Nuclear Gene Trees and the Phylogenetic Relationships of the Mangabeys (Primates: Papionini)

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Phylogenetic relationships of mangabeys within the Old World monkey tribe Papionini are inferred from analyses of nuclear DNA sequences from five unlinked loci. The following conclusions are strongly supported, based on congruence among trees derived for the five separate gene regions: (1) mangabeys are polyphyletic within the Papionini; (2) *Cercocebus* is the sister taxon to the genus *Mandrillus*; and (3) *Lophocebus* belongs to a clade with *Papio* and *Theropithecus,* with *Papio* as its most likely sister taxon. Morphologically based phylogenies positing mangabey monophyly were evaluated by mapping the sequences for each locus on these trees. The data seem to fit these trees poorly in both maximum-parsimony and likelihood analyses. Incongruence among nuclear gene trees occurred in the interrelationships among *Lophocebus, Papio,* and *Theropithecus*. Several factors that may account for this incongruence are discussed, including sampling error, random lineage sorting, and introgression.

Introduction

The Old World monkey tribe Papionini includes baboons (*Papio*), geladas (*Theropithecus*), drills and mandrills (*Mandrillus*), and the mangabeys (*Cercocebus* and *Lophocebus*), all largely distributed in Africa, as well as the macaques (*Macaca*), predominantly found in Asia. As a whole, this tribe represents the most extensively studied group of Old World monkeys with regard to anatomy, ecology, behavior, and genetics, which makes them an ideal group for comparative biological studies. Despite extensive study, the phylogenetic relationships of this group at both the species and genus levels remain uncertain.

Within the papionins, significant phylogenetic controversy surrounds evolutionary relationships among mangabeys. Mangabeys comprise four species (Schwarz 1928; Napier 1981) of medium- to large-bodied arboreal to semiterrestrial monkeys distributed across the African equatorial belt from east to west. Two species groups, the *torquatus-galeritus* and the *albigena-aterrimus* groups, have commonly been recognized based on a number of morphological differences in the skull and ecological differences in substrate preference (Schwarz 1928; Napier 1981; Napier and Napier 1985). Traditional phylogenies based on morphology have supported mangabey monophyly (see fig. 1*a* and *b*), due to their similarity in size and close overall phenetic resemblance in external appearance, including moderate prognathism and the presence of deep suborbital maxillary fossae. Mangabey phenotypic uniformity is particularly pronounced when mangabeys are contrasted with other genera of African papionins (*Papio, Theropithecus,* and *Mandrillus*), which are considerably larger in body size, possess longer faces, and are strongly terrestrial.

Molecular phylogenies, in contrast, suggest that mangabeys are polyphyletic within the Papionini (i.e., the two species groups form two unrelated lineages). The genus names *Cercocebus* and *Lophocebus* have been proposed for these two mangabey groups (see Groves 1978). These phylogenies are derived from analyses of immunology, chromosome structure, amino acid sequences, and mtDNA sequences (Cronin and Sarich 1976; Hewett-Emmett, Cook, and Barnicot 1976; Dutrillaux, Fosse, and Chauvier 1979; Disotell 1994; Van der Kuyl et al. 1994). Despite the consistent indication of polyphyly, molecular phylogenies show a considerable amount of disagreement over the exact relationship of each mangabey genus. Perhaps the strongest hypothesis of their relationships, however, comes from analyses of mtDNA sequences from the COII and 12S rRNA genes (Disotell 1994; van der Kuyl et al. 1994), in which *Lophocebus* is proposed to be closely related to *Papio* and *Theropithecus,* whereas *Cercocebus* is found to be the sister taxon to *Mandrillus* (see fig. 1*c* for the mtDNA COII gene tree).

The current research analyzes new data in the form of nuclear DNA sequences from regions of the CD4, interstitial retinol-binding protein (IRBP), testis-specific protein (TSPY), α 1,3 galactosyltransferase (α 1,3 GT) genes, and the ψη-δ-globin intergenic region with the objective of further clarifying the relationships of mangabeys within the papionin tribe. Theory predicts, and empirical studies show, that gene trees can have different evolutionary histories from their species trees (Nei 1987). Because all five gene regions are inferred to fall on different chromosomes in papionins, they will have independently segregated during the evolutionary history of this group, thus forming unlinked loci. Therefore, estimates of species' relationships become stronger when concordance is found between several gene trees derived from unlinked regions (Nei 1987; Pamilo and Nei 1988; Saitou and Nei 1986; Miyamoto and Fitch 1995). Additionally, DNA sequences previously collected for the papionin group consist entirely of mtDNA sequences. While the analysis of mtDNA has certain advantages for the reconstruction of phylogeny, including relatively fast mutation rate, smaller effective population size, and no recombination (see Moore 1995), it
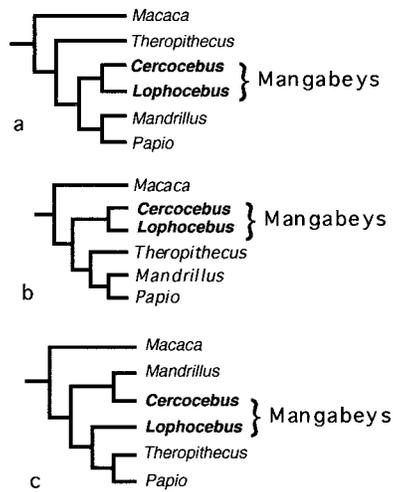
FIG. 1.—Proposed phylogenies of the tribe Papionini. Trees *a* (from Kuhn 1967; Szalay and Delson 1979; Strasser and Delson 1987) and *b* (adapted from Delson and Dean 1993) are based on morphology and posit monophyly of the mangabeys. Tree *c,* based on mtDNA COII sequences (Disotell 1994), supports polyphyly of the mangabeys.

