Molecular Evolution of the Nuclear von Willebrand Factor Gene in Mammals and the Phylogeny of Rodents

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Nucleotide sequences of exon 28 of the von Willebrand Factor (vWF) were analyzed for a representative sampling of rodent families and eutherian orders, with one marsupial sequence as outgroup. The aim of this study was to test if inclusion of an increased taxonomic diversity in molecular analyses would shed light on three uncertainties concerning rodent phylogeny: (1) relationships between rodent families, (2) Rodentia monophyly, and (3) the sister group relationship of rodents and lagomorphs. The results did not give evidence of any particular rodent pattern of molecular evolution relative to a general eutherian pattern. Base compositions and rates of evolution of vWF sequences of rodents were in the range of placental variation. The 10 rodent families studied here cluster in five clades: Hystricognathi, Sciuridae and Aplodontidae (Sciuroidea), Muridae, Dipodidae, and Gliridae. Among hystricognaths, the following conclusions are drawn: a single colonization event in South America by Caviomorpha, a paraphyly of Old World and New World porcupines, and an African origin for Old World porcupines. Despite a broader taxonomic sampling diversity, we did not obtain a robust answer to the question of Rodentia monophyly, but in the absence of any other alternative, we cannot reject the hypothesis of a single origin of rodents. Moreover, the phylogenetic position of Lagomorpha remains totally unsettled.

Introduction

The order Rodentia represents almost half of the mammalian diversity, with more than 2,000 species organized into 29 families (Wilson and Reeder 1993). Rodents include animals with very different morphologies, life histories, population structures, and demographic variables. Because of this diversity, rodents are a key taxon for the study of biogeography, ecology, and even patterns of DNA evolution. Numerous studies have focused only on mice and rats, but little is known about the overall rodent diversity. The Rodentia phylogeny is still controversial and provides an illustration of the conflicts between morphological and molecular approaches.

At the subordinal level, Brandt (1855) used the insertion pattern of masseter muscles to define myomorph, sciuromorph, and hystricomorph rodents, but this myological character has been shown to be homoplastic (Hartenberger 1985; Nedbal, Honeycutt, and Schlitter 1996). Tullberg (1899) used the plane of incisor insertion to split rodents into Sciurognathi and Hystricognathi, and this is the current classification (Wilson and Reeder 1993). Whereas Sciurognathi may be paraphyletic, Hystricognathi is recognized as monophyletic and includes Old World phiomorphs and New World caviomorphs. The lack of straightforward, nonhomoplastic morphological characters to define suprafamilial clades among rodents is explained by the bushlike radiation of rodent families associated with convergent adaptations in similar environments (Jaeger 1988; Hartenberger 1996).

Key words: evolution, phylogeny, mammals, rodents, Lagomorpha, von Willebrand Factor (vWF).

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Molecular approaches offered a promising alternative to morphology for investigating rodent evolution, but they complicated the subject by calling Rodentia monophyly into question. Whereas morphological data support the monophyly of rodents (e.g., Luckett and Hartenberger 1993), several molecular studies suggested that Rodentia is at least diphyletic with two clades: guinea pig plus dormouse, and mouse plus rat (Graur, Hide, and Li 1991; Li et al. 1992; Ma et al. 1993; D'Erchia et al. 1996; Reyes, Pesole, and Saccone 1998). Actually, extensive investigations on those data sets indicate that monophyly and paraphyly of rodents are equally likely (Hasegawa et al. 1992; Cao, Okada, and Hasegawa 1997; Sullivan and Swofford 1997). Rodentia paraphyly may result from: (1) the fast molecular evolutionary rate of murids (Wu and Li 1985; Catzeflis et al. 1987; Li et al. 1996), which could lead to a long-branch attraction phenomenon (Philippe 1997); (2) the use of the ''quartet approach'' (three taxa and an outgroup), which appears unreliable (Philippe and Douzery 1994; Adachi and Hasegawa 1996*a*; Philippe 1997); or (3) an inadequate species sampling among both rodents and other placentals (Luckett and Hartenberger 1993; Philippe 1997; Sullivan and Swofford 1997). Because phylogenetic reconstructions are very sensitive to species sampling (Lecointre et al. 1993; Philippe and Douzery 1994), it is puzzling that no molecular study has yet explored both rodent and placental diversities.

The question of rodent monophyly also raises the one of their sister group. Morphological studies cluster Rodentia and Lagomorpha (rabbits, hares, and pikas) in a Glires clade (e.g., Luckett and Hartenberger 1993). However, dental and cranioskeletal inferences may be based on homoplastic characters, as revealed by a larger outgroup consideration (Lopez Martinez 1985), and molecular studies failed to clearly elucidate the Glires affinities (e.g., Graur, Duret, and Gouy 1996 [but see Halanych 1998]; Porter, Goodman, and Stanhope 1996; Madsen et al. 1997; Springer et al. 1997*a*, 1997*b*).

Molecular investigations of the relationships between rodent families focused on three mitochondrial genes: 12S rRNA (Catzeflis et al. 1995; Nedbal, Honeycutt, and Schlitter 1996), cytochrome oxydase II (Adkins, Honeycutt, and Disotell 1996), and cytochrome *b* (Matthee and Robinson 1997). Despite the use of ribosomal or protein-encoding mitochondrial markers, a general lack of phylogenetic resolution was observed, which could be the consequence of the rapid evolutionary rate of the mitochondrial genome. To explore the rodent phylogeny, the use of single-copy nuclear markers, e.g., exonic regions of the von Willebrand Factor gene (vWF; Porter, Goodman, and Stanhope 1996) or the lecithin cholesterol acyltransferase gene (LCAT; Robinson et al. 1997), appeared to be inescapable alternatives to mitochondrial studies.

The human vWF gene consists of a single functional nuclear copy with 52 exons spanning 180 kb on chromosome 12 (Mancuso et al. 1989). Exon 28 is the longest, with 1,379 bp corresponding to amino acids 463–921 of the mature subunit. The vWF gene codes for a plasma glycoprotein, constituted by multimerized 270-kDa subunits and produced by megakaryocytes and endothelial cells. It plays two major roles in haemostasis: it mediates platelet adhesion to damaged vessels and stabilizes blood coagulation factor VIII in the circulation (review in Sadler 1991).

Recently, Porter, Goodman, and Stanhope (1996) suggested that vWF exon 28 could be a powerful tool with which to investigate the eutherian phylogeny. In their analysis, the monophyly of three rodent species (*Mus, Spalax,* and *Dasyprocta*) was highly supported, but their location in the placental bushlike radiation remained unresolved (Porter, Goodman, and Stanhope 1996; Springer et al. 1997*b*). The large database now available for vWF exon 28 calls for further studies, especially for the exploration of the rodent diversity, together with the identification of a much-needed marsupial outgroup. Three interdependent questions are therefore asked: (1) What are the relationships between the main rodent families? (2) Is Rodentia a monophyletic order? (3) What are the closest living relatives of rodents?

To explore the Rodentia phylogeny in the context of eutherian evolution, we sequenced vWF exon 28 for representative rodent and lagomorph species and for a marsupial outgroup. The molecular evolution of this newly described phylogenetic marker is considered, and its contribution to the rodent phylogeny is evaluated at both familial and ordinal levels.

Materials and Methods

Species Sampling

We increased the mammalian data matrix of vWF sequences of Porter, Goodman, and Stanhope (1996) and Springer et al. (1997*b*) with of 12 new rodents sequences, 2 new lagomorph sequences, and 1 new marsupial sequence (table 1). For each Sciurognathi family considered (Muridae, Dipodidae, Aplodontidae, Sciuridae, Gliridae), we analyzed, whenever possible, two species from different subfamilies in order to reduce a potential long-branch attraction phenomenon (Felsenstein 1978). For Hystricognathi, whose subfamilial diversity is less important, we studied one species for two Phiomopha and three Caviomorpha families. In order to test for the Glires concept, three lagomorph sequences were considered: two Leporidae and one Ochotonidae. The sequence of a marsupial (the eastern gray kangaroo, *Macropus giganteus*) was determined in order to root the eutherian tree without ambiguity.

