Amino Acid Sequence versus Morphological Data and the Interordinal Relationships of Mammals

André R. Wyss,*† Michael J. Novacek,* and Malcolm C. McKenna*†
*Department of Vertebrate Paleontology, American Museum of Natural History; and
†Department of Geology, Columbia University

To a large extent, the mutual affinities of the mammalian orders continue to puzzle systematists, even though comparative anatomy and amino acid sequencing offer a massive data base from which these relationships could potentially be adduced. In the present paper the consistency index—the number of character states less the number of characters in a data set, divided by the total number of changes in the character states on a cladogram—was used to examine the relative resolving powers of recently published morphological and molecular-sequence data. Consistency indices were calculated for previously published alpha crystallin A chain and myoglobin amino acid-sequence cladograms and for four original amino acid-sequence cladograms (alpha crystallin A, myoglobin, and alpha and beta hemoglobin); these were found to be comparable to the consistency indices of morphologically based cladograms. Qualitative comparisons between the morphologically based and molecularly based trees were also made; only moderate congruence between the two was observed. Moreover, there was a general lack of congruence between the cladograms specified by each of the four proteins. Amino acid–sequence and morphological data agreed on the placement of edentates as an early eutherian offshoot and on the grouping of hyracoids, proboscideans, and sirenians. Otherwise there was only limited congruence: morphology strongly supported the grouping of lagomorphs and rodents and the alliance of pholidotes and edentates, but sequence analyses did not. The placement of tubulidentates differed widely among proteins. Morphology indicated the close association of sirenians with proboscideans; proteins suggested a pairing of sirenians with hyracoids. Sequence data did not identify many (morphologically well-diagnosed) orders as monophyletic (e.g., Lagomorpha). Because the cladograms specified by the four proteins herein considered were widely incongruent, we question how much confidence can be placed in the results of tandem-alignment analyses. Numerous published, apparently completely bifurcating cladograms based on amino acid sequences obscure the fact that significant problems in higher-level eutherian systematics remain.

Introduction

Morphologic and molecular data relevant to questions of higher-level mammalian systematics have greatly improved during the past several years. In particular, an effective sampling of phylogenetically informative amino acid sequences has been achieved during this period. Several authors (e.g., Romero-Herrera 1978; Goodman et al. 1985), using a variety of parsimony algorithms, have generated numerous phy-
logenetic interpretations of this wealth of data. In the present paper we wish to compare the relative stability and degree of resolution of these molecularly based conclusions with results of recently completed mammalian phylogenies based on comparative anatomy. Furthermore, we wish to examine the congruence of the cladograms derived from these two kinds of data. Finally, the question of relationship between parsimony and the credibility of phylogenetic hypotheses will be addressed.

Although a basic dichotomy between prototherian (=monotreme) and eutherian (="placental") plus marsupial mammals is generally accepted, major subgroupings within these divisions represent a serious problem to systematists. Relationships among the major lineages of eutherian mammals have not been completely sorted out. Consequently, since the end of the nineteenth century, the prevailing depiction of eutherian evolution has been one of a bushlike radiation. Untangling this phylogenetic knot has, by and large, resisted the attempts of numerous comparative anatomists and paleontologists (Gregory 1910; Simpson 1945; McKenna 1975; Szalay 1977; Novacek 1982). It is not true, however, as Goodman et al. (1985) commented regarding Novacek's (1982) work, that "only a bush could be drawn to depict the consensus of cladistic arrangements proposed for eutherian phylogeny, from traditional paleontological and comparative anatomical evidence." Morphological treatments of eutherian phylogeny have resulted in several proposed ordinal pairings and eutherian subdivisions. Although the branching pattern is more certain than is often portrayed, in most such studies a substantial residuum of polytomous splitting does remain. Goodman et al. (1985) make the reasonable prediction that the developing molecular analysis of phylogeny may eventually resolve what they see as a bush or what we see as a few points of uncertainty. It is timely, then, to consider what protein sequence data have already contributed to our understanding of the relationships of the mammalian orders. Then we can critically examine the apparently widely held perception that such information is providing a resolved pattern of relationships that has not been obtained through morphological studies.

At issue here is not whether morphological or molecular studies have succeeded in converting the eutherian bush into completely bifurcating branching diagrams; parsimony algorithms can, after all, produce such resolution even for randomly generated data. Comparative-anatomical (Shoshani 1986) and biomolecular (see, e.g., Goodman et al. 1985) evidence have both been used to deduce apparently completely resolved cladograms of the eutherian orders. What is at issue is the relative effectiveness of these data in identifying eutherian subgroups and the degree of confidence that can be placed in such results.