provides only a single estimate of relationships, because it is inherited as a linked unit. An mtDNA tree provides an account of the evolutionary history of the mitochondrial genome, which is not necessarily the same as the evolutionary history of the species. For this reason, we collected sequences from independent regions of the nuclear genome to complement previously derived mitochondrial gene trees.

Materials and Methods

Gene regions sequenced consist largely of noncoding sequences from introns, pseudogenes, or intergenic regions. We sequenced 717 bp of intron 4 and portions of exons 4 and 5 of CD4. The human CD4 gene codes for the CD4 receptor molecule, a recognition protein on T cells for HIV-1. The gene maps to the chromosomal location 12p12–pter (Ansari-Lari et al. 1996). The TSPY gene region consists of 689 bp spanning intron 1 and short regions of exons 1 and 2, which overlap entirely with the published sequences for hominoids (Kim and Takenaka 1996). In humans, the TSPY gene is located on the Y chromosome in the sex-specific region (Ypter–p11.2) (Arnemann et al. 1991; Zhang et al. 1992), and thus it is a male-specific marker. In primates, this region is evolutionarily conserved in the sex-specific region of the Y chromosome in the great apes and is likely to be sex-specific in Old World monkeys as well (Guttenbach, Müller, and Schmid 1992).

The ψη-δ globin intergenic region consists of a 672-bp DNA segment which is part of a larger intergenic segment sequenced by Maeda et al. (1988) in hominoids and several Old and New World monkeys. This subregion contains all of an *Alu* element, a member of a family of repeat elements, and 310 bases beyond it. In humans, the β-globin cluster which contains the ψη-δ globin intergenic region falls on chromosome 11 (approximately region 11p15.4–15.5; Fong et al. 1990).

The 687-bp region of IRBP extends from the very tail end of exon 1 and includes 38% of intron 1, overlapping entirely with the sequences collected by Harada et al. (1995) for a number of platyrrhine primates. In humans, IRBP falls on chromosome 10 (region 10p12). A 514-bp region of the α 1,3 GT gene was also sequenced which maps to chromosome 9 (region 9q33–q34) in humans. This gene is functional in most mammals but is apparently inactive in all catarrhines, Old World monkeys, and apes. This sequence overlaps entirely with the region sequenced by Galili and Swanson (1991) for a number of primate species, including Old World monkeys, apes, and New World monkeys.

We believe that the five regions represent unlinked loci in papionins, as in humans. When the human chromosomal positions of these regions are compared with homologous chromosomal positions in *Macaca fuscata* (Weinberg et al. 1992), they are inferred to fall (except for α 1,3 GT) on the same numbered chromosomes in *M. fuscata.* The α 1,3 GT region, however, most likely maps to chromosome 14 in papionins, as judged by the general homology between human chromosome 9 and chromosome 14 in *M. fuscata.* Because all living papionins have a diploid complement of 42 chromosomes and display overall chromosomal homogeneity (Dutrillaux et al. 1982; Stanyon et al. 1988), these gene regions probably fall on the same chromosome in all other papionin species as well.

DNA Extraction, Amplification, and Sequencing

Samples consisted of whole blood, white blood cells, blood serum, or liver tissue. Genomic DNA was extracted using either a phenol/chloroform protocol (see Maniatis, Fritsch, and Sambrook 1982) or a Pure Gene (Gentra Systems, Inc.) extraction kit. Specimens used in this study and sample sizes are as follows: *Papio hamadryas anubis* (2), *Macaca mulatta* (2), *Theropithecus gelada* (2), *Mandrillus sphinx* (1), *Mandrillus leucophaeus* (1), *Lophocebus albigena albigena* (2), *Lophocebus aterrimus* (2), *Cercocebus torquatus atys* (2), *Cercocebus torquatus lunulatus* (2), *Cercocebus galeritus chrysogaster* (2), *Cercopithecus mitis* (2), and *Cercopithecus aethiops aethiops* (6).

