitively considered a tribe of the Murinae subfamily, as already suggested by several morphological studies (Tullberg 1899; Miller and Gidley 1918; Simpson 1945; Carleton 1984).

Differences Between Datings Estimated by Fossils and Molecules

Although showing a trend toward overvaluation, most of the separation times estimated on the basis of the molecular data are in good agreement with those obtained with the fossil records (table 3). However, some of them seem more unclear. As already observed in Michaux and Catzeflis (2000), the divergence time estimation based on our molecular data suggests that the separation between *Myospalax* and the other Cricetinae appeared earlier (Late Miocene–Early Pliocene: 5–6 MYA) than the Pleistocene. Further paleontological investigations on this genus should be very useful to test our hypotheses.

Moreover, the value of 8.0–9.3 (standard error = 0.6) MYA for the split between the Arvicolinae *Clethrionomys* and *Dicrostonyx* is much older than the estimate of 4–6.0 MYA suggested by Chaline and Graf (1988). Our estimate is similar to the one calculated by Michaux and Catzeflis (2000), obtained with the nuclear LCAT gene only, but is at odds with other molecular studies based on DNA/DNA hybridization (Catzeflis et al. 1987) or on the nuclear ribonuclease gene sequences (Dubois, Catzeflis, and Beintema 1999). The relative-rate tests for the combined molecular data showed that the two Arvicolinae taxa do not evolve at a particular rate of evolution with regard to the other Muridae. Thus, the molecular clock of the LCAT and vWF genes seems also valid for these taxa. New paleontological investigations are needed to confirm this older *Dicrostonyx*/*Clethrionomys* divergence.

Conclusions

The present molecular study was performed on two nuclear genes sequenced for representatives of 14 among 17 Muridae subfamilies and four of the seven Dipodidae subfamilies.

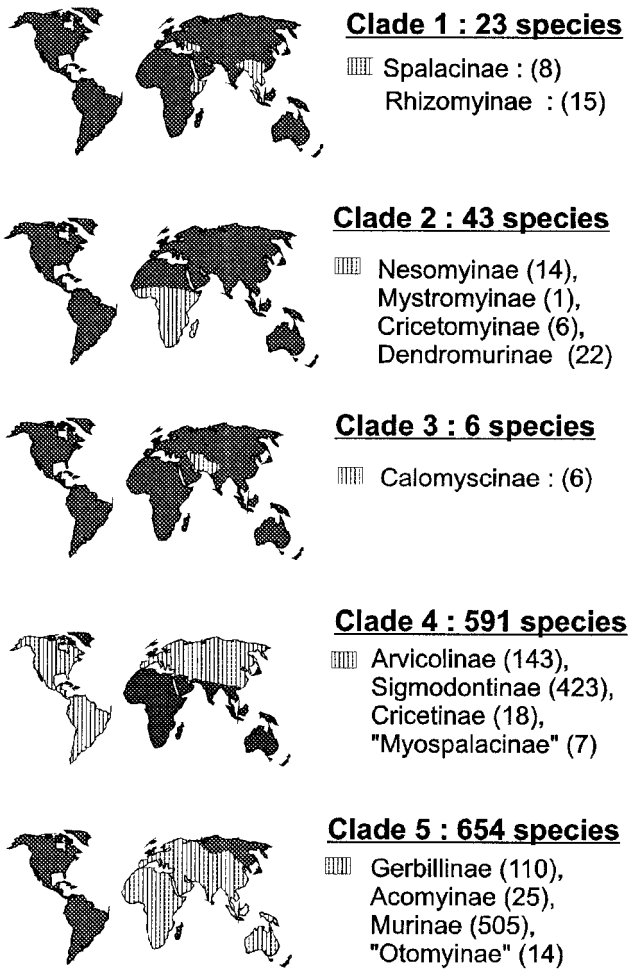

FIG. 5.—Approximate geographic distribution (from data in Wilson and Reeder 1993) of living species in each major lineage within Muridae. The numbers of species are those derived from Musser and Carleton (1993) assuming that each subfamily is monophyletic.

This taxonomic sampling led to evidence that from the base composition and rate-of-evolution points of view, muroids would be characterized by a shift in base composition toward GC-poorer DNA when compared with the pattern observed in nonmurid rodents and all other mammals (general pattern). The LCAT gene shows lower GC_3 values than vWF, regardless of the taxa under consideration (Muridae, Dipodidae, or humans), and is more conserved at both the amino acid and the nucleotide levels than is vWF. No apparent relation between taxonomy (or systematics) and GC_3 content is readily observable.

The sampling also led to evidence that the studied subfamilies of muroids are organized into five major lineages: a first isolation unites the Spalacinae and Rhizomyinae with a strong robustness; the second one includes the African taxa representing nesomyines, dendromurines, cricetomyines, and the sole living member of mystromyines; the third one comprises only the genus *Calomyscus*; the fourth one represents the cricetines, myospalacine, sigmodontines, and arvicolines; and the fifth one comprises four ''traditional'' subfamilies (Gerbillinae, Murinae, Otomyinae, and Acomyines).

Also evidenced by the present study were the monophyly of almost all studied subfamilies, including the Spalacinae, the Rhizomyinae, the Nesomyinae, the Dendromurinae (as understood by Michaux and Catzeflis 2000), the Cricetomyinae, the Arvicolinae, the Sigmodontinae, the Cricetinae, the Gerbillinae, the Acomyinae (as defined in Michaux and Catzeflis 2000), and the Murinae; an isolated position of *Calomyscus* with regard to the other modern subfamilies; a sister group relationship of the Acomyinae and the Gerbillinae; confirmation of a nested position of Myospalacinae within Cricetinae and Otomyinae within Murinae; and a close relationship between the South African *Mystromys* and the African murids (Nesomyinae, Cricetomyinae, Dendromurinae).

Future studies including members of the remaining subfamilies listed by Musser and Carleton (1993) (Lophiomyinae, Petromyscinae, and Platacanthomyinae), as well as some important missing genera for several subfamilies considered in our study (i.e., Murinae, Arvicolinae, Sigmodontinae), will confirm definitively the reliability of these results.

From the biogeographical point of view, an examination of the recent distribution of members of those five major lineages (fig. 5) allows the following comments, assuming that all muroids originated, spread, and radiated from Asia with different degrees of success: the first three groups radiated in a limited number of species (23, 43, and one, respectively) and genera (five, 18, and one, respectively) (Wilson and Reeder 1993) localized in some regions, whereas the last two groups literally exploded in a wide variety of species (591 and 654) and genera (109 and 139) dispersed all over the world.

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