DNA Sequencing of vWF Exon 28

Tissue samples came from the Collection of Preserved Mammalian Tissues of the Institut des Sciences de l'Evolution de Montpellier (France) (Catzeflis 1991). Taxonomy, origins, and references of the tissues are shown in table 1. 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 with primers V1 (5′-TGTCAACCTCACCTGTGA AGCCTG-3′) and W1 (5′-TGCAGGACCAGGTCAG-GAGCCTCTC-3′), constructed from the alignment of human, murine, canine, and bovine ortholog sequences. PCR reactions were performed using the following parameters: one cycle of 95°C denaturation (5 min), 50–60°C annealing (1 min), 72°C extension (2 min); 25 cycles of 95°C denaturation (45 s), 50–60°C annealing (45 s), 72°C extension (2 min); one cycle of 72°C final extension (15 min). A minimum of two independent PCR products were pooled and fragments were purified on a NuSieve (FMC Bioproduct) agarose gel (1% in 1 × TAE). PCR products of the expected size (1,314 bp) were excised, purified with the Wizard™ PCR preps DNA purification kit (Promega), and cloned in the pCR™ 2.1 plasmid vector. The *Escherichia coli* strain INVαF′ was used for transformations according to the Original TA cloning kit (Invitrogen). Recombinant plasmids were sequenced using the dideoxy chain termination method (Sanger, Nicklen, and Coulson 1977) with [α ^{35}S]dATP and the ^{T7}Sequencing™ mixes kit (Pharmacia Biotech). Sequencing of vWF inserts was conducted on both strands with the V1 and W1 external primers, and with internal primers V2 (5′-CCCTCAGA GCTGCGGCGCAT-3′), W2 (5′-ACGTCCATGCGCTG GATCACCT-3′), V3 (5′-TCCATGGTTCTGGATGTG GT-3′), W3 (5′-GTGTACTTCAAGACCTCACTGG-3′), V4 (5′-AAGCAGGCCCCTGAAAACAA-3′), W4 (5′-TTGTTTTCAGGGGCCTGCTT-3′), and W5 (5′-GGAGCCRTCCAGCAGGAA-3′). Internal primers were determined from the alignment of Porter, Goodman, and Stanhope (1996). For each species, a minimum of two clones was sequenced. The procedure was slightly modified for *Dryomys nitedula* and *Allactaga elater*. Fragments of 1,300 bp were reamplified with pairs of external and internal primers, and smaller overlapping fragments V1/W2 (903 bp), V2/W1 (992 bp), or V4/W1 (713 bp) were cloned and sequenced according to the protocol previously described. The sequence of *M. giganteus* was obtained by direct sequencing of the two overlapping V1/W2 and V2/W1 reamplified PCR frag-

Table 1
Taxonomic Frame Following Wilson and Reeder (1993) for the Rodent, Lagomorph, and Marsupial Taxa Studied

Rodentia Sciurognathi	
Muridae	
Murinae	*Rattus norvegicus* (Norway rat); Yale Medical School; F. Catzeflis (T1413)/AJ224673; this paper
Murinae	*Mus musculus* (domestic mouse); BALB/C strain/U27810; Nichols et al. (1994)
Spalacinae	*Spalax polonicus* (Ukranian blind mole-rat); origin unknown/U31621; Porter, Goodman, and Stanhope (1996)
Dipodidae	
Dipodinae	*Dipus sagitta* (northern three-toed jerboa); Caucasus, Georgia; P. Gambarian (T869)/AJ224665; this paper
Allactaginae	*Allactaga elater* (small five-toed jerboa); Turbat Jam, Iran; M. Zadé (T1045)/AJ224661; this paper
Sciuridae	
Sciurinae	*Marmota monax* (woodchuck); Yukon, Canada; R. S. Hoffmann (T137)/AJ224671; this paper
Petauristinae	*Glaucomys volans* (eastern flying squirrel); Audubon Zoo, New Orleans, Louisiana; R. M. Zink and D. Reynolds (T1110)/AJ224667; this paper
Aplodontidae	*Aplodontia rufa* (mountain beaver); Washington; D. Nolta (T1462)/AJ224662; this paper
Gliridae	
Glirinae	*Glis glis* (fat dormouse); Forel, Switzerland; F. Catzeflis (T1220)/AJ224668; this paper
Leithiinae	*Dryomys nitedula* (forest dormouse); Caucasus, Georgia; M. Baskevich (T768)/AJ224666; this paper
Rodentia Hystricognathi	
Thryonomyidae (Phiomorpha)	*Thryonomys swinderianus* (cane rat); Koubatchi, Congo; L. Granjon (T809)/AJ224674; this paper
Hystricidae (Phiomorpha)	*Trichys fasciculata* (long tailed porcupine); Sabah, Borneo; R. Stuebing (T726)/AJ224675; this paper
Erethizontidae (Caviomorpha)	*Coendou melanurus* (prehensile tailed porcupine); Kourou, French Guiana; J.-C. Vié (T1559)/AJ224664; this paper
Caviidae (Caviomorpha)	*Cavia porcellus* (guinea pig); breeding colony; P. Perret (T1575)/AJ224663; this paper
Dasyproctidae (Caviomorpha)	*Dasyprocta leporina* (agouti); origin unkown/U31607; Porter, Goodman, and Stanhope (1996)
Lagomorpha	
Leporidae	*Lepus crawshayi* (hare); Yono Féré, Senegal; J.-M. Duplantier and L. Granjon (T480)/AJ224669; this paper *Oryctolagus cuniculus* (rabbit); origin unknown/U31618; Porter, Goodman, and Stanhope (1996)
Ochotonidae	*Ochotona princeps* (pika); Albuquerque, New Mexico; T. Yates (T1691)/AJ224672; this paper
Diprotodontia	
Macropodidae	*Macropus giganteus* (eastern gray kangaroo); Zoo La Palmyre, France; T. Petit (T727)/AJ224670; this paper

NOTE.—The following information is also provided: latin name (common name), origin of the animal, name of the tissue collector (catalog number in the collection of mammalian tissues of the Institut des Sciences de l'Evolution de Montpellier; Catzeflis 1991)/EMBL Data Bank accession numbers for the sequences of the exon 28 of the von Willebrand Factor gene, and reference of the corresponding works.

ments. Direct sequencing was realized with [α ^{33}P]ddNTP and using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham).

Sequence Alignment

Sequences were aligned by hand with the ED editor (MUST package; Philippe 1993). The alignment was unequivocal; a single insertion of one codon was required for the two Gliridae species, and a deletion of one codon was introduced for the *Macropus* sequence. In the subsequent analyses, gaps were coded as missing data.

Phylogenetic Reconstructions

Data were analyzed by distance, maximum-parsimony (MP) and maximum-likelihood (ML) methods. The neighbor-joining method (NJ; Saitou and Nei 1987) was performed on Tamura and Nei (1993) distances with the NJ program (MUST package; Philippe 1993). MP analyses were conducted with PAUP 3.1.1 (Swofford 1993). Heuristic searches were realized with the tree bisection-reconnection (TBR) branch-swapping option and 100 random-addition sequences. ML trees were constructed with MOLPHY 2.3b3 (Adachi and Hasegawa

1996*b*) and with the quartet puzzling procedure of Strimmer and von Haeseler (1996) using PUZZLE 4.0. The Tamura and Nei (1993) and JTT (Jones, Taylor, and Thornton 1992) models of sequence evolution were chosen for nucleotide and amino acid matrices, respectively. The gamma distribution with four categories was used to describe the substitution rate heterogeneities (Yang 1996). Because of the computation time limitations of MOLPHY 2.3b3, orders represented by only one species were discarded (Artiodactyla, Dermoptera, Pholidota, and Xenarthra), as were primates and carnivores, which were represented only by two closely related taxa. All rodent and lagomorph species were kept with two perissodactyls, two bats (the Microchiroptera *Tonatia* and the Megachiroptera *Dobsonia*), two divergent Paenungulata *sensu lato* (*Amblysomus* and *Elephas*) and the kangaroo (*Macropus*). The MOLPHY 2.3b3 search for the best dichotomous ML tree for this limited data set of 25 species was realized in four steps: (1) a constrained topology was defined (i.e., the quartet puzzling tree; see below and fig. 2), with unresolved relationships (multifurcations) left free to vary; (2) an exhaustive search was done with the ProtML program, and the best 1,000 amino acid trees were retained by the approximate-likelihood criterion; (3) the log-likelihood of these 1,000 starting trees was computed with NucML for each codon position separately; and (4) the log-likelihoods were summed up over the three positions with TotalML.

The robustness of trees was assessed by bootstrap percentages (BP; Felsenstein 1985) after 1,000 replications for NJ and MP. In the case of ML, reliability percentages (RP; Strimmer and von Haeseler 1996) estimated the occurrence of the nodes in the quartet puzzling trees after 10,000 puzzling steps. Bremer's (1988) support indices (BSIs) were calculated on the most parsimonious trees with enforcement of topological constraints. Likelihoods of alternative topologies were compared with MOLPHY 2.3b3 (Adachi and Hasegawa 1996*b*). According to Kishino and Hasegawa (1989), an alternative hypothesis was rejected when $\Delta\text{lnL} > 1.96$ SE, where ΔlnL is the difference between the log-likelihoods of the best and the evaluated trees, and SE is the standard error of this difference.

