Mitochondrial DNA Phylogeny of the Old-World Monkey Tribe Papionini[1]

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The evolution of the Old World monkey tribe Papionini, composed of macaques, baboons, mandrills, drills, and mangabeys, was examined using mitochondrial DNA (mtDNA) sequence data on the cytochrome oxidase subunit II gene. When analyzed cladistically, these data support a baboon clade of savannah (*Papio*) plus gelada (*Theropithecus*) baboons, as well as a clade containing drill (*Mandrillus*) plus mangabey (*Cercocebus*) genera. This result stands in opposition to most morphological phylogenies, which break up the baboon clade by placing *Papio* and *Mandrillus* as sister taxa and *Theropithecus* as a more distantly related lineage. Analyses of COII gene sequences also suggest that the papionin ancestral stock divided into two lineages, one leading to macaques and the other to the purely African genera. From a molecular evolutionary perspective, the papionin COII gene sequences reveal a pattern of amino acid replacements concentrated in the regions spanning the mitochondrial membrane.

Introduction

Phylogenetic relationships among hominoid primates have been addressed in more detail than those of any other group of mammals, yet a complete resolution of conflicts over the interpretation of morphological and molecular synapomorphies has not been realized. If one accepts a *Homo/Pan* association to the exclusion of *Gorilla* (e.g., see Holmquist et al. 1988; Ruvolo et al. 1991), then presumed morphological synapomorphies between chimpanzees and gorillas, such as thin tooth enamel and postcranial characters associated with knuckle-walking, would have to be interpreted either as parallelisms or as retained plesiomorphic (ancestral) conditions. What is not often realized when problems associated with hominoid systematics are considered is that patterns of morphological and molecular evolution in the Old-World monkeys (superfamily Cercopithecoidea), the sister group to the Hominoidea, are just as complex.

This is especially true of the relatively speciose cercopithecine tribe Papionini, comprising macaques (*Macaca*), savannah (*Papio*), and gelada (Theropithecus) baboons; drills and mandrills (*Mandrillus*); and mangabeys (*Cercocebus* and *Lophocebus*). The papionins radiated during the Late Miocene to Plio-Pleistocene, leaving behind a sizable fossil record abundant in part because many of the fossil species are found in association with early hominid sites (Szalay and Delson 1979, p. 335). The

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genus *Macaca* has a range extending from Morocco to Japan, while the rest of the papionin genera are almost exclusively confined to Africa, with one *Papio* species found in southern Arabia. Thus the tribe inhabits a broad range of ecological zones including savannah, rain forest, desert, mountain, and temperate regions. The tribe is quite heterogeneous in both body size and sexual dimorphism patterns. Papionin locomotor and dietary adaptations show considerable variation from terrestrial omnivory to arboreal frugivory. Together, these features make the Papionini an ideal group for the study of primate evolutionary patterns.

Previous systematic studies of cercopithecoid monkeys by using morphological and molecular data have produced incongruent results, as in the hominoids. The Papionini are considered to be monophyletic, on the basis of morphological features (Groves 1978, 1989, pp. 132–146; Szalay and Delson 1979, pp. 332–381), cytogenetic features (Giusto and Margulis 1981; Dutrillaux et al. 1982), molecular distances (Sarich and Cronin 1976), and molecular characters (Hewett-Emmett et al. 1976; Nelkin et al. 1980). However, intergeneric relationships within the group are not clearly known. According to Delson (1975, p. 210), who investigated morphological characters, "not enough is known of the early history of the dentally typical African Papionini to support more than a 'guestimate' of genus-group relationships." While *T. gelada* is commonly known as the gelada baboon, the widespread perception among morphologists is that it is not the sister taxon to the savannah baboons of the genus *Papio*. Knowledge of intrageneric relationships within the Papionini is similarly tenuous, and this is especially true for the savannah baboons of the genus *Papio*. There is some evidence that the mangabeys are paraphyletic (Cronin and Sarich 1976; Hewett-Emmet et al. 1976; Groves 1978), in which case the *albigena/atterimus* species group remains in the genus *Cercocebus*, while the *galeritus/torquatus* species group is placed in the genus *Lophocebus* (Groves 1978) (this terminology is followed herein). Finally, the interspecies relationships of the genus *Macaca* are poorly understood, and it has even been suggested that the more divergent species may be paraphyletic (Groves 1989, pp. 140–143). Thus, our current hypotheses of both intergeneric and intrageneric papionin relationships are provisional at best.

