



Origin of the Sulawesi macaques (Cercopithecidae: *Macaca*) as suggested by mitochondrial DNA phylogeny

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One of the sharpest biogeographical transitions in the world occurs between the Indonesian islands of Borneo and Sulawesi; this transition is demarcated by Wallace's line. Macaque monkeys represent an interesting anomaly to faunal distributions in this region as they occur on both sides of Wallace's line, with *Macaca fascicularis*, *M. nemestrina* and other species to the west and seven Sulawesi species to the east. We have investigated macaque evolution and dispersal in the Sunda region and Sulawesi using phylogenetic analysis of mitochondrial DNA sequences. Female philopatry of macaques, which causes sharp geographic clustering of maternally inherited mitochondrial DNA haplotypes, makes mitochondrial phylogenies particularly useful for investigating ancient patterns of dispersal. Results of this study suggest the following: (1) *M. fascicularis* is not a sister taxon to any species of Sulawesi macaque; (2) haplotypes of some *M. nemestrina* have a sister relationship to northern and central Sulawesi macaques, while haplotypes of other *M. nemestrina* have a sister relationship to southern Sulawesi macaques; (3) Sulawesi was probably colonized by macaques twice, once to the base of the northern peninsula now occupied by *M. hecki* and once to the southwestern peninsula now occupied by *M. maura*; and (4) within north/central and southern Sulawesi, patterns of dispersal are largely consistent with contemporary and past geography of the island, with the exception of a geographically discontinuous relationship between *M. nigra* and a portion of *M. tonkeana* from a region in northwest central Sulawesi.

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ADDITIONAL KEY WORDS:—biogeography – dispersal – evolution – philopatry – Sunda shelf.

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INTRODUCTION

The Indonesian island of Sulawesi is situated between the Sunda continental shelf of South East Asia and the Sahul shelf of northwest Australia. The faunal composition of Sulawesi is a depauperate admixture of Asian and, to a lesser extent, Australian stock and is highly endemic as a result of an extended history of biogeographical isolation from neighbouring islands. Only one representative of Australia's marsupials is found on Sulawesi and many of Asia's large terrestrial mammals, including members of the order Primates (*Pongo*, *Hylobates*, *Presbytis*, *Trachypithecus*, *Nasalis*), occur on Borneo, Java, and/or Sumatra but not on Sulawesi. The stark contrast between these faunal compositions is generally attributed to climatic fluctuations which linked islands on each continental shelf to Asia or Australia during times of low sea level while Sulawesi remained isolated by deep oceanic trenches (Audley-Charles, 1981). The Philippine islands have been similarly isolated, though less so than Sulawesi (Heaney, 1985). A conspicuous exception to the faunal differences between Sulawesi and the Sunda shelf, separated by what has been referred to as Wallace's line (Wallace, 1876; George, 1981), is Sulawesi's seven species of endemic macaque monkey.

Macaques, which have the largest distribution of all non-human primates, are the only papionins with extant members outside of Africa. Recent paleontological findings indicate that macaques dispersed into Europe by the late Miocene, reaching China soon after (possibly as early as 5.5 Mya), and Indonesia by the mid-Pleistocene (Delson, 1980, 1996). Sulawesi is now surrounded by two species of macaque, either of which (based on proximity) could be the sister taxon to the Sulawesi macaques. *Macaca fascicularis* occurs on continental Asia, Borneo, Sumatra, Java, the Lesser Sunda Islands and the Philippines, while *M. nemestrina* is found on continental Asia, Borneo, and Sumatra. Fossil evidence suggests that *M. nemestrina* may have existed on Java at one time (Fooden, 1975) though this has been debated (Hooijer, 1962). There is some consensus that *M. nemestrina* is the sister taxon to all of the Sulawesi macaques based on morphology, mitochondrial DNA (mtDNA), and proteins coded by nuclear DNA (Fooden, 1969; Cronin, Cann & Sarich, 1980; Delson, 1980; Melnick & Kidd, 1985; Fa, 1989; Hoelzer & Melnick, 1996; Morales & Melnick, 1998).

