

Mitochondrial evidence for the origin of hamadryas baboons

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Abstract

Baboons (Mammalia: Primates, *Papio*) are found primarily on the continent of Africa, but the range of hamadryas baboons (*Papio hamadryas*) extends to the Arabian Peninsula, and the origin of Arabian populations is unclear. To estimate the timing of the divergence between Arabian and African hamadryas populations we analyzed mitochondrial DNA (mtDNA) sequences from individuals of Arabian and African origin, and from representatives of the other major baboon taxa. The oldest hamadryas mitochondrial lineages in the Arabian Peninsula form an ancient trichotomy with the two major African lineages. This suggests that Arabia was colonized by hamadryas very soon after the appearance of the distinctive hamadryas phenotype, both events perhaps coinciding with a mid-Pleistocene stage of dry climate and low sea-level. The most closely related Arabian and African mtDNA haplotypes coalesce at approximately 35 ka, suggesting that no gene flow between African and Arabian baboons has occurred since the end of the last ice age, when a land bridge at the southern sill of the Red Sea was submerged. The mitochondrial paraphyly of Ethiopian hamadryas and anubis (*P. anubis*) baboons suggests an extensive and complex history of sex-specific introgression.

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1. Introduction

Five living species are commonly distinguished in the baboon genus *Papio*, although many more morphologically distinct allotaxa are recognizable (Groves, 2001; Jolly, 1993). Of these, the hamadryas baboon (*Papio hamadryas*) is distinguished by a suite of behavioral and morphological autapomorphies (Jolly, 2001; Kummer, 1968). In spite of this distinctiveness, hamadryas baboons hybridize readily with anubis baboons (*Papio anubis*) where their ranges meet in Ethiopia, and surveys

in the hybrid zone document local genetic introgression. (Nagel, 1973; Newman, 1997; Phillips-Conroy et al., 1991; Shotake et al., 1977; Woolley-Barker, 1999). Previous molecular-genetic studies have concluded that hamadryas baboons comprise a sister group of a clade consisting of anubis (*Papio anubis*) and yellow (*Papio cynocephalus*) baboons (Wildman, 2000; Williams-Blangero et al., 1990), and that Ethiopian anubis and hamadryas baboons have been separate lineages for approximately 350 ka. (Nakamura et al., 1983; Newman et al., 2003; Shotake et al., 1977; Wildman, 2000).

The hamadryas is the only *Papio* baboon whose range extends beyond the African continent, being found both in the Horn of Africa and across the Red

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Sea in the Arabian countries of the Republic of Yemen and the Kingdom of Saudi Arabia (Fig. 1). One possibility is that this distribution, like that of other vertebrates such as the ostrich (Robinson and Mathee, 1999) consists of remnants of a natural range known to have extended from Africa to Arabia via Sinai and the Levant. *Papio* is, however, apparently absent from the well-known Pleistocene fauna of the Eastern Mediterranean region (Tchernov, 1998). Hamadryas were, of course, well-known in Egypt from dynastic to Ptolemaic times (Osborn, 1998), but there is no evidence that they were native to the country, any more than were the many other sub-Saharan mammals depicted in paintings of the period, which include anubis baboons, vervet monkeys (*Chlorocebus aethiops*), and patas monkeys (*Erythrocebus patas*). If numerous hamadryas baboons were shipped up the Red Sea from sub-Saharan Africa by Egyptians of the dynastic period, it is possible that, as some authors have speculated (Kummer, 1981, 1995), Arabian hamadryas are descended entirely from founders imported deliberately or accidentally from Africa.

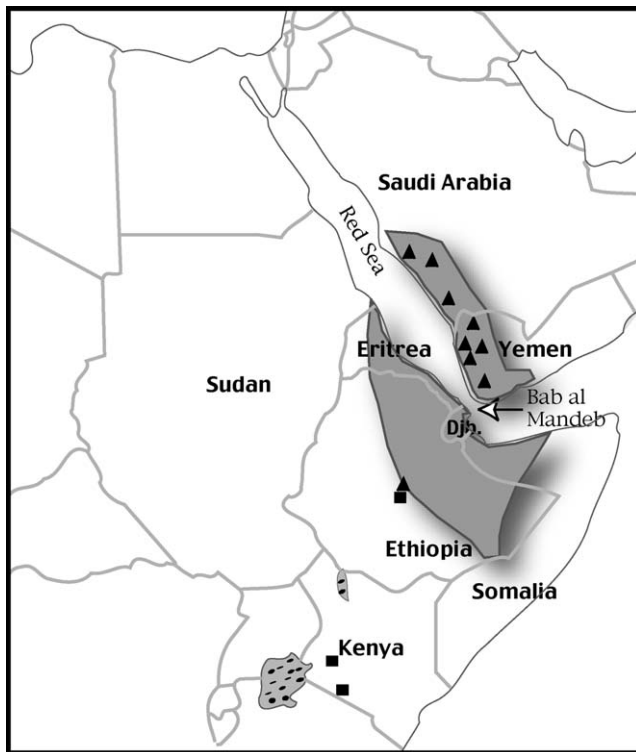


Fig. 1. A map of the Arabian Peninsula and northeastern Africa showing localities for hamadryas and anubis baboons. *Papio hamadryas* (hamadryas baboon) is found only in northeast Africa and the Arabian Peninsula. The estimated current geographic range of hamadryas baboons is shaded. Triangles represent provenienced sampling localities of *P. hamadryas* individuals, and squares represent provenienced *P. anubis* (anubis or olive baboon) samples. Detailed locality names, geographic coordinates, and GenBank Accession numbers for all of the study samples (Arabian and African) are given in Table 1. Djib. = Djibouti. Stippled, shaded areas indicate lakes.