Without a clear picture of how extant taxa are related, the identification of unambiguous derived traits or apomorphies within particular lineages is not possible. Such traits are essential in assigning fossil forms to particular lineages. A phylogeny is also important in interpreting biomedical studies, which have used papionin monkeys (macaques and baboons) more than species from any other primate group (Williams-Blangero et al. 1990). Molecular data can be useful therefore in interpreting morphological studies of both extant and fossil taxa. The results presented herein provide a phylogenetic framework for representatives of the extant Papionini which may help interpretation of their evolutionary history.

Our approach to generating new hypotheses about papionin phylogeny uses mitochondrial DNA (mtDNA) sequences of the cytochrome oxidase subunit II (COII) gene analyzed in a cladistic framework. The COII sequences can be aligned straightforwardly because they are from a protein-coding region. This is not true of noncoding regions, such as the mtDNA control region, which evolves rapidly and undergoes many small insertions and deletions. Since alignments are actually hypotheses of character homology, using protein-coding regions minimizes the need for initial alignment hypotheses. Furthermore, the COII gene provides a high level of resolution for hominoid phylogeny (Ruvolo et al. 1991). Since the hominoids and papionins probably

radiated during the same range of time, the COII gene seemed appropriate for papionin research.

Material and Methods

Data Collection

The 684-bp mitochondrially encoded COII gene was sequenced in six cercopithecoids: *Macaca mulatta* (rhesus macaque), *Papio anubis* (olive baboon), *P. hamadryas* (sacred baboon), *Theropithecus gelada* (gelada baboon), *Cercocebus galeritus* (golden-bellied mangabey), and *Mandrillus leucophaeus* (drill). *Macaca fascicularis* (crab-eating macaque) and *Cercopithecus aethiops* (green monkey) sequences reported elsewhere (Ruvolo et al. 1991) are used in the present analysis. A previously reported *Macaca fascicularis* COII gene sequence (Ramharack and Deeley 1987) was not used because of the almost certain errors in its generation or reporting (T. R. Disotell, unpublished data, as described in Ruvolo et al. 1991). mtDNA was isolated from organ tissues (heart, liver, and kidney) of *Macaca mulatta, P. anubis, P. hamadryas, T. gelada, Cercocebus galeritus,* and *Mandrillus leucophaeus* by using cesium chloride propidium iodide gradient centrifugation according to the method of Honeycutt et al. (1987) and was resuspended in 10 mM Tris-HCl, pH 8.0/1 mM ethylenediaminetetraacetate (EDTA).

The polymerase chain reaction (PCR) was used to amplify COII genes from mtDNA of the above taxa. Oligonucleotide primers were designed for the aspartic acid (5′-AACCATTTCATAACTTTGTCAA-3′) and lysine (5′-CTCTTAATCTTTAACTTAAAAG-3′) tRNAs flanking the COII gene (Ruvolo et al. 1991); their 3′ ends are located at positions 7552 and 8321 of the human genome (Anderson et al. 1981). Three additional internal primers were used to amplify portions of the gene, depending on the taxon under investigation.

A sample of mtDNA was subjected to 25–30 cycles of amplification in a 50-μl reaction volume with 1.5–2.0 units of *Taq* DNA polymerase (Perkin Elmer—Cetus and Promega) to form double-stranded DNA of either the entire COII gene or two overlapping halves, by using a cycle of 1 min at 95°C for denaturation, 1 min at 51°C–55°C for annealing, and 1 min at 72°C for extension. After agarose-gel verification, half of the remaining sample was reamplified by following the procedure of Allard et al. (1991) for 25–30 cycles. The resulting single-stranded DNA was centrifuged three times with 10 mM Tris-HCl, pH 8.0/0.1 mM EDTA in either Centricon-30 tubes (Amicon) or Ultrafree-MC30 tubes (Millipore), according to the manufacturer's directions. One-quarter to one-half of the resultant ssDNA was sequenced on both strands by the dideoxy method of Sanger et al. (1977), by using the Sequenase kit (United States Biochemical) according to the manufacturer's protocol. The sequence was read using an IBI MacVector gel reader and sequence-analysis software.