Homoplasy and Saturation Analysis

Pairwise numbers of observed nucleotide differences were plotted against changes inferred by MP for each of the six types of substitutions at the three codon positions (Hassanin, Lecointre, and Tiller 1998). Each of the 18 resulting plots (obtained through the ''homoplasy matrix'' option of PAUP 3.1.1) was characterized by the slope (*S*) of the linear regression between observed and inferred mutational events and by the consistency index (CI; excluding uninformative characters) of the corresponding most-parsimonious trees. The character-state changes were then weighted according to the product $CI \times S$ of the homoplasy and saturation parameters in the weighted MP (MP_w) reconstructions (cited in Hassanin, Lecointre, and Tiller 1998).

Relative-Rate Test

The two-cluster test and the branch-length test (Takezaki, Rzhetsky, and Nei 1995) were conducted with the LINTRE package (http://www.bio.psu.edu/People/Faculty/Nei/Lab/) to evaluate the clocklike behavior of the data. The Tamura and Nei (1993) and amino acid distances with gamma rates were, respectively, applied on nucleotide and amino acid matrices (the rate heterogeneity parameter α was estimated by PUZZLE 4.0). The topology used for the tests was the MP_w tree (see below and fig. 1).

For comparisons between lineages, relative-rate tests were conducted with RRTree, version 0.5 (Robinson et al. 1998; ftp://pbil.univ-lyon1.fr/pub/datasets/MBE98/), which improves the test of Wu and Li (1985) by taking into account several sequences per lineage under focus, and introducing a topological weighting. Relative-rate tests were performed for different taxonomic levels (supra- and infrafamilial comparisons), and the ML tree computed by quartet puzzling was chosen as the reference phylogeny (see below and fig. 2). In each case, the outgroup chosen was diversified, slow-evolving, and closely related to the ingroup. Such properties are more prone to reveal a rate heterogeneity (Robinson et al. 1998). Relative-rate tests on nucleotide sequences were performed on the proportions of synonymous (K_s) and nonsynonymous (K_a) substitutions.

Results

Nucleotide and Amino Acid Characteristics of vWF Exon 28

Nucleotides

The third codon position of exon 28 exhibited the highest variability, the highest bias against transversions, and the lowest substitution rate heterogeneity (table 2). The strongest bias in nucleotide composition was also found at the third position of the codon (table 2). By comparison with the human sequence, a standard used for isochore studies (e.g., Robinson, Gautier, and Mouchiroud 1997), eutherians grouped in three categories following their G+C content at the third codon position (GC3): (1) ΔGC3 (i.e., $GC3_{human} - GC3_{others}$) ranged from 0 to +10% for Chiroptera, Artiodactyla, Xenarthra, Pholidota, Dermoptera, Leporidae, *Thryonomys*, *Dipus*, and the two Gliridae; (2) ΔGC3 ranged from 0 to −10% for Perissodactyla, Carnivora, Tethytheria, Ochotonidae, the two Sciuridae, the Aplodontidae, *Allactaga*, and the two porcupines; (3) ΔGC3 exceeded −10% for *Cavia*, *Dasyprocta*, the three Muridae, and the four Paenungulata *s.l.* (*Procavia*, *Orycteropus*, *Elephantulus*, *Amblysomus*). Relative to the placental orthologs, the marsupial (*M. giganteus*) vWF sequence showed a strongly deviating ΔGC3 (+23%).

Amino Acids

The vWF amino acid composition indicated that exon 28 encodes a hydrophobic region, and the most frequent amino acids were valine (11.1%), leucine (8.7%), arginine (7.9%), serine (7.9%), and glutamine (7.2%). Variable sites of the polypeptide were mainly

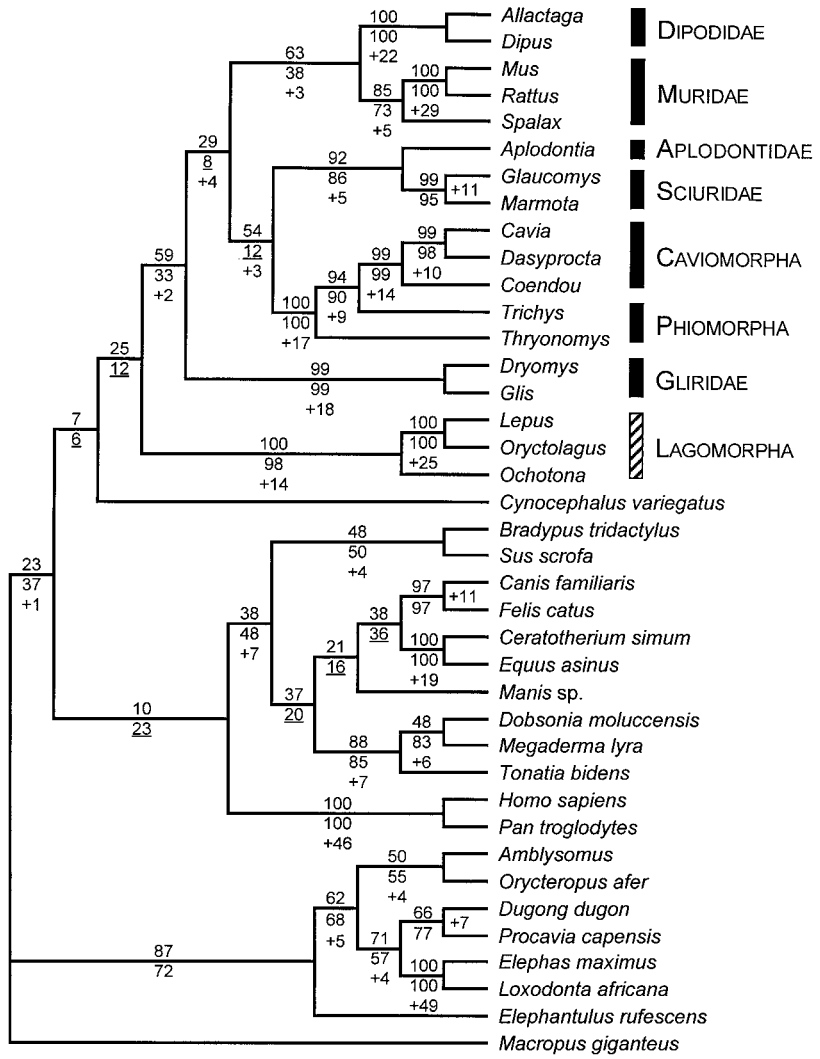

FIG. 1.—Maximum-parsimony (MP) analysis of 38 placental DNA sequences of the vWF exon 28. The majority-rule consensus tree was reconstructed from 1,000 bootstrap replicates and rooted by the marsupial sequence (*Macropus giganteus*). Bootstrap percentages (BPs) are indicated above (MP weighted by consistency and saturation indices) and below (standard MP) the internal branches, and residual BPs are underlined. Positive Bremer support indices computed under equally weighted MP are given below branches. Under standard MP, the four most-parsimonious cladograms were 3,656 steps long (consistency index excluding uninformative characters = 0.34; retention index = 0.66). Rodentia species belonging to the same taxonomic group are indicated with black bars and Lagomorpha species are indicated by the hatched bar.

restricted to the 20 first and last amino acid (aa) positions, and in a short region corresponding to aa 702–733 (the human sequence was used as a reference for aa numbering). A 1–2 aa insertion was, respectively, detected for the two glirids and the cow in this region.

