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General relationships of mammalian orders and evolutionary development of primates inferred from best-fit α-globin phylogenies

SHAMA BARNABAS*, R. USHA, T. N. GURU ROW and JOHN BARNABAS

Molecular Evolution Unit, National Chemical Laboratory, Pune 411 008, India

*Post Graduate School for Biological Studies, Ahmednagar College, Ahmednagar 414 001, India

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Abstracts. A best-fit α-globin phylogeny was identified by means of a global minimization approach from among the topologies generated by a parsimony strategy. Zip parsimony method was used to derive a set of near-parsimonious trees. For each of these topologies, a difference matrix was computed; and the topology with the best goodness of fit with the original matrix was retained as the best tree. Based on this phylogenetic scheme inter-relationships among eutherian orders and the evolutionary development of primates has been discussed.

Keywords. α-Globin phylogeny; zip parsimony method; global minimization approach; eutherian order inter-relationships; primate lineage.

Introduction

The living mammals consist of monotremes, marsupials and placentals. Their origins can be traced to Triassic times to a group of mammal-like reptiles called therapsids. In the middle Permian and lower and middle Triassic age, therapsids evolved along two lines (Romer, 1966). One was herbivorous and consisted of the dinocephalians and the dicynodonts. The other consisted of the theriodonts (*e.g.* Cynognathus) a group of active carnivores which preyed upon the herbivorous forms (Howgate, 1983). It is these predatory forms which through a little known group of theriodonts, the ictidosaurs from late Triassic and early to middle Jurassic have given rise to the mammals. The Ictidosauria (*e.g.* Diarthrognathus from South Africa) compared to other theriodonts were more mammal-like in their characters and exhibited a double jaw articulation, with a reptilian type of joint between the reduced quadrate and articular, and a new mammalian type of joint between the squamosal and dentary bones (Colbert, 1970).

Information about the early mammals is based on the study of fossils from the late Triassic – early Jurassic age from places throughout the old World (Jenkins and Crompton, 1983). For a long time the early mammalian evolution was regarded as being dichotomous consisting of the morganucodontids (non-therians) and Kuehneotheriids (therians) (Crompton and Jenkins, 1979). But study of fossils like Dinnetherium (Ord. Trichonodonta, family: incertae sides) from the late Triassic – early Jurassic of Kayenta formation in Arizona, shows that the early evolution of mammals may have been more complex than the simple dichotomy visualized ear-

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Abbreviation used: APSD, Average per cent standard deviation.

lier. It has been suggested that Dinnetherium which shows clear differences from the other mammals of that time represents an intermediate stage in the evolution of mammalian jaw masculature and movement; and in the development of the angular region, it is more advanced than these mammals (Jenkins and Crompton, 1983).

Marsupials and placentals appear to be the end products of a complex probably common North American stock (Clemen 1979; Kielan-Jawarowska *et al.,* 1979) which diverged before the early late Cretaceous resulting in these groups undergoing separate adaptive radiations to form the two dominant mammalian groups.

At the Cretaceous-Tertiary (C-T) boundary, there was mass extinction of organisms and the dinosaurs which had dominated the earth for one hundred million years of Mesozoic history became extinct. Consequently, the ecological niches abandoned by the dinosaurs became available to the surviving primitive mammals which rapidly radiated along many evolutionary lines giving rise to the varied and successful mammals of Tertiary, Quaternary and recent times.

The tempo and mode of evolution of mammals can be captured in part, from the geneological analysis of sequences of informational macromolecules. In this paper, we present basic pattern of evolution of eutherian orders and that of primates through a best-fit globin phylogeny. The primate phylogeny has been discussed in the context of available information based on protein immunology, protein electrophoresis, DNA hybridization and macromolecular sequence data. Evidences from paleontology and other biological disciplines have also been incorporated in our discussions.