Data Analysis

We chose to examine our data by using the character-based phylogenetic analysis techniques of maximum parsimony and maximum likelihood. Unlike phenetic or distance-matrix methods, these cladistic approaches do not result in the loss of information when character data are converted to distances (Penny 1982; Felsenstein 1988). Most important, maximum-parsimony methods partition similarity into putative synapomorphies (shared-derived traits), symplesiomorphies (shared-ancestral traits), and homoplasies (parallelisms), of which only the first are used to define clades. This

avoids the assumption inherent in distance methods—i.e., that overall similarity invariably indicates propinquity of descent. Furthermore, character-based methods are not weakened by the fact that nonadditivity of distances increases as sequences diverge; the latter property violates one of the basic assumptions of distance techniques (Farris 1986). The neighbor-joining method is recommended over other distance techniques because of its efficiency in recovering simulated trees (Sourdis and Nei 1988; Jin and Nei 1990). However, the neighbor-joining algorithm yields a single tree, which is an exact result only when the data are a perfect fit (Saitou and Nei 1987; Studier and Keppler 1988). If the data do not meet the additivity assumption, we cannot be confident about the behavior of the algorithm (Farris 1986; Swofford and Olsen 1990). This algorithm also precludes the examination of multiple topological hypotheses, several of which may closely fit the observed data. For these reasons, we have chosen to analyze the data by using character-based parsimony and likelihood methods.

In the present study, the maximum-parsimony method was implemented using the microcomputer programs PAUP 3.0 (Swofford 1990), HENNIG86 (Farris 1988), and MacClade 3.0 (Maddison and Maddison 1992). Trees were constructed using total base substitutions, as well as comparatively rare transversions only. Because of their infrequency, transversions have a smaller chance of occurring multiple times at any given site than do transitions. Amino acid sequences inferred from COII DNA sequences were also used to construct maximum-parsimony trees. Trees were further examined—and alternative topologies were produced—using MacClade 3.0 (Maddison and Maddison 1992).

Two variants of the parsimony approach that incorporate character weighting and/or character-state-transformation models were also applied. For both, prior knowledge of character-state-transformation frequencies (e.g., the probability of an adenine changing to a guanine) is derived either empirically from the data set under study or from another source, and these frequencies are used in tree building. The first variant is the combinatorial weighting model proposed by Wheeler (1990). Probabilities of all possible nucleotide transformations are calculated on the basis of observed co-occurrences of each nucleotide at each position. In this analysis, each codon position was considered separately, in an attempt to prevent codon position effects from biasing the analysis. This method is less simplistic than one in which transversions and transitions are weighted differently; within transition and transversion classes there may in fact be quite different probabilities of character-state change. In the combinatorial weighting method, observed associations are tabulated on the basis of minimum number of substitutions required at a given position and are normalized, log transformed, and then used as the character-state-transformation cost matrix (Wheeler 1990). As a consequence, if many adenines and guanines, for example, are observed at a particular codon position, then the A-to-G and the G-to-A transformation costs (not necessarily equal) will be weighted less than are transformation costs between less frequently observed co-occurring nucleotides.