Material and Methods

There are many not always clearly explicit criteria for evaluating the strength of a particular phylogenetic result. Criteria that address the strength of a hypothesis of character change or the diversity of traits sampled in various organisms may not be equally applied to anatomical, amino acid-sequence, or other kinds of data sets. A common, more quantitative, and more generally applicable means of judging the relative quality of data sets and their resultant cladograms is the consistency index (CI) (Kluge and Farris 1969), a measure of the degree to which character-state changes on a given cladogram are minimal. The CI is the number of character states minus one, summed over all characters in the data set, divided by the total number of character-state changes displayed by the cladogram in question. It has a value of one if
Proteins versus Morphology 101

and only if there are no parallel or reversed character-state changes, and it moves
toward zero the more such changes there are. We have calculated CIs for previously
published cladograms based on myoglobin (McKenna 1987) and alpha crystallin A
(De Jong 1982; McKenna 1987) amino acid sequences. In addition we have performed
similar calculations for four new cladograms (alpha crystallin A, myoglobin, and alpha
and beta hemoglobin) generated here.

CIs are regularly calculated in morphologic studies. However, aside from cal-
culations of the frequency of parallel mutations in myoglobin (Romero-Herrera et al.
1978), such indices have generally not been calculated for studies of sequence data.
Sequence-data cladograms typically are presented as simple branching diagrams ac-
companied by a single number—or numbers along each branch—denoting the number
of nucleotide substitutions required to account for observed sequence differences be-
tween the amino acid chains being compared. The detailed arrangement of these
amino acid replacements on the cladograms, however, is usually left unspecified. The
cladograms indicated above were chosen for this analysis because they are some of
the few available for which such distributional data, essential to calculating CIs, are
presented. Our evaluation of these molecular cladograms is compared with the
morphologic cladograms of Novacek (1986), Shoshani (1986), and Novacek and
Wyss (1986).

Difficulties arise when an attempt is made to quantify comparisons between cur-
rently available morphologic and biochemical cladograms. First there is the incom-
parability of data sets that arises from differences in sampling. The ordinal sampling
in molecular studies is uneven (Dermoptera [flying lemur] is not known from any
sequence information, and pholidotes [scaly anteaters], sireniens [sea cows], and mac-
roscelidoids [elephant shrews] are known from only a single protein—in contrast to
the well-sampled primates), whereas the morphological information extends across
all extant orders.

Second, there is the problem of differences in scope of the two types of cladograms
considered here. Those that are morphologically based extend only to characters per-
tinent to interordinal comparisons while those based on sequences extend, in many
cases, as far as the subgeneric level. To account for this discrepancy we have, in addition
to deriving the molecular cladograms noted above, calculated a series of CIs for the
molecularly based results of De Jong (1982) and McKenna (1987) on the basis of a
stepwise reduction of these cladograms to their salient interordinal nodes (see below).

Those cladograms derived in the present paper are the result of a complete re-
analysis of the available mammalian sequences for alpha crystallin A, myoglobin, and
alpha and beta hemoglobin. Lists of the taxa for which amino acid sequences were
examined appear in De Jong (1982), Goodman et al. (1982), and McKenna (1987).
Character polarities were determined using the outgroup parsimony algorithms of
Maddison et al. (1984); these provide parsimonious estimates for the “ancestral” state
of a character for a particular group given the distribution of this feature within its
known outgroups. Outgroups employed in this study included, as sequences were
available, monotremes, marsupials, squamates, birds, chelonians, lissamphibians, and
teleosts. Characters were evaluated at the ordinal level; where there was some question,
and where the intraordinal relationships are sufficiently understood, the morphotypic
conditions were determined by the aforementioned outgroup algorithms. In this man-
ner sequence data were transformed into binary characters from which parsimonious
phylogenetic solutions were derived using the global branch-swapping subroutine of
the phylogenetic analysis using parsimony (PAUP) and consensus-tree (CONTREE) pro-
grams (Swofford 1985). Points of ambiguity are noted in the data matrix (Appendix B) by a question mark. They are treated by PAUP as missing data and are accounted for in the CI calculations by regarding the character as what it most parsimoniously could have been, given the phylogenetic placement of the taxon (Swofford 1985).

Results

The results of these calculations are presented in table 1 and the procedures by which they are derived follow. Figures in column one represent the CI determined from the published molecular cladograms taken at face value; all characters plotted for each cladogram were included in the calculation. Each amino acid replacement (and in some cases nucleotide substitution—see explanation of Appendix A) was treated as a unique event.

In column two all features that are autapomorphic at the terminal taxon level have been removed. The necessity of this step points to a weakness in the CI measure. Inclusion of characters defining only terminal taxa deceptively boosts CI values without directly reflecting the stability of characters supporting branch points. As expected, because many of the apomorphic replacements counted in the previous calculation occur only in isolated taxa, this culling of characters results in a general drop in the CIs of column one (but note the exception for the alpha crystallin A from De Jong [1982]).