An alternative, natural dispersal route was, however, intermittently available during the Pleistocene, via a landbridge at the southern end of the Red Sea. Geomorphological, paleoclimatic, zoogeographic, and paleoanthropological data point to the existence of a direct land connection at this point during glaciations (Clark, 1954; Delaney, 1989; Kingdon, 1991; Lahr, 1996; Rohling, 1994). The seafloor at the Bab al Mandeb lies 137 m below present sea level. Rohling (1994) and Rohling et al. (1998) have shown that sea level was at least 139 m lower during Oxygen-isotopic stage (OIS) 12 (approximately 440 ka), and these data suggest that an isthmus linking Arabia and Africa may also have formed at 340 ka (OIS 10), 130 ka (OIS 6), and 20 ka (OIS 2). Arid intervals at approximately 1.7 million years ago (Mega annum = Ma) and 1.0 Ma (deMenocal, 1995) may correspond to additional, earlier events of low sea level and opportunities for biotic exchange.

In this paper we use evidence from the mitochondrial genome to examine the timing and place of origin of hamadryas baboons, and the relationships between Arabian and African hamadryas populations.

2. Materials and methods

We examined 47 *Papio* baboons, including representatives of all the *Papio* species recognized by Groves (2001). A gelada (*Theropithecus gelada*), a mandrill (*Mandrillus sphinx*), a Red-capped mangabey (*Cercocebus torquatus*) and a Barbary macaque (*Macaca sylvanus*) served as outgroup taxa. Wildman collected *P. hamadryas* samples in Yemen in 1997 under a research plan approved by the Yemeni Center for Research and Science (YCRS). Some samples consisted of tissue, collected from animals shot by farmers, while others consisted of hair collected non-invasively (Valderrama et al., 1999). Samples from Saudi Arabia were derived from the collection of Dr R. Hammond. Ethiopian *P. hamadryas* and *P. anubis* samples were collected as part of the Awash National Park Baboon Research Project (AN-PBRP, co-directed by Jolly and Phillips-Conroy). Additional samples were drawn from the collection of the Molecular Anthropology Laboratory of New York University, the Southwest Foundation for Biomedical Research (Dr. J. Rogers), and Dr. I. DeVore (Harvard University). Sample type, location, provenience, and GenBank Accession numbers are given in Table 1, and sample collection sites are shown in Fig. 1. As shown in Table 1, we prepared DNA from blood, hair, or liver using standard phenol/chloroform DNA extractions and ethanol precipitation (Sambrook et al., 1989), or Qia-Amp tissue and blood kits (Qiagen). Difficult templates (hair) were amplified using a nested PCR procedure (Valderrama et al., 1999). Dilution of the initial PCR product for re-amplification varied from 1:1 to 1:1000.