Oligonucleotide primer pair sequences and PCR conditions are given in table 1. Amplifications were done with *Thermus aquaticus* (*Taq*) DNA polymerase in 100-μl total volumes in a 2400 Perkin Elmer Thermocycler and subsequently vacuum-centrifuged to 30–40 μl and loaded into 1.5% agarose gels for electrophoresis. Target bands were excised, and the DNA was purified using the Qiaquick Gel Extraction Kit (Qiagen Inc.). Quantification of the DNA template employed a DyNA QUANT fluorometer (Hoefer Pharmacia Biotech Inc.). For each 10-μl sequencing reaction, 30–45 ng of PCR template was used.

Double-stranded PCR template was directly sequenced using cycle sequencing and employing the dye terminator sequencing chemistry with Amplitaq FS-polymerase (Applied Biosystems Inc.). Forward and reverse strands were sequenced to verify results. Labeled fragments were analyzed on either an ABI 310 or an

Table 1
Oligonucleotide Primer Pairs Used in this Study and their PCR Conditions

Gene Region	Forward/Reverse Primer Sequences	PCR Conditions[a]
CD4.........	CD4e4f1 5′-CAAGAATCTTAAGATAGAAGACTCAG-3′ CD4e5r1 5′-CACCACGATGTCTATTTTGAACTCC-3′	1.5 mM 61°C
TSPY	TSPYforint 5′-GGAAAAGATGGAGCGGAGGCG-3′ TSPYrevint 5′-CAGTGATCAGGGCTGACATCTG-3′	2.0 mM 57°C
ψη-δ globin intergenic...	Psietafor 5′-CTTATCCTGGCTGGATGCGGTGGC-3′ Psietarev 5′-GAATACAACCAGGTCAAGAATACAG-3′	2.5 mM 57°C
IRBP........	IRBPf4 5′-GAGAATGCCAAGGACCGCATTCCTGG-3′ IRBPr1 5′-CCTGGCTTCAGCAGAACAGCTTTGAG-3′	3.0 mM 54°C
α 1,3 GT.....	GAL 559 5′-GTCATATTTTACATCATGGTGGAT-3′ GAL 1121 5′-TATCTGAAGGCAGGCCTATATGAT-3′	2.0 mM 50°C

[a] mM quantities refer to the MgCl$_2$ concentrations in the PCR reactions; centigrade temperatures are for annealing of primers. Thermocycling parameters were 35 cycles of denaturation at 94°C, 45 s; annealing at variable temperatures, 45 s (given above); and extension at 72°C, 45 s.

ABI 377 automated DNA sequencer (Perkin Elmer–Applied Biosystems). DNA sequences were assembled using the Factura (version 1.2.0) and AutoAssembler (version 1.3.0) computer programs (Applied Biosystems Inc.) and were aligned by eye. All sequences have been deposited in GenBank under accession numbers AF057381–AF057448.

Phylogenetic Methods

Phylogenetic methods included maximum parsimony (MP) using PAUP version 3.1 (Swofford 1993) and maximum likelihood (ML) using the DNAML computer program within the PHYLIP Phylogeny Inference Package, version 3.572 (Felsenstein 1993). All MP analyses employed PAUP's branch-and-bound search option. Deletions were coded as gaps and treated as fifth character states in phylogenetic analyses. Multiple-base deletions were treated as single events. Three weighting schemes were used, including uniform, a priori, and a posteriori weighting. The a priori method used a 2:1 transition-transversion (Ts/Tv) ratio, reflecting empirically observed asymmetries in nucleotide substitutions (see table 2; Kimura 1980; Nei 1987; Ruvolo 1997). The a posteriori method (or successive weighting) employed a weighting scheme (Farris 1969) in which characters were reweighted in successive analyses based on their mean consistency indices, derived from an initial branch-and-bound search. Identical results (for both tree number and topology) were usually found using the different weighting schemes; when differences occur, they are noted.

Relative branch support was measured using a bootstrap resampling technique of 500 replications. Bootstrap proportions are provided as a heuristic guide to clade support (Felsenstein 1985b). Decay indices (DIs) were calculated for each branch on a tree using AutoDecay version 2.9.6 (Eriksson 1996). These values represent the difference in tree length between the most-parsimonious tree possessing a particular clade and the most-parsimonious trees at which the clade collapses (Bremer 1994).

In all ML analyses, DNAML options were set as follows: empirical base frequencies option (F) was maintained, transition-transversion ratio option (T) was

Table 2
Comparison of Five Nuclear Gene Regions Sequenced

Gene Region	Chromosomal Location (humans/papionins)[a]	Variable Sites, N (%)	Phylogenetically Informative Sites, N (%)[b]	Intraspecific Variability, Range (mean)[c]	Transitions/Transversions[d]	GC Content (%)[e]
TSPY...................	(Ypter–p11.2)/Y	49 (7.1)	33 (4.8)	0.000–0.003 (0.00043)	1.77	54.40
ψη-δ globin intergenic.....	(11p15.4–15.5)/11	45 (6.7)	22 (3.3)	0.000–0.001 (0.00025)	2.64	39.06
CD4...................	(12p13)/12	35 (4.9)	20 (2.8)	0.000	2.00	57.18
α 1,3 GT...............	(9q33–q34)/14	31 (6.0)	8 (1.5)	0.000	1.31	47.05
IRBP	(10p12)/10	30 (4.4)	12 (1.8)	0.000	2.33	53.32

[a] Human chromosomal locations were obtained either from literature sources or from one of several computer databases: The Human Genome Data Base, The Online Inheritance in Man, GenBank, and The Human Genome Map, NCBI. Locations in papionins were inferred from chromosomal homology studies between humans and *Macaca fuscata* (Weinberg et al. 1992).