CIs in column three were calculated from only the characters judged to be relevant to the interordinal level of comparison. These include characters defining Eutheria (placental mammals) or uniting Eutheria and Metatheria (marsupials). Again, CIs might appear lower in cases in which the stable characters supporting Eutheria and Metatheria are omitted (see, e.g., the data of Novacek [1986] in table 1). Derived replacements shared between orders are not necessarily invariant within orders but must at least have been judged to be morphotypic (=ancestral or primitive) for the

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Cladograms Calculated for Cladograms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLADOGRAM</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Alpha crystallin A:</td>
</tr>
<tr>
<td>McKenna (1987)</td>
</tr>
<tr>
<td>De Jong (1982)</td>
</tr>
<tr>
<td>Present paper</td>
</tr>
<tr>
<td>Myoglobin</td>
</tr>
<tr>
<td>McKenna (1987)</td>
</tr>
<tr>
<td>Present paper</td>
</tr>
<tr>
<td>Alpha hemoglobin</td>
</tr>
<tr>
<td>Beta hemoglobin</td>
</tr>
<tr>
<td>Morphology:</td>
</tr>
<tr>
<td>Novacek (1986)</td>
</tr>
<tr>
<td>Shoshani (1986)</td>
</tr>
<tr>
<td>Novacek and Wyss (1986)</td>
</tr>
</tbody>
</table>
orders being compared. In cases in which determination of the morphotypic condition of an order proved ambiguous (e.g., when only two members of an order have been sampled and differ in an amino acid at a particular sequence position), the convention adopted herein has been to preserve a maximum CI value. If, for example, two orders potentially shared a unique amino acid replacement but one of these orders was only ambiguously morphotypic (because of intraordinal variance) for this feature, and if, on the basis of other information, these orders were determined to be sister groups, then the potentially unique amino acid replacement was scored as a synapomorphy. This requires a secondary reversal within the order where there is ambiguity, but this step was not counted at the interordinal level of comparison. Similarly, if the two orders were found not to be sister groups, the condition in the ambiguous group was regarded as an uncounted secondary intraordinal acquisition. A replacement was counted more than once only when it occurred morphotypically in two or more unrelated orders. Thus, our determinations of the sequence-based CIs of the cladograms of De Jong (1982) and McKenna (1987) are actually maximum estimates. CIs for the original cladograms presented here are also given in table 1.

These calculations demonstrate that, in quantitative terms of consistency, sequence-based trees fared comparably with those based on morphology (table 1). We have not presented CI values as the last word in the comparison of sequence-based and morphological cladograms but offer them simply as one expedient. A more qualitative approach is taken below.

The original cladograms discussed here are summarized in figure 1; the characters on which they are based appear in Appendix A. Our representations of previously published cladograms reduce those cladograms to the depicted pattern of branching between orders (fig. 2).

How do these sequence-based cladograms compare to those generated from comparative anatomy, and what degree of resolution do they offer that morphological studies do not? A consideration of the relationships suggested by the morphological and sequence data points to general congruence in branching pattern but also to some important differences.

Note that for none of the cladograms derived here was a unique most-parsimonious solution identified. The myoglobin data produced nine equally most parsimonious trees—the alpha crystallin A and the alpha and beta hemoglobin—each > 100. Many of these variants involved swapping of taxa within polytomous branch points or within isolated clades, and a few percent were the result of character shuffling with no rearrangement of topology. Some elements of the branching pattern, however, did remain stable. These were identified using CONTREE, and it is these consensus results that we wish to emphasize. The version of PAUP utilized here analyzes only the first 100 equally most parsimonious trees it encounters. In all probability, the data analyzed here would yield more trees than this. Therefore, our consensus results could represent greater resolution than the data warrant. Nevertheless, for the three proteins for which at least 100 equally most-parsimonious solutions were identified, we compared the consensus trees defined from the first 50 of these solutions with the consensus trees derived from all 100. In each pairwise comparison we found an exact congruence. Therefore, we believe that the consensus trees considered here serve as good approximations of the consensus trees that would be derived from all possible most-parsimonious solutions. Note that the trees considered here are strict consensus trees—that is, they preserve only those topologies common to all equally most-parsimonious trees being compared.
For nearly 2 centuries, morphologists have emphasized a three-part division of mammals, recognizing monotremes as the sister group of a clade encompassing Metatheria and Eutheria. Although it is sometimes poorly accommodated by their results (see Discussion), sequence workers accept this arrangement. Both sequence (except alpha hemoglobin) and morphological evidence strongly indicate the monophyly of Eutheria.

When relationships within Eutheria are considered, both types of evidence suggest that edentates (sloths, armadillos, and anteaters) represent an early offshoot within the group. Morphological studies place edentates as the sister group to all remaining eutherians, here termed epitheres (after McKenna 1975). Molecularly based interpretations differ somewhat on this point. De Jong (1982) has suggested that paenungulates (in those studies taken to include tubulidentates [aardvarks], hyracoids, sirensians, and proboscideans) form the outgroup to edentates plus the remaining epitheres. On the basis of alpha crystallin A, McKenna (1987) has suggested a trifurcation of macros-
celidids, edentates, and paenungulates as the outgroup to the remaining epitheres. McKenna and De Jong's cladograms differ slightly in their arrangement of the members of the clump comprising Lipotyphla (true insectivores), Tupaiidae (tree shrews), Rodentia, Lagomorpha (rabbits and pikas), and Primates but are otherwise in essential agreement. A consensus of these two cladograms is presented in figure 2. The myoglobin data cannot address this point because edentates have not been sampled. In our analysis of alpha crystallin A sequences, edentates and macroscelidids fall as the unresolved outgroups to all remaining epitheres. It is worth noting, however, that at present the macroscelidid sequence is only very incompletely known; its apparent basal position on the consensus tree almost certainly results from incomplete sampling. Edentates fall within an unresolved basal eutherian clump in our alpha hemoglobin analysis and as the sister group to epitheres in our beta hemoglobin analysis. Thus, although there is not a precise protein-sequence consensus, there is relatively close agreement between morphology and protein data on the position of the Edentata.