Sparse representation of non-volant Asian mammals on Sulawesi suggests that invading late Pliocene and Pleistocene taxa, such as macaques, were probably hindered by a sea barrier (Cranbrook, 1981; Musser, 1987). Proposed routes for macaque colonization of Sulawesi from Borneo include the Doangdoang Shoals in the southern Makassar Strait (Sartono, 1973; Audley-Charles, 1981) and the drainage basin of the Mahakan River in eastern Borneo (Herrenschmidt, 1990; Froehlich, Supriatna & Muskita, 1996). The Sangihe Islands offer a plausible faunal link between the Philippines and Sulawesi (de Beaufort, 1951; Hooijer, 1958, 1962) and a subsided landbridge (Sartono, 1973; Groves, 1980) or island chain from the Kangean Islands (Sartono, 1973; Somadikarta, 1977) could have linked Java and Sulawesi. Fossil remains of Pliocene taxa that seem related to Javan forms support an ancient faunal link between Java and southwest Sulawesi during the Pliocene (4.6–3 Mya) (Groves, 1985). Upon arrival, dispersal and evolution of Sulawesi's fauna may have been influenced by a higher sea level and/or lower elevation of Sulawesi, making it an intermittent archipelago in the past (Fooden, 1969; Whitten, Mustafõ & Henderson, 1987).

Studies of cranial morphology suggest that *M. maura* is primitive relative to other Sulawesi macaques and that within Sulawesi, morphological patterns bifurcate (Albrecht, 1978). A major dispersal is postulated to have spread from *M. maura* in the southwest peninsula to *M. ochreata* and *M. brunnescens* in the southeast peninsula and distal islands. This dispersal is reflected, for example, in the development of narrow, concave supramaxillary ridges in these species (Albrecht, 1978: 102). A second dispersal is proposed to have branched north from *M. maura* into central and northern Sulawesi, now occupied by *M. tonkeana*, *M. hecki*, *M. nigrescens*, and *M. nigra*. This radiation is reflected by the development of broad supramaxillary ridges in northern species (Albrecht, 1978: 102). Fooden (1969) proposes a colonization of a *M. nemestrina*-like ancestor to central Sulawesi, which is now occupied by *M. tonkeana*. This hypothesis is based on the proximity of this region to Borneo during times of low sea level, and on morphological characteristics that appear primitive in *M. tonkeana* such as the suboval form of the ischial callosities, relatively long tail vestige, relatively unspecialized skull, and baculum most like that of Bornean *M. n. nemestrina* (Fooden, 1969: 64). Albrecht (1978: 89) and Groves (1980) dispute this interpretation. Multiple dispersal events of macaques to Sulawesi have been postulated to explain the distribution of different beta chain amino acid sequences in Sulawesi macaques (Takenaka *et al.*, 1987) and variation in fingerprints (Froehlich *et al.*, 1996).

Mitochondrial DNA phylogenies do not necessarily reflect current phylogenetic relationships among the nuclear genome of macaques, which are influenced by male migration (Melnick & Hoelzer, 1992). This is particularly true in Sulawesi macaques which have small population sizes (Sugardjito *et al.*, 1989; Supriatna *et al.*, 1992), a complex ecological and geological history, and possible gene flow across contact zones (Fooden, 1969; Groves, 1980; Ciani *et al.*, 1989; Supriatna, 1991; Supriatna *et al.*, 1992). On the other hand, strict female philopatry of macaques causes sharp geographic clustering of maternally inherited mtDNA (Melnick & Hoelzer, 1992; Hoelzer *et al.*, 1994). Consequently, phylogenetic analysis of mtDNA sequences is an effective tool with which to test hypotheses about macaque evolution and dispersal (Melnick & Hoelzer, 1993).

We have generated a molecular phylogeny from mtDNA sequences of a broad geographic representation of macaques on Southeast Asia, the Sunda shelf, and Sulawesi. We use this mtDNA phylogeny to test hypotheses regarding (1) the identity

of the sister taxon of the Sulawesi macaques, (2) whether Sulawesi macaques arose from a single or multiple colonizations, and (3) patterns of macaque dispersal in the Sunda region and Sulawesi.

MATERIAL AND METHODS

Sampling

Thirty wild-caught and captive animals were sampled for this study, representing ten of the 19 species in the genus *Macaca* and including *M. fascicularis*, *M. nemestrina*, and the Sulawesi macaques (Table 1, Fig. 1). Six of these samples (#5–10, sample numbers from Table 1 and Fig. 1) were used in a previous study (Rosenblum, Supriatna & Melnick, 1997). *M. sylvanus*, the mtDNA haplotype and morphology of which is primitive among the macaques (Delson, 1980; Hayasaka, Fujii & Horal, 1996; Morales & Melnick, 1998), was used as an outgroup. Since many pet macaques were sampled within Sulawesi, we were usually able to select animals that were caught (not bought) by the owner or a family member and thus of known accurate origin. To avoid possible phylogenetic complexities associated with hybridization, individuals were selected from locations outside known or suspected contact zones among species (Groves, 1980; Ciani *et al.*, 1989; Supriatna *et al.*, 1992). Redundant locality sequencing was carried out on *M. nigra* and on *M. tonkeana* from northwest central Sulawesi. In each case, individuals reputed to be from the same region showed close relationships. In order to best sample the mtDNA haplotypes of philopatric females, we used females or juvenile males (which are assumed to have not yet migrated far from their natal group) whenever possible. Age of monkeys was estimated from the stage of eruption of the third molar (absent in juveniles, present in subadults) and by the size and sharpness of canines and molars (large and sharp for adults, less so for subadults).