[b] A nucleotide position is phylogenetically informative if it favors some trees over others under a maximum-parsimony optimality criterion. In parentheses is the percentage of total nucleotide sites that are phylogenetically informative.

[c] Calculated using PAUP 3.1.1 (Swofford 1993). Mean distances between multiple individuals within a species and/or subspecies are the percentages of bases differing between pairs.

[d] Calculated based on the most-parsimonious tree(s) in PAUP 3.1.1 (Swofford 1993).

[e] Calculated using DNAsis-Mac v2.0 program (Hitachi Software Engineering Co.). Value is the mean GC content averaged over all taxa.
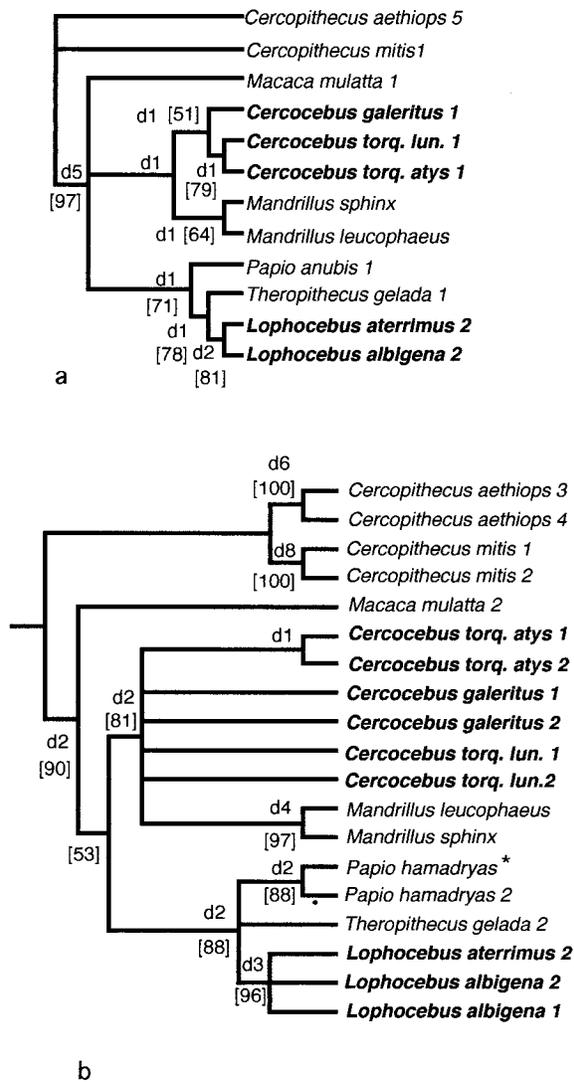
Fig. 2.—*a*, Fifty percent majority-rule tree of the MP trees found for the CD4 sequences. *b*, Fifty percent majority-rule tree of the MP trees found for the TSPY sequences. Mangabey species are in boldface. Bootstrap percentages (in brackets) and decay indices (denoted by "d") are placed along branches. * Sequence is published in Kim and Takenaka (1996).

maintained at its default setting of 2:1, and the jumble option (J) was set to randomize the input order of taxa with ten replicates.

Results
CD4

For CD4, MP analyses yielded three most-parsimonious trees, all of which support mangabey polyphyly. A 50% majority-rule consensus tree of the most-parsimonious trees is shown in figure 2*a* (length = 42, retention index [RI] = 0.789, homoplasy index [HI] = 0.286). A *Lophocebus, Papio,* and *Theropithecus* clade is supported by three putative synapomorphies (DI = 1). The *Cercocebus* and *Mandrillus* clade is supported by one inferred synapomorphy (DI = 1). In addition, *Lophocebus* is inferred to be the sister taxon of *Theropithecus* (DI = 1 with two putative synapomorphies).

The ML tree shows the same relationships for each mangabey genus as the consensus tree from the MP analysis. The branch lengths leading to the *Lophocebus-Papio-Theropithecus* clade and the *Cercocebus-Mandrillus* clade are significantly positive ($P < 0.01$) using Felsenstein's (1993) criteria, which he notes are only approximate.