From this point onward in the cladogram there appear several notable differences between the alpha crystallin A trees of McKenna, De Jong, and the present study. Many of the discrepant features between these cladograms arise from differences in interpretation of single characters. For example, a replacement at position 146 of the alpha crystallin A chain is regarded by McKenna as a synapomorphy linking artiodactyls (even-toed ungulates) and perissodactyls (odd-toed ungulates) with subsequent loss in *Tapirus* and *Sus*. De Jong, on the other hand, viewed this mutation as an independent acquisition in *Equus*, *Ceratotherium*, and higher artiodactyls, a transformation scheme also requiring three steps. Rather than enumerate such interpretive differences, our comparisons focus on the qualitative differences of the branching patterns seen in the various morphological and molecular cladograms presented. These are often rather substantial. The lack of resolution resulting from the analysis of a single protein and the lack of congruence of branching pattern between proteins are best appreciated by comparing the consensus cladograms presented in figures 1 and 2; highlights are enumerated below.

As was mentioned previously, the alpha crystallin A sequences suggest a close association of tubulidentates, hyracoids, sirenians, and proboscideans. Morphology strongly corroborates the alliance of hyracoids, sirenians, and proboscideans but is moot as to the affinities of tubulidentates. Within the hyracoid-sirenian-proboscidean cluster, however, the alpha crystallin A solution departs significantly from that suggested by morphology. Morphologists have long recognized (1) a close alliance of sirenians and proboscideans, to the exclusion of other putative paenungulates, and (2) hyracoids as the nearest relative of this pair (see Gregory 1910, p. 468 [fig. 32]). The alpha crystallin A data suggest the alternative association of hyracoids and sirenians (plus tubulidentates), to the exclusion of proboscideans.

The alliance of tubulidentates and proboscideans (although they represent the only paenungulates sampled) is not even weakly supported by the myoglobin information. The myoglobin data suggest that tubulidentates, chiropterans (bats), and a group including artiodactyls, perissodactyls, and proboscideans plus cetaceans (as sister groups) form an unresolved trichotomy. (Alpha crystallin A information suggests that cetaceans group with a carnivore-plus-pholidote and artiodactyl-plus-perissodactyl assembly!) Both hemoglobin sequences support the pairing of hyracoids with proboscideans.

Shoshani’s (1986) morphological study suggests a group comprising paenungulates (minus tubulidentates) and cetaceans as the outgroup to remaining epitheres (minus
pholidotes). Morphological evidence has otherwise not been used to suggest the outgroup or near-outgroup position of paenungulates relative to other eutherians. Neither do most of our molecular analyses reveal such a branching scheme (note the exception for beta hemoglobin mentioned below). In the consensus cladograms for alpha crystallin A and alpha hemoglobin, paenungulates may only be construed as representing an epitheria outgroup, inasmuch as they group in an unresolved cluster at the level of Eu- or Epitheria (figs. 1, 2). As was indicated above, with respect to myoglobin, paenungulates (represented only by a proboscidean), excluding tubulidentates, fall well within Epitheria as the sister group of cetaceans.

In a broad sense, then, comparative anatomy and protein sequences agree at least in the clumping of hyracoids, sirenians, and proboscideans but differ significantly in the details of the alliances within this group. Furthermore, there is major disagreement among the protein data sets with regard to the placement of tubulidentates.

The protein consensus cladograms suggest several additional interordinal groupings. Of the four proteins analyzed here, beta hemoglobin is clearly the least effective at resolving the eutherian tree. This protein yields only three superordinal groupings. It identifies Epitheria (minus proboscideans and hyracoids) and two ordinal pairings, the linking of hyracoids with proboscideans and the linking of lipotyphlan insectivores with carnivores. The former pairing we have previously discussed, but the latter merits further attention. Most morphological cladograms depict carnivores and lipotyphlans as early eutherian offshoots, but the precise mutual relationships of these two orders are usually left unspecified. There appears to be an equal degree of uncertainty in our protein-based results. Carnivores and lipotyphlans fall in widely dissimilar places on the three remaining protein-based cladograms. From these data it would be difficult to suggest that these groups share anything but an extremely distant relationship.

The alpha hemoglobin consensus tree shows a considerably better degree of resolution than does the beta hemoglobin tree, but it also has the lowest CI (0.49) of any of the protein-based trees compared. Alpha hemoglobin groups rodents, lagomorphs, and cetaceans (!) in an unresolved trifurcation with carnivores as its outgroup. A grouping of ungulates is identified with perissodactyls forming the outgroup to a hyracoid/proboscidean pairing and with artiodactyls representing the outgroup to all three.