Phylogenetic Reconstructions

Equally Weighted Maximum Parsimony

MP analyses based on equal weighting of each nucleotide substitution strongly supported the monophyly of representative species of the three Rodentia families Dipodidae, Sciuridae, and Gliridae (fig. 1; BP > 95% and BSI > +11). Lower support was evidenced for the three Muridae species (*Mus, Rattus,* and *Spalax*; BP = 73% and BSI = +5).

vWF exon 28 provided marked support for several suprafamilial clades: Sciuroidea (Sciuridae + Aplodontidae; BP = 86% and BSI = +5), Caviomorpha (BP = 99% and BSI = +14), Caviomorpha + Hystricidae (BP = 90% and BSI = +9), and Hystricognathi (the latter two taxa plus Thryonomyidae; BP = 100% and BSI = +17). Within caviomorphs, *Coendou* (Erethizontidae) emerged first relative to the two other South American taxa. The two Phiomorpha representatives (*Thryonomys* and *Trichys*) constituted a paraphyletic assemblage. Five rodent lineages (Dipodidae, Muridae, Sciuroidea, Hystricognathi, and Gliridae) were therefore clearly defined (fig. 1), and relationships between them were not resolved. Rodentia appeared to be monophyletic, but the support was very weak (BP = 33% and BSI = +2). Lagomorpha was monophyletic (BP = 98% and BSI = +14), and the two Leporidae clustered together (BP = 100% and BSI = +25) with respect to the Ochotonidae species. Negligible support was observed for the monophyly of Glires (i.e., Rodentia + Lagomorpha). Basal relationships between eutherian orders received only

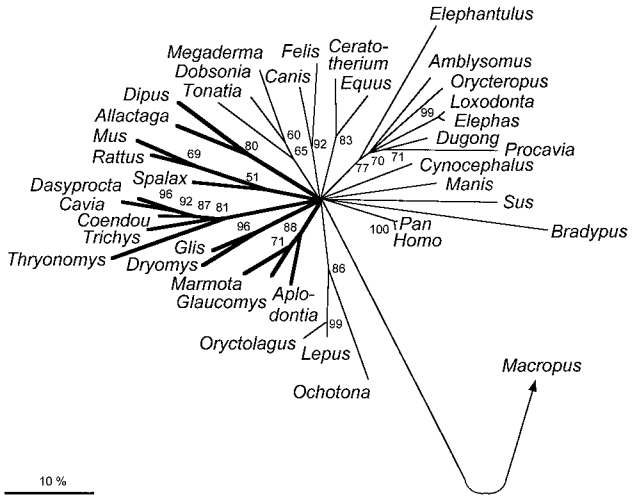

FIG. 2.—Maximum-likelihood phylogram (lnL = −17,831.8) calculated by quartet puzzling after 10,000 replicates (PUZZLE 4.0 program) on 38 placental DNA sequences of vWF exon 28 and rooted by the marsupial sequence. Reliability percentages are reported near the corresponding nodes, and nodes supported by less than 50% are collapsed into multifurcations. Branch lengths are proportional to the estimated number of substitutions per site. The star tree presentation has been adopted to highlight the superimposition of the bushlike radiations of rodent families and placental orders. All rodent taxa branches are thickened, and the arrow is directed toward the outgroup (*Macropus giganteus*).

Table 2
Mean Base Composition and Number of Sites Compared (total, variable, and phylogenetically informative) for von Willebrand Factor Exon 28 Sequences of 1 Marsupial and 38 Placentals at Each Codon Position and for the Whole Molecule

	Position 1	Position 2	Position 3	All Positions
% A	24.1	31.0	8.6	21.2
% C	27.8	21.8	39.1	29.6
% G	35.5	16.9	39.1	30.5
% T	12.7	30.3	13.3	18.8
% G+C	63.3	38.7	78.2	60.1
Number of sites				
Total	412	412	412	1236
Variable	255	210	392	857
Informative	167	128	341	636
Ti/Tv	1.39	2.38	4.26	2.44
α parameter	0.48	0.36	2.25	0.52

NOTE.—The maximum-likelihood estimates of the transitions/transversions (Ti/Tv) ratio and the substitution rate heterogeneity parameter (α) are also given (PUZZLE 4.0 program, using the Tamura and Nei [1993] model of sequence evolution).

very weak support, except for a clade comprising Xenarthra, Artiodactyla, Carnivora, Perissodactyla, Pholidota, and Chiroptera, which was defined by a high BSI (+7) but a low BP (48). Other relationships concerning placental orders (e.g., Chiroptera or Paenungulata) were similar to those described by Porter, Goodman, and Stanhope (1996) and Springer et al. (1997*b*).

To check if one of the 38 placental vWF sequences behaved randomly (see, e.g., Sullivan and Swofford 1997), 100 random sequences were generated using MacClade 3.04 (Maddison and Maddison 1992), constraining each codon position to respect the mean placental vWF base composition. Due to computing time limitations, 100 MP analyses were run, each with one random sequence, and BPs after 100 replications were approximated without branch swapping. Most of the random sequences weakly clustered with either the kangaroo (67 cases; mean BP = 57 ± 17) or the sloth (28 cases; BP = 34 ± 11).

Weighted Maximum Parsimony

For each of the six substitution types and at each of the three codon positions, the number of differences observed between each pair of taxa was plotted against the number of substitutions inferred by MP to evaluate the homoplasy and saturation level of the sequences. No contrasted differences in substitution pattern were evident between the three codon positions; all sites were kept in the phylogenetic analyses. However, transitions appeared more homoplastic (range of CI at the three positions: 0.24–0.42) and saturated (range of *S*: 0.46–0.64, except for the less-saturated C-T substitutions at first positions) than transversions (CI and *S* range, respectively, from 0.46 to 0.70 and from 0.77 to 0.99, except for the more noisy C-G substitutions at third positions). Weighting the MP reconstructions by the CI × *S* product of homoplasy and saturation indices improved the robustness of most nodes (fig. 1), particularly Myodonta (Muridae plus Dipodidae; BP = 63 vs. 38), Rodentia (BP = 59 vs. 33), and Paenungulata (BP = 71 vs. 57).

Maximum Likelihood

The ML tree obtained with the PUZZLE 4.0 program is presented in figure 2. All the nodes that were strongly supported in the MP tree were retrieved, and the five rodent clades and the other placental orders emerged in the same multifurcation.

The highest-likelihood tree computed with 25 species showed the monophyly of rodents but not that of Glires (fig. 3). Among rodents, Myodonta (Muridae + Dipodidae) was monophyletic, and sciuroids clustered with glirids. Moreover, the relationships between placental orders were depicted by very short branches, and of the first 1,000 highest-likelihood trees, as many as 720 were significantly worse relative to the best tree (fig. 3). Among the 280 remaining trees, Rodentia as well as Myodonta appeared to be monophyletic in the 100 best phylograms, and the Glires monophyly was not statistically rejected.

The Alternatives to the Best Trees

Only two main alternatives to Rodentia monophyly were retrieved by both NJ and MP bootstrap analyses as well as in the 280 most likely trees: (1) Lagomorpha clustered with either Gliridae, Dipodidae or both; (2) Muridae or Myodonta, and then the other rodent clades, were sister group to all other placentals (i.e., the eutherian tree was rooted by *Macropus* on the Muridae or Myodonta nodes).

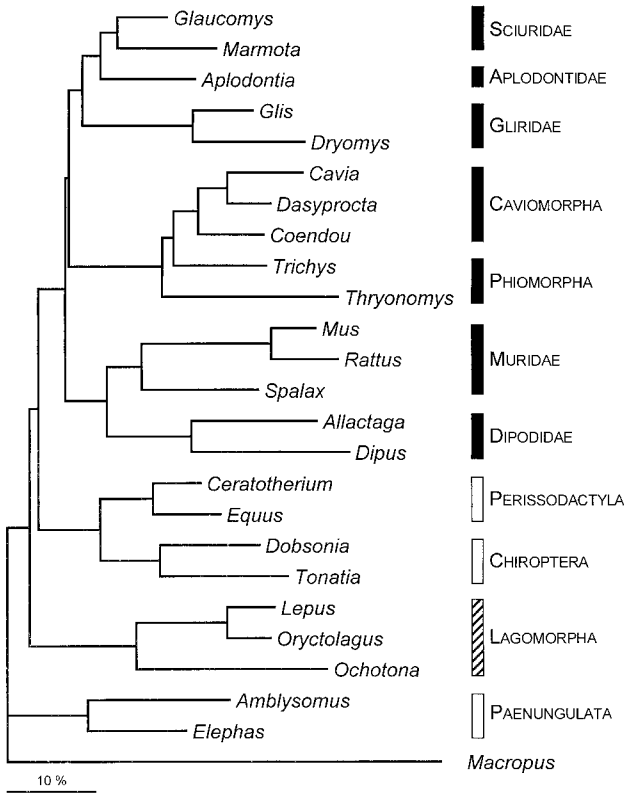

FIG. 3.—Maximum-likelihood phylogram computed with MOLPHY 2.3b3 on 24 eutherian vWF nucleotide sequences and rooted by a marsupial sequence (*Macropus giganteus*). This tree was identified among the 1,000 best ML trees (computed on amino acid sequences). Its log-likelihood (lnL = −12,228.4) is the sum of the log-likelihoods at each codon position. Branch lengths are proportional to the estimated number of substitutions per site. Taxonomic frames for Rodentia, Lagomorpha, and other placentals are indicated by full, hatched, and empty bars, respectively.

The highest-likelihood tree (fig. 3) was then used as the reference topology to investigate all 105 relationships connecting the five hystricognaths. Only two topologies did not exhibit significantly worse log-likelihood: (1) the Phiomorpha and Caviomorpha monophylies (+0.67 SE) and (2) the exchange of the relative positions of *Trichys* and *Thryonomys* (+0.55 SE).

