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The alpha crystallin A chain of the eye lens and mammalian phylogeny

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A new cladogram of (living) higher mammalian relationships is presented for 52 OTUs, based upon analysis of the alpha crystallin A chain, a lens protein of the eye. Pangolins and (other) edentates are weakly united, and they, plus elephant shrews (partial analysis) and paenungulates, appear to be the sisters of the rest of the placental mammals. These results are in agreement with some morphological analyses but not with others. Among rodents, *Spalax* offers evidence that the "molecular clock" does not run very accurately above the DNA level. No one approach has a monopoly on "the truth", but it is hoped that morphologists and molecular biologists will continue to cooperate in an effort to bring as many perspectives as possible to bear on phylogenetic analysis of the Mammalia.

1. Introduction

There are two ways to reconstruct history after the fact, should we ourselves have been absent as observers. One way is to dig through stratified accumulations of data, firm in the belief that, unless disturbed, the oldest information will be at the bottom, as in most stacks of last week's newspapers. Paleontologists often do that, stringing together a vertical sequence of known events like "connect-the-dots" art and downplaying the possibility of ignorance (for critique, see McKenna et al. 1977; Lillegraven et al. 1981: fig. 55). The other belated approach to history is to retrodict what ought to have happened in order to lead to a particular situation, keeping the story as simple as possible. Most neontologists do that, including molecular biologists. Both methods have pitfalls, some of which are shared and some are unique to the particular method. Both methods need temporal calibration from some external discipline, such as radioisotopic dating. Although newspapers have dates written on them, biological data do not. The dates must be supplied, especially if we are to consider calibrated rates of evolution. Paleontologists and geophysicists often work together to provide dates for rocks that contain the fossils, but, even when obtained, the dates merely document that an organism was in existence at the time sampled — a minimum number. It is uncertain how long before that date the organism was in existence but unsampled. However, if a sister-group is known to have occurred earlier, then the gap can be narrowed.