The maximum-parsimony solution for myoglobin consistently groups leporid lagomorphs (rabbits) with tupaiids and ochotonid lagomorphs (pikas) with carnivores in a clade representing the outgroup to the remaining eutherians. The myoglobin information shows another highly unusual grouping of proboscideans and cetaceans, which appears in a trichotomy including artiodactyls and perissodactyls. Even more unusual is the placement of Primates as the sister group of this triple branch point.

The alpha crystallin A chain consensus tree shows only a moderate degree of resolution but has the highest CI (0.63) among the molecular trees sampled. It pairs carnivores with pholidotes and artiodactyls with perissodactyls in a clade including cetaceans. As discussed above, it strongly supports the grouping of paenungulates (including tubulidentates).

The salient features of morphologically based phylogenies considered in the present paper are (1) the outgroup position of Edentata, (2) the monophyly of Paenungulata sans Tubulidentata, and (3) the monophyly of Glires (lagomorphs + rodents). Although independent studies of sequence data show some support for this grouping (Penny and Hendy 1985), none of the protein-based consensus trees considered above groups lagomorphs and rodents, a pattern strongly supported by some morphological studies.
(see, e.g., Luckett 1985; Novacek 1985). In addition, Novacek (1986), Novacek and Wyss (1986), and Shoshani (1986) advocate a close relationship between edentates and pholidotes, a grouping not corroborated by protein-based results. Also, Novacek and Wyss (1986) acknowledge some evidence for the Archonta (including primates, dermopterans, chiropterans, and tupaiids), although, again, this is not supported by molecular sequence–based trees.

**Discussion**

It is appropriate here to offer a few general comments regarding sequence-based cladograms and a few suggestions to make the information contained within them more directly accessible. The reduction of enormous amounts of sequence information to a single branching-solution diagram, although certainly a desirable objective, deemphasizes an essential aspect of phylogenetic systematics—that is, character transformation analysis. We would urge purveyors of future sequence-based trees to strengthen this aspect of their investigations. Strict outgroup procedures should be applied for the assessment of polarity, and nodes on cladograms should be supported explicitly by discrete sets of characters. Such procedures make cladograms more tangible; they point to character conflicts and provide a better indication of the support for specific nodes than do assurances that branch points are upheld by the overall parsimony scheme.

When polarity determinations for a given locus are ambiguous for a given level of analysis, either the character should be regarded as uninformative and be discarded or alternatives should be presented. Likewise, if a given position on an amino acid sequence is shown to be highly variable for a given level of comparison, it will never serve as a reliable character on a cladogram. At least in the first step of the analysis, such a character is better disregarded. The use of such characters only impedes detection of the phylogenetic signal carried by other, more stable positions. It scarcely requires mention that not all data are pertinent to all levels of phylogenetic analysis. Just as in morphological studies in which a character might be appropriate to a lower level of comparison but not to a higher one, it is probably futile to apply all sequence information to the question of interordinal relationships. For example, the variation at position 132 of myoglobin within Primates nearly equals that between all other orders combined. Thus, it can hardly be considered reliable at a higher level of analysis.

Given the discrepancies between the resultant trees of different sequences, it is worthwhile to note the problem of "tandem alignment" solutions. In such analyses the proteins under investigation are treated as if they formed a single composite sequence linked end to end. Comparisons between such linked sequences have the drawback of obscuring the incongruent branching patterns specified by the sequences' component parts by averaging them out. It must be remembered, however, that the conflicts still exist. In attempting to elucidate the relationships of the major groups of tetrapods on the basis of several sets of protein sequences, Maeda and Fitch (1981) reached the same conclusion. Concluding their final paragraph, they write: "While 'averaging' across several proteins may reduce the noise, this effectively reduces the data to 1 large sequence and we no longer see the phylogenetic variability in the results from the different sequences. The so-called improved estimate is bought at the price of being much less certain of the degree of relative confidence one can place in alternative phylogenetic hypotheses."

A further problem confronting molecular systematists concerns the issue of typology. By practical necessity, molecular studies are often forced to ascribe to whole
species or higher taxa the character state of particular specimens analyzed. Thus, we are left without knowledge about variation or the extent of molecular polymorphism. Such a typological rationale may be necessary initially, but at some point it needs to be replaced by more adequate sampling, either among individuals of species or among a greater diversity of species within higher taxa.

In the push for maximum resolution and maximum parsimony, the reliability of results is often sacrificed. Although we endorse the application of parsimony in phylogenetic analyses, we would also hope that the inherent limitations of the data themselves will not be overlooked.