Reconstructions on Amino Acids

Plots of the number of observed aa differences against inferred substitutions did not reveal that variable regions were experiencing multiple aa replacements or saturation (data not shown). For this reason, all sites were kept in the phylogenetic reconstructions. Amino acid analyses showed congruent but more unresolved results (table 3), and did not support the clade comprising Xenarthra, Pholidota, Chiroptera, Carnivora, Perissodactyla, and Artiodactyla.

Altogether, the results obtained with standard MP and ML on nucleotide and aa matrices were congruent with those obtained with weighted MP and NJ (except for weak Sciuroidea support with the distance approach) (table 3).

Relative-Rate Tests

A branch length test indicated that half of the 39 nucleotide and amino acid sequences evolved with an evolutionary rate significantly different from the average ($P < 0.05$). A two-cluster test showed that, for both data sets, five robustly supported nodes exhibited significantly different rates in their descent lineages. *Mus* and *Rattus*, *Thryonomys*, *Felis*, *Procavia*, and *Elephantulus*, respectively, evolved faster than *Spalax*, the remaining hystricognaths, *Canis*, *Dugong*, and the other paenungulates *s.l.*

To identify taxa involved in the vWF substitution rate heterogeneities, relative-rate tests were conducted

Table 3
Indices of Robustness for Various Nodes of the Phylogenetic Trees Reconstructed from DNA and Amino Acid vWF Exon 28 sequences Using Maximum-Parsimony (MP), Distance, and Maximum-likelihood (ML) Analyses

	NUCLEOTIDE ANALYSIS					AMINO ACID ANALYSIS			
CLADES	BSI	MP	MP_w	NJ	ML	BSI	MP	NJ	ML
Murinae	+29	100	100	100	69	+10	100	100	72
Muridae	+5	73	85	87	51	—	—	48	—
Dipodidae	+22	100	100	86	80	+3	92	99	73
Myodonta	+3	38	63	65	—	—	24	58	—
Sciuridae	+11	95	99	62	71	—	54	92	68
Sciuroidea	+5	86	92	22	88	—	37	74	78
Gliridae	+18	99	99	89	96	+4	92	100	90
Cavidae + Dasyproctidae	+10	98	99	94	96	+3	74	58	97
Caviomorpha	+14	99	99	81	92	+6	96	99	94
Caviomorpha + Hystricidae	+9	90	94	91	87	+1	66	84	84
Hystricognathi	+17	100	100	100	81	+11	100	100	87
Rodentia monophyly	+2	33	59	18	—	—	27	34	—
Rodentia + Lagomorpha	—	12	25	—	—	—	—	13	—

NOTE.—Bremer support indices (BSIs), i.e., numbers of extra steps required to break the corresponding nodes under the MP criterion, are first given. Then, bootstrap percentages computed after standard MP (i.e., with equal weighting), MP weighted by consistency indices and slopes of saturation profiles (MP_w, for DNA analysis only), and neighbor-joining (NJ) on Tamura and Nei (1993) distances with gamma rates ($\alpha = 0.52$ for DNA and 0.64 for amino acids) are reported. Reliability percentages deduced from the quartet puzzling ML method are also given. A dash indicates that the node does not appear in the corresponding majority-rule consensus bootstrap tree.

with each of the 15 placental clades (cf. fig. 2) against the remaining groups. Whereas neither K_s nor amino acid comparisons showed significant contrast, K_a comparisons evidenced marked differences in evolutionary rates: Sciuroidea were slowly evolving ($P < 0.01$), and Hystricidae and Xenarthra were fast-evolving ($P < 0.05$). Muridae did not have a particularly fast rate of evolution. Differences in behavior between K_s and K_a could reflect the fact that synonymous substitutions saturated at the ordinal level for eutherians. The lack of significant results at the amino acid level could also result from the lower number of sites analyzed.

Relative-rate tests were then performed within each rodent family, with the slow-evolving sciuroids as outgroup. Comparisons within murids confirmed that *Spalax* evolved more slowly than did *Mus* and *Rattus* ($P < 0.05$ for K_s comparisons; $P < 0.01$ for K_a comparisons). Within hystricognaths, significant K_s comparison involved a fast-evolving *Thryonomys* relative to *Coendou* ($P < 0.05$). Then, *Thryonomys* appeared to be the fastest-evolving hystricognath, as evidenced in the two-cluster test (see also branch length in figs. 2 and 3). Neither DNA comparisons within sciuroids, glirids, or dipodids nor amino acid comparisons with each rodent family were significant.

Discussion

vWF Exon 28 Structure and Function

Medical studies revealed that two different bleeding disorders result from point mutations in two regions of exon 28 (review in Ginsburg and Sadler 1993). IIb mutations are responsible for spontaneous platelet aggregation and occur between aa 497 and 698. This region includes a disulphide loop between Cys(509) and Cys(695) that could contain the sites involved in vWF ability to react with platelets (e.g., Matsushita and Sadler 1995). IIa mutations occur between Gly(742) and Glu(875) and decrease the half-life of the vWF protein in blood circulation (Berkowitz et al. 1987). Our vWF sequence comparisons across mammals suggest that: (1) the cysteine loop and the IIa mutations domain and its surrounding sites are characterized by a high conservation of aa sites, in agreement with medical works which suggest a major functional role of these two regions; and (2) the short Cys(695)–Pro(709) domain, including part of the IIb region, is more variable, although it has been suggested that it may play an important role in vWF adhesion to platelets. The latter observation agrees with the results of Matsushita and Sadler (1995), which moderate the functional importance of the aa 695–709 domain.

Evolution of vWF Exon 28 in Mammals: Are Rodents a Molecular Exception?

It has been suggested that some rodents have many molecular peculiarities relative to other eutherians. For example, Muridae possess a more homogeneous genome base composition than do phylogenetically distant placental species which represent the ''general mammalian pattern'' (Mouchiroud and Bernardi 1993; Sabeur et al. 1993; Robinson, Gautier, and Mouchiroud 1997). It has also been claimed that rodents have a fast-evolving genome because they have short generation times, small adult body sizes, and/or high metabolic rates (Wu and Li 1985; Catzeflis et al. 1987; Ohta 1993; Li et al. 1996). These results, however, were deduced from a restricted set of murid species (mouse, rat, hamster).

Base Composition

The GC3 composition of vWF (ranging from 69% to 90% in eutherians) indicates that this gene belongs to the richest class of isochores in the genome. This class is characterized by the most contrasted differences between murid and human base compositions (Mouchiroud and Gautier 1990). Comparison of hominid (82% GC3), murid (69%–70%), and caviomorph (69%–77%) vWF sequences shows that their GC3 compositions agree with the hypothesis of a GC homogenization, and/or reordering, in murine and guinea pig genomes (Robinson, Gautier, and Mouchiroud 1997). One should also note that some paenungulates show GC3 contents similar to those of murids. Generalizations are thus premature, as our taxonomic sampling indicates that in the same rodent family, two different genera can have strikingly contrasted base compositions: among Dipodidae, ΔGC3 is -5.8% for *Dipus* versus $+5.7\%$ for *Allactaga*. The same contrast in ΔGC3 values is observed for some eutherian orders, such as Lagomorpha (*Ochotona* $+2.6\%$, *Lepus* -7.8%).

Molecular Evolutionary Rates

Relative-rate tests indicate that vWF exon 28 of rodents is not especially fast-evolving compared with those of other eutherian species. Faster-evolving clades (Hystricognathi), as well as slower-evolving clades (Sciuroidea), are evidenced among rodents. Moreover, animals with relatively similar life history traits, such as South American porcupines (*Coendou*) and African cane rats (*Thryonomys*), exhibit significantly different rates of evolution. These results weaken the hypothesis suggesting that rates of molecular evolution are mainly correlated to generation times (e.g., Li et al. 1996), and they suggest that other parameters, such as metabolic rates and paleodemographic evolutions, may be acting (Martin and Palumbi 1993; Easteal and Collet 1994; Douzery, Lebreton, and Catzeflis 1995; Matthee and Robinson 1997).

Within rodents, murid sequences are not especially fast-evolving, and this finding contrasts with other nuclear and mitochondrial studies (e.g., Catzeflis et al. 1987; Philippe 1997). This result is probably the consequence of the use of a ''topology-weighting'' procedure in the computation of the relative-rate test (Robinson et al. 1998). *Spalax* is a slow-evolving genus, and its lineage has a topological weight of one-half among the three Muridae sequences. However, the mouse and the rat remain fast-evolving species relative to *Spalax*. It is also noteworthy that *Thryonomys* is a particularly fast evolving species. Such a fast rate of evolution for the cane rat was also reported after mitochondrial cytochrome *b* studies (Matthee and Robinson 1997). Those

observations may suggest a correlation between rates of evolution of nuclear and mitochondrial genomes.