Table 1
Samples, localities, and Accession numbers

Taxon	Specimen ID	Animal origin	Coordinates	Animal, tissue	Haplotype ^a	GenBank
<i>Papio anubis</i>	BE73142	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A7	AY212086
<i>P. anubis</i>	BE73076	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A8	AY212087
<i>P. anubis</i>	BE73165	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A9	AY212088
<i>P. anubis</i>	BE73005	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A10	AY212089
<i>P. anubis</i>	BE73121	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A11	AY212090
<i>P. anubis</i>	BE73138	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	A12	AY212091
<i>P. anubis</i>	BE93089	Highlands, Ethiopia	Unknown	Addis Ababa (export)	A13	AY212092
<i>P. anubis</i>	BE93090	Highlands, Ethiopia	Unknown	Addis Ababa (export)	A14	AY212093
<i>P. anubis</i>	A2SW76	Kenya	Unknown	Unknown, DNA	A3	AY212095
<i>P. anubis</i>	A1SW331	Kenya	Unknown	Unknown, DNA	A15	AY212094
<i>P. anubis</i>	[SWF]14710	Gilgil, Kenya	0° 35' S, 36° 21' E	SWF, blood	A4	AY212098
<i>P. anubis</i>	[SWF]14722	Gilgil, Kenya	0° 35' S, 36° 21' E	SWF, blood	A5	AY212097
<i>P. anubis</i>	[SWF]14739	Gilgil, Kenya	0° 35' S, 36° 21' E	SWF, blood	A6	AY212099
<i>P. anubis</i>	RC233	Masai Mara, Kenya	1° 49' S, 35° 40' S	Wild, blood	A6	AY212096
<i>P. cynocephalus</i>	1K05mk	Mikumi, Tanzania	7° S, 36° E	Wild, blood	Y4	AY212100
<i>P. cynocephalus</i>	3117mk	Mikumi, Tanzania	7° S, 36° E	Wild, blood	Y5	AY212047
<i>P. cynocephalus</i>	2008mk	Mikumi, Tanzania	7° S, 36° E	Wild, blood	Y6	AY212102
<i>P. hamadryas</i>	DW-37	Jabal Raymah, Yemen	14° 40' N, 43° 26' E	Wild, hair	H14	AY212061
<i>P. hamadryas</i>	DW-104	Jabal Iraf, Yemen	13° 07' N, 44° 15' E	Wild, liver	H15	AY212062
<i>P. hamadryas</i>	DW-55	Taiz, Yemen	13° 35' N, 44° 02' E	Taiz (zoo), hair	H16	AY212063
<i>P. hamadryas</i>	DW-57	Taiz, Yemen	13° 35' N, 44° 02' E	Taiz (zoo), hair	H16	AY212064
<i>P. hamadryas</i>	DW-58	Taiz, Yemen	13° 35' N, 44° 02' E	Taiz (zoo), hair	H17	AY212065
<i>P. hamadryas</i>	DW-106	Sana'a, Yemen	17° N, 44° E	Near Sada (pet), hair	H18	AY212066
<i>P. hamadryas</i>	DW-152	Sana'a, Yemen	15° 23' N, 44° 15' E	Pet, hair	H17	AY212067
<i>P. hamadryas</i>	DW-157	Wadi Mur, Yemen	15° 44' N, 43° 25' E	Wild, hair	H19	AY212068
<i>P. hamadryas</i>	DW-158	Wadi Mur, Yemen	15° 44' N, 43° 25' E	Wild, hair	H20	AY212069
<i>P. hamadryas</i>	AB-21	Abha, Saudi Arabia	18° N, 42° E	Wild, DNA	H17	AY212073
<i>P. hamadryas</i>	AB-31	Abha, Saudi Arabia	18° N, 42° E	Wild, DNA	H17	AY212074
<i>P. hamadryas</i>	BA-01	Baha, Saudi Arabia	20° N, 42° E	Wild, DNA	H17	AY212075
<i>P. hamadryas</i>	BA-23	Baha, Saudi Arabia	20° N, 42° E	Wild, DNA	H17	AY212076
<i>P. hamadryas</i>	TA-03	Taif, Saudi Arabia	21° N, 40° E	Wild, DNA	H17	AY212077
<i>P. hamadryas</i>	TA-23	Taif, Saudi Arabia	21° N, 40° E	Wild, DNA	H17	AY212078
<i>P. hamadryas</i>	BE73222	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H7	AY212079
<i>P. hamadryas</i>	BE73275	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H8	AY212080
<i>P. hamadryas</i>	BE73360	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H9	AY212081
<i>P. hamadryas</i>	BE73357	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H10	AY212082
<i>P. hamadryas</i>	BE73498	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H5	AY212083
<i>P. hamadryas</i>	BE73274	Awash, Ethiopia	8° 54' N, 39° 55' E	Wild, blood	H7	AY212084
<i>P. hamadryas</i>	BE94003	Ethiopia (not Awash)	Unknown	Addis Ababa(zoo), blood	H11	AY212085
<i>P. hamadryas</i>	BE97070	Awash, Ethiopia	9° N, 40° E	Wild, blood	H7	AY212072
<i>P. hamadryas</i>	BE97119	Awash, Ethiopia	9° N, 40° E	Wild, blood	H12	AY212071
<i>P. hamadryas</i>	BE97122	Awash, Ethiopia	9° N, 40° E	Wild, blood	H13	AY212070
<i>P. hamadryas</i>	Arnason et al. (1998)	Africa/Arabia	Unknown	Published	H5	Y18001
<i>P. hamadryas</i>	Hayasaka et al. (1996)	Africa/Arabia	Unknown	Published	H13	D85290
<i>P. papio/P. anubis</i>	PGU031	Sierra Leone	Unknown	Wild	G9	AY212104
<i>P. papio</i>	BD003	Senegal	Unknown	Wild	G9	AY212103
<i>P. ursinus</i>	H677 = TM40952	South Africa	22° 12' S, 29° 23' E	mtDNA	U5	AY212105
<i>Theropithecus gelada</i>	871162	Ethiopia	Unknown	Bronx zoo, blood	T2	AY488130
<i>Cercocebus torquatus</i>	CTOR	Africa	Unknown	Blood	NA	AY488131
<i>Mandrillus sphinx</i>	MAN25	Lope, Gabon	Unknown	Blood	NA	AY488132
<i>Macaca sylvanus</i>	Arnason et al. (2000);	N. Africa	Unknown	Published	NA	NC002764

^a Haplotype system refers to Newman et al. (2003); and is an extension of that system.

We then amplified and sequenced a stretch of the mtDNA genome which we call the Brown region (Brown et al., 1982). It is bracketed by two *Hin* dIII restriction sites (Brown et al., 1982; Hayasaka et al., 1988, 1996). In most baboons, the Brown region comprises 457 bp of the 3' end of the NADH dehydrogenase subunit IV (*ND4*) gene, the tRNA genes for histidine (His), serine (Ser), and leucine (Leu), and 239 of the 5' end of the NADH dehydrogenase subunit V (*ND5*) gene. In the hamadryas baboon reference sequence (Arnason et al., 1998), it begins at position 11,103 and ends at position 11,999, corresponding to positions 11,680 through 12,576 in the human reference sequence (Anderson et al., 1981).