TSPY

For TSPY, MP analyses yielded six most-parsimonious trees, all positing mangabey polyphyly. The 50% majority-rule consensus of these trees is given in figure 2*b* (length = 51, RI = 0.959, HI = 0.059). *Lophocebus* forms a clade with *Papio* and *Theropithecus,* supported by three putative synapomorphies (DI = 2); *Cercocebus* forms a clade with *M. sphinx* and *M. leucophaeus,* supported by two inferred synapomorphies (DI = 2).

Overall, nine mangabeys were sequenced for TSPY: three individuals belonging to the *Lophocebus* group (two from *L. albigena* and one from *L. aterrimus*) and six individuals belonging to the *Cercocebus* group (two individuals each from *C. t. atys, C. t. lunulatus,* and *C. galeritus chrysogaster*). Species belonging to each of the two mangabey genera cluster together, forming distinct generic-level clades. A *Lophocebus* clade is supported by three putative synapomorphies (DI = 3), and a *Cercocebus* clade is supported by one. Within each of the two mangabey clades, little resolution is found regarding mangabey species relationships.

The topology of the ML tree is essentially congruent with that of the MP consensus tree. The *Lophocebus-Papio-Theropithecus* and *Cercocebus-Mandrillus* clades are well supported by branch lengths that are significantly positive ($P < 0.01$). Certain clades are further resolved than in the MP tree: *Papio* and *Theropithecus* form a clade, and the genus *Cercocebus* is found to be polyphyletic with respect to *Mandrillus,* although these clades are not supported based on Felsenstein's (1993) branch length test.

ψη-δ Globin Intergenic Region

Uniform and 2:1 Ts/Tv MP analyses of the ψη-δ globin intergenic region yielded a single most-parsimonious tree (length = 43, RI = 0.966, HI = 0.083; see fig. 3*a*). *Lophocebus* falls within a relatively strongly supported clade including *Papio* and *Theropithecus* (DI = 6). In contrast to the CD4 tree, in which *Lophocebus* is the sister taxon of *Theropithecus,* the ψη-δ globin intergenic tree groups *Lophocebus* with *Papio* (DI = 1). The individuals within the species of *Lophocebus* do not cluster together into species-specific clades. *Cercocebus* clusters with mandrills and drills more weakly (DI = 1), with a trichotomy including a *Mandrillus* clade (DI = 2) and *C. galeritus*– (DI = 3), and *C. torquatus*–specific (DI = 2) clades. The tree found with succesive weighting differs slightly, with *Cercocebus* and *Mandrillus* unresolved at the base of the papionin tree.

The ML tree is similar to the MP tree, except that mangabeys of the genus *Cercocebus* appear to be polyphyletic, with *C. galeritus* falling outside a *C. torqua-*
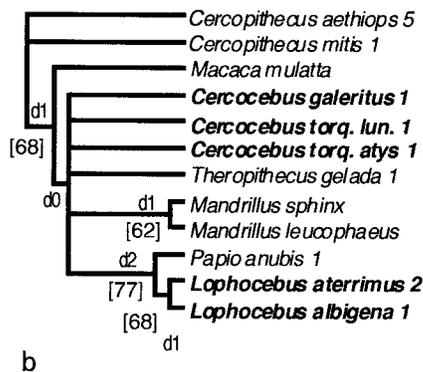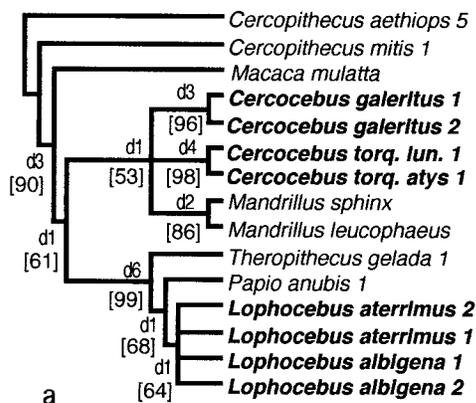
FIG. 4.—Fifty percent majority-rule tree of the MP trees found for the IRBP nuclear gene region. Decay indices (denoted by ''d'') and bootstrap percentages (in brackets) are placed along branches.

FIG. 3.—*a,* The single MP tree found for the $\psi\eta$-δ globin intergenic region. *b,* Fifty percent majority-rule consensus tree of the MP trees found for the α 1,3 GT sequences. Bootstrap percentages (in brackets) and decay indices (denoted by ''d'') are placed along branches. The sequence of *Macaca mulatta* in the $\psi\eta$-δ globin integenic tree is from Maeda et al. (1988), and that in the α 1,3 GT tree is from Galili and Swanson (1991).

tus–Mandrillus clade. However, the branch lengths separating these three groups are not significantly positive, and the species clades of *Cercocebus* alternate positions with each other when multiple analyses are done (with DNAML's jumble (J) option set, and when different *Cercopithecus* species are used as outgroups).