Some of these shortcomings are highlighted by a review of a few published results of maximum-parsimony analyses. A summary of the mammalian portion of figure 3 of Goodman et al. (1985) is presented in figure 3 here. Of several most-parsimonious cladograms for the tandem alignment of as many as seven protein sequences, it represents the one showing the fewest contradictions with morphologically well-established monophyletic groupings. Nevertheless, it exhibits five such disagreements: the nonmonophyly of (1) Theria (monotremes placed as the sister group to most eutherians, to the exclusion of metatherians), (2) Eutheria (proboscideans plus edentates placed as the sister group to monotremes plus other eutherians), (3) Artiodactyla and (4) ruminant artiodactyls (both including perissodactyls and cetaceans), and (5) Camelidae (including cetaceans). A consensus tree derived from the same data set for the 13 most-parsimonious trees (Goodman et al. 1985, fig. 1) shows a sixth contradiction, the nonmonophyly of Lipotyphla (the group includes Chiroptera or vice versa). These undesirable results have been circumvented by broadening the parsimony procedure to include tabulation of postulated extracurricular genetic events in the form of hypothetical gene duplication and expression events (Goodman et al. 1982). In effect, this is an a posteriori means of character weighting that insures ordinal and infrordinal monophyly for the above problem groups. As Fitch (1979, p. 375) cogently observed, "misused, [this] weighting system is nothing more than a way of assuring the result comes out the way you want it. Clearly, if the weight or penalty for a duplication is sufficiently high, the only possible outcome is the tree of your choice." Hence, to what degree can these data (and algorithms) reliably identify the more obscure eutherian

![Diagram of Eutherian interordinal relationships](https://example.com/diagram.png)

**Fig. 3.**—Eutherian interordinal relationships depicted by Goodman et al. (1985, fig. 3). Abbreviations are as in fig. 1.
branching patterns when they fail to identify consistently the constituent orders and fail to reproduce the well-established groups at or above the level of Eutheria? What is to preclude the occurrence of other extracurricular genetic events that might alter branching sequences at other less certain nodes within Eutheria? A more consistent rationale would be to present the data-based—albeit biologically unacceptable—result without the accoutrements of hypothetical genetic events or related weighting factors.

As discussed above, the putative nonmonophyly of certain well-established orders poses another significant problem for molecular-sequence data. The alpha crystallin A chain–based interpretations of McKenna (1987) and De Jong (1982) do not demonstrate the monophyly of several orders (artiodactyls, carnivores, primates, rodents, and lagomorphs). In addition, if parsimony is rigorously pursued, pholidotes form the sister group of ursids (bears)—or at least Melursus—resulting in a paraphyletic Carnivora (sharing the unique replacement at position 74 F-Y).

In this analysis we have generally assumed ordinal monophyly because (1) this study pertains to interordinal relationships and (2) there is nearly incontrovertible morphological evidence that the orders considered here are indeed monophyletic. This should be emphasized because molecular-sequence studies are developed from the premise that such groupings are clearly supported.

The monophyly of lagomorphs is no less certain than is that of other eutherian orders, but, because it was convenient, we have treated the two lagomorphs sampled—a leporid and an ochotonid—separately. We find it remarkable that for myoglobin, the two lagomorphs sampled were never identified as each other’s nearest relatives in any of the nine equally most-parsimonious trees produced. Moreover, only six of the 100 most-parsimonious alpha crystallin A–based cladograms analyzed paired these two families.

Conclusion

Goodman et al. (1985, p. 171) have advanced the opinion that traditional anatomical evidence is “currently incapable of converting the phylogenetic ‘bush’ traditionally portrayed for the 15 or so extant eutherian orders into a consistently bifurcating tree.” If this passage can be taken to mean that morphological data are incapable of providing such “consistently bifurcating” resolution reliably, then we would submit that protein-sequence data have much the same problem.

For any difficult phylogenetic problem, the task of a systematist is one of seeking a resolved pattern for incompatible sets of apparent homologies. That parsimony should dictate the preference for one of several competing phylogenetic hypotheses is a premise grounded on sound logic. The search for the shortest possible trees is an appropriate aim. Parsimony affirms nothing, however, about how much reliance may be placed in the chosen hypothesis. Although published, fully resolved, protein-sequence–based cladograms abound, it should not be considered that biochemical systematics has achieved a definitive resolution of the riddle of eutherian interrelationships. Many ordinal linkings proposed on biochemical grounds are extremely precarious and are beset by the same uncertainties as those encountered in morphological studies. The contrast between the polytomous cladograms of most morphologists and the fully bifurcating trees of molecular systematists is more a reflection of the comparative optimism of the investigators than it is of the quality of their respective data sets. Apparently, comparative anatomists have tended to be less sanguine about phylogenetic hypotheses deduced from bewildering arrays of discordant character information than
have their colleagues working in molecular systematics. Therefore, it is not that traditional methods are incapable of fully resolving the relationships of the eutherian orders but simply that most workers have been reluctant to ascribe as much significance to the relationships that a maximum-parsimony solution might specify. This attitude is clearly reflected in a concluding comment to Gregory's (1910, p. 462) classic treatise on the mammalian orders; he cautions: "the history of classification warns us against taking superordinal groupings too seriously." This does not, of course, imply that the morphologists' view of moderation (if it is, in fact, an accurate generalization) is necessarily correct.