In conclusion, our observations indicate that rodent biodiversity cannot be summarized by just a few exemplar sequences. When all available taxonomic diversity is considered, rodents do not appear to have a particular pattern of evolution, as they are included in the range of the eutherian diversity. We here caution that these inferences are derived from a single nuclear gene, and we do not generalize our results to the whole genome.

Phylogenetic Relationships Between Rodentia Families

Phylogenetic analyses of the vWF DNA sequences evidence 5 distinct clades among the 10 rodent families studied: Hystricognathi, Sciuroidea (Sciuridae and Aplodontidae), Muridae, Dipodidae, and Gliridae. The interrelationships between these five clades are not robustly resolved (figs. 1–3). Such a lack of phylogenetic resolution between Rodentia families has also been observed with mitochondrial 12S rRNA (Nedbal, Honeycutt, and Schlitter 1996) and nuclear LCAT comparisons (Robinson et al. 1997).

Hystricognathi

Hystricognathi is the best-supported clade in the present analyses. Comparison of 38 placental vWF amino acid sequences and 1 marsupial vWF amino acid sequence indicates that hystricognaths are defined by six exclusive synapomorphic replacements of residues: Q by S (position 626, referring to the complete vWF human sequence), K by Q (position 662), L by H (position 699), E by A (position 762), R by Q (position 815), and H by G (position 873).

Among hystricognaths, living Caviomorpha are restricted to South America, where they suddenly appear in the fossil record at the Late Eocene–Early Oligocene transition, between 37.5 and 31.5 MYA (Wyss et al. 1993). The biogeographical origin of caviomorphs relative to phiomorphs remains controversial. Either the hystricognath radiation took place in North America (Wood 1985), with subsequent colonization of South America by caviomorphs and Africa by phiomorphs, or South American caviomorphs originated from an African phiomorph stock (Lavocat 1969). These two hypotheses, respectively, involve either the monophyly of Phiomorpha (here, *Thryonomys* + *Trichys*) or their paraphyly.

Phylogenetic analyses of vWF data evidence an unexpected result: the sister group relationship between Old World porcupines (Hystricidae) and Caviomorpha (table 2). This is the consequence of the basal position of Thryonomyidae among hystricognaths and could be the result of a long-branch attraction phenomenon (Felsenstein 1978), as the cane rat (*Thryonomys*) exhibits the fastest-evolving and richest GC3 hystricognath sequence. Alternative topologies are not significantly different from an ML point of view: log-likelihoods of trees either clustering *Thryonomys* with Caviomorpha or clustering *Thryonomys* with *Trichys* are, respectively, 0.55 SE and 0.67 SE ($P > 0.50$) lower than that of the best tree (which groups *Trichys* with Caviomorpha; fig. 3). Our results therefore favor the hypothesis of Lavocat (1969) but cannot statistically reject the Wood (1985) hypothesis. Current paleontological evidence assumes an African origin of Caviomorpha before 37 MYA (Lavocat 1969; Wyss et al. 1993), while extant Thryonomyidae and Hystricidae are located, respectively, in Africa and in Asia plus Africa. Identification of a Caviomorpha + *Trichys* clade (table 2 and figs. 1–3, but see above) suggests an African location of Old World porcupines ancestors, possibly at the end of the Eocene. The latter observation agrees with Jaeger, Denys, and Coiffait (1985), even if available hystricid fossils are recorded only in South Asia Miocene deposits. One should also note that our comparisons include representatives of only two of the four Phiomorpha families. Improving the taxonomic diversity for phiomorphs, i.e., adding Petromuridae and Bathyergidae taxa, could help to break the long Thryonomyidae branch, to assess the question of Phiomorpha monophyly, and to test for the phylogenetic affinities of Hystricidae.

South American porcupines (Erethizontidae) are, from a morphological point of view, a rogue taxon. Despite their extant American location, these porcupines are sometimes considered a distinct caviomorph lineage, possibly grouped with Old World porcupines (e.g., Lavocat and Parent 1985), or considered the deepest originating hystricognath family (e.g., Woods and Hermanson 1985; Bryant and McKenna 1995). All of the 102 topologies involving the paraphyly of caviomorphs are significantly less likely than the three showing their monophyly (SE = 1.97–4.09, $P < 0.05$). Therefore, in the highest-likelihood vWF phylogenies, (1) erethizontids are true caviomorphs; (2) *Coendou* is a sister group to *Cavia*/*Dasyprocta*; (3) ''porcupines'' are paraphyletic.

Sciuridae and Aplodontidae

The Aplodontidae are currently represented by a single species highly adapted to a semifossorial way of life. Morphological studies conflict about Aplodontidae relationships, because extant mountain beavers mix autapomorphic and plesiomorphic traits (e.g., Bugge 1985). MP and ML analyses of vWF characters strongly cluster Aplodontidae with Sciuridae into a Sciuroidea clade (table 3). This agrees with conclusions based on mitochondrial data (Nedbal, Honeycutt, and Schlitter 1996) and with some morphological plus paleontological analyses (review in Luckett and Hartenberger 1985).

Muridae and Dipodidae

Support for a Myodonta clade (Muridae + Dipodidae) is provided by studies on morphological, myological, and embryological (review in Luckett and Hartenberger 1985) and molecular retroposon (Serdobova and Kramerov 1998) characters. Conversely, 12S rDNA and vWF sequence data weakly support the monophyly of Myodonta (Nedbal, Honeycutt, and Schlitter 1996; table 2). This weak support could be the result of a deep divergence between the two clades (possibly as old as the Late Eocene; Flynn, Jacobs, and Lindsay 1985) com-

bined with contrasted base compositions between murid and dipodid DNA sequences.

Gliridae

The Gliridae were traditionally grouped with Muridae and Dipodidae because they show a myomorph pattern, but it has been inferred from paleontological data that glirids actually display a pseudomyomorph pattern (Vianey-Liaud 1985). Other analyses clustered Gliridae either with Sciuridae (Bugge 1985; Lavocat and Parent 1985) or with Hystricognathi (Reyes, Pesole, and Saccone 1998 [sciurids were not included in their mitochondrial DNA study]). The vWF data suggest the grouping of Gliridae with Sciuroidea (fig. 3). However, there is no significant difference in likelihood among the three alternatives: log-likelihoods of trees displaying the Gliridae/Hystricognathi and Gliridae/Myodonta associations are, respectively, 0.12 SE and 0.69 SE ($P > 0.49$) lower than that of the best ML tree and its Gliridae/Sciuroidea node (fig. 3). Gliridae vWF polypeptides are also characterized by an insertion of one Pro or Tyr residue between human positions 706 and 707, and this character could allow identification of the glirid sister clade among other rodent families.

Rodentia in the Context of the Eutherian Ordinal Radiation

The Question of Rodentia Monophyly

Rodentia appeared monophyletic in the MP (fig. 1) and ML (fig. 3) phylogenetic trees, but the support indices were very weak (table 2). The same results were obtained with 12S rRNA sequences (Nedbal, Honeycutt, and Schlitter 1996). Conversely, Luckett and Hartenberger (1993) listed seven exclusive morphological synapomorphies to define Rodentia. The fossil record indicates that an explosive radiation took place just after the emergence of the order and produced an average number of 2.8 families per million years (Hartenberger 1996). These subsequent radiations probably precluded the accumulation of synapomorphies in molecular markers, and consequently, phylogenetic analyses cannot robustly resolve the questions of rodent monophyly and most of relationships between rodent families. The discrepancy between the numbers of morphological and molecular synapomorphies defining Rodentia remains a conundrum.

In this study, the increased taxonomic diversity for rodents leads to one conclusion drawn by Porter, Goodman, and Stanhope (1996). In their vWF analysis, the monophyly of Rodentia was highly supported, but only three representatives were included: *Dasyprocta* (Caviomorpha), *Mus,* and *Spalax* (Muridae). Our results indicate that their high support for rodent monophyly was based on homoplastic changes. More generally, given the tremendous Rodentia biodiversity, phylogenetic conclusions concerning the order cannot be inferred without ambiguity with only three taxa.