α 1,3 Galactosyltransferase

For α 1,3 galactosyltransferase, uniform and 2:1 Ts/Tv MP analyses yielded five most parsimonious trees. The 50% majority-rule consensus of these five trees is shown in figure 3*b* (length = 33, RI = 0.846, HI = 0.167). Successive weighting yields three trees with a consensus that is identical. The tree shows relatively poor resolution of the relationships among African papionin genera when compared with trees derived from the TSPY, IRBP, CD4, and psi eta intergenic DNA regions. *Papio* and *Lophocebus* are the only two genera that form a clade (DI = 1). This is undoubtedly due to the small number of phylogenetically informative sites within the α 1,3 GT sequences (1.5%) compared with the other gene regions sequenced (which range from 1.8% to 4.6%; see table 2).
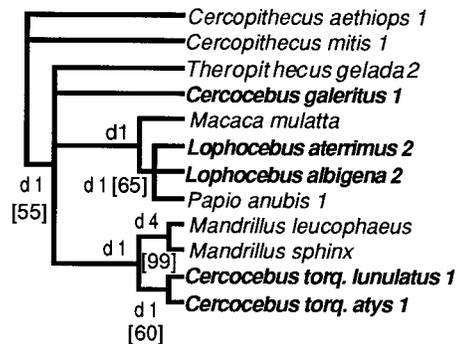
The ML tree is more resolved than the 50% majority-rule MP tree, although many of its internodal branches are not significantly positive. Among the few significantly positive branches is the one leading to a clade containing *Papio* and *Lophocebus* ($P < 0.01$).

IRBP

For IRBP, MP analysis with uniform and successive weighting each yielded two most-parsimonious trees. A consensus of the two trees is shown in figure 4 (length = 30, RI = 0.762, HI = 0.294). Within the papionins, two separate clades are found which have equivalent levels of support (DI = 1). One clade is composed of *Macaca, Lophocebus,* and *Papio*; the other clade includes *Cercocebus torquatus* and *Mandrillus.* The positions of *T. gelada* and *Cercocebus galeritus* with respect to these clades are unresolved. The two species of *Lophocebus* form a trichotomous clade with *Papio.* The subspecies of *C. torquatus* form a distinct clade (DI = 1) that is the sister group to a strongly supported *Mandrillus* clade composed of the mandrill and drill (DI = 4).

The 2:1 Ts/Tv weighted analysis yields a single most-parsimonious tree identical to one of the most-parsimonious trees using the other weighting schemes. *Cercocebus galeritus* appears as the sister taxon to a *Mandrillus–C. torquatus* clade, and *T. gelada* appears as the sister taxon to a *Macaca* and *Lophocebus-Papio* clade. The ML tree is almost identical to the 2:1 Ts/Tv weighted tree. The different clades to which *Lophocebus* and *Cercocebus* belong are both significantly positive ($P < 0.01$).

Tests of Morphologically Based Phylogenies

Each of the five sequence data sets was ''mapped'' onto two morphological trees of the papionin tribe which appear in the literature (Kuhn 1967; Szalay and Delson 1979; Strasser and Delson 1987 [see fig. 1*a*]; Delson and Dean 1993 [see fig. 1*b*]) and evaluated using PAUP 3.1.1 (Swofford 1993). The morphological trees, both of which postulate mangabey monophyly, are very poorly supported by any of the five sequence data sets (see results for trees a and b in table 3). They range in length from 3 to 14 steps greater than the most-parsimonious trees and require levels of homoplasy that

Table 3
Morphologically Based Trees Evaluated Using MP and ML Statistical Tests[a] After Each of the Five Nuclear Gene Sequences Were "Mapped" onto them

Gene Region	Tree Length	SGMP[b]	Homoplasy Index[c]	Synapomorphies: Mangabey Monophyly	Rejected by Maximum-Parsimony Test?	Rejected by Maximum-Likelihood Test?
CD4[d]	(42)		(0.286)	0		
Tree *a*[e].	51	9	0.459	0	Yes	Yes
Tree *b*	51	9	0.459	0	Yes	Yes
TSPY	(51)		(0.059)	0		
Tree *a*	57	6	0.227	0	No	No
Tree *b*	57	6	0.227	0	No	No
ψη-δ	(43)		(0.083)	0		
Tree *a*	57	14	0.421	0	Yes	Yes
Tree *b*	57	14	0.421	0	Yes	Yes
α 1,3 GT. . .	(33)		(0.167)	0		
Tree *a*	34	3	0.333	0	—	No
Tree *b*	34	3	0.333	0	—	No
IRBP	(30)		(0.294)	0		
Tree *a*	35	5	0.400	0	No	No
Tree *b*	35	5	0.400	0	No	No

[a] Statistical comparisons used the pairwise parsimony method of Templeton (1983) as implemented by Felsenstein (1993) in DNAPARS, and the Kishino-Hasegawa (1989) log-likelihood ratio test implemented in DNAML (Felsenstein 1993).

[b] SGMP = steps greater than the maximum-parsimony tree.

[c] Homoplasy index was obtained using PAUP 3.0 (Swofford 1993) and excluded uninformative characters.

[d] The values in parentheses describe the MP tree for each gene region.