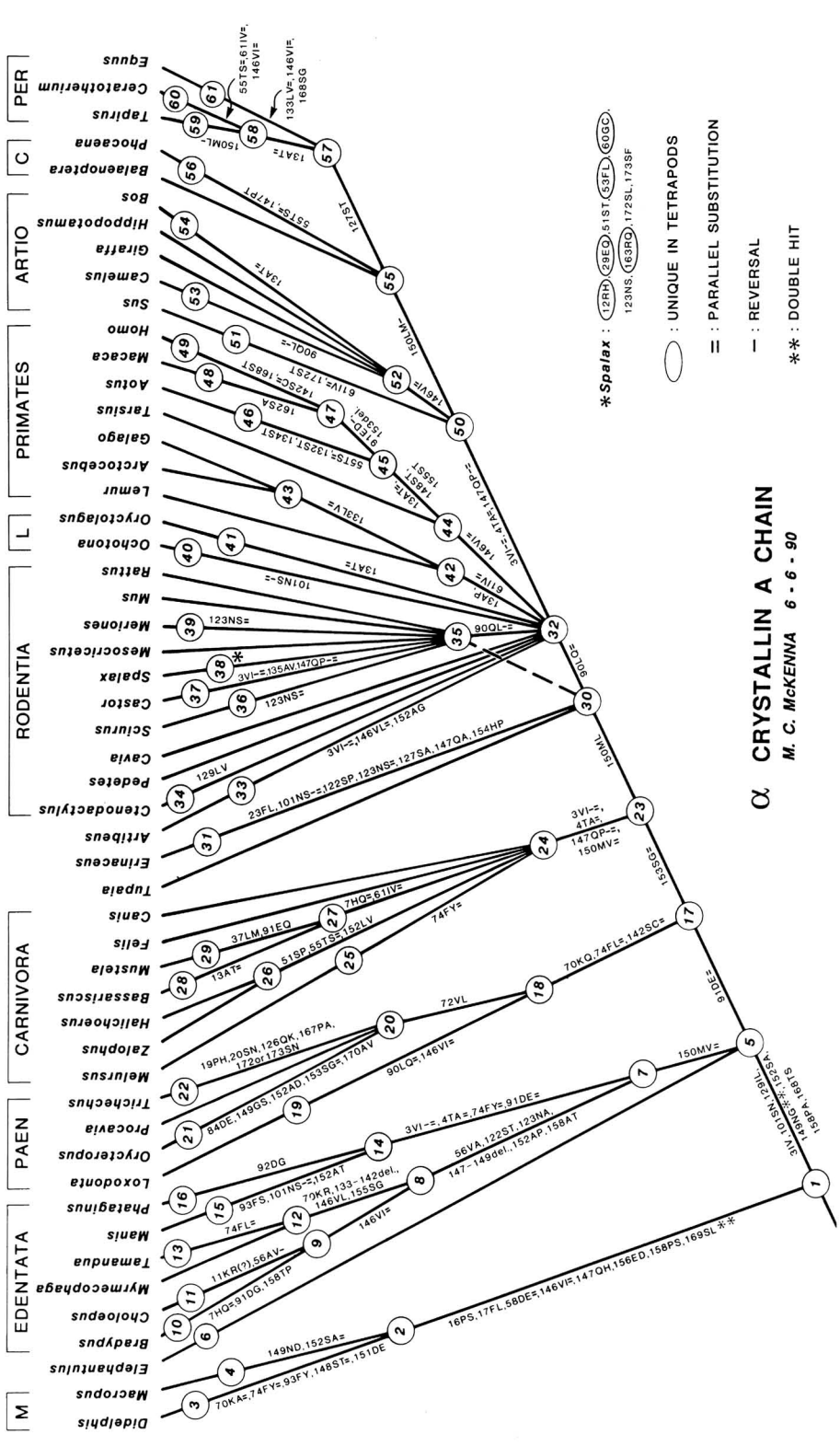

Fig. 1. Cladogram of mammalian genealogy based upon analysis of currently available amino acid sequence data for the alpha crystallin A chain, a lens protein of the eye. Taxonomic abbreviations: M = marsupials; PAEN = paenungulates (plus *Orycteropus)*; L = lagomorphs (rabbits, hares, pikas); ARTIO = artiodactyls; C = cetaceans (whales, dolphins); PER = perissodactyls (tapirs, rhinos, horses). Hypothesized apomorphies for all nodes possessing them (except node 1) are listed immediately before the appropriate node. If *Elephantulus* is to join *Procavia* (de Jong et al., in press), then a reversal (91 ED-) is required for *Elephantulus*.

After comparison and testing, both methods of historical reconstruction can be used together. That is why molecular biologists and paleontologists have been cooperating in recent years.

In the postulated phylogenetic history of a mammalian lens protein that follows, I have assumed the same plesiomorph tetrapod outgroups as those assumed by McKenna (1987): frogs (*Rana esculenta*), turtles (*Graptemys geographica*), lizards (*Tupinambis teguixin*), alligators (*Alligator mississippiensis*), and five genera of birds (*Dromaeus*, *Rhea*, *Struthio*, *Gallus*, and *Aptenodytes*). I have also assumed the same plesiomorphous character states for the 173 amino acid loci of the alpha crystallin A chain of the eye lens (Tables 1, 2). However, Hendriks et al. (1987) would have leucine rather than isoleucine occur at locus 129. There is also a possibility that at locus 55 threonine is not actually the plesiomorphous condition (because of glycine at locus 55 in *Rana*). These changes, whether correct or not, would not affect the topology of the mammalian alpha crystallin A chain cladogram developed here (Fig. 1).

Table 1. Genetic code. For convenience, the genetic code for Crick's (1958) magic twenty biologically active amino acids is given here, together with the one-letter and three-letter abbreviations for these amino acids. Note that third codon states in alanine, glycine, leucine (CU–), proline, arginine (CG–), serine (UC–), threonine, and valine are indeterminate.

Alanine	(A, ala)	GCU GCC GCA GCG
Cysteine	(C, cys)	UGU UGC
Aspartic acid	(D, asp)	GAU GAC
Glutamic acid	(E, glu)	GAA GAG
Phenylalanine	(F, phe)	UUU UUC
Glycine	(G, gly)	GGU GGC GGA GGG
Histidine	(H, his)	CAU CAC
Isoleucine	(I, ile)	AUU AUC AUA
Lysine	(K, lys)	AAA AAG
Leucine	(L, leu)	UUA UUG CUU CUC CUA CUG
Methionine	(M, met)	AUG
Asparagine	(N, asn)	AAU AAC
Proline	(P, pro)	CCU CCC CCA CCG
Glutamine	(Q, gln)	CAA CAG
Arginine	(R, arg)	CGU CGG CGA CGC AGA AGG
Serine	(S, ser)	UCU UCC UCA UCG AGU AGC
Threonine	(T, thr)	ACU ACC ACA ACG
Valine	(V, val)	GUU GUC GUA GUG
Tyrosine	(Y, tyr)	UAU UAC
Tryptophan	(W, trp)	UGG

The new cladogram (Fig. 1) has been constructed by the same principles as the cladogram given by McKenna (1987: fig. 3.1–3.6). Like its predecessor, and for the same reasons having to do with the amount of computer time required for useful results, it is a "manual" solution to a very complex problem. As before, reversals are accepted where indicated by parsimony analysis, multifurcations are not artificially broken up into forced dichotomies, and postulated replacements are listed at the requisite cladogram nodes to facilitate criticism. The method of "tandem alignment" with other molecular results is avoided because it produces a muddle of cladistic and phenetic interpretation. As in my earlier paper, I warn the reader of the typological nature of most amino acid sequenced samples. There simply has not been either time or funding for biochemists to analyse multiple examples from populations of all taxa. Polymorphism may remain undetected.