We are not discounting the importance of sequence-based systematics but in fact welcome it as a useful test of morphology. Sequence data have made and will continue to make significant contributions to the study of mammalian phylogeny. We believe, however, that, at present, morphology remains the most powerful comparative tool available for identifying eutherian orders and their mutual affinities. Our intent here is simply to point out that many of the phylogenetic conclusions based on amino acid–sequence techniques are no more secure than are those based on comparative anatomy—and to emphasize that the interordinal relationships of eutherian mammals are still far from being adequately resolved.

**APPENDIX A**

This is a specification of the characters appearing in the data matrices that follow in appendix B. The number at the left of each line identifies the position of a given replacement in an amino acid chain. The replacements themselves are usually represented as pairs of letters, the first one denoting the primitive amino acid residue for that position and the second one denoting its derived counterpart. Question marks represent the primitive amino acid for a given position in cases in which it is not precisely determinable but in which the derivative nature of the second amino acid may still be directly inferred. When possible, in cases of multiple hits (when there is more than a single amino acid replacement at a given position), we have treated the character at the level of unique nucleotide substitutions at positions in the triplet code; these are specified. Thus, while it was impossible to define the primitive amino acid character state at these loci, from outgroup comparison it was possible to establish that the occurrence of a specific nucleotide at a given position in the triplet code was derived. We have employed the standard amino acid abbreviations, which are as follows: A, alanine; C, cystine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, Isoleucine; K, Lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.

**Alpha Crystallin A**

(3) I-V  
(4) T-A  
(70) K-Q  
(72) V-L  
(74) F-L  
(90) L-Q  
(91) D-E  
(101) S-N  
(129) I-L  
(142) S-C  
(146) I-V  
(147) ?-P, T, or A. Cytosine in second triplet position derived.
(149) ?-G. Guanine in second triplet position derived.
(150) ?-L. Uracil in first triplet position derived.
(153) S-G
(158) ?-A. Guanine in first triplet position derived.
(168) T-S

Myoglobin

(5) G-A
(12) ?-N (seen in no outgroups)
(22) ?-A
(34) K-T
(51) T-S
(53) D-A
(57) A-G. Lipotyphla (S) is equally likely derived from G or A.
(66) ?-N, H, or D. Adenine in second triplet position derived.
(70) T-S
(74) ?-G or A. Guanine in first triplet position derived (this replacement requires a
double hit).
(86) L-I
(103) F-Y
(109) E-D
(116) Q-H
(121) A-G
(122) D-E
(129) A-G
(142) M-I
(149) F-L

Alpha Hemoglobin

(4) ?-P. Cytosine in first triplet position derived. Rodents (S) and tupaiids (T) have
replacements at this position, but they are not necessarily related to the one noted
here.
(10) V-I
(13) ?-T. Cytosine in first triplet position derived.
(15) G-D, N, or E. Adenine in second triplet position derived.
(17) ?-I. A in first triplet position derived.
(49a) S-T
(49b) S-G. (49a+b are not necessarily related replacements.)
(63) A-G or S. Guanine in second triplet position derived.
(68) ?-N or K. Adenine in second triplet position derived.
(78) ?-D or N. Adenine in second triplet position derived.
(82) ?-D or E. Guanine and adenine in first and second triplet positions derived.
(89) H-Y
(108) V-T
(110) A-S
(115) ?-S or T. Adenine in first triplet position derived.
(130) ?-S, N, or T. Adenine in first triplet position derived.
(131) ?-N. Adenine in first triplet position derived (T in Lipotyphla probably a
related replacement.

Beta Hemoglobin

(2) H-N
(4) T-S
(5) ?-D. Adenine in second triplet position derived.
(6) ?-A. Cytosine in second triplet position derived.
(9) -A. Guanine in first triplet position derived.
(10a+10b) A-L or Q. Carnivores and lipotyphlans share L, a double hit; proboscideans have Q (all three groups share cytosine in first triplet position).
(13) -G. Guanine in second triplet position derived.
(19) -K. Adenine or guanine in second triplet position possibly derived.
(23) -V. Guanine in first triplet position derived.
(44) S-H
(52) -S. Uracil or adenine in first triplet position derived.
(54) V-I
(56) -N, H, or K. Adenine in second triplet position derived.
(61) -L. Uracil in second triplet position derived.
(65) A-K, Q, or E. Adenine in second triplet position derived.
(68) L-A (double hit)
(70) S-A
(72) G-S
(74) A-G
(112) -C or R. Guanine in second triplet position derived.
(115) A-S
(116) -H (seen in no outgroups)
(125) -Q or P. Cytosine in second triplet position derived.
(126) -L. Cytosine in first triplet position derived.
(130) W-Y or F. Uracil or cytosine in third triplet position derived.
(133) L-V

APPENDIX B

Distribution of the characters supporting cladograms in figure 1. 0, primitive; 1, derived; and ?, equivocal. Numbers at the top are the positions of that protein’s sequence being utilized and correspond to those in Appendix A.