Methodology

Construction of evolutionary trees

The phylogenetic relationships of organisms are traditionally derived on the basis of evidences from fossil record, morphology, ultrastructure, cytology, and other biological disciplines. In recent years, an entirely new body of evidences has come from the phylogenies based on relevant proteins and nucleic acids. Protein immunology, protein electrophoresis, nucleic acid restriction analysis, DNA hybridization and sequence data of proteins and nucleic acids are increasingly being used for discerning the evolutionary relationships of organisms. We have used sequence data of globins to construct phylogenetic trees. Two types of methods are currently available for this purpose. One is based on matrix numbers representing non-matching residues in the aligned set, and the other, is based on the reconstruction of ancestral sequences which generate descendant sequences through minimum number of mutations. In the matrix methods, one clustering strategy which is generally used to give a first approximation of phylogeny is unweighted pair group method (Sokal and Sneath, 1963). Among the other matrix methods, the weighted invariant step strategy of Farris (1970), additive tree approach of Moore *et al.* (1973a) and the least squares matrix approach of Dayhoff (1976) are also useful for deriving phylogeny.

Ancestral sequence methods involve double minimization of sum of values of branch lengths on the one hand, and topological configurations on the other. Many variations of the ancestral sequence method including those of Fitch (1971), Dayhoff (1972), Moore *et al.* (1973b), Barnabas *et al.* (1978, 1980) are available for deriving phylogenetic trees.

Matrix methods have an underlying assumption that genes evolve at a uniform rate. Though genes do not evolve at a stochastically constant rate, average rates of evolution over long periods and over many genes may be uniform. This would suggest that matrix methods which are based on uniform rate of genic evolution, may be methods of choice for distantly related species. On the other hand, ancestral sequence methods which capture back mutations are ideal for closely related species.

In this paper, we follow an approach which captures facets of both the above methods. First, we generate a phylogenetic tree using zip method of Barnabas *et al.* (1980). This method is based on the reconstruction of ancestral sequences at the interior points of a network (unrooted) where exterior points represent contemporary sequences. Here, initially by means of an operation called 'zipping' amino acids and their underlying codons are identified at the interior points of a given network which have the potential of minimising the total length of the network which is $\Sigma_{mr,}$ where mr is the length of the r^{th} link. Next by means of "unzipping" operation, final solution set is established at interior points such that Σ_{mr} is minimum possible. Alternate networks are tried and the one which gives a minimum network length is retained. Nearly parsimonius trees are also retained for further analysis. Based on the branch lengths of each network, mutation distances are calculated. These form the upper triangle matrix elements R_{ji} . The lower triangle matrix elements O_{ij} are computed based on the differences on aligned sequences in terms of codons of all N sequences. Next for each topology, average per cent standard deviation (APSD) coefficient, a test function defined by Fitch and Margoliash (1967) is calculated:

$$\left[\frac{\sum_{i=1}^{N-1}\sum_{j=i+1}^{N}[(O_{ij}-R_{ji})/O_{ij}]^2}{\frac{[N(N-1)]}{2}-1}\right]^{1/2}$$

The topology with the lowest APSD identifies the best-fit tree.

Results and discussion

Higher vertebrates possess tetrameric haemoglobins which are encoded by a variety of globin genes belonging to two families, the α and the β (Efstratiadis *et al.,* 1980). The structural organization of globin gene clusters of a few mammalian species is now known. In man, α-globin genes are present as a cluster on chromosome 16 and consist of two sets of genes (Lauer *et al.,* 1980). One set has a gene (ζ), which is functional in early embryo and is present along with a pseudogene ($\psi\zeta$). The other set has two identical α-genes, which are expressed both in the foetus and in the adult. Located between the two sets is a non-functional α-pseudogene (Proudfoot *et al.,* 1980). Gene duplication at the α-chain locus first observed by us (Balani and Barnabas, 1965) is now known to be widespread in mammals. Interestingly, although the origin of this duplication can be traced to an amniote ancestor, yet there is little or no divergence among within-species duplicate set of α-globins. Such events have been recently reconciled by assuming extensive within-species homogenisation (Zimmer *et al.,* 1980).