Primer sequences are shown in Table 2. One hundred microliter PCR were conducted using 20 μ l of 10 \times buffer, 2.5 mM MgCl₂, and 200 mM dNTPs (primer concentration varied according to template DNA yield). PCRs were carried out on Perkin–Elmer 2400 thermocyclers with an initial denaturation hold at 96 °C for 2 min; followed by 35 cycles of: denaturing at 94 °C for 30 s; annealing at 56 °C for 30 s; and extension at 72 °C for 1 min. All reactions were completed with a final extension at 72 °C for 7 min.

PCR products were visualized on a 1.5% agarose gel, then excised and purified using Qiagen Gel extraction kits. Purified product was cycle sequenced with either ABI Prism BigDye or dRhodamine terminators using standard manufacturer's protocols. Sequences were obtained with automated (ABI 310 and/or ABI 377) protocols. Both the heavy and light strands of mtDNA were sequenced in all samples.

We considered the possibility that sequences resulted from misamplification of pseudogenes or other nuclear insertions, 'numts' (nuclear insertions of mtDNA) (Bensasson et al., 2001). We checked sequences by performing protein translations, and, in some instances, by cloning PCR products. Additionally, all sequences were checked for frameshifts against the published mitochondrial genomes of papionin primates (Arnason et al., 1998, 2000). All sequences obtained were compared with a sample from *P. ursinus* (U5, Accession No.

AY212105) that was isolated from purified mitochondrial DNA. The fact that all *P. hamadryas* sequences we obtained were less similar to this *P. ursinus* sequence than to the published mtDNA genome of *P. hamadryas* (H5, Accession No. Y18001), increases our confidence that mtDNA rather than numtDNA was indeed sequenced in all individuals analyzed. However, all *Papio* sequences were so similar (>95% pairwise similarity in most cases) that they would be informative even if some were in fact derived from numtDNA, since the nuclear pseudogenes involved would be very recent in origin.

Bases were considered ambiguous until confirmed by bi-directional sequences from multiple products, and sequences were aligned by eye.

We used maximum parsimony (MP), minimum evolution (ME), and maximum likelihood (ML) methods in PAUP* (version 4.0b10, Swofford, 2002) to infer phylogenetic relationships among haplotypes, and measured topological reliability by performing 500 random addition sequence heuristic (TBR) bootstrap replicates, with resampling. Log likelihood ratio tests were performed to test models of nucleotide substitution for ME and ML analyses (Modeltest v3.06, Posada and Crandall, 1998), and the HKY+ Γ model (transition/transversion = 14.3363, α -shape = 0.3414) was chosen for phylogeny inference.

Tests for substitution rate heterogeneity both among individuals and among nucleotide sites were conducted both to determine the reliability of the divergence dates among taxa, and to examine the pattern of molecular evolution of the electron transport chain genes studied. Saturation analyses (Brown et al., 1982; Griffiths, 1997; Irwin et al., 1991) were conducted in order to determine whether excessive homoplasy resulting from multiple hits was likely to cause divergence dates to be underestimated. To test whether natural selection affected the phylogeographic results, we calculated K_a/K_s ratios of nonsynonymous (amino-acid changing) to synonymous (silent changes) nucleotide substitution by the method of Li (1993) as implemented in the program FENS (De Koning et al., 1998). Divergence dates were calculated using pairwise HKY+ Γ distances, calibrated upon a

Table 2
Mitochondrial primers^a

Primer name	Amp/Seq ^b	Genomic location ^c	5' to 3'
FE1	Amp	10,766	TCCACGTAAGCCCATAGCCCTA
FE2	Amp/Seq	11,090	GTAATTGTAGCTTCCCCTCA
F11	Seq	11,425	ACAACGAGGAGCGCTCACACACC
R11	Seq	11,583	ATAGTTAGATTCACAGTCTAACG
R12	Seq	11,740	GTTACTTTTATTTGGAGTTGCAC
RE3	Amp/Seq	12,039	ATAGACCAGGTAATGAATAGT
RE2	Amp/Seq	12,035	ATAGACCAGGTAATGAATAGTGCCA
RE1	Amp/Seq	12,050	GTGAGAATTCTATGATAGACCAGG

^a Primers used for amplification and sequencing. Other primers used are described in Newman et al. (2003).

^b Amp = primer used for initial PCR amplification; Seq = primer used for sequencing.

^c 5' nucleotide position based on *P. hamadryas* mtDNA genome (Arnason et al., 1998).

paleontologically-documented split between both *Papio* and *Theropithecus* dated at c. 4 million years ago (mya) (Delson, 1993; Goodman et al., 1998; Gundling and Hill, 2000). Mismatch distributions were calculated to infer demographic structure within hamadryas baboons (Harpending et al., 1998).

3. Results

The 47 *Papio* baboons carried 31 different mitochondrial haplotypes (Table 1). The 27 hamadryas baboons yielded 15 haplotypes, of which 7 were found only in Arabian baboons, and 8 only in African individuals. The reference *P. hamadryas* (H5, Accession No. Y18001) is identical to that of a hamadryas baboon from the Awash National Park (H5, BE73498; Accession No. AY212083). Additionally, the other previously published hamadryas sequence (H13, Hayasaka et al., 1996; Accession No. D85290) is identical to the sequence obtained for the Ethiopian hamadryas specimen BE97122 (H13, Accession No. AY212070).