Table 2. Alpha crystallin A chain sequence hypothesized to be primitive for tetrapods (McKenna 1987).

1	M	30	G	59	S	88	K	117	R	146	V
2	D	31	L	60	G	89	V	118	Y	147	Q
3	I	32	F	61	I	90	L	119	R	148	S
4	T	33	E	62	S	91	D	120	L	149	N
5	I	34	Y	63	E	92	D	121	P	150	M
6	Q	35	D	64	V	93	F	122	S	151	D
7	H	36	L	65	R	94	V	123	N	152	S
8	P	37	L	66	S	95	E	124	V	153	S
9	W	38	P	67	D	96	I	125	D	154	H
10	F	39	F	68	R	97	H	126	Q	155	S
11	K	40	L	69	D	98	G	127	S	156	E
12	R	41	S	70	K	99	K	128	A	157	R
13	A	42	S	71	F	100	H	129	I	158	P
14	L	43	T	72	V	101	S	130	S	159	I
15	G	44	I	73	I	102	E	131	C	160	P
16	P	45	S	74	F	103	R	132	S	161	V
17	F	46	P	75	L	104	Q	133	L	162	S
18	Y	47	Y	76	D	105	D	134	S	163	R
19	P	48	Y	77	V	106	D	135	A	164	E
20	S	49	R	78	K	107	H	136	D	165	E
21	R	50	Q	79	H	108	G	137	G	166	K
22	L	51	S	80	F	109	Y	138	M	167	P
23	F	52	L	81	S	110	I	139	L	168	T
24	D	53	F	82	P	111	S	140	T	169	S
25	Q	54	R	83	E	112	R	141	F	170	A
26	F	55	T	84	D	113	E	142	S	171	P
27	F	56	V	85	L	114	F	143	G	172	S
28	G	57	L	86	T	115	H	144	P	173	S
29	E	58	D	87	V	116	R	145	K		

In contrast to my earlier alpha crystallin A chain results (McKenna 1987), several hypothesized pathways emerge differently (e.g., in edentates and carnivorans) and are in closer accord with the current morphological consensus. Nonetheless, other conflicts remain.

In the present cladogram, replacements are depicted by the single-letter convention: e.g., 70KQ stands for a replacement from lysine to glutamine at locus 70. Parallelisms are indicated by $^{=}$, reversals by $^{-}$, and double hits by **. As before, postulated replacements have been checked for plausibility at the codon level, but only two double hits were required. When additional marsupial diversity is sampled, one of the double hits (169SL** in marsupials) might prove to have been acquired sequentially.

2. Analysis of mammalian alpha crystallin phylogeny

The alpha crystallin A chain has now been studied in more than 50 mammalian species, distributed over a number of orders. Nevertheless, significant gaps remain, such as Monotremata and Dermoptera. Within orders, taxic diversity remains to be sampled adequately in several, such as among the Marsupials, Macroscelidea, Lipotyphla, Chiroptera, and Cetacea. Even Rodentia and Artiodactyla are much in need of broader taxic sampling. In the rush to reach ever deeper levels close to the genome, protein sequencing is now being leap-frogged by many molecular phylogenists, but the results obtained so far from the alpha crystallin A chain have been encouraging, even though some aspects are puzzling. Phylogenetic analysis of the distribution of amino acid replacements in this chain has yielded a stimulating amount of resolution in mammalian phylogenetic reconstruction thus far, and much more seems possible. I therefore take this opportunity to update an earlier analysis (McKenna 1987).

As just noted, the alpha crystallin A chain of monotremes remains unstudied. This is unfortunate, for several reasons. Nearly all other kinds of studies report monotremes as an outgroup to both marsupials and placentals. Determination of the molecular sequences of their alpha crystallin A chains thus would have a strong bearing on the determination of the plesiomorphous condition in the alpha crystallin A chain for all other living mammals. The next living outgroup beyond the monotremes is remote — true reptiles — with a branch point away from the line leading to mammals that would have been in the mid-Paleozoic Era. Thus, knowledge of the various monotreme conditions would be most useful. Moreover, monotremes may not be so remote from therian mammals as once was supposed, although they appear to predate the acquisition of truly tribosphenic teeth. This became evident with the description of the Australian Cretaceous toothed monotreme *Steropodon* (Archer et al. 1985), whose dentition is not very different from that of primitive tribosphenic therians close to the marsupial/placental dichotomy (Kielan-Jaworowska et al. 1987), but would seem to have branched away from the line leading to other living mammals somewhat before the rest became tribosphenic. *Steropodon* occurs in rocks about 110 million years old. Moreover, the platypus and the echidnas are phenetically rather different, despite many shared-derived morphological characters, possibly representing two orders. There are three living genera, and each cries out for study.