The second variant is the weighted parsimony model of Williams and Fitch (1989). It has the advantage over the combinatorial weighting model in that, in determining transformation costs, tree-based observations of character-state change replace the initial character-state observations. In addition, character weightings are calculated for each nucleotide position in the sequence; consequently, hypervariable nucleotide positions are weighted less, and common nucleotide transformations are given less cost. This method requires an input tree from which initial transformation

costs and character weights are determined. On each pass of the algorithm, transformation costs and character weights are recalculated, from which procedure a new tree is generated. This algorithm iteratively continues until a stable topology is reached. Multiple starting topologies were used, all of which made the reasonable assumptions that the *Papio* and *Macaca* genera are monophyletic and that *Cercopithecus* is the outgroup of the papionins. Both linear ($1/n$) and quadratic ($1/n^2$) character-weighting schemes were used, where n is the number of times a position has had an inferred nucleotide substitution. An asymmetric character-state-transformation matrix was allowed, so that the A-to-T and T-to-A frequencies could be different.

An additional tree was produced using the maximum-likelihood approach as implemented by Felsenstein (1990), by using the DNAml program of the PHYLIP 3.3 package. This model contains many of the advantages of character-based techniques, even though it does not partition similarity into a cladistic framework. In the maximum-likelihood approach, one begins with observed data and a particular model of how change occurs. Given the model, the tree with the greatest likelihood (conditional probability) of producing the observed data is generated. For the COII data analysis, the underlying model was determined from the empirically observed base frequencies (F option); the total pool of nucleotides from gene sequences in all taxa is used for calculating frequencies, ignoring codon and nucleotide position. This variant of the model begins with a "star" or "explosion" phylogeny and therefore errs conservatively in estimating unknown frequencies of the ancestral base composition.

Results

Maximum-Parsimony Results

Based on the newly derived DNA sequence data (fig. 1), the maximum-parsimony tree (fig. 2) calculated with unweighted and unordered characters clearly demonstrates a sister-group relationship between *Theropithecus* and *Papio,* on one hand, and between *Mandrillus* and *Cercocebus* on the other. The maximum-parsimony tree is 290 events long when total substitutions are used, revealing 130 phylogenetically informative characters. The *Papio+Theropithecus* clade is supported by 20 unambiguous (minimum number) inferred substitutions, including one transversion event. The *Cercocebus+Mandrillus* clade is supported by 16 unambiguous putative synapomorphies, minimally including three transversion events. In contrast, the morphology-based tree (fig. 3d) hypothesizing neither of these clades is much less parsimonious; when nucleotide sequences are mapped onto it, the total tree length is 335, or 15% longer than the best-fit tree. The branch separating these two clades from the *Macaca* clade is minimally supported by six putative synapomorphies, with at least one transversion. Two slightly less parsimonious trees either group the *Cercocebus+Mandrillus* clade with the *Macaca* clade (fig. 3b, tree length 291) or group the *Papio+Theropithecus* clade with the *Macaca* clade (fig. 3c, tree length 292).

The second analysis applied to the nucleotide data was the combinatorial weighting model (Wheeler 1990). The topology of the shortest tree by this method is identical to that in the unordered unweighted parsimony analysis (fig. 2). The second and third shortest trees by the combinatorial weighting method match the third and second shortest trees by unweighted parsimony. In this case, categorization of differences into only transitions and transversions would have resulted in a loss of information, because of the heterogeneity of probabilities calculated within the two classes. Weighted parsimony analyses (Williams and Fitch 1989) using the conditions described above also