[e] Trees *a* and *b* appear in figure 1.

range from about one-half to four times greater than that required by the most-parsimonious tree. Furthermore, there are no putative synapomorphies found in any of the data sets that support a monophyletic mangabey clade composed of *Cercocebus* and *Lophocebus.*

Two different statistical tests, implemented in DNAPARS and DNAML of PHYLIP (Felsenstein 1985*a*, 1993), were used to compare the best trees obtained from MP and the ML analyses with the two different morphological trees (see table 3). Templeton's (1983) pairwise sequence test is a nonparametric method that compares the number of characters that favor each tree being compared and tests the results against a binomial distribution. The Kishino and Hasegawa (1989) test is a parametric test which uses the mean and variance of the log-likelihood differences between trees, taken across sites, to determine whether the trees are significantly different.

Using these tests (see table 3), the CD4 and ψη-δ globin intergenic molecular trees were both supported as significantly better than the two morphological trees. The tests failed to reject the morphological trees for TSPY, α 1,3 GT, and IRBP.

Discussion

We collected and analyzed sequence data from five unlinked nuclear loci, our rationale being that congru-

ence among gene trees with potentially different histories is important evidence of the actual species tree, an approach advocated by Miyamoto and Fitch (1995) and others. An alternative approach, expressed by Kluge (1989), recommends combining all the data to maximize the explanatory power of the data. However, for DNA sequence data, there exists a considerable body of population genetics theory which explains how and why different genetic loci can have different histories and the significance of the gene tree patterns that emerge for understanding molecular and speciation processes (Pamilo and Nei 1988; Hey 1994; Maddison 1997). To combine mutilocus data would therefore result in losing significant insight regarding such processes. For the nuclear gene trees derived in this study, we note points of incongruence which we believe to be significant for understanding the evolutionary history of the papionins (see below). Therefore, we do not present a combined analysis.

For each gene region, gene trees derived using MP and ML methods are essentially in agreement, especially in their support of a polyphyletic relationship of the mangabeys. Agreement among these methods is expected when sequence divergence is small, as it is in the present study (Tateno, Takezaki, and Nei 1994). Points of disagreement between trees are primarily cases in which the ML trees show greater resolution than the MP trees. This is not surprising, because methods that use explicit models of nucleotide change can extract more information from a given set of sequences. However, when greater resolution is obtained by ML, the branches are usually not highly supported according to the confidence limits test employed in DNAML (Felsenstein 1993).

Study of the congruence among the five nuclear gene trees indicates strong support for mangabey polyphyly. That is, mangabeys of the genus *Lophocebus* are observed in all trees to be more closely related to *Papio* and/or *Theropithecus* than to *Cercocebus,* and all analyses but one (for α 1,3 GT) show at least some *Cercocebus* to be more closely related to *Mandrillus* than to *Lophocebus.* The particular relationships of the genus *Lophocebus* are less clear. In the MP trees, it is placed variously as the sister taxon to *Papio* or *Theropithecus.* Because a *Lophocebus-Papio* clade is the most frequently found clade (in three of the five nuclear gene trees), a sister group relationship between these taxa appears to be favored by the nuclear gene trees. This result, however, is in disagreement with papionin trees derived from mtDNA COII DNA sequences (Disotell 1994) and immunological distances (Cronin and Sarich 1976) in which *Papio* and *Theropithecus* are inferred to be sister taxa.

Statistical Evaluation of Traditional Papionin Trees

Of the five gene regions, both the CD4 and the ψη-δ globin gene regions were able to reject all three alternative phylogenies (see table 3) using both the Kishino-Hasegawa (1989) ML test and the Templeton (1983) parsimony test. Tests of TSPY, α 1,3 GT, and IRBP were not always able to reject the alternative phylogenies. The

tests' failure to reject all three alternative trees for several of the gene regions is in accord with previous theoretical and empirical findings that these tests are fairly conservative (see Felsenstein 1985a; Tateno, Takezaki, and Nei 1994; Fitch, Bugaj-Gaweda, and Emmons 1995). Their failure to reject the alternative trees for several of the gene regions may be related to the overall low levels of nucleotide diversity exhibited by the gene regions leading to unusually large standard deviations.

Gene Tree Incongruence

Two basic points of incongruence were observed among the nuclear gene trees. The first concerns the position of *Lophocebus* with respect to *Papio* and *Theropithecus,* in which alternative pairings are supported. The second incongruency is the anomalous placement of *Macaca* within the African papionins in the IRBP gene tree. Incongruence between gene trees may indicate actual differences between the evolutionary history of genes or may result from sampling error. Sampling error refers to the fact that a sampled stretch of DNA may contain a biased number of homoplasious sites that mislead the estimation of the actual gene tree. The risk of this error is greater when the regions sequenced are relatively short (Saitou and Nei 1986; Cummings, Otto, and Wakeley 1995).