The β-globin cluster in man is present on chromosome 11 and contains 5 functional genes plus a pseudogene ($\psi\eta$). The 5 functional genes are: embryonic ϵ-gene, two fetal γ-genes, adult δ- and β-genes (Lauer *et al.,* 1980). The organisation of loci in β-chain gene clusters of mouse (Jahn *et al.,* 1980), rabbit (Hardison, 1984), lemur (Barrie *et al.,* 1981), catarrhine primate (Barrie *et al.,* 1981) and goat (Shapiro *et al.,* 1983) suggests that the adult β-gene has undergone both gene duplications and contractions in the course of evolution. Also, hybridization and comparative sequence analysis of η-genes show that this gene is ancient in origin and is maintained as a pseudogene in primates and may be functional in non-primates (Harris *et al.,* 1984). In fact, Goodman *et al.* (1984) have inferred from phylogenetic studies that η-gene was functional in early eutherians.and continued to be functional in artiodactyls but became a pseudogene ($\psi\eta$) in protoprimates and was lost in rodents and lagomorphs. The δ-gene is a pseudogene in old World monkeys (Kimura and Takagi, 1963; Martin *et al.,* 1983) and has undergone homogenisation with the β-gene over the 5' region of the gene (Efstratiadis *et al.,* 1980; Jeffrey *et al.,* 1982; Martin *et al.,* 1983). Similarly, human γ^{A}- and γ^{G}-genes have undergone multiple localised gene conversions in recent evolution (Slightom *et al.,* 1980; Scott *et al.,* 1984).

Clearly, α-globin genes are less variable in their profile over evolutionary time than the β-globin genes. Moreover, though the duplication at the α-globin locus is widespread in many species (Barnabas, 1979), these genes have evolved in concert (Zimmer *et al.,* 1980). This fact makes α-globin sequences dependable data sets for phylogenetic systematics. In our analysis, we have utilised relevant orthologous sets of α-globin sequences for deriving the pattern of evolution of eutherian orders as well as of primates. It may be noted that globin genes show three exon-two intron structure. Eaton (1980) by analysing the range of haemoglobin functions in terms of 3 exons has shown that the central exon-encoded fragment has most of the functionally important sites such as $\alpha_1\beta_2$ contact sites and heme contact sites, whereas $\alpha_1\beta_1$ contact sites are located predominently in the right exon-encoded fragment. These sites are conserved during evolution; but it appears that functionally silent sites diverge rapidly and evolve at a constant rate (Miyata *et al.,* 1980; Kimura, 1981). We have also used a set of functionally silent amino acids residues as a comparative measure.

General relationships of eutherian orders

There are suggestions that many mass extinctions, similar to the one at the C-T boundary have occurred during the history of evolution of life. Based on their analysis of fossil record of marine animals, Raup and Sepkoski (1984) have suggested that during the last 250 million years or so there has been a regular periodicity of 26 million years in the occurrence of mass extinctions. Many interpretations have been made to explain this cyclic phenomenon. Benton (1985) has summarised 4 such interpretations. The first being that each cyclic event was due to some external factor. This view is supported by a number of controversial publications (Davies *et al.,* 1984; Whitmori and Jackson, 1984; Schwartz and James, 1984; Rampino and Stothers, 1984). The other interpretations of cyclic mass extinctions are: they are related to some cyclic physical phenomenon such as fluctuations in sea-level or temperature,

they are caused by factors related to the internal dynamics of the biological systems, they are artefacts due to patchy nature of the fossil record. Based on the presence of iridium and other noble metals in the clay layers at the C-T boundary, Alvarez *et al.* (1980) have suggested that these elements were products of an asteriod impact and that the terrestrial dust generated by the impact could have blotted out sunlight for several weeks causing extinction of dinosaurs and plankton. More recently, utilising a combination of the analytical results and time-structure analysis of the C-T event on the Gosaw basin Austria, Preisinger *et al.* (1986) suggest that about 66·7 million years ago, an asteroid impact took place producing world-wide geochemical and minerological anomoly within a short stretch of time, causing conditions for mass extinctions of large quantities of biodata.