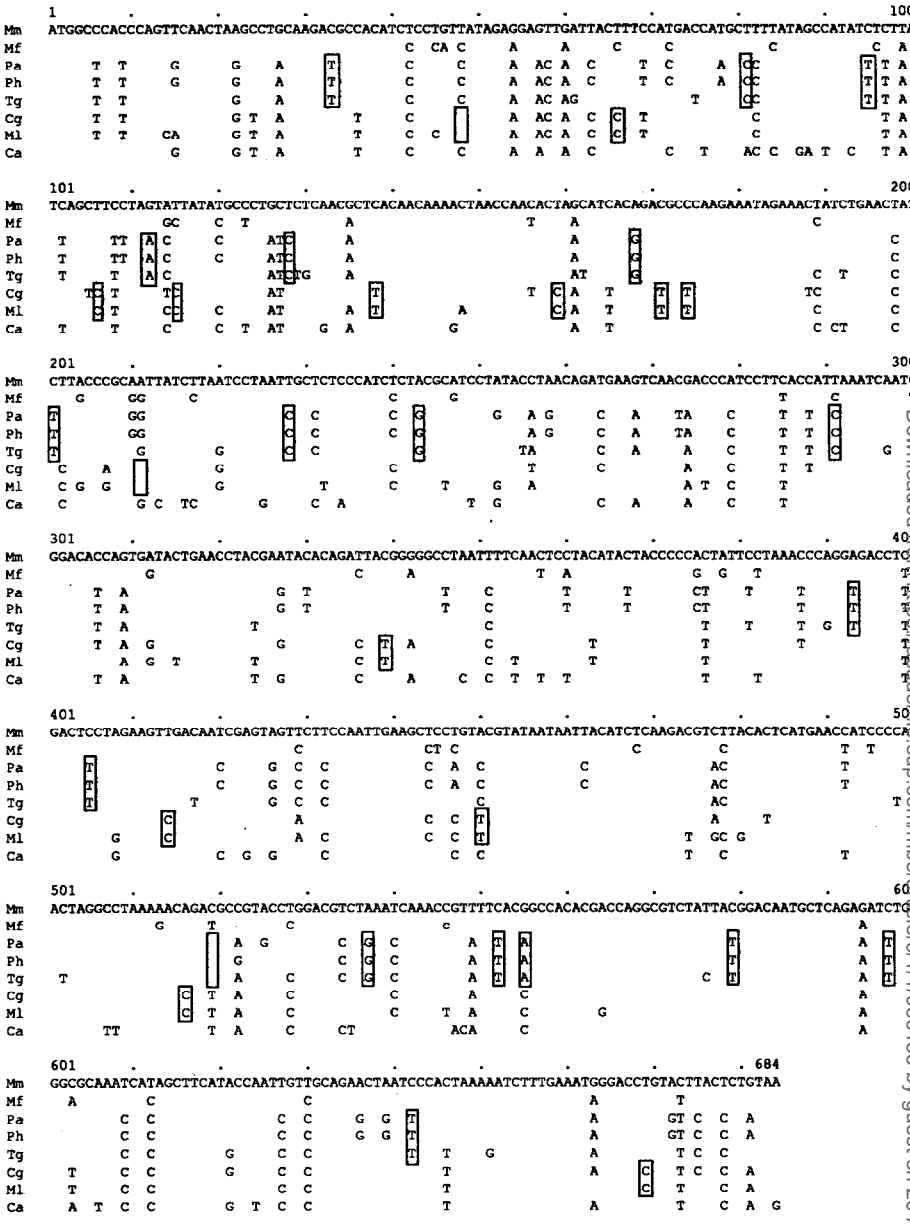


FIG. 1.—Nucleotide sequences of mitochondrial COII gene of eight Old-World monkeys of tribe Papionini. Mm = *Macaca mulatta;* Mf = *M. fascicularis;* Pa = *Papio anubis;* Ph = *P. hamadryas;* Tg = *Theropithecus gelada;* Cg = *Cercocebus galeritus;* Ml = *Mandrillus leucophaeus;* and Ca = *Cercopithecus aethiops.* The *Macaca fascicularis* and *Cercopithecus aethiops* sequences are from Ruvolo et al. (1991). The complete nucleotide sequence is shown only for *Macaca mulatta.* A nucleotide is shown in the other taxa only if it differs from that in *Macaca mulatta.* Boxes represent the unambiguous putative synapomorphies [as determined by the phylogenetic analysis (PAUP 3.0)] which support the most-parsimonious tree for both the *Papio+Theropithecus* and *Mandrillus+Cercocebus* clades.

