Evidence on Primate Phylogeny from \(\varepsilon\)-Globin Gene Sequences and Flanking Regions

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Abstract. Phylogenetic relationships among various primate groups were examined based on sequences of \(\varepsilon\)-globin genes. \(\varepsilon\)-globin genes were sequenced from five species of strepsirhine primates. These sequences were aligned and compared with other known primate \(\varepsilon\)-globin sequences, including data from two additional strepsirhine species, one species of New World monkeys (representing all extant genera), and five species of catarrhines. In addition, a 2-kb segment upstream of the \(\varepsilon\)-globin gene was sequenced in two of the five strepsirhines examined. This upstream sequence was aligned with five other species of primates for which data are available in this segment. Domestic rabbit and goat were used as outgroups. This analysis supports the monophyly of order Primates but does not support the traditional prosimian grouping of tarsiers, lorisoids, and lemuroids; rather it supports the sister grouping of tarsiers and anthropoids into Haplorhini and the sister grouping of lorisoids and lemuroids into Strepsirhini. The mouse lemur (Microcebus murinus) and dwarf lemur (Cheirogaleus medius) appear to be most closely related to each other, forming a clade with the lemuroids, and are probably not closely related to the lorisoids, as suggested by some morphological studies. Analysis of the \(\varepsilon\)-globin data supports the hypothesis that the aye-aye (Daubentonia madagascariensis) shares a sister-group relationship with other Malagasy strepsirhines (all being classified as lemuroids). Relationships among ceboids agree with findings from a previous \(\varepsilon\)-globin study in which fewer outgroup taxa were employed. Rates of molecular evolution were higher in lorisoids than in lemuroids.

Key words: Primates — Strepsirhines — Aye-aye — Lemurs — Phylogeny — \(\varepsilon\)-globin gene — Molecular evolution

Introduction

The order Primates has traditionally been divided into the suborders Prosimii and Anthropoidea (Simpson 1945; Fleagle 1988). The prosimians include the so-called “lower” primates—lemurs, lorisises, and tarsiers. The remaining “higher” primates are classified as anthropoids and divide into platyrrhines (ceboids or New World monkeys) and catarrhines. The catarrhines divide into cercopithecoids (Old World monkeys) and hominoids (apes and humans). An alternative arrangement has been supported by many workers, which divides the order into strepsirhine and haplorhine primates. In this arrangement, the tarsiers are grouped with anthropoids in the suborder Haplorhini, while the Strepsirhini includes the lemuroid and lorisoid primates (Nowak 1991). Some phylogenetic studies have supported the latter classification, with the tarsiers being the sister clade to the anthropoids, rather than being allied with the lemurs and lorisises.

Sister-group relationships within the Strepsirhini have
also been controversial. Traditional studies have supported the phylogenetic classification of African and Asian species as lorisoids and Malagasy species as lemuroids. This classification suggests that prior to the introduction of humans, only a single primate lineage was able to cross the 400-km Mozambique Channel between Africa and Madagascar. However, more recent studies have questioned the traditional view and have suggested that some Malagasy primates are more closely related to the African lorisoids than to other primates of Madagascar.

Extant species of Malagasy strepsirhines are classified into four or five families (Corbet and Hill 1991; Fleagle 1988; Honacki et al. 1982; Martin 1990; Nowak 1991; Wilson and Reeder 1993). The family Cheirogaleidae includes four or five genera of small primates known as dwarf lemurs and mouse lemurs. The remaining species of extant Malagasy primates are included in the families Lemuridae (four or five genera, one of which is sometimes placed in its own family), Indriidae (three genera), and Daubentoniidae (one monotypic genus). The aye-aye (Daubentonia madagascariensis) is morphologically quite distinct from other primate groups, and is placed in its own monotypic family (Daubentoniidae). Daubentonia has been variously allied as a sister group to indriid lemurs (Schwartz and Tattersall 1985), to all lemuroids (Dene et al. 1976, 1980; Yoder 1994), or to the lemuroid-lorisoid clade (Groves 1989; Adkins and Honeycutt 1994).

Some ecological and behavioral similarities have been noted between cheirogaleids and lorisoids (Charles-Dominique and Martin 1970), and cladistic studies of morphological characters (Szalay and Katz 1973; Groves 1974; Schwartz and Tattersall 1985; Dagosto 1986) have questioned the placement of cheirogaleids with the lemuroids. Certain derived morphological characters are shared between cheirogaleids and lorisoids, suggesting that these organisms shared a common ancestor subsequent to their divergence from the lemuroids. If this interpretation is correct, it would indicate that at least two separate lineages of strepsirhines invaded Madagascar. However, Yoder (1994) examined both molecular and morphological characters and concluded that cheirogaleids are more closely related to the Malagasy primates than to any continental primate group.

In primates, the β-globin gene cluster consists of an ε-globin gene, a γ-globin gene (duplicated in anthropoids), the pseudogene Ψγ, and the δ and β globin genes. The ε-globin gene is the 5'-most member of the cluster and arose from a duplication of the original β-globin gene (Goodman et al. 1984). The ε-globin gene, which is active in primate embryos, was used in this study. The structure of the ε-globin gene is shown in Fig. 1.

A number of studies have examined rates of DNA sequence evolution in primates. Koop et al. (1989) found that different primate lineages had considerable variability in the rates of nucleotide substitution in noncoding sequences of the β-globin cluster. Substitution rates appear to have been considerably reduced in the hominoid lineage, while the most rapid molecular evolution has occurred in the early anthropoids and in the lorisoid lineage. This study provides additional data on rates of molecular evolution in noncoding sequences associated with the ε-globin gene in several primate species.

The purposes of this study were (1) to use sequence data from the ε-globin gene and its upstream flanking region to help elucidate the phylogenetic positions of strepsirhine primate taxa in relationship to one another and to the tarsiers and anthropoids and (2) to determine rates of molecular evolution in various primate lineages. Nineteen species of New World monkeys representing all 16 extant genera of the superfamily Ceboidea were included in the study. The relationships among these species have been a matter of some dispute, and Schneider et al. (1993) have previously used ε-globin sequence data to help resolve cebid relationships.

Materials and Methods

Species Examined. The ε-globin gene was sequenced in Daubentonia madagascariensis, Microcebus murinus, Cheirogaleus medius, Propithecus verreauxi, and Nycticebus coucang. These sequences were compared with those previously known from other species of primates (references in Table 1). The domesticated species of rabbit (Oryctolagus cuniculus) and goat (Capra hircus) were used as outgroups. Tissues were obtained from the Duke University Primate Center. DNA was isolated from Cheirogaleus and Microcebus using the procedures of Longmire et al. (1988), with the modification that frozen liver tissue was macerated and used in place of blood. DNA samples of Daubentonia, Propithecus, and Nycticebus were isolated using the procedures of Sambrook et al. (1989).

Sequence Amplification by PCR. A fragment of approximately 1.8 kb, including the ε-globin gene was amplified by means of the polymerase chain reaction (PCR) from Propithecus, Microcebus, and Cheirogaleus using primers A and B of Bailey et al. (1992a). These primers were unsuccessful in amplifying the gene in Daubentonia and Nycticebus; therefore, in these species, a new primer designated as primer E was used in place of B. Primer E (5' AAGAGGGCTCAAGTG-TTGGCCTGGA GCGTGA 3'), when used in conjunction with A, produced a fragment which extends from just upstream of exon 1 to the extreme 5' end of exon 3 (Fig. 1).

Primers 15 (5' GAGTCATACTAAGTCAGGTAAAGACTTCC 3') and 16 (5' AGCACTTTCTTGGCCTGGA 3') were designed