In my own reconstruction of myoglobin phylogeny (McKenna 1987; based on Fisher & Thompson 1976), monotremes fell into place in the position predicted by nearly all morphological analysis. Nevertheless, Goodman et al. (1985) came up with a molecular interpretation that placed monotremes well within placental mammals, a stimulating result questioned by Wyss et al. (1987). However, in a morphological analysis of 20 mammalian OTUs by Novacek (1989: fig. 1), five out of the six most parsimonious trees he found place monotremes outside the marsupial/placental dichotomy.

Monotremes aside, in this analysis marsupials and placentals (Fig. 1; node 1) are clearly mammalian sister-groups possessing unique sets of replacements in the alpha crystalline A chain. Marsupials (node 2) are thus far represented only by two widely different and long isolated taxa, *Didelphis* (opossum) and *Macropus* (kangaroo), but they share eight hypothesized replacements. Of the eight, several occur also within the

placentals at minor taxonomic levels, but most are unique (including one double hit). However, loci 149 and 152 of *Macropus* could possibly be interpreted differently, in view of their hypothesized involvement deep in the placental phylogeny. Moreover, further sampling in marsupials might suggest sequential acquisition of the marsupial synapomorphies. The present analysis of the marsupial-placental split is identical to my previous results (Mckenna 1987) and agrees with morphological analysis. Undoubted marsupials occur in Cenomanian Cretaceous sediments of Utah (*Pariadens* Cifelli & Eaton 1987) about 90 to 95 million years old, and a disputed genus, *Holoclemensia*, occurs in Albian Cretaceous rocks in Texas, between about 95 and 107 million years old. A presumed placental mammal, *Prokennalestes*, occurs in rocks of approximately Albian age in Mongolia. This would suggest but certainly not prove that the marsupial/placental split occurred a little over 100 million years ago, or shortly after the known occurrence of *Steropodon.* It could have occurred before then.

Placentals (Eutheria of Huxley 1880; node 5) are held together by seven postulated replacements (31V, 101SN, 129IL, 149NG**, 152SA, 158PA, & 168TS) involving eight hits. However, as noted above concerning *Macropus,* possibly loci 149 and 152 can be interpreted in some different way. Knowledge of the situation (or situations) in monotremes could help to resolve this problem. Within the placentals, it is evident that several groups are ancient, but few synapomorphies occur and some are subject to alternative interpretation. Nonetheless, among the placental mammals for which alpha crystallin A chain sequences are available, elephant shrews (Macroscelidea; known from an incomplete sequence, node 6) and edentates *(sensu lato:* including pangolins; node 7) are suggested on the basis of this evidence to be the earliest of these divergent lines leading away from other placentals.

Elephant shrews once were allied with tupaiids (tree shrews) as "Menotyphla" (Haeckel 1866). From the standpoint of the incompletely analysed alpha crystallin A chain of *Elephantulus rufescens* (de Jong 1985; no reversals assumed), elephant shrews are apparently more ancient than tupaiids and have to be placed at an early node in the cladogram, but their fossil record has remained elusive. Elephant shrews occur in the African early Tertiary, however, and may have some sort of vague relationship to the anagalid radiation of Asia, as Evans (1942) and I (McKenna 1975) have adumbrated. If that is true, elephant shrews and their allies can be traced back to the Paleocene. However, because of analytical problems, the amino acids at active loci 3, 4, 70, 74 and 142 are not yet published for *Elephantulus,* although they soon will be (de Jong et al., in preparation). Neither elephant "shrews" nor tree "shrews" have any demonstrated close relationship to true shrews, which are lipotyphlans. It would be better simply to call these animals, respectively, macroscelideans, tupaiids, and soricids. On the present cladogram (Fig. 1) macroscelideans must be depicted as simply placentals, *incertae sedis.* Inasmuch as *Elephantulus* is concluded to be plesiomorphous at loci 91, 150, and 153, it would be useful to know conditions at loci 3, 4, 70, 74, and 142. All of these uncharacterized loci are active at nearby cladogram nodes (5, 18, 24, 30). Because Novacek et al. (1988) and Novacek (1989) conclude on morphological grounds that macroscelideans are the closest sister-group of Glires (rodents and lagomorphs) a conflict therefore exists between the current morphological and the alpha crystallin A chain interpretations. Interestingly, as pointed out by de Jong (1985), macroscelideans display no known alpha crystallin A chain autapomorphies, but this situation could change when the complete *Elephantulus* alpha crystallin A chain sequence is published. On the serological front, Sarich (1985) has reported strong precipitin crossreaction between *Sylvilagus/Oryctolagus* and the macroscelidean genus *Rhynchocyon.*