Pet and wild caught animals were anaesthetized with ketamine hydrochloride based on the estimated weight of the individual. Blood was drawn from the femoral artery using a disposable 21 gauge needle and syringe and preserved by mixing it with an equal volume of a lysis buffer containing 100 mM EDTA, 100 mM Tris pH 8, and 1% SDS. This mixture was kept at room temperature until it could be frozen.

DNA sequencing

Total genomic DNA was extracted from preserved blood using the Quiagen DNA extraction kit (#29306). A 2254 base pair region of mtDNA spanning the tRNA^{Gly}, the ND3 gene, tRNA^{Arg}, the ND4L gene, the ND4 gene, and tRNA^{His}, tRNA^{Ser}, and tRNA^{Leu} was amplified with the polymerase chain reaction (PCR) using primer PIB (5' TAC TGA CAC TTT GTA GAY GTT GTC TG 3') (Wang *et al.*, 1997) and PII (5' TAC TTT TAC TTG GAG TTG CA 3'). One hundred microliter amplifications were carried out with 35 PCR cycles, each with a denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 3 min, followed by a final extension at 72°C for 3 min. A fragment larger than the region that was sequenced was amplified to reduce the chance of concurrently amplifying

TABLE 1. Details of genetic samples, ? indicates unknown information

| # | Taxon | Origin | Sex | Age* | Code | Comments |
|-----------------------|---------------------------------------|--|-----|------|--------------|---|
| NON-SULAWESI MACAQUES | | | | | | |
| 1. | <i>M. sylvanus</i> | North Africa | ? | ? | ? | |
| 2. | <i>M. fascicularis fascicularis</i> | Tubuwau near Kalaha River, east Borneo | M | J | PM666 | captive individual at the Bronx Zoo, New York |
| 3. | <i>M. fascicularis fascicularis</i> | Java, precise location unknown | M | ? | Mfasc34 | pet caught by owners with hands |
| 4. | <i>M. fascicularis philippinensis</i> | Sibuyan Island, Philippines | M | ? | FMNH#135714 | wild caught individual |
| 5. | <i>M. nemestrina leonina</i> | Yunnan Province, South China, precise location unknown | ? | ? | ZAS | wild caught, donated by the FMNH from Kunming Institute of Zoology, Yunnan, China |
| 6. | <i>M. nemestrina leonina</i> | Northern Thailand, precise location unknown | ? | ? | 2AB | Dusit Zoo in Bangkok, Thailand |
| 7. | <i>M. nemestrina pagensis</i> | Siberut Island, Mentawai Islands | M | ? | Mncmpag 1040 | pet sampled July, 1994 |
| 8. | <i>M. nemestrina nemestrina</i> | Ngasang, near Lake Mantengau, north Sumatra | F | ? | SW#10 | pet sampled July, 1994 |
| 9. | <i>M. nemestrina nemestrina</i> | Tambung, near Lampung, south Sumatra | F | ? | S#1 | pet sampled July, 1994 |
| 10. | <i>M. nemestrina nemestrina</i> | Sarawak, Malaysia, north Borneo | ? | ? | Mncmsar | |
| 11. | <i>M. nemestrina nemestrina</i> | Sepilok Nature Reserve, Malasia, northeast Borneo | F | SA | gumgum | pet sampled by Dr. Edwin Bosi, Aug 1997 |
| 12. | <i>M. nemestrina nemestrina</i> | Karlabarun, Kalimantan, east Borneo | M | J | PM665 | caught by owner when it fell out of a tree |
| SULAWESI MACAQUES | | | | | | |
| 13. | <i>M. nigra</i> | Klabat Mtn. North Sulawesi | F | J | PF660 | pet caught by owner |
| 14. | <i>M. nigra</i> | Klabat Mtn. North Sulawesi | M | J | PM661 | pet caught by owner (same owner as PF660) |
| 15. | <i>M. nigrescens</i> | Bilungala, near Gorontalo, North Sulawesi | M | J | PM655 | caught by owner with hands |
| 16. | <i>M. hechi</i> | Sahagan, near Toi Toi, Central Sulawesi | F | J | PF648 | caught by owner's father |
| 17. | <i>M. hechi</i> | Toribulu, north of Palu, Central Sulawesi | F | SA | PF644 | caught by owner with a chicken trap |
| 18. | <i>M. tonkeana</i> | Towali, west of Palu, Central Sulawesi | F | A | PF599 | caught by owner's brother after being injured with a gun |
| 19. | <i>M. tonkeana</i> | Lore Lindu National Park, Central Sulawesi | F | ? | WF128 | wild caught by Jatna Supriatna, 1988 |
| 20. | <i>M. tonkeana</i> | Tagolu, near Poso, Central Sulawesi | M | SA | PM561 | caught by owner's dog |
| 21. | <i>M. tonkeana</i> | Bunta, Central Sulawesi | F | A | PF536 | caught by owner |
| 22. | <i>M. tonkeana/logeanus</i> | Ranga Ranga forest, near Bonenobakal, Central Sulawesi | M | SA | PM547 | caught by owner's relative |
| 23. | <i>M. tonkeana</i> | Prona, Central Sulawesi | M | J | PM569 | caught by owner |
| 24. | <i>M. tonkeana</i> | Mamasa, Central Sulawesi | F | A | PF597 | caught by owner's son |
| 25. | <i>M. tonkeana</i> | Enrekang, South Sulawesi | M | A | PM604 | caught by owner's father |
| 26. | <i>M. maura</i> | Karenta, South Sulawesi | M | ? | WM024 | wild caught by Jatna Supriatna, 1988 |
| 27. | <i>M. maura</i> | Bontobohari, South Sulawesi | F | ? | WF001 | wild caught by Jatna Supriatna, 1988 |
| 28. | <i>M. ochreata</i> | Saklta forest, near Bungku, Central Sulawesi | F | J | PF575 | caught by owner's son |
| 29. | <i>M. ochreata</i> | near Kolaka, Southeast Sulawesi | F | A | PF704 | caught by owner with monkey trap |
| 30. | <i>M. brunescens</i> | near Waruruma, Buton Island, Southeast Sulawesi | F | A | PF707 | caught by a friend |