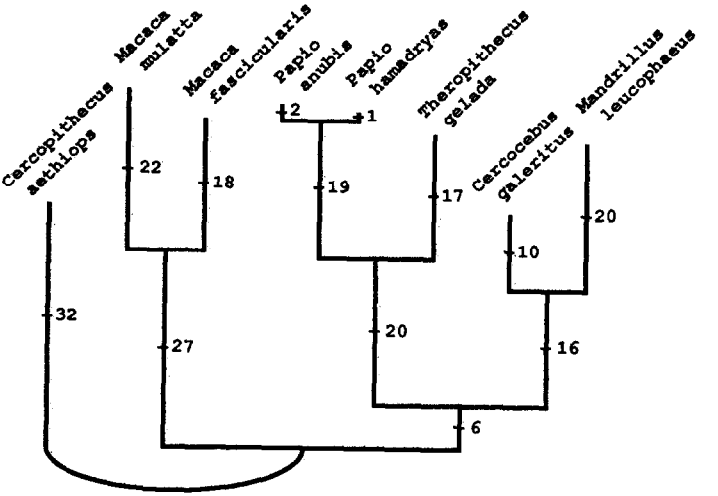
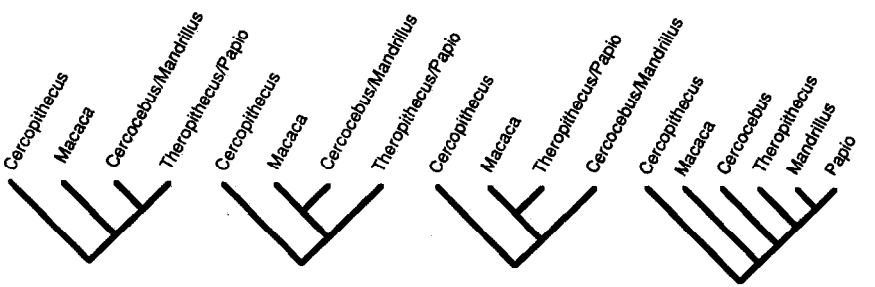


FIG. 2.—Maximum-parsimony tree constructed by using total nucleotide substitutions. The tree is drawn to scale and uses only the unambigous changes, with uniform weighting of unordered characters. The length of the tree is 290. If there were no homoplasy, the sequences would require a tree length of 215.

produced the same most-parsimonious topology (fig. 2), identical to that found by the other methods. Maximum-parsimony analysis of inferred amino acid sequences, using the minimum number of amino acid replacements, yields a family of four nearly equal trees differing, at most, by one step. In all cases, the *Papio+Theropithecus* and *Cercocebus+Mandrillus* clades are present.



	a	b	c	d
Maximum parsimony unweighted and unordered	290	291	292	335
combinatorial model	1039.7	1056.3	1053.0	1157.0
weighted model	1682	1698	1691	1914
Maximum likelihood	-2268.96	-2270.64	-2311.47*	-2381.84*

FIG. 3.—Tree lengths and likelihood values obtained by different phylogenetic analysis techniques. Trees a–c were the three most-parsimonious results, on the basis of analysis of the COII gene. Tree d is the tree derived from morphological criteria (E. Delson, personal communication). An asterisk (*) indicates that the value is significantly worse than the first two values, by the Kishino and Hasegawa (1989) criterion.

Maximum-Likelihood Results

The maximum-likelihood method yields the same best-fit topology as do the above parsimony analyses (fig. 2). On the basis of branch lengths, the *Papio+Theropithecus* and *Cercocebus+Mandrillus* clades are strongly supported, with shared ancestral branches that are significantly positive ($P < 0.01$), when the Kishino and Hasegawa (1989) criterion is applied. When the second and third most-parsimonious trees from the parsimony analyses and the morphology-based tree were tested against the most likely tree, only the second most-parsimonious tree (fig. 3b) was deemed not significantly different statistically (fig. 3).