In this analysis, edentates include Pholidota, the pangolins (Novacek et al. 1988). In contrast to my earlier interpretations (McKenna 1975, 1987), I have now found a parsimonious arrangement that weakly unites pangolins (scaly anteaters) with edentates and does not place them in Preptotheria, *incertae sedis,* or with the arctoid carnivoran *Melursus.* In accord with some but not all of the morphological evidence used by Novacek (Novacek & Wyss 1986, Novacek et al. 1988), pangolins can be tied weakly to the edentates by breaking apart alpha crystallin A

chain node 102 (of McKenna 1987: fig. 3.2) so that locus 150MV$^{=}$ becomes an edentate-pangolin synapomorphy (node 7 of the present cladogram) parallel with carnivorans. This requires loci 74FY$^{=}$ and 91 DE$^{=}$, two of the three postulated synapomorphies that unite *Manis* with *Phataginus,* to be parallelisms with all remaining placentals (91DE$^{=}$) or with *Melursus* (74FY$^{=}$). However, locus 150 no longer need progress from 150M to 150L to 150V as was required in my earlier interpretation (McKenna 1987). In fact, 150MV$^{=}$ now can be used weakly to support carnivoran monophyly. Moreover, locus 153 is no longer required to back-mutate to serine in pangolins, although locus 3 reverses to isoleucine and locus 4 must change from threonine to alanine. These modifications are in accord with recent morphological studies (Novacek et al. 1988, Novacek 1989), but the linkage is not strongly supported. Within the edentates, sloths separate from anteaters as expected, but we have no information yet concerning the alpha crystallin A chain of armadillos. Their eye lenses are very small and therefore difficult to test.

The fossil record of edentates clearly extends to the Paleocene in South America, but some South American paleontologists hold that the group is even more ancient there. In South America the Campanian Cretaceous genus *Gondwanatherium* and the post-Salamancan Paleocene genus *Sudamerica* have been identified by some workers as early edentates (Scillato-Yané & Pascual 1985, Bonaparte 1986, Mones 1987), but the evidence is extremely weak. Unquestioned edentates were in South America at least as far back as the late Paleocene, about 60 million years ago, when the first known armadillos occur. Both "true" anteaters and pangolins have been reported from the medial Eocene of Germany, and palaeanodonts, a group placed by many paleontologists either in or near the edentates and pangolins, occur in the early Tertiary of Asia and North America. The availability or non-availability of a dispersal route to explain the German occurrence of what would usually be regarded as a South American anteater may possibly shed some light on edentate antiquity. While it is tempting to suggest a route via the ancestral Lesser Antilles up the east coast of North America and from there across the North Atlantic gap at a time when proto-Iceland choked the North Atlantic in the late Paleocene, evidence for dispersal between North and South America in the late Paleocene or Eocene is very weak (Cifelli et al. 1989). Rather, interchange probably occurred earlier, at about the beginning of the Paleocene, or still earlier. The older the interchange, the narrower the South Atlantic gap between Africa's bulge and Brazil's, so that an African route between South America and Europe in the Late Cretaceous, when Africa and South America were still sliding past each other along the Romanche transform fault and other nearby transform faults, needs to be considered. All this is weak evidence that edentates may have occurred in the Cretaceous, even if *Gondwanatherium* should prove not to be one.

After the phylogenetic departure of the edentates, a replacement at locus 91 from aspartic acid to glutamic acid is interpreted to characterize all other placentals (Epitheria, node 17), but the replacement at this locus is subject to parallelism (in pangolins) and is later reversed in Old World higher primates. It is therefore not very certain whether a three-way or four-way (with paenungulates added) split occurs early in placental evolution (possible collapse of node 17 into node 5).