It is known that the development of two main mammalian groups took place in the early Cretaceous and that the diversification of placentals occurred at the end of Cretaceous and continued into the early Tertiary (Van Valen and Sloan, 1965). The 9 eutherian orders included in our study are: Primate, Carnivora, Lagomorpha, Rodentia, Chiroptera, Insectivora, Proboscidea, Artiodactyla and Perissodactyla (table 1). It can be seen from figure 1 that scuffling of branches does not appreciably alter the total network lengths. In fact, a number of alternate branching

Table 1. The mammalian species and their taxonomic groups.

Homo sapiens (human)	Primates
Pan troglodytes (chimpanzee)	–"–
Gorilla gorilla (gorilla)	–"–
Pongo pygmaeus (orangutan)	–"–
Colobus badius (red colobus)	–"–
Cercopithecus aethiops (Savanah monkey)	–"–
Macaca mulatta (rhesus monkey)	–"–
Papio anubis (baboon)	–"–
Cercocebus atys (mangabey)	–"–
Callithrix argentatus (silvery marmoset)	–"–
Saquinus oedipus (cotton-headed tamarin)	–"–
Cebus apella (capuchin monkey)	–"–
Ateles geoffroyi (spider monkey)	–"–
Tarsius bancus (tarsier)	–"–
Lemur fulvus fulvus (brown lemur)	–"–
Galago crassicaudatus (grand galago)	–"–
Loris tardigradus (slender loris)	–"–
Nycticebus coucang (slow loris)	–"–
Tupaia glis (tree shrew)	Tupaioidea
Canis familiaris (dog)	Carnivora
Oryctolagus cuniculus (rabbit)	Lagomorpha
Mus musculus (mouse C57BL)	Rodentia
Rousettus aegyptiacus (bat)	Chiroptera
Suncus murinus (musk shrew)	Insectivora
Elephas maximas (elephant)	Proboscidae
Bos taurus (bovine)	Artiodactyla
Equus caballus (horse)	Perissodactyl
Macropus giganteus (Kangaroo)	Marsupialia

The α-globin sequences of these animal species were taken from the Data Base of Proteins and Nucleic Acids (National Biomedical Research Foundation, Georgetown University Medical Centre, Washington DC).

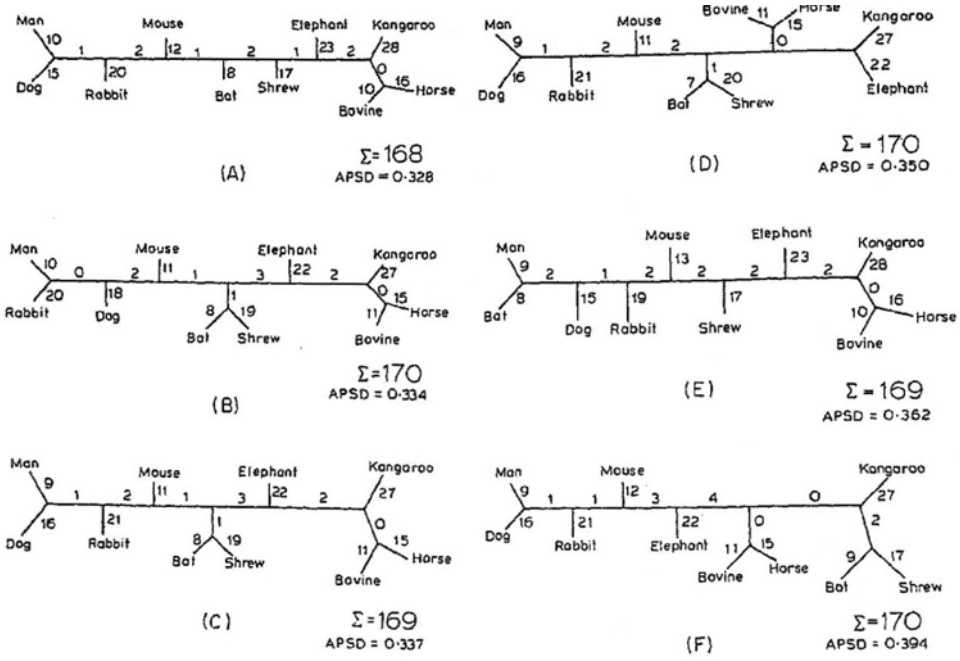

Figure 1. Parsimonious network obtained by the application of zip method. Numbers denote link lengths. (A) is the 'best-fit' network.