A key point in favor of rodent monophyly is that there is no obvious alternative against it. Moreover, vWF alternatives are not equivalent to those proposed by Graur, Hide, and Li (1991), D'Erchia et al. (1996), or Reyes, Pesole, and Saccone (1998) (but see Philippe 1997; Sullivan and Swofford 1997; Halanych 1998). To discover which rodent lineage may emerge first among placentals, we constrained each rodent clade of the best tree (fig. 3) to be the sister group of the remaining placentals. All resulting trees had significantly lower log-likelihoods, except when Hystricognathi or Gliridae were displaced. Contrasting with results derived from complete mitochondrial genome comparisons (D'Erchia et al. 1996; Reyes, Pesole, and Saccone 1998), the topology was significantly worse when Muridae alone (i.e., without the other rodents) or Myodonta alone were moved at the base of the tree.

One should also note that vWF alternatives cluster species having extreme GC levels. These alternatives could be the consequence of sensitivity to base composition of reconstruction methods (e.g., Galtier and Gouy 1995). Hence, we consider that the traditional hypothesis of Rodentia monophyly is not yet rejected—nor confirmed—by comparative molecular approaches.

The Bushlike Radiation of Placental Orders

The comparison of 39 vWF sequences of placentals and a marsupial illustrates the rapid cladogenesis of Rodentia families and its superimposition on the bushlike radiation of placental orders (fig. 2). The additional sampling of Lagomorpha biodiversity did not allow us to identify the sister clade of rabbits, hares, and pikas among eutherians. Although Luckett and Hartenberger (1993) found evidence of 12 morphological and embryological synapomorphies for Glires, no strong vWF signal was found for the association of Lagomorpha with Rodentia (table 3).

Other eutherian relationships obtained with exon 28 of the vWF gene were previously discussed by Porter, Goodman, and Stanhope (1996). Moreover, the presence of the marsupial sequence allowed investigation of the phylogenetic position of Xenarthra. It was repeatedly proposed that armadillos, anteaters, and sloths may represent the oldest extant eutherian order (Novacek 1992), and xenarthran sequences were commonly used to root molecular trees (e.g., Porter, Goodman, and Stanhope 1996; Madsen et al. 1997; Springer et al. 1997*b*). Recent molecular studies suggested that Xenarthra could cluster with a Ferungulata clade including Cetartiodactyla, Carnivora, and Perissodactyla (Arnason, Gullberg, and Janke 1997) or with Paenungulata *s.l.* (Springer et al. 1997*a*). Our analysis of mammalian vWF sequences reveals that among eutherians, the xenarthran sequence repeatedly clusters with random sequences (see *Results*). Consequently, its relative phylogenetic position could be the result of random behavior. Phylogenetic conclusions about the order Xenarthra will be doubtful without additional sampling within its four families.

Conclusion

Our results strengthen the importance of considering taxonomic diversity before reaching molecular conclusions in such a difficult area of phylogeny as the evolution of rodents. First, it appears that mouse and rat

sequences do not adequately represent the rodent or even the murid pattern of evolution. Second, embracing several well-chosen representatives helps to avoid biased phylogenetic conclusions (Lecointre et al. 1993; Philippe and Douzery 1994; Adachi and Hasegawa 1996*a*).

Further research in the field of rodent phylogeny will identify the total number of major extant clades through the sampling of additional families (e.g., Ctenodactylidae, Castoridae, Pedetidae, Bathyergidae) and test morphological and paleontological hypotheses. From this perspective, exon 28 of the vWF gene appears to be a promising phylogenetic tool, which has already answered questions on hystricognath evolution and revealed molecular signatures for some clades.

The improvement of the taxonomic diversity has not permitted us to solve the basal dichotomies within rodents, probably because of their bushlike radiation. Such a tremenduous radiation (as documented by paleontology; Hartenberger 1996) may be at the limit of the resolving power of sequence comparisons. Other molecular approaches, such as the analysis of repeated sequences in various parts of the genome (Shimamura et al. 1997; Verneau, Catzeflis, and Furano 1997; Serdobova and Kramerov 1998), should be investigated to resolve the radiation of rodent families.

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LITERATURE CITED

ADACHI, J., and M. HASEGAWA. 1996*a*. Instabilility of quartet analyses of molecular sequence data by the maximum likelihood method: the Cetacea/Artiodactyla relationships. Mol. Phylogenet. Evol. **6**:72–76.

———. 1996*b*. MOLPHY version 2.3: programs for molecular phylogenetics based on maximum likelihood. Comput. Sci. Monogr. **28**:1–150.

ADKINS, R. M., R. L. HONEYCUTT, and T. R. DISOTELL. 1996. Evolution of eutherian cytochrome *c* oxidase subunit II: heterogeneous rates of protein evolution and altered interaction with cytochrome *c*. Mol. Biol. Evol. **13**:1393–1404.

ARNASON, U., A. GULLBERG, and A. JANKE. 1997. Phylogenetic analyses of mitochondrial DNA suggest a sister group relationship between Xenarthra (Edentata) and Ferungulates. Mol. Biol. Evol. **14**:762–768.

BERKOWITZ, S. D., J. DENT, J. ROBERTS, Y. FUJIMURA, E. F. PLOW, K. TITANI, Z. M. RUGGERI, and T. S. ZIMMERMAN. 1987. Epitope mapping of the von Willebrand factor subunit distinguishes fragments present in normal and type IIA von Willebrand disease from those generated by plasmin. J. Clin. Invest. **79**:524–531.

BRANDT, J. F. 1855. Beiträge zur nahern Kenntniss der Säugethiere Russlands. Mem. Acad. Imp. St. Petersbourg Ser. **69**:1–375.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42**:795–803.

BRYANT, J. D., and M. C. MCKENNA. 1995. Cranial anatomy and phylogenetic position of *Tsaganomys altaicus* (Mammalia: Rodentia) from the Hsanda Gol Formation (Oligocene), Mongolia. Am. Mus. Novit. **3156**:1–42.

BUGGE, J. 1985. Systematic value of the carotid arterial pattern in rodents. Pp. 381–402 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

CAO, Y., N. OKADA, and M. HASEGAWA. 1997. Phylogenetic position of guinea pigs revisited. Mol. Biol. Evol. **14**:461–464.

CATZEFLIS, F. M. 1991. Animal tissue collections for molecular genetics and systematics. Trends Ecol. Evol. **6**:168.

CATZEFLIS, F. M., C. HÄNNI, P. SOURROUILLE, and E. DOUZERY. 1995. Molecular systematics of hystricognath rodents: the contribution of sciurognath mitochondrial 12S rRNA sequences. Mol. Phylogenet. Evol. **4**:357–360.

CATZEFLIS, F. M., F. H. SHELDON, J. E. AHLQUIST, and C. G. SIBLEY. 1987. DNA-DNA hybridization evidence of the rapid rate of muroid rodent DNA evolution. Mol. Biol. Evol. **4**:242–253.

D'ERCHIA, A. M., C. GISSI, G. PESOLE, C. SACCONE, and U. ARNASON. 1996. The guinea-pig is not a rodent. Nature **381**:597–600.

DOUZERY, E., J.-D. LEBRETON, and F. M. CATZEFLIS. 1995. Testing the generation time hypothesis using DNA/DNA hybridization between artiodactyls. J. Evol. Biol. **8**:511–529.

EASTEAL, S., and C. COLLET. 1994. Consistent variation in amino-acid substitution rate, despite uniformity of mutation rate: protein evolution in mammals is not neutral. Mol. Biol. Evol. **11**:643–647.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. **27**: 401–410.

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

FLYNN, L. J., L. L. JACOBS, and E. H. LINDSAY. 1985. Problems in muroids phylogeny: relationship to other rodents and origin of major groups. Pp. 589–616 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

GALTIER, N., and M. GOUY. 1995. Inferring phylogenies from DNA sequences of unequal base compositions. Proc. Natl. Acad. Sci. USA **92**:11317–11321.

GINSBURG, D., and J. E. SADLER. 1993. Von Willebrand disease: a database of point mutations, insertions, and deletions. Thromb. Haemost. **69**:177–184.

GRAUR, D., L. DURET, and M. GOUY. 1996. Phylogenetic position of the order Lagomorpha (rabbits, hares and allies). Nature **379**:333–335.

GRAUR, D., W. A. HIDE, and W.-H. LI. 1991. Is the guinea-pig a rodent? Nature **351**:649–652.

HALANYCH, K. M. 1998. Lagomorphs misplaced by more characters and fewer taxa. Syst. Biol. **47**:138–146.

HARTENBERGER, J.-L. 1985. The order Rodentia: major questions on their evolutionary origin, relationships and suprafamilial systematics. Pp. 1–33 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

———. 1996. Les débuts de la radiation adaptative des Rodentia (Mammalia). C. R. Acad. Sci., Paris, Série IIa **323**: 631–637.

HASEGAWA, M., Y. CAO, J. ADACHI, and T. YANO. 1992. Rodent polyphyly? Nature **355**:595.