Alpha Crystallin A

| Metatheria | 000000000000000000000000 |
| Tubulidentata | 1011001111010111 |
| Hyracoidea | 1011001111010111 |
| Sirenia | 1011001111010111 |
| Proboscidea | 1010110110010111 |
| Edentata | 1000000110?010001 |
| Rodentia | 10000?1110101111 |
| Leporidae | 1000011101011111 |
| Ochotonidae | 1000001101010111 |
| Primates | 1000011101010111 |
| Lipotyphla | 1000001010111111 |
| Tupaiidae | 10000011110101111 |
| Chiroptera | 0000011101010111 |
| Carnivora | 0100001111011011 |
| Pholidota | 0100001111011011 |
| Artiodactyla | 0100011100110111 |
| Cetacea | 0100011110111011 |
| Perissodactyla | 0100011110011011 |
| Macroscelidia | ??000000?010?001? |
### Myoglobin

<table>
<thead>
<tr>
<th>Metatheria</th>
<th>010010000000?01000?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubulidentata</td>
<td>1100000000111001011</td>
</tr>
<tr>
<td>Proboscidea</td>
<td>0001010001110110111</td>
</tr>
<tr>
<td>Leporidae</td>
<td>0000000000111001011</td>
</tr>
<tr>
<td>Ochotonidae</td>
<td>1101010101111010101</td>
</tr>
<tr>
<td>Primates</td>
<td>0101?00001010010?11</td>
</tr>
<tr>
<td>Tupaiidae</td>
<td>1100000111110010011</td>
</tr>
<tr>
<td>Chiroptera</td>
<td>0100000001010010111</td>
</tr>
<tr>
<td>Carnivora</td>
<td>0110?01101011010101</td>
</tr>
<tr>
<td>Artiodactyla</td>
<td>0111?10101011?110101</td>
</tr>
<tr>
<td>Cetacea</td>
<td>0110010101010110111</td>
</tr>
<tr>
<td>Perissodactyla</td>
<td>0110100010111011111</td>
</tr>
<tr>
<td>Lipotyphla</td>
<td>010010?001?10010111</td>
</tr>
</tbody>
</table>

### Alpha Hemoglobin

<table>
<thead>
<tr>
<th>Metatheria</th>
<th>000000000000010000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edentata</td>
<td>000000000000010000</td>
</tr>
<tr>
<td>Chiroptera</td>
<td>010100010100011000</td>
</tr>
<tr>
<td>Scandentia</td>
<td>110001000000011000</td>
</tr>
<tr>
<td>Lipotyphla</td>
<td>000010000000010000</td>
</tr>
<tr>
<td>Primates</td>
<td>100100000000010000</td>
</tr>
<tr>
<td>Carnivora</td>
<td>1?1100000000110000</td>
</tr>
<tr>
<td>Lagomorpha</td>
<td>110110000010001000</td>
</tr>
<tr>
<td>Rodentia</td>
<td>?11100000010001000</td>
</tr>
<tr>
<td>Artiodactyla</td>
<td>000000001000100?1</td>
</tr>
<tr>
<td>Hyracoidea</td>
<td>000100001110101111</td>
</tr>
<tr>
<td>Perissodactyla</td>
<td>100000010000000000</td>
</tr>
<tr>
<td>Proboscidea</td>
<td>001000011101111111</td>
</tr>
<tr>
<td>Cetacea</td>
<td>101010101111010010</td>
</tr>
</tbody>
</table>

### Beta Hemoglobin

<table>
<thead>
<tr>
<th>Metatheria</th>
<th>000000000000010000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edentata</td>
<td>100000000000010000</td>
</tr>
<tr>
<td>Chiroptera</td>
<td>0100100010100010001</td>
</tr>
<tr>
<td>Scandentia</td>
<td>0100100010100010001</td>
</tr>
<tr>
<td>Lipotyphla</td>
<td>00000011?1010001001000</td>
</tr>
<tr>
<td>Primates</td>
<td>00000000001000110111</td>
</tr>
<tr>
<td>Carnivora</td>
<td>00000111010001001110101</td>
</tr>
<tr>
<td>Lagomorpha</td>
<td>010000000100001111101101</td>
</tr>
<tr>
<td>Rodentia</td>
<td>01010001010100010011</td>
</tr>
<tr>
<td>Artiodactyla</td>
<td>01000001000010101001</td>
</tr>
<tr>
<td>Hyracoidea</td>
<td>001100011010111000011</td>
</tr>
</tbody>
</table>

Downloaded from https://academic.oup.com/mbe/article-abstract/4/2/99/1184670 by guest on 09 February 2019
Acknowledgments

We are indebted to Dr. Morris Goodman for generously providing a catalogue of the sequence information analyzed here. We thank W. Fitch, J. Cracraft, and three anonymous reviewers for their helpful comments; P. Cannell and J. Cracraft for assistance with PAUP; and L. Lomauro for final preparation of the figures. This work was supported by a Columbia University faculty fellowship to A.R.W. and by the Frick Laboratory Endowment Fund in Vertebrate Paleontology, American Museum of Natural History.

LITERATURE CITED


WALTER M. FITCH, reviewing editor

Received June 6, 1986; revision received October 3, 1986.