

Phylogenetic position of the order Lagomorpha (rabbits, hares and allies)

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EVER since they have been classified as ruminants in the Old Testament (Leviticus 11:6, Deuteronomy 14:7) and equated with hyraxes in the vulgate Latin translation, rabbits and their relatives (order Lagomorpha) have frequently experienced radical changes in taxonomic rank. By using 91 orthologous protein sequences, we have attempted to answer the classical question "What, if anything, is a rabbit?"¹. Here we show that Lagomorpha is significantly more closely related to Primates and Scandentia (tree shrews) than it is to rodents. This newly determined phylogenetic position invalidates the superordinal taxon Glires (Lagomorpha + Rodentia), and indicates that the morphological 'synapomorphies' previously used to cluster rodents and lagomorphs into Glires^{2,3}, may actually represent symplesiomorphies or homoplasies that are of no phylogenetic value. This raises the possibility that the ancestral eutherian morphotype may have possessed many rodent-like morphological characters.

Linnaeus classified the lagomorphs as a family (Leporidae) within the order Rodentia. Subsequently, Lagomorpha has been

elevated twice in taxonomic rank, first to the rank of suborder (Duplicidentata) within Rodentia, and then to the rank of order¹. The monophyletic status of Lagomorpha is undisputed, and has been supported most recently by molecular data^{4,5}. The evolutionary affinities of Lagomorpha to the other eutherian orders, however, remain controversial, and on morphological grounds the order has been variably placed as a sister group of Rodentia within cohort Glires⁶, a sister group of Macroscelidea (elephant shrews) within cohort Anagalidia⁷, or in an indeterminate position relative to the other eutherian orders¹. Previous molecular attempts to identify the phylogenetic position of rabbits within the eutherian tree have cast serious doubt on the validity of Glires, but have failed to provide strong positive evidence concerning the superordinal affinities of Lagomorpha⁸⁻¹¹. Here we have used orthologous protein sequences to assess the evolutionary relationships of Lagomorpha to other eutherian taxa.

We analysed 88 protein sequences for which data from Sciuromognathi, Lagomorpha, Primates and an outgroup species are available (Table 1). With three ingroup taxa and an outgroup, there are three possible phylogenetic trees (Fig. 1), of which the traditional phylogeny is shown in Fig. 1a. All reconstruction methods result in the topology given in Fig. 1b, that is, primates are phylogenetically closer to lagomorphs than either taxa are to sciuriforms. The internal branch, although short, was significantly different from 0 ($P < 0.001$), and was supported in the neighbour-joining analysis by all 1,000 bootstrap replicates. In parsimony analysis the length of the tree in Fig. 1b is 10,328 amino acid replacements, shorter by 121 replacements than the traditional tree (Fig. 1a). Interestingly, even the second unorthodox tree (Fig. 1c) was shorter by 47 replacements than the traditional tree. The log-likelihood bootstrap probability of Fig. 1b tree is 0.71, as opposed to only 0.15 for the Fig. 1a tree. With all three methods of phylogenetic reconstruction, identical results were

TABLE 1 Neighbour-joining trees for Lagomorpha, Primates, an outgroup and a test eutherian taxon

Test eutherian taxon	Number of proteins	Number of aligned amino-acid sites	Sister taxon of Primates in the four-taxon analysis	Bootstrap value (%)*
Sciurognathi	88	24,865	Lagomorpha	100
Hystricognathi	21	3,528	Lagomorpha	98
Artiodactyla + Cetacea	62	14,492	Lagomorpha	99
Carnivora	32	7,057	Lagomorpha	100
Edentata	4	682	Lagomorpha	99
Pholidota	1	172	Lagomorpha	100
Sirenia	3	459	Lagomorpha	95
Tubulidentata	2	325	Lagomorpha	95
Macroscelidea	1	172	Lagomorpha	100
Hyracoidea	3	459	Lagomorpha	85
Insectivora	3	440	Lagomorpha	86
Proboscidea	7	1,118	Lagomorpha	88
Chiroptera	9	1,427	Lagomorpha	63
Perissodactyla	23	4,114	Lagomorpha	66
Dermoptera	3	629	Dermoptera	55
Scandentia	7	1,151	Outgroup	62