We next come to the "paenungulate question." Simpson (1945) subdivided the ungulates (except whales) into four superordinal groups: Protungulata, Paenungulata, Mesaxonia (=Perissodactyla), and Paraxonia (=Artiodactyla). Protungulata contained the extinct condylarths, litopterns, notoungulates, astrapotheres, and the still-living aardvarks *(Orycteropus,* placed in Tubulidentata). As originally proposed, the paenungulates were a paraphyletic or polyphyletic lot, including the extinct pantodonts, uintatheres (Dinocerata), pyrotheres, and arsinoitheres (Embrithopoda), and the still-living Proboscidea, Sirenia (including the extinct Desmostylia), and Hyracoidea. However, some or all of the still-living paenungulates are now usually regarded as monophyletic. McKenna (1975) united the Sirenia, Desmostylia (no longer subsumed within Sirenia), and Proboscidea in a monophyletic taxon that he named Tethytheria. The Hyracoidea, together with perissodactyls and condylarths, were placed by McKenna (1975) in a taxon that he

named Phenacodonta. McKenna allocated the remainder of Simpson's paenungulates to other monophyletic taxa. Thus, among living mammals once placed in the Paenungulata, the Tethytheria were considered monophyletic and the Phenacodonta (including the still-living perissodactyls and hyracoids but not the artiodactyls) were considered one of several polytomous sister-groups.

McKenna's (1975) Tethytheria was regarded as an unresolved ordinal tritomy in 1975, but, more recently, Domning et al. (1986) placed Desmostylia closer to Proboscidea than to Sirenia. However, Novacek & Wyss (1987) maintained the original tethytherian three-way split, suggesting that the amastoid condition of the brain case and the serial condition of the tarsus might nevertheless unite living paenungulates — in other words, that Hyracoidea may well be the still-living sister-group of the Tethytheria. Hyracoids and tethytheres together would represent a larger monophyletic group, the living paenungulates. Perissodactyla would represent a close outgroup. Such a system would constitute resolution of node four of the cladogram presented by McKenna & Manning (1977: fig. 1). Thus, while related to perissodactyls collaterally, Hyracoidea are nonetheless members of a monophyletic Paenungulata if the paenungulates are defined by living taxa and various fossil groups like pantodonts are placed elsewhere.

From an alpha crystallin A chain perspective (de Jong et al. 1981, McKenna 1987: fig. 3.2) as well as a serological perspective (Sarich 1985), living paenungulates (*Loxodonta, Trichechus, Procavia*) plus *Orycteropus* appear to represent an early split (node 17) from other placentals. Three synapomorphies (70KQ, 74FL$^{=}$, 142SC$^{=}$) unite them, but 74 FL$^{=}$ is paralleled in *Tamandua* and 142SC$^{=}$ occurs as well in *Homo*. If these synapomorphies hold, this would resolve slightly the multifurcation required in the cladogram presented by Novacek et al. (1988: fig. 3.3). Paenungulates and *Orycteropus* would not only be separate from "true" ungulates (artiodactyls, perissodactyls, whales; whether this grouping is monophyletic or not), but also would be at least as ancient as the branches leading to carnivorans, insectivorans, tupaiids, bats, rodents, lagomorphs and primates. This would imply an origin of paenungulates early in the Paleocene or even in the Cretaceous. However, this interpretation contradicts some recent anatomical and paleontological work (Fischer 1986, 1989a, Prothero et al. 1988, reviewed by Novacek 1989), which suggests close similarities and cladistic alliance of hyraxes with perissodactyls. Fischer (1986, 1989a) and Prothero et al. (1988) even subsume the hyraxes *within* the Perissodactyla as Sir Richard Owen proposed over a century ago. But if the hyrax *Procavia is* genealogically allied with perissodactyls, then ten alpha crystallin A chain replacements (exclusive of locus 150) called for in the present analysis would have to be reversed somehow, which would have profound effects on the topology of almost the entire cladogram. From the viewpoint of my alpha crystallin A chain analysis, this seems unlikely, although conceivably some mechanism such as neoteny might be invoked.

McKenna et al. (1989) described a new late Paleocene phenacolophid mammal genus from China, *Radinskya*. It seems to be morphologically intermediate between paenungulates and perissodactyls. The known morphology of *Radinskya* seems to support the cladistic topology of perissodactyl and paenungulate genealogy arrived at by McKenna & Manning (1977): i.e., perissodactyls and paenungulates are related, but hyracoids are not to be placed within Perissodactyla. Fischer's (1989b) point of view seems to be close to my own, but his analyses are still in a state of flux. Obviously, additional analysis is in order, but I favor the placement of the Hyracoidea with the Tethytheria in a restricted Paenungulata that is stripped of some of the fossil orders [e.g., Pantodonta, Dinocerata, Pyrotheria and Xenungulata (then regarded as Pyrotheria)] originally placed in it by Simpson (1945).

The position of Tubulidentata (aardvarks: *Orycteropus*) close to paenungulates (de Jong et al. 1981) is questionable on the basis of morphological studies (Thewissen 1985, Novacek et al. 1988) and analysis of myoglobin sequence data (McKenna 1987, Wyss et al. 1987). The problem of tubulidentate relationship is worth much additional study.