arrangements could be accommodated within 3 nucleotide changes of the total network length. This would suggest that the eutherian orders probably arose in a spurt during a relatively short evolutionary time. The widespread diversification of placental orders occurred only after the C-T event. It must be mentioned here that in our calculation cycles, whenever there are alternate parsimony choices, we have distinguished Ά' and 'B' solutions as outlined by Barnabas *et al.* (1972). The Ά' solution which we have retained directs the mutations to those exterior points with fewest intervening points, whereas the 'B' solution directs the mutations in just the opposite way to the link paths most populated with interior points.

The best-fit network using our global minimization approach is identical to the maximum parsimony network, although both these approaches do not necessarily run parallel (figure 1). A set of functionally silent sites also gave the same best-fit topology. Our results indicate that the primate branch arose close in time to the carnivore branch, which in turn arose close in time to the lagomorph branch. Rodent branch appears as an independent branch close to the lagomorph branch. It can also be seen that branches of ungulates and sub-ungulates were probably among the pearliest branches to arise during eutherian evolution since they are relatively closer to the marsupial branch than to others. Chiropterans represented by fruit-bat arose independent of insectivores. More recently, Pettigrew (1986) based on electrophysiological and neuroanatomical studies showed that fruit-bats have primate-like pattern of retinotectal organisation, and suggested that it is a derived character shared in both fruit-bats and primates. The α-globin phylogeny does not support the idea that ancestors of fruit bats and primates arose close in time. In fact,

shifting the bat branch close to the primate branch increases both the Σ_{mr} and APSD values (refer figure 1. Here numbers in network refers to link lengths and Σ refers to total link lengths).

Evolution of primates in the descent of man

Molecular evolutionary biology of primates continues to be a subject of current interest in many laboratories as more and more sequences both at the protein level and at the gene level are becoming available. The evolution of primates based on α-globin sequences using a maximum parsimony approach was first reported by Barnabas *et al.* (1972). Subsequently, phylogeny reconstruction and fitting gene lineage into species phylogeny through a parsimony strategy have been studied in great detail by Goodman *et al.* (1978). Our discussion on primate evolution in this paper is a continuation of our earlier work (Barnabas *et al.,* 1978) with additional new sequences.

Two outgroup non-primates belonging to the order. Insectivora (hedge hog and musk shrew) have been included in our network (figure 2). Here, tree shrew separates next to the insectivore branch. There is a controversy whether or not tree shrew is a primate (Napier, 1972). Our network indicates a non-primate origin of tree shrew. When we introduced *α*-globin sequences of dog and rabbit in the network, the rabbit branch joined the tree shrew branch whereas the dog branch took an intermediate position between primates and tree shrew. Paleontological evidences (Romer, 1966) indicate that there was an initial radiation of primates in the Paleocene and Eocene, represented by lemurs, lorises and tarsiers. This was followed by another radiation in the Upper Eocene and following geologic periods, represented by monkeys, apes and man. Based on these evidences, we have identified the ancestor of primates in the rootless parsimonious network (marked by arrow in figure 2). Hence the discussions that follow will have ancestor-descendant orientation.

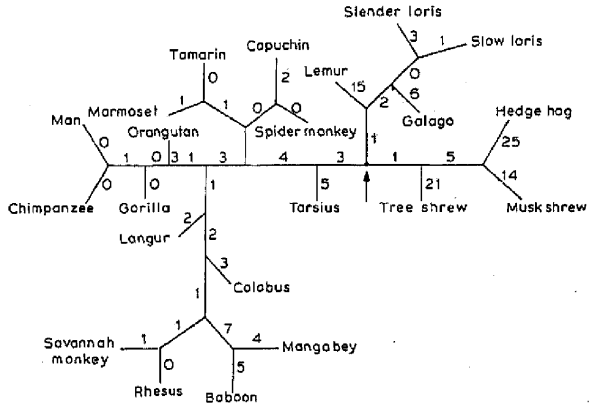

Figure 2. The parsimonious network of 22 *α*-globin sequences of mammals. Numbers denote link lengths.