While incongruence may be due to sampling error, it may also indicate real differences in the evolutionary histories of these gene regions. If the homology of the gene regions among taxa is ensured, there are at least two other ways that unlinked gene regions can have gene trees that differ from the species tree. First, differential sorting of ancestral DNA sequences can represent a significant problem when the times between divergences are relatively short, as they may have been in the papionins. Second, reticulation between lineages due to hybridization can lead to incongruence among gene trees.

With regard to lineage sorting, it is important to keep in mind the mtDNA encoded COII tree, positing a *Papio* and *Theropithecus* clade exclusive of *Lophocebus,* an arrangement not seen in the nuclear MP gene trees. Considering this tree along with the nuclear gene trees is important, because the overall pattern observed is such that all alternative pairings of these three genera are supported (as well as a trichotomy in the TSPY MP tree). Such a pattern is predicted by population genetics theory under circumstances when the interval of time between the divergences of three species is relatively short. This is because the DNA lineages sampled from each of these species are more likely to have had an origin predating the divergences for all three species (see Nei 1987; Pamilo and Nei 1988). In such a situation, the gene lineages for the loci will have randomly sorted into all three descendant species lineages, yielding a random tree. When short internodes exist, Moore (1995) predicts that mtDNA gene trees will usually track the species relationships better than nuclear trees because of their faster mutation rate and smaller effective population size. While this may be the case, the level of confidence of the *Papio-Theropithecus* clade in the COII tree, which has a bootstrap confidence level of less than 50%, does not provide strong confidence that this particular relationship is necessarily favored over any of the nuclear trees, all of which indicate higher bootstrap levels for the different clades they support. In this situation, it appears that further DNA sequence data from unlinked loci will be needed to resolve this troublesome part of the papionin tree.

Hybridization between papionin species with subsequent introgression of nuclear alleles (or mtDNA haplotypes) from one population to another is another factor that may lead genera to falsely appear as sister taxa (Ferris et al. 1983). For the papionins, introgression is speculative but could conceivably have occurred at any time in the evolutionary history of these genera, although it may be expected to have occurred early in their evolutionary divergence. It is significant that hybridization has been reported to occur in wild and captive populations of *Papio* and *Theropithecus* (see Dunbar and Dunbar 1974a; Jolly et al. 1997). Furthermore, offspring from backcrosses are viable (Jolly et al. 1997). Because these genera have occupied sympatric ranges in the past, both in east and south Africa, and the ranges of the gelada (*T. gelada*) and the olive baboon (*P. hamadryas anubis*) overlap today in the Ethiopian Highlands (Dunbar and Dunbar 1974b; Mori and Belay 1990; Jolly et al. 1997), it is possible that free hybridization has occurred between these two genera periodically during their evolutionary histories (Jolly et al. 1997). Thus, the COII gene tree showing *Theropithecus* and *Papio* as sister taxa, in conflict with the majority of the nuclear gene trees, could perhaps be the result of mtDNA introgression between them. If this is the case, we would expect to find this sister group relationship supported by other mtDNA gene trees, since the mitochondrial genome is inherited without recombination. The 12S rRNA tree for papionins (van der Kuyl et al. 1994) does not allow us to test this prediction, because a sequence for *T. gelada* was not reported by these authors.

The Evolution of Morphological Characters in the Papionins

The strong support for mangabey polyphyly found here is in disagreement with traditional phylogenies of the mangabeys based on morphological evidence for monophyly. The overall close morphological resemblance between the species belonging to the two separate lineages of mangabeys is therefore quite intriguing. In contrast to the other papionin genera, both mangabey genera share moderately prognathic faces, deep suborbital maxillary fossae, long tails, a medium to large body size, and an arboreal to semiterrestrially adapted postcranium. At least two interpretations of the evolution of these features are possible under the present view of their relationships. Either they evolved in parallel in the two separate mangabey lineages or they are retained characters from the common ancestor of the African papionins.

Furthermore, the finding of mangabey polyphyly has been pivotal in changing our understanding of the relationships of the long-faced, terrestrially adapted pa-

pionins, particularly *Papio* and *Mandrillus,* which are now shown to belong to two unrelated lineages. The long faces shared by these genera (traditionally assumed to be shared derived features) apparently either evolved independently in these two genera or are retained from their common African papionin ancestor. This first interpretation would seem to be supported by the general allometric trend exhibited by the large-bodied papionins, in which disproportionate lengthening of the face is correlated with increasing body size (see Freedman 1962; Jolly 1970). The second interpretation, that long faces may be primitive for all African papionins, however, has been suggested by Groves (1978) and, more recently, by Kingdon (1997). According to this view, the development of the suborbital maxillary fossae in mangabeys is interpreted as a result of independent shortening of the faces in these genera. Another finding of the current study is that *Theropithecus* may not be the sister taxon to *Papio.* This may indicate that the features shared by these genera—terrestrially adapted postcrania, large body size, and long (although differently shaped) faces—may also be independent acquisitions. Sorting out the polarity of change of these various features within the papionins will require futher detailed morphological studies, but will be aided by the findings of molecular systematics.

Acknowledgments

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