We have collected 91 orthologous protein sequence sets for which orthologous sequence data exist for lagomorphs and at least two other eutherian orders and an outgroup (a marsupial, a monotreme, a bird or a reptile) using the HOVERGEN¹³ and PIR¹⁴ databases. The list of proteins is available on World Wide Web at <http://acnuc.univ-lyon1.fr/data/lagomorpha.phylo.html>. Whenever possible, a mammalian outgroup was preferred to a reptilian or an avian one. Orthology, as opposed to paralogy, was ascertained by either consulting the primary literature or by using HOVERGEN to separate gene families into orthologous sets. It is expected that those few paralogous sequences that may have remained in our database will merely increase the random phylogenetic noise, rather than yield consistent erroneous phylogenies. The orthologous sets were aligned by using the CLUSTAL W program¹⁵. Ambiguous parts in the alignments (as judged by visual inspection), as well as gaps, have been removed from further analysis. If a certain eutherian order was represented in a sequence set by two or more species, we constructed a neighbour-joining phylogenetic tree, and selected the sequences with the shortest branch length to represent the order in question. Implicit in this procedure is the assumption that each taxon dealt with in this study is monophyletic, and therefore valid. Because of possible paraphyly of Rodentia^{16,17}, its two constituent suborders, Sciurognathi (including mice and squirrels) and Hystricognathi (including guinea pigs), have been treated as separate taxa. The results are, however, unaffected by the phyletic status of Rodentia. On grounds of well-established phylogenetic intimacy, the orders Cetacea and Artiodactyla were treated as a single taxon¹⁸. The eutherian taxa used were: Lagomorpha, Primates, Artiodactyla + Cetacea, Carnivora, Chiroptera, Dermoptera, Edentata, Hyracoidea, Insectivora, Perissodactyla, Macroscelidea, Pholidota, Proboscidea, Scandentia, Sirenia, Tubulidentata, Sciurognathi, and Hystricognathi. To maximize the use of the molecular data, we dealt with four taxa at a time.

* Based on 1000 replicates.

obtained by using the second suborder of rodents, Hystricognathi. The length of the internal branch is significantly different from 0, and was supported by 980 out of 1,000 bootstrap replicates. In the parsimony analysis, the trees in Fig. 1b and 1c were found to be shorter by 37 and 3 amino acid replacements, respectively, than that in Fig. 1a. The bootstrap probability of the Fig. 1b tree under the maximum-likelihood method is 0.90 as opposed to 0.09 for the tree in Fig. 1a. These results contradict the taxonomic validity of Glires.

This raises the question of whether primates are the closest relatives of the lagomorphs or whether other eutherian orders are closer to Lagomorpha than are the primates. Each taxon in our

compilation was phylogenetically tested against Lagomorpha, Primates and an outgroup. With the exception of Dermoptera and Scandentia, all other orders emerged as outgroups of a Lagomorpha-Primates clade. Bootstrap values were larger than 85% for 12 orders (Table 1). The inferred close phylogenetic affinity between lagomorphs and primates to the exclusion of Macroscelidea, although based on a single sequence, argues against a phylogenetic proximity between rabbits and elephant shrews. Similarly, on the basis of two amino acid sequences, Macroscelidea was shown to be unrelated to either suborder of rodents (data not shown). Indeed, published data suggests that elephant shrews are related to the paenungulates¹², a conclusion

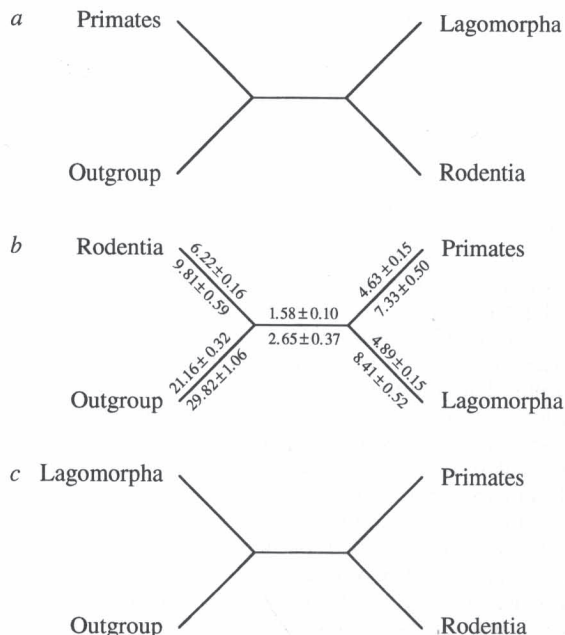


FIG. 1 Three possible phylogenetic trees for Lagomorpha, Primates, a rodent, and an outgroup. a, The tree represents traditional morphological taxonomy. b, On the basis of molecular data, this tree emerges as the most-supported phylogenetic hypothesis. Maximum-likelihood branch lengths in units of amino-acid replacements per 100 amino-acid sites (\pm s.e.) are given. Upper values, Sciurognathi (88 proteins); lower values, Hystricognathi (21 proteins). c, Unorthodox tree.