Fig. 2 shows the majority rule bootstrap consensus of the MP analysis (500 TBR replicates). The optimal ML tree (100,000 quartet puzzling steps) and the ME tree (neighbor-joining using the previously described HKY model parameters) are slightly more resolved than the MP consensus. We prefer the more conservative approach of presenting only the MP consensus in view of the high pairwise sequence similarity (>97% on average) between individuals. The optimal MP trees ($n = 16$) have a score of 407 steps (all characters unordered with equal weights), the optimal ML tree has score of $-\ln L = 3080.6$, and the optimal ME tree has score of 0.70. The MP consensus tree is not significantly different (KH test, $P = 0.5$) from the optimal ML tree. No statistically significant differences (likelihood ratio test) were found for the tree (Fig. 2) whether the molecular clock was enforced ($-\ln L = 3110.9$) or not ($-\ln L = 3086.6$). Therefore, we were able to use the HKY distances for dating divergences between haplotypes.

As in a previous analysis of the same taxa (Newman et al., 2003), which was based on different individuals, the most divergent sequence was found in *P. ursinus*, and the haplotype from *P. papio* is the sister to all the remaining non-*ursinus* haplotypes. All the hamadryas, anubis and yellow baboon (*P. cynocephalus*) sequences from Arabia, Ethiopia, Kenya, and Tanzania form a well-supported clade. Within it, four similarly well-supported clades can be distinguished. These do not correspond either to the recognized taxa or to the Arabia/Africa distinction. Clade I includes all haplotypes from anubis and yellow baboons of Kenyan and Tanzanian origin. Clade IIA consists only of two haplotypes from Arabian hamadryas. Clade IIB includes hamadryas haplotypes from both Arabia and Ethiopia.

Clade IIC includes haplotypes from Ethiopian hamadryas and anubis baboons. All but two of them fall into two clear subclades, which include, respectively, haplotypes from hamadryas and anubis baboons originating from the Awash National Park. The other two haplotypes/individuals (A13, BE93089; Accession No. AY212092 and A14, BE93090; Accession No. AY212093) were both captured in the Ethiopian highlands, west of Addis Ababa. Parsimony analysis shows that they belong within Clade II, rather than with anubis baboons from Kenya, since placing them in Clade I lengthens the tree by six steps. Within Clade II, however, they can be situated equally parsimoniously within either of the main clusters, or, with only one extra step, as another separate lineage within Clade II.

Haplotypes from Arabian hamadryas baboons do not form a single clade in the bootstrap consensus tree, and if Arabian monophyly is constrained in parsimony analysis, the resulting trees are longer by 4 steps.

The maximum pairwise ML corrected sequence divergence between individuals within the genus *Papio* is 7.4%. The highest observed transition to transversion ratio among pairs of *Papio* individuals is 20:1. The maximum pairwise distance between hamadryas individuals is 1.5%, and this divergence is between an Arabian (H19, DW-157, Accession No. AY212068) and an Ethiopian (H9, BE73360, Accession No. AY212081) individual. Saturation analysis using the techniques of Hassanin and Douzery (1999) reveal no evidence of a significant number of multiple hits at individual nucleotide positions. None of the comparisons between the protein coding regions of *ND4* and *ND5* (either combined or treated separately) exhibits an increase in nonsynonymous substitution as would be indicated by a Ka/Ks ratio higher than 1.0 (data not shown) which could suggest positive Darwinian selection acting upon these molecules (Messier and Stewart, 1997). These observations, taken together with the statistical support for the molecular clock, suggest that the distance data are appropriate for estimating divergence dates. Pairwise HKY+ Γ distances were used to correct for site-specific rate heterogeneity when inferring divergence dates between clades.

Pairwise comparisons using the HKY+ Γ distances yielded the estimated divergence dates shown in Fig. 2. The distances between Arabian and African haplotypes ranged from 0.0011 to 0.0153, yielding estimated Afro-Arabian divergence dates from a minimum of 37 ka to a maximum of 511 ka. This 37 ka minimum coalescent date neglects the cladistic structure in Fig. 2. Alternatively, the phylogenetic tree suggests the minimum divergence between African and Arabian hamadryas baboons was 74 ka. If the uncorrected pairwise distances are plotted in a histogram, the resulting mismatch distribution reveals two peaks (Fig. 3). These peaks correspond to modes of 0.1 and 0.9%, and thus may