After the branch (or branches) leading to paenungulate-like mammals, remaining placentals (node 23) acquired 153SG$^{=}$ (paralleled in

Orycteropus). Within these placentals, the carnivorans become the outgroup to the others. Four alpha crystallin A chain synapomorphies (3VI$^{-=}$, 4TA$^{=}$, 147QP$^{-=}$, 150MV$^{=}$) unite Carnivora as an order (node 24), but, as currently interpreted, all four of these replacements are paralleled elsewhere and therefore their significance is lessened. The fossil record of carnivorans goes back to the medial Paleocene, where an arbitrary taxonomic break separates them from earlier cimolestids of the Paleocene and Late Cretaceous. If the node leading to carnivorans is younger than that leading to paenungulates, this would be evidence for pushing back the origin of node 17 at least as far.

At this point in the hypothetical phylogeny (node 30) a postulated synapomorphy (150ML) characterizes what appears to be a monophyletic remaining assemblage (Fig. 1) comprising tupaiids, hedgehogs, bats, rodents, lagomorphs, primates, and "true" ungulates. However, 150ML may either be unstable or possibly wrongly interpreted. *Tupaia* and *Erinaceus* are interpreted on the basis of very weak evidence (involving locus 90) as outgroups to a ten-fold fragmentation (node 32) characterized by 90LQ$^{=}$, but this locus is evidently highly unstable and only minimal weight should be placed on its interpretation. Nonetheless, it is interesting to contemplate a collocation of rodents (not demonstrated to be monophyletic by the alpha crystallin A chain), lagomorphs (ditto), bats (ditto), primates (ditto), and true ungulates (node 50; 3 synapomorphies paralleled elsewhere): artiodactyls (not demonstrated to be monophyletic), whales (ditto), and perissodactyls (one synapomorphy: 127ST). Whales and perissodactyls are interpreted here to have the reversal 150LM^{-} in common (node 55), but in the alpha crystallin A chain, locus 150 would appear to be about as unstable as locus 90.

Although the alpha crystallin A chain does not resolve the ten-fold fragmentation (node 32) that follows the departure of *Tupaia* and *Erinaceus,* it does make remotely allied sisters of mammals that are ordinarily called rodents, lagomorphs, bats, primates, and "true" ungulates. Analysis of the alpha crystallin A chain does not support Glires or Archonta as supraordinal constructs, but, among the ungulates supported by reversals 3VI$^{-=}$, 4TA$^{=}$, and 147QP$^{-=}$, it weakly allies cetaceans with perissodactyls (unstable reversal 150LM^{-}; node 55). This last alliance is in agreement with Novacek's (1989) morphological results. Among rodents, myomorph, castorimorph, and sciuromorph rodents are weakly united by the single back-mutation 90QL$^{-=}$, whereas *Ctenodactylus, Pedetes,* and *Cavia* are characterized by 90Q. Where comparable, this is in agreement with differences in pancreatic ribonucleases studied by Beintema & Lenstra (1982) and with the occurrence of a unique additional alpha crystallin chain in muroids (de Jong 1985; *Castor* and *Sciurus* not yet tested for it). Lagomorphs are not united by alpha crystallin A chain synapomorphies and indeed the chain is not helpful in evaluating lagomorph relationship to other orders of mammals represented within the ten-fold fragmentation at node 32 of the cladogram. Some current morphological studies claim support for the Glires concept (Li & Ting 1985, Li 1989, Luckett 1985, Novacek 1985, 1989), whereas other studies of both morphology and molecular information are less sanguine (McKenna 1961, Wood 1962, Shewale et al. 1984, Bleefeld & McKenna 1985, Lopez Martinez 1985, Sarich 1985, Shoshani et al. 1985:fig. 2, Butler 1985). Molecular work on lactalbumins would have lagomorphs be an outgroup to a combination of rodents, primates, and artiodactyls (Shewale et al. 1984). On the basis of sequences obtained from globin genes, Easteal (1988, 1990) obtained a resolution of the multifurcation at node 32 that would have rodents branch off first, followed by ungulates, and then a sister pair comprising lagomorphs and primates. Other molecular work would even link lagomorphs with bats (Shoshani et al. 1985). Sarich (1985), primarily a serologist, is certainly the most vehement exponent of the "anti-Glires school," but his paper does not demonstrate shared-derived characters of lagomorphs with any other order, although he demonstrates that in precipitin cross-reactions anti-(*Sylvilagus/Oryctolagus*) antiserum made in chickens reacts more strongly with the macroscelidean *Rhynchocyon* than with the lagomorph *Ochotona*, and much more strongly than with any rodent tested. In view of these exceedingly diverse molecular results, the conservative view afforded by the alpha crystallin

A chain can serve for the moment as a rather uninformative molecular consensus.