Summary of Systematic Results

Both maximum-parsimony-based and maximum-likelihood analyses, using COII nucleotide sequence data, yield the same tree topology (fig. 2). The three major clades—macaques, *Papio+Theropithecus,* and *Cercocebus+Mandrillus*—are supported by all of the above methods of phylogenetic analysis. In all methods of calculation, any tree including a *Papio+Mandrillus* clade (an association often hypothesized in the morphological literature) is less parsimonious, whether all bases, only transversions, empirically determined weighting schemes, or inferred amino acid sequence are used. It is also clear from these analyses that the Papionini are monophyletic with respect to *Cercopithecus.* Macaques form a lineage separate from the exclusively African Papionini, and this split is followed by a split into the mangabeys and mandrills, on the one hand, and the baboons, including the gelada, on the other. This sequence of divergences at the base of the papionin tree is only slightly favored over the other two alternatives (fig. 3b and c).

Molecular Results

Among the papionins, the first 41% of the inferred amino acid sequence contains 88% of the observed variable residues among the Old-World monkey species studied here (fig. 4). In terms of the anatomy of the molecule (Capaldi et al. 1983), the cytoplasmic portions (residues 1–26 and 83–227) have 44% of the variable residues, the two alpha helices traversing the membrane (residues 27–48 and 63–82) have 48%, and the internal mitochondrial portion (residues 49–62) has 7%. The expected proportions (based on lengths of these regions) are 75%, 19%, and 6%, respectively.

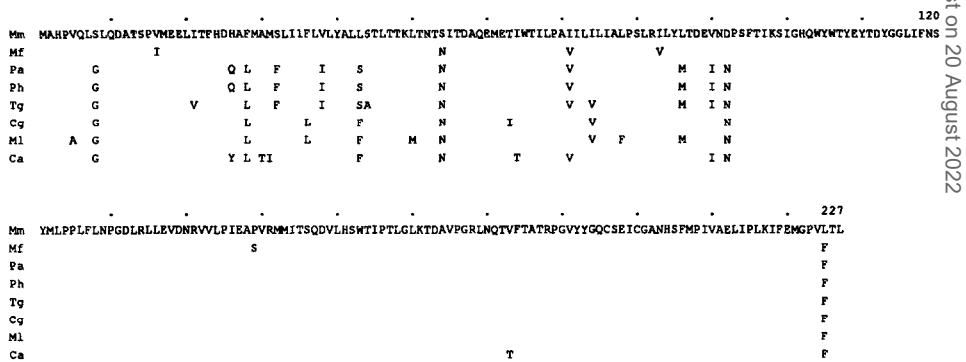


FIG. 4.—Inferred amino acid sequences of mtDNA COII gene of eight Old-World monkeys of tribe Papionini. Abbreviations are as in the legend to fig. 1.

Therefore the membrane-spanning alpha helices show proportionately greater amino acid differences, while cytoplasmic portions show fewer than do other parts of the molecule. This is similar to the pattern noted by Irwin et al. (1991) for cytochrome *b*, in which a higher frequency of amino acid replacements occur in the molecule's transmembrane region, thought to be composed of alpha helices (Capaldi et al. 1983). Most of the replacements in the COII transmembrane region are among leucine, isoleucine, phenylalanine, and valine, all of which are hydrophobic residues. The paucity of replacements in the cytoplasmic carboxyl half of the protein may be due to functional constraints associated with copper-binding sites and with sites involved in binding to cytochrome *c*. In contrast, the overall pattern of nucleotide differences, measured as the average of pairwise differences between the outgroup *Cercopithecus* and the other species, shows an approximately even distribution throughout these regions.

Discussion

From a molecular phylogenetic perspective, two conclusions can be drawn from analyses of the COII sequence data. First, *Theropithecus gelada* is most closely related to the genus *Papio* (fig. 2). Second, *Mandrillus* is most closely related to the genus *Cercocebus*. Both of these results are in accordance with other molecular-based studies and contradict the proposed phylogeny (fig. 3d) based on morphological features.