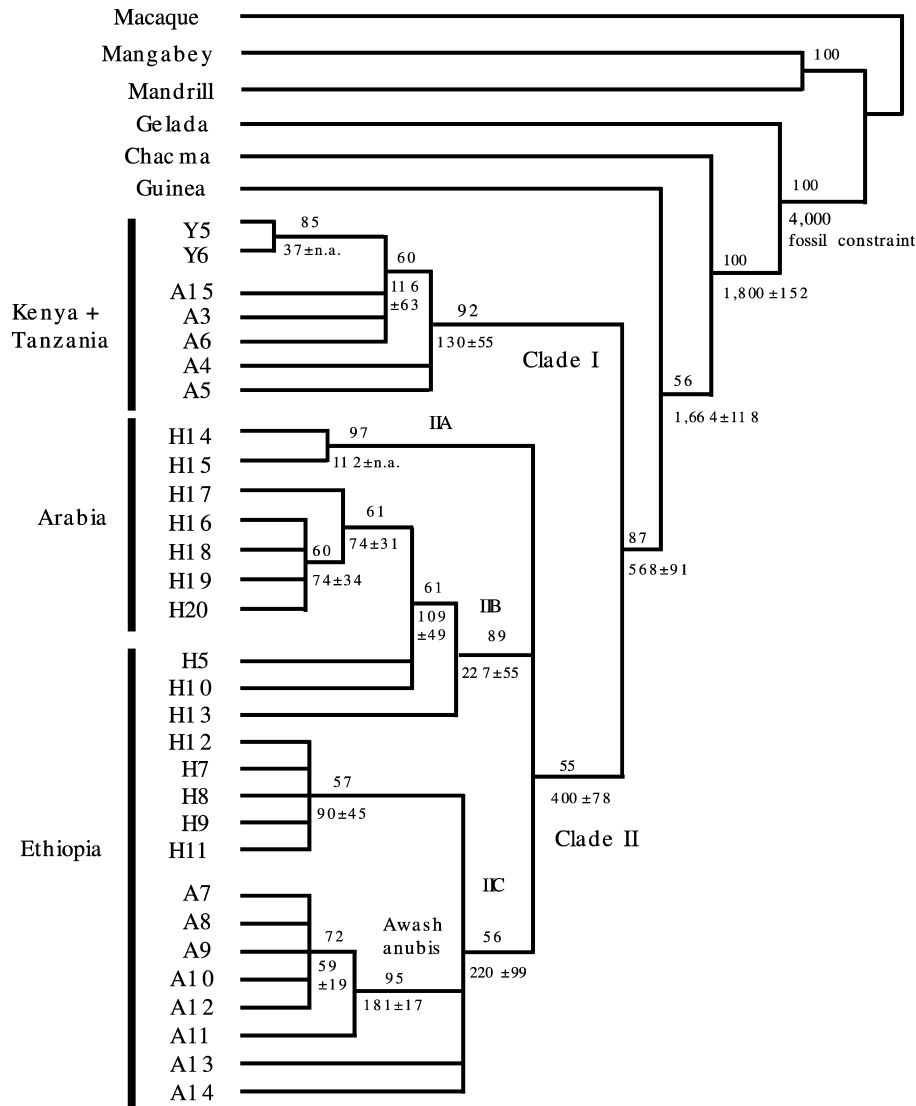


Fig. 2. Mitochondrial phylogeny and divergence dates within *Papio*. Shown is a consensus of the optimal MP trees. This tree is nearly equivalent to the optimal ME and ML trees (see text for details). Parsimony bootstrap values (500 random addition sequence replicates with TBR branch swapping and replacement with all characters unordered and equally weighted) greater than 50 are reported above branches. The MP topology was unaffected (although bootstrap support values change slightly) with the transition:transversion ratio set to 10:1, 15:1, or 20:1. Only unique haplotypes were included in the analysis. Specimen names and geographic localities for each haplotype are given in Table 1. Haplotype and common name designations are: H = *Papio hamadryas* (Hamadryas baboon), A = *Papio anubis* (Anubis baboon), Y = *Papio cynocephalus* (Yellow baboon), Guinea = *Papio papio* (Guinea baboon), Chacma = *Papio ursinus* (Chacma baboon), Gelada = *Theropithecus gelada* (Gelada baboon), Mandrill = *Mandrillus sphinx*, Mangabey = *Cercocebus torquatus* (Red-capped mangabey), and Macaque = *Macaca sylvanus* (Barbary macaque). Divergence dates for the clades were calculated from the pairwise maximum likelihood distances (see methods for parameter values). Dates were calculated by assuming an age for the most recent common ancestor of *Papio* and *Theropithecus* of 4 Ma based on fossil evidence. Mean divergence dates (\pm SD) in thousands of years are given below each relevant branch. N.A. indicates that a standard deviation was not calculated because only one pair of taxa was compared for that branch.

indicate a complex population history without marked expansion within hamadryas (Harpending et al., 1998).

Clades I and II, respectively, of haplotypes from Kenya/Tanzania and Ethiopia/Arabia, separated about half a million years ago (568 ± 91 ka).

Within Arabia, there was some observed geographic structure of the haplotype distribution. Clade IIA consists solely of wild-sampled Arabian individuals, and all are from south of latitude 15° N. Conversely, all sam-

pled wild individuals from clade IIB are from north of latitude 15° N.

4. Discussion

Our results are not consistent with the suggestion that Arabian hamadryas baboons are descended from animals introduced from Africa in historic times. If this