METHODS. Phylogenetic trees were constructed by using three reconstruction methods: neighbour-joining¹⁹ by means of the CLUSTAL W program¹⁵, maximum-parsimony with the PROTPARS program in the PHYLIP package²⁰, and maximum-likelihood with PROTML²¹. Genetic distances between amino acid sequences were computed by correcting for multiple hits²². Reliability of internal branches of neighbour-joining trees has been ascertained by bootstrap analysis²³ and Li's test²⁴. To evaluate the extent to which a maximum-likelihood tree is a significantly better representation of the true tree than the alternative possible trees, the relative bootstrap probabilities of all the possible trees were estimated²⁵.

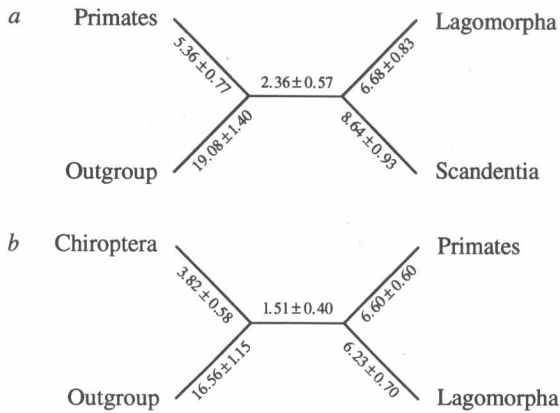


FIG. 2 Maximum-likelihood trees for Lagomorpha, Primates, an outgroup, and Scandentia (a) or Chiroptera (b). Maximum-likelihood branch lengths (\pm s.e.) are given in units of amino-acid replacements per 100 amino-acid sites. a, The tree is based on 7 orthologous protein sequences (1,157 amino acids). b, This tree is based 9 proteins (1,433 amino acids).

strongly supported by analyses of complete mitochondrial DNA sequences (Ú. Árnason, personal communication). Therefore, cohort Anagalidia or the positioning of Macroscelidea as an immediate outgroup to a monophyletic Glires are invalidated by the molecular data. We can also reject the monophyly of Glires by noting that four taxa other than Primates, these being Artiodactyla + Cetacea, Carnivora, Scandentia, and Dermoptera, are significantly closer phylogenetically to Lagomorpha than are the rodents (data not shown).

Finally, we tried to pinpoint more accurately the phylogenetic position of Lagomorpha. Three orders are thought on morphological grounds to be closely related to the primates: Scandentia, Dermoptera and Chiroptera⁶, which together are included within a superordinal taxon called Archonta. Scandentia is considered particularly close phylogenetically to Primates, and the two orders are joined in morphological systems of classification within a superorder called Primatomorpha, whereas Chiroptera and Dermoptera form a sister superordinal taxon called Volitantia⁶. In a maximum-likelihood analysis, Scandentia was found to cluster with Lagomorpha to the exclusion of Primates and the outgroup (Fig. 2a), with a log-likelihood bootstrap probability of 0.80, as opposed to 0.17 for the tree in which Primatomorpha is monophyletic. Primates clustered with Lagomorpha to the exclusion of Chiroptera, a phylogenetic arrangement supported by a log-likelihood bootstrap probability of 0.74, as opposed to 0.07 for the tree in which Archonta is monophyletic (Fig. 2b). These results add to the accumulating molecular evidence against the monophyly of Archonta¹⁰. The phylogenetic position of Dermoptera relative to Primates and Lagomorpha could not be resolved with the available data (3 proteins, 629 amino acids). Pending a significant increase in the database for dermopterans and scandentians, we conclude tentatively that Scandentia and Lagomorpha are sister orders.

About a dozen anatomical 'synapomorphies' form the morphological basis for the clustering of Rodentia and Lagomorpha³. Some of them, such as 'orbitosphenoid relatively large' or 'incisive foramina enlarged' are so vaguely defined as to defy objective analysis. Other traits, such as a 'blastocyst attachment invasive', also typify other eutherian orders, but these conditions are usually brushed aside as being 'derived secondarily'. Many character-state resemblances between rodents and lagomorphs, for example, the absence of canines, are related to specializations due to the evolution of gnawing in both orders, and as such may be subject to rampant parallelism¹. Finally, the polarities of morphological character states as either 'derived' or 'primitive' are decided on the basis of comparisons with a eutherian 'morphotype', an artificial construct based on assumptions about hypothetical

ancestral taxa. Errors in polarity identification may alter the phylogenetic tree considerably. Our molecular results indicate that a reassessment of the morphological evidence is needed. One simple and intriguing possible resolution of the conflict between morphological and molecular data is to assume that many morphological 'synapomorphies' used in support of Glires are actually ancestral character states that have been retained in some mammalian orders but were lost in others. If this reversal of character-state polarity proves valid, then the ancestral eutherian morphotype may have resembled a rodent species much more closely than is currently recognized in the morphopalaentological literature. □

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