The relationship of *Theropithecus* to other Papionini has been the subject of several types of molecular studies, including protein electrophoresis (Barnicot and Wade 1970), immunodiffusion (Baba et al. 1976; Dene et al. 1976), microcomplement fixation (Sarich and Cronin 1976), DNA-DNA hybridization (Benveniste and Todaro 1976; Gillespie 1977), amino acid sequencing (Hewett-Emmett et al. 1976), and nuclear RNA restriction mapping (Nelkin et al. 1980). These molecular-based analyses all place *Theropithecus* as the sister taxon of *Papio*. This congruence of seven independent molecular data sources, rather than just the congruence of different methods of analyzing the same data, supports this phylogenetic hypothesis. In contrast, morphologists have not generally accepted this grouping (Jolly 1966, 1970; Delson 1975; Szalay and Delson 1979, pp. 332–382; Strasser and Delson 1987), except when it is analyzed in light of molecular studies (Cronin and Meikle 1979; Groves 1989, pp. 132–146).

More interesting is the apparent close relationship of *Cercocebus* and *Mandrillus*. Chromosomal banding patterns reveal that these two taxa share a rather complex reorganization (Dutrillaux et al. 1982; Stanyon et al. 1988) which is unlikely to have evolved twice. Immunodiffusion data are consistent with these clades being more closely related to each other than either is to the baboon clade (Baba et al. 1976; Dene et al. 1976). Amino acid sequence analysis of alpha-globin chains reveals at least three forms in *Cercocebus* and two in *Mandrillus,* while only one is found in other Old-World monkeys (Hewett-Emmett et al. 1976). Parsimony analysis reveals that some forms of the *Cercocebus* and *Mandrillus* alpha chains clearly form sister-group relationships. Morphologically, it is perhaps significant that some authors (Hill 1974, pp. 13; Groves 1978) have remarked that both genera display an almost identical pattern of female perineal swelling during estrus, a pattern distinct from that shown in the other Papionini.

The molecular data show separate origins for *Mandrillus* and *Papio*. One can understand, however, why morphologists have often classified them together. Morphologically, *Mandrillus* and *Papio* display a strong phenetic similarity, as evidenced

by their long faces, deep maxillary and mandibular fossae, mandibular shape, and body proportions. Szalay and Delson (1979, p. 336) argue that these forms are congeneric and that savannah baboons should be assigned to the subgenus *Chaeropithecus,* with mandrills and drills left in the subgenus *Papio.* However, few if any clear synapomorphies have been described which link these genera to the exclusion of others. A cladistic study by Strasser and Delson (1987) groups *Papio* with *Mandrillus* to the exclusion of *Theropithecus* based on a single character, "substrate preference," found to be highly homoplasic when mapped onto their proposed tree (T. R. Disotell, unpublished analysis). The similarity between *Mandrillus* and *Papio* is heightened when the uniquely derived (autapomorphic) features of *Theropithecus* morphology are considered. In several morphometric and behavioral characters, *Papio* displays features intermediate in relative size between those of *Mandrillus* and those of *Theropithecus,* and these clines are generally taken to indicate the derived condition of *Papio* and *Mandrillus* with respect to *Theropithecus.* Alternatively, the clines could equally be viewed either as evidence for a *Papio+Theropithecus* clade or, as suggested by Jolly (1970), as functional adaptations that do not necessarily have any phylogenetic significance.

At the base of the most parsimonious tree, macaques diverge first away from the exclusively African clades. However, the COII data do not strongly rule out other alternatives. Two possibilities exist. First, the common stem of the papionins may be very short because of a rather broad basal radiation of these monkeys sometime during the mid to late Miocene, making it difficult if not impossible to differentiate fully the individual lineages from their common background. Second, the present nucleotide sequences may be of insufficient length and/or of too few taxa to resolve confidently the basal trichotomy.

The determination of which similarities are sympleisiomorphic and therefore of no phylogenetic value is very difficult within the Papionini, given the current state of morphological interpretation. A parallel between the evolution of the baboons and mandrills and that of the African hominoids is evident in the difficulty in interpreting morphological similarities of living taxa, given the known pattern of genetic differences. By establishing a well-supported phylogeny of the living taxa, the morphological systems may be reevaluated, and polarities and patterns of change not previously hypothesized might be considered. Additional morphological features—including nonskeletal features—of these living taxa also should be evaluated.

Sequence Availability

The newly reported COII gene sequences for the six species presented here have been deposited in GenBank under accession numbers M74004–M74009.

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