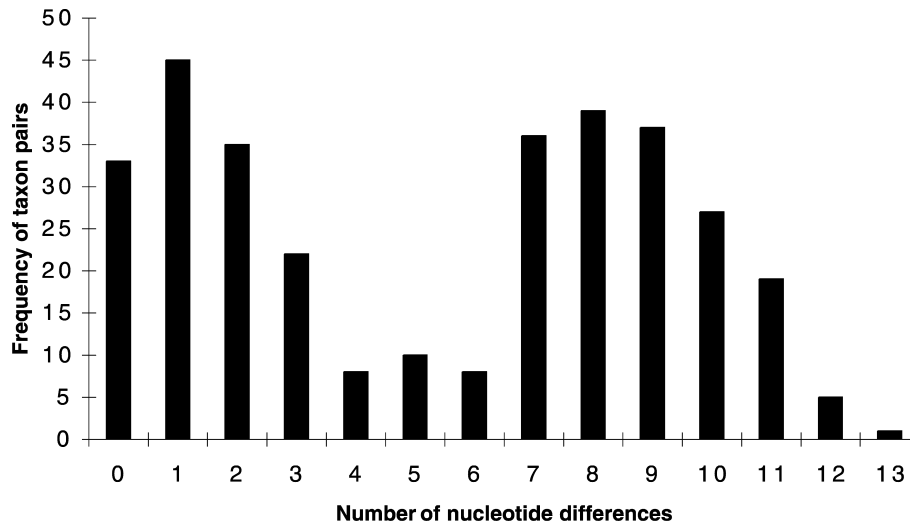


Fig. 3. Frequency of nucleotide substitutions between pairs of hamadryas baboons. A mismatch distribution plotting the number of nucleotide substitutions (X axis) vs. the frequency of the individual pairs of hamadryas baboons (Y axis). The bimodal distribution indicates a complex population history within hamadryas baboons.

were the case, one would expect individual haplotypes from both sides of the Red Sea to be closely related, or even identical, and genetic diversity in hamadryas to be greater in Africa than in Arabia. In fact, no haplotype was common to Arabia and Africa, diversity is as deep in Arabia as in Africa, and the most recent divergence between an Arabian and an African haplotype (H17 and H5, respectively) is estimated between 37 and 74 ka, an order of magnitude older than the time of Red Sea navigation and transport of baboons from the Horn of Africa to Egypt in Dynastic and Ptolemaic times.

If, as seems very probable, the ancestors of today's Arabian baboons crossed the Red Sea on dry land, and long before modern humans evolved, two distinct questions arise: first, when, where and in which direction did the crossing(s) occur; and second, were all baboons making the crossing already phenotypically hamadryas. The distinctive, derived hamadryas phenotype includes co-adapted pelage and behavioral elements proposed to be related to life in a comparatively arid habitat with dispersed resources (Jolly, 1963; Kummer, 1995). The phylogeography of mitochondrial haplotypes cannot directly document the evolution of this complex, but it does bear on the plausibility of alternative scenarios.

The present-day distribution of haplotypes in hamadryas suggests that baboon groups, including females, crossed the Red Sea several times during the Pleistocene. The first ancestors of Arabian hamadryas probably left Africa in the Middle Pleistocene, perhaps during a low sea-level event during OIS 10, 8, or 6. They would have carried a haplotype ancestral to Clade IIA, which had apparently diverged from its closest African relatives by about 400 ka.

Since all three major subclades of Clade II include phenotypically hamadryas individuals, it seems most

parsimonious to suppose that the last common ancestor of Clade II was itself phenotypically hamadryas. Of the major subclades of Clade II, IIA is entirely Arabian, IIC entirely African, and IIB includes both Arabian and African haplotypes. They form a trichotomy, with statistically indistinguishable divergence dates clustering around 400 ka. The mitochondrial evidence thus makes unlikely any scenario that sees the first hamadryas reaching Arabia long after the origin of the taxon, and in fact suggests that the origin of hamadryas baboons occurred almost simultaneously with their first crossing of the Red Sea. It does not, however, indicate whether Clade II's ancestral hamadryas population lived on the African or Arabian side of the Red Sea.

There are two possible scenarios. In the first, a population ancestral to Clade II, but not yet phenotypically hamadryas, crossed the Red Sea to Arabia, where it was isolated by rising sea levels. In the Arabian environment it evolved the hamadryas phenotypic complex, then, during a later low sea-level phase, sent colonizing populations back into Africa, bearing mitochondria ancestral to Clades IIB and IIC. In the second scenario, the hamadryas phenotype evolved in Ethiopia during a dry climatic event, and a colonizing subpopulation of this population then entered Arabia, bearing mitochondria ancestral to Clade IIA. No back-migration is necessary.

On balance, it seems less likely that the hamadryas phenotype evolved on the Arabian side. In several ways, it is less parsimonious. First, it involves an extra Red Sea crossing in the mid-Pleistocene. Second, it has to postulate an intrinsically unlikely population replacement in Ethiopia. As hamadryas spread back into Ethiopia, they would have had to displace (not absorb) a less specialized, ancestral "pre-hamadryas" baboon population from which they were previously derived,

and of which there is no genetic or phenetic trace. (As discussed below, Ethiopian anubis baboons are not survivors of such a population). It is also inconsistent with the mitochondrial data. Although mitochondrial variation in Arabia is ancient, it is no more ancient than that seen in African hamadryas. Significantly greater diversity would be expected on the Arabian side if the hamadryas population had spent tens of thousands of years in isolation, between low-sea-level events, before sending migrants back to Africa.

If the African-origin scenario is correct, it is not unreasonable to suppose that it was the *same*, severe, mid-Pleistocene cold, dry period that isolated a pre-hamadryas baboon population on the Ethiopian massif, favored the evolution and fixation of the hamadryas adaptive complex in that population, and also opened the dry land crossing that enabled them to extend their range into Arabia.

According to this scheme, hamadryas were the only baboons inhabiting the Ethiopian massif until the moister conditions of a full interglacial re-established connections with other well-watered areas in East Africa. This climatic swing, whose date has yet to be determined, presumably allowed anubis baboons to spread into Ethiopia from the west. The divergence between mitochondrial clades IIB and IIC, and most of the diversification within these clades, probably occurred in the African hamadryas population during its period of isolation. Finally, during a low sea-level stand early in the last glaciation, another influx from Africa apparently brought IIB mitochondria to Arabia, where they diversified into the H16–H20 cluster about 70 ka. We have no evidence that haplotypes of Clade IIC ever reached Arabia.