Nodes 30 and 32 probably occurred about 75 or 80 million years ago (Ma) in the Late Cretaceous, but the affinities of the nominally Campanian (= approximately Judithian, about 76 Ma) Late Cretaceous genera *Zalambdalestes* and *Barunlestes* of Mongolia (Kielan-Jaworowska 1984) may have a bearing on this and may ultimately force node 32 (and all others before it) back into the Cretaceous as far as the Judithian (for problems of dating see Lillegraven & McKenna 1986).

The topology obtained for primate synapomorphies is identical to that of de Jong & Goodman (1988) except that their fig. 2 erroneously attributes aspartic acid to rhesus monkeys and humans at their locus 101 rather than 91 (correctly shown in their fig. 3; de Jong, pers. comm.). However, primates as a whole, like rodents, lagomorphs, and artiodactyls, are still not united by an alpha crystallin A chain synapomorphy. The topology of the cladogram of the myoglobin molecule, on the other hand, does unite the Primates and yields the morphologically expected phylogeny (Romero-Herrera et al. 1973, 1978, McKenna 1987: fig. 3.6).

The alpha crystallin A chain is not very helpful in resolving artiodactyl phylogeny. In fact, as just noted, it does not even establish that the order is monophyletic. It does suggest (node 52), however, that *Hippopotamus* should be placed with higher artiodactyls rather than with suids or just *incertae sedis.* This is an improvement over the less resolved scheme of McKenna (1987: fig. 3.5).

Among perissodactyls, tapirs and rhinos appear to share a more recent ancestor (node 58) than they do with *Equus,* but the replacement involved, 13 $AT^{=}$, is paralleled extensively elsewhere and is thus not very convincing evidence. The result, however, is in keeping with the morphological consensus.

Morphological evidence and known ranges of included taxa suggest that nodes 50, 55, and 57 occurred close together in time, well back in the Paleocene at the latest. Thus, it is not surprising that we are having such a difficult time resolving the multifurcations that characterize conservatively constructed molecular cladograms.

3. Significance of *Spalax* (blind mole rat) for molecular clock hypotheses and phenetic analysis

Alpha crystalline A chain has been sequenced in ten species of rodents, yet the only synapomorphy found is a somewhat dubious reversal at locus 90 for a subset of rodents comprising *Sciurus*, *Castor*, and five muroids but excluding the presumably more primitive genera *Ctenodactylus*, *Pedetes*, and *Cavia*. Among the rodents apparently united by $90QL^{-=}$ (node 35), *Mus*, *Mesocricetus*, and *Rattus* have no further apomorphies, *Meriones* and *Sciurus* modify a single locus ($123NS^{=}$: probably homoplasy), and *Castor* has three replacements, two of which are reversals. However, the muroid genus *Spalax* (node 38) is characterized by nine replacements, a relatively larger number than in any other mammal tested (Hendriks et al. 1987, Gould 1989). This amount of replacement is noteworthy even when compared with *Didelphis* (five), *Orycteropus* (five), *Trichechus* (five), and *Erinaceus* (seven), because when close relatives of these terminal taxa are studied some of their replacements can be expected to be distributed among ancient nodes in a more branching cladogram. Those of *Spalax* are more recently acquired, after *Spalax* differentiated from other muroid rodents in the Miocene (Catzeflis et al. 1989) .

Not only is the number of replacements high in *Spalax,* but also the replacements themselves are noteworthy: five of the nine (12RH, 29EQ, 53FL, 60GC, and 163RQ) occur nowhere else among tetrapods tested. However, a satisfactory explanation is available (Hendriks et al. 1987; de Jong et al. 1989). *Spalax,* even though it has an eye lens, is blind. Replacements in its alpha crystallin A chain are therefore lessened in importance and can increase in number. An indefinite number of deleterious or neutral replacements can be present. The implication is that amino acid replacements are normally under the control of selection in mammals, but in the case of blind *Spalax* selection has been relaxed somewhat and replacements have proliferated accordingly. This in turn has a deeper significance: if selection normally operates at the codon level, then would-be molecular clocks at this level (or higher) will

be modulated by selection and will not run reliably. Clocklike behaviour may exist in DNA pseudogenes not under selection, or may be a tendency approached by combining large amounts of data, but the evidence from *Spalax* strongly suggests that selection normally modulates or overrides any clocklike behaviour that might otherwise occur at the codon level or above in tetrapods (Joysey 1988).

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