HASSANIN, A., G. LECOINTRE, and S. TILLIER. 1998. The ''evolutionary signal'' of homoplasy in protein-coding gene sequences and its consequences for a priori weighting in phylogeny. C. R. Acad. Sci. Paris, Life Sci. **321**:611–620.

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. Clarendon Press, Oxford.

JAEGER, J.-J., C. DENYS, and B. COIFFAIT. 1985. New Phiomorpha and Anomaluridae from the late Eocene of North-West Africa: phylogenetic implications. Pp. 567–588 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

JONES, D. T., W. D. TAYLOR, and J. M. THORNTON. 1992. The rapid generation of mutation data matrices from protein sequences. CABIOS **8**:275–282.

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.

LAVOCAT, R. 1969. La systématique des Rongeurs hystricomorphes et la dérive des continents. C. R. Acad. Sci., Paris, Série D **269**:1496–1497.

LAVOCAT, R., and J.-P. PARENT. 1985. Phylogenetic analyses of middle ear features in fossil and living rodents. Pp. 333–354 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

LECOINTRE, G., H. PHILIPPE, H. L. V. LÊ, and H. LE GUYADER. 1993. Species sampling has a major impact on phylogenetic inference. Mol. Phylogenet. Evol. **2**:205–224.

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., W. A. HIDE, A. ZHARKIKH, D.-P. MA, and D. GRAUR. 1992. The molecular taxonomy and evolution of the guinea pig. J. Hered. **83**:174–181.

LOPEZ MARTINEZ, N. 1985. Reconstruction of ancestral cranioskeletal features in the order Lagomorpha. Pp. 151–190 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

LUCKETT, W. P., and J.-L. HARTENBERGER. 1985. Evolutionary relationships among rodents: comment and conclusions. Pp. 227–276 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

———. 1993. Monophyly or polyphyly of the order Rodentia: possible conflict between morphological and molecular interpretations. J. Mamm. Evol. **1**:127–147.

MA, D.-P., A. ZHARKIKH, D. GRAUR, J. L. VANDEBERG, and W.-H. LI. 1993. Structure and evolution of opossum, guinea pig, and porcupine cytochrome *b* genes. J. Mol. Evol. **36**: 327–334.

MADDISON, W. P., and D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.04. Sinauer, Sunderland, Mass.

MADSEN, O., P. M. T. DEEN, G. PESOLE, C. SACCONE, and W. W. DE JONG. 1997. Molecular evolution of mammalian aquaporin-2: further evidence that elephant shrew and aardvark join the paenungulate clade. Mol. Biol. Evol. **14**:363–371.

MANCUSO, D. J., E. A. TULEY, L. A. WESTFIELD, N. K. WORRALL, B. B. SHELTON-INLOES, J. M. SORACE, Y. G. ALEVY, and J. E. SADLER. 1989. Structure of the gene for human von Willebrand factor. J. Biol. Chem. **264**:19514–19527.

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.

MATSUSHITA, T., and J. E. SADLER. 1995. Identification of amino acid residues essential for von Willebrand factor binding to platelet glycoprotein Ib. J. Biol. Chem. **270**:13406–13414.

MATTHEE, C. A., and T. J. ROBINSON. 1997. Molecular phylogeny of the springhare, *Pedetes capensis*, based on mitochondrial DNA sequences. Mol. Biol. Evol. **14**:20–29.

MOUCHIROUD, D., and G. BERNARDI. 1993. Compositional properties of coding sequences and mammalian phylogeny. J. Mol. Evol. **37**:109–116.

MOUCHIROUD, D., and C. GAUTIER. 1990. Codon usage changes and sequence dissimilarity between human and rat. J. Mol. Evol. **31**:81–91.

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.

NICHOLS, W. C., K. A. COONEY, K. L. MOHLKE, J. D. BALLEW, A. YANG, M. E. BRUCK, M. REDDINGTON, E. K. NOVAK, R. T. SWANK, and D. GINSBURG. 1994. von Willebrand disease in the RIIIS/J mouse is caused by a defect outside of the von Willebrand factor gene. Blood **83**:3225–3231.

NOVACEK, M. J. 1992. Mammalian phylogeny: shaking the tree. Nature **356**:121–125.

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

PHILIPPE, H. 1993. MUST: a computer package of management utilities for sequences and trees. Nucleic Acids Res. **21**: 5264–5272.

———. 1997. Rodent monophyly: pitfalls of molecular phylogenies. J. Mol. Evol. **45**:712–715.

PHILIPPE, H., and E. DOUZERY. 1994. The pitfalls of molecular phylogeny based on four species as illustrated by the Cetacea/Artiodactyla relationships. J. Mamm. Evol. **2**:133–152.

PORTER, A. P., M. GOODMAN, and M. J. STANHOPE. 1996. Evidence on mammalian phylogeny from sequences of exon 28 of the von Willebrand Factor gene. Mol. Phylogenet. Evol. **5**:89–101.

REYES, A., G. PESOLE, and C. SACCONE. 1998. Complete mitochondrial DNA sequence of the fat dormouse, *Glis glis*:

further evidence of rodent paraphyly. Mol. Biol. Evol. **5**: 499–505.

ROBINSON, M., F. CATZEFLIS, J. BRIOLAY, and D. MOUCHIROUD. 1997. Molecular phylogeny of rodents, with special emphasis on murids: evidence from nuclear gene LCAT. Mol. Phylogenet. Evol. **8**:423–434.

ROBINSON, M., C. GAUTIER, and D. MOUCHIROUD. 1997. Evolution of isochores in rodents. Mol. Biol. Evol. **14**:823–828.

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

SABEUR, G., G. MACAYA, F. KADI, and G. BERNARDI. 1993. The isochore patterns of mammalian genomes and their phylogenetic implications. J. Mol. Evol. **37**:93–108.

SADLER, J. E. 1991. von Willebrand factor. J. Biol. Chem. **266**: 22777–22780.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York.

SANGER, F., S. NICKLEN, and A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.

SERDOBOVA, I. M., and D. A. KRAMEROV. 1998. Short retroposons of the B2 superfamily: evolution and application for the study of rodent phylogeny. J. Mol. Evol. **46**:202–214.

SHIMAMURA, M., H. YASUE, K. OHSHIMA, H. ABE, H. KATO, T. KISHIRO, M. GOTO, I. MUNECHIKA, and N. OKADA. 1997. Molecular evidence from retroposon that whales form a clade within even-toed ungulata. Nature **388**:666–670.

SPRINGER, M. S., A. BURK, J. R. KAVANAGH, V. G. WADDELL, and M. J. STANHOPE. 1997*a*. The Interphotoreceptor Retinoid Binding Protein gene in therian mammals: implications for higher level relationships and evidence for loss of function in the marsupial mole. Proc. Natl. Acad. Sci. USA **94**: 13754–13759.

SPRINGER, M. S., G. C. CLEVEN, O. MADSEN, W. W. DE JONG, V. G. WADDELL, H. M. AMRINE, and M. J. STANHOPE. 1997*b*. Endemic African mammals shake the phylogenetic tree. Nature **388**:61–64.

STRIMMER, K., and A. VON HAESELER. 1996. Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. Mol. Biol. Evol. **13**:964–969.

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. 1993. PAUP 3.1.1: phylogenetic analysis using parsimony. Illinois Natural History Survey, Champaign.

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

TAMURA, K., and M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10**:512–529.

TULLBERG, T. 1899. Ueber das System der Nagetiere: eine phylogenetische Studie. Nova Acta Reg. Soc. Sci. Upsala Ser. 3 **18**:1–514.

VERNEAU, O., F. CATZEFLIS, and A. V. FURANO. 1997. Determination of the evolutionary relationships in *Rattus* sensu lato (Rodentia: Muridae) using L1 (Lines-1) amplification events. J. Mol. Evol. **45**:424–436.

VIANEY-LIAUD, M. 1985. Possible evolutionary relationships among Eocene and lower Oligocene rodents of Asia, Europe and North America. Pp. 277–309 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

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

WOOD, A. E. 1985. The relationships, origin and dispersal of the hystricognathous rodents. Pp. 475–513 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

WOODS, C. A., and J. W. HERMANSON. 1985. Myology of hystricognath rodents: an analysis of form, function, and phylogeny. Pp. 515–548 *in* W. P. LUCKETT and J.-L. HARTENBERGER, eds. Evolutionary relationships among rodents: a multidisciplinary analysis. Plenum Press, New York.

WU, C.-I., and W.-H. LI. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proc. Natl. Acad. Sci. USA **82**:1741–1745.

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. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends Ecol. Evol. **11**:367–372.

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