An explanatory scenario must also accommodate the fact that Clade IIC includes, along with hamadryas haplotypes, all haplotypes found in Ethiopian anubis baboons (A7–A14). A similar mtDNA phylogeny was described by Hapke et al. (2001) in baboons from Eritrea. Although their results are based upon D-loop sequences and thus are not directly comparable to ours, they too detected two major mitochondrial clades in African hamadryas, and found a cluster of anubis baboon haplotypes nested within one of them. Hapke et al. (2001) suggest that this situation probably arose as female anubis baboons, or hybrids carrying anubis haplotypes, were incorporated into hamadryas populations. Transfer of individual females or small matrilineages from anubis or hybrid groups to hamadryas troops at the inter-species boundary has been observed or inferred in the contemporary hybrid zone (Beyene, 1998; Kummer, 1968), and such movements would indeed bring anubis mitochondria into hamadryas populations, as Hapke et al. (2001) suggest. The weakness of this scenario lies in its assumption that the common ancestor of Clade IIC haplotypes was carried by anubis baboons.

Because Clade IIC is not closely related to haplotypes carried by Kenyan anubis baboons (Clade I), and is rooted securely among haplotypes of hamadryas origin, we have suggested (above) that the carrier of its stem haplotype (in fact the stem haplotype of all Clade II) was a hamadryas baboon. It follows that all Ethiopian (and probably Eritrean) anubis baboons so far surveyed carry mitochondria of hamadryas origin.

This apparently counter-intuitive result could easily be produced by powerful, sexually-differentiated introgression of anubis nuclear genes into a marginal hamadryas population. We envision a situation in which subadult males dispersing from marginal troops of an expanding anubis population encountered and mated with female hamadryas. The resulting male hybrids were probably smaller in body size than immigrant male anubis (Phillips-Conroy and Jolly, 2004), and perhaps less behaviorally consistent (Suguwara, 1979, 1988). Female hybrids, all carrying hamadryas mitochondria, thus tended to backcross preferentially to the numerous, available anubis and anubis-like males, producing still more anubis-like offspring. In each generation, up to 50% of hamadryas autosomal genes could be replaced by alleles derived from anubis. Eventually, the hybridized groups would become phenotypically anubis, with their mtDNA the only obvious evidence of genetic input from their hamadryas parentage. The introgression scenario is consistent with the respective sex-dispersal patterns characteristic of the two baboon taxa (anubis males normally disperse, while most hamadryas males are philopatric). It is also analogous to the situation recently described in warblers (Aves, Parulidae) in western North America (Rohwer and Wood, 2001). We therefore adopt it as a working hypothesis that requires further testing, especially with paternally inherited genetic markers.

Both in Eritrea (Hapke et al., 2001) and in Awash National Park (haplotypes A7–A12) haplotypes of anubis baboons form related clusters, rather than being independently derived from among the many different African hamadryas haplotypes. (Until orthologous sequences are compared, we cannot determine whether the Eritrean hybridization represents a separate occurrence.) This pattern suggests that the introgressive hybridization scenario was not played out everywhere that anubis and hamadryas came into contact, but rather that anubis populations of hybrid origin expanded from a few initial foci.

Since the relative advantage of anubis and hamadryas baboons is habitat-specific, such expansions presumably occurred whenever climate favored the spread of more mesic savannas, woodlands and highland forest at the expense of semi-arid hamadryas habitat. Judging from their diversity, haplotypes of the youngest Awash National Park anubis cluster (A7, A8, A9, A10, and A12) diverged from a common ancestor about 60 ka. The

other two Ethiopian anubis haplotypes, A13 and A14, also belong to clade IIC, but their divergence from other stems in the clade, and from each other, is evidently ancient, presumably representing an early cycle of introgression. It is significant that these animals were captured far to the west of the present-day anubis-hamadryas contact zone, deep in anubis territory. Their hamadryas-derived mitochondria support the view that hamadryas once occupied all of the Ethiopian massif, but much more extensive sampling of anubis haplotypes in western Ethiopia and Sudan is required to explore this speculation.

Interestingly, the many plant and animal taxa shared between the Horn of Africa and Arabia Felix (Yemen) also point to a Pleistocene connection across the straits. At least 62 mammalian species, representing nine orders, occur on both sides of the Red Sea (Harrison and Bates, 1991; Yalden et al., 1996). Comparison of their mtDNA phylogeography with hamadryas baboons would provide a powerful test of our biogeographic hypotheses (Avice, 2000; Wildman, 2000).

Further, if Pleistocene faunal exchanges occurred via a southern connection, early humans are likely to have participated. Tishkoff et al. (1996) have shown that genetic variation among present-day human populations of the Horn of Africa approximates that expected for the source population of all non-Africans. Other studies (reviewed in Disotell, 1999) postulate a southern route of human dispersal to Asia, mirroring to some extent the pattern of dispersal and vicariance in other Afro-Arabian mammals. The complex genetic processes underlying biogeographic patterns in the Afro-Arabian region present a formidable puzzle, but one whose solution is likely to throw light on a critical period in human evolution.

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