In the primate line of descent, the first branch to separate is that of prosimians represented by lemur, galago and loris. This is followed by the tarsier branch which

occupies an intermediate position between prosimians and anthropoids. The position of tarsiers in primate classification is controversial. From the morphological evidences tarsiers appear to be the closest living relatives of simians (Martin, 1985). Next to separate from the primate stem are the new World monkeys. The evolutionary relationships of ceboid monkeys cannot be discerned clearly. For example, at position 19, cotton-headed tamarin has serine whereas silvery marmoset has either glycine or serine at the same position (Maita *et al.,* 1984). With glycine at position 19, tamarin joins the branches of spider monkey and capuchin monkey. With serine at position 19, however, tamarin joins the marmoset branch (figure 2).

In the cercopithecoid line, langur separates first followed by colobus monkey. Next to separate from the cercopithecoid stem are two branches: one containing Savanah monkey and rhesus monkey, and the other containing mangabey and baboon. Interestingly, both mangabey and baboon show high rate of evolutionary change (figure 2). In the hominoid line of descent, orangutan separates first followed by African apes and man. However, the separation of gorilla, chimpanzee and man is not well resolved in the globin tree, since α-globins of chimpanzee and man have identical sequences and that of gorilla differs from these sequences by only one amino acid replacement. In fact, there is no unanimity of opinion regarding the relative branching order of gorilla, chimpanzee and man. The globin data indicate chimpanzee to be closer to man than to gorilla. Similarly, DNA-DNA hybridization studies indicate that chimpanzee and human lineages have most recently diverged (Sibley and Ahlquist, 1984). Templeton (1983) on the basis of statistical analysis of the data of Ferris *et al.* (1981) on the cleavage maps of mitochondrial DNAs of hominoids, has suggested that chimpanzee and gorilla have recently diverged. Interestingly, comparison of sequences and structure of 12S rRNA genes of mitochondrial DNAs of hominoids show approximately equivalent relationships among gorilla chimpanzee and man (Hixson and Brown, 1986). Thus the relationship of these 3 hominoids is still an unresolved trichotomy. In all these studies, orangutan is an outgroup hominoid. However, Schwartz (1984) based on overall morphological characters argues that among the moderns apes, orangutan is the closest relative of man. It is generally accepted that the man-orangutan split occurred around 16 million years ago (Pilbeam, 1982). Orangutan shares a number of derived characters with *Sivapithecus,* the middle to late Miocene hominoid (Andrews and Cronin 1982).

The primate paleontologists now agree that man-African apes split occurred around 5-10 million years ago (Andrews, 1982; Pilbeam, 1984). The record of early human begins in Africa (Delson, 1986) as is evident from the fossil remains of *Australopithecus afarensis* found in the Pliocene sites of Hadar in Ethiopia (3·3-3·0 million years old) and Laetolil in Tanzania (about 3·7 million years old). A number of later *Australopithecus* species have also been identified which include the 'Gracile' *A. africanus* (3·3-2·3 million years old), *A. robustus* (1·9-1·6 million years old) and *A. bosei* (2·0-1·3 million years old). Various views are currently being expressed regarding the antecedents of man. Most agree that *A. afarensis* is close to the ancestor which led to two main early human lineages. One led to the robust species through the 'Gracile' stage and the other led to 'Homo' whose 1·76 million years old remains (*Homo habilis)* have been found in northern Tanzania, Kenya and Ethiopia (Susman and Stem 1982). Recently, a 2·5 million years old fossil of *A. bosei* (KNM 17000) which shares a number of primitive characters with *A. afarensis* has been discovered from west of lake Turkana in Kenya (Walker *et alb.,* 1986). The evolution

of anatomically modern man (*Homo sapiens*) also appears to have taken place in Africa where his earliest fossils have been recovered (Stringen, 1984).

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