* age (Juvenile, SubAdult, or Adult) assessed as described in text.

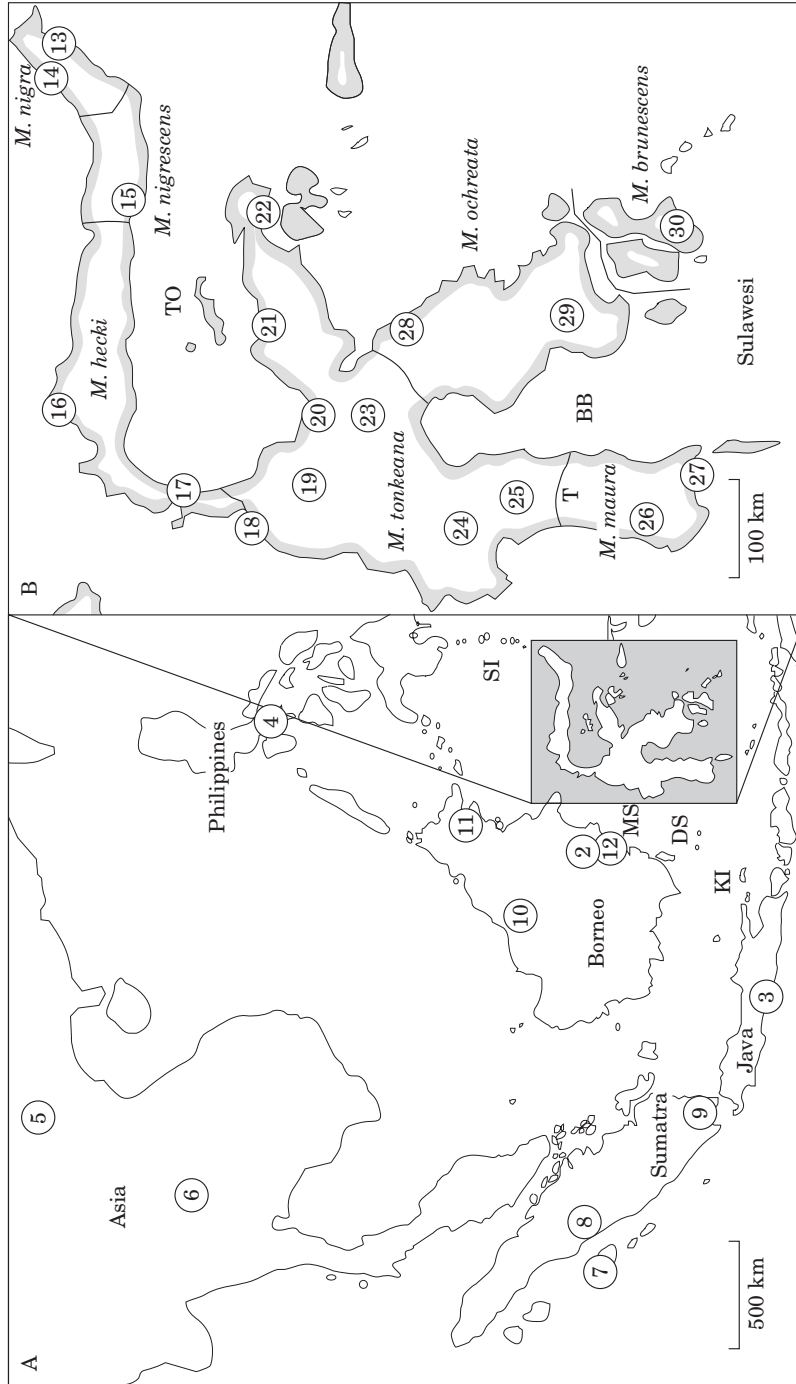


Figure 1. Map of ingroup samples used in this study (*M. gylbanus* outgroup not shown). Numbers inside circles refer to those in Table 1. Regions mentioned in the text are indicated with abbreviations: MS = Makassar Strait, T = Tempe depression, DS = Doangloang Shoals, SI = Sangihe Islands, TO = Togian Islands, BB = Bay of Bone. A, samples from southeast Asia and the Sumda shelf. B, locations of Sulawesi species contact zones are indicated with a solid line.

an equally sized paralogous region inserted in the nuclear genome (Collura & Stewart, 1995). Within the amplified fragment, a 1325 base pair region including part of the tRNA^{Gly}, the ND3 gene, tRNA^{Arg}, the ND4L gene, and 574 base pairs of the ND4 gene, was sequenced using primers PIB, ND4SREV2 (5' AAG AAT TAT TTT TAG CAT TG RAG TA 3'), MACF2 (5' GGC ATA YTA ACC TAC CGC TCA CA 3'), MACR2 (5' ATG AAT GTC AGA ATT ARG GTG ATT TG 3'), ND4#1 (5' CTT CTA ACA CTR ACC GCC TGA CT 3'), and NAP2M (5' GGA GCT TCA ACG TGG GCT TT 3') (Wang *et al.*, 1997) and ABI FS dyedeoxy cycle sequencing kit (#402090, Applied Biosystems, Inc.) according to the manufacturer's protocol. PCR amplifications and cycle sequence reactions were purified as in Wang *et al.* (1997) and sequence reactions were run on an ABI 377 automated sequencer. Sequences were compiled with the AutoAssembler program (Applied Biosystems, Inc., Perkin Elmer, 1994), and aligned manually using the homologous sequence in humans (Anderson *et al.*, 1981) as a reference for codon and tRNA positions. These sequences have been submitted to Genbank (access # AF091400–AF091429).

Analyses

Levels of saturation of first, second, and third codon positions and of tRNAs were evaluated by plotting the number of transitions and transversions against pairwise uncorrected p-distance among mtDNA haplotypes; regressions of these parameters were performed with the JMP program, version 3.1 (SAS Institute Inc., 1995). Phylogenetic relationships among aligned mtDNA sequences were examined with maximum parsimony and with likelihood analyses. These approaches both attempt to reconstruct evolutionary history, but do so in different ways. Parsimony analysis infers a hierarchical distribution of shared derived character states (synapomorphies) that minimizes the number of changes in character state to explain the observed data; a branching topology (tree) that requires fewer changes in character states to explain the observed data is better than one that requires more. Likelihood analysis chooses a phylogeny for which it is most likely to observe the data, given a particular model of evolution (Maddison & Maddison, 1992). Computer simulations of evolution suggest that parsimony and likelihood analyses perform comparably under simple models of evolution; under more complex models which include variation in substitution rates, the likelihood method tends to do better in recovering a true topology (Yang, 1996). Heuristic searches for the most parsimonious trees were conducted using 10 replicates of random addition of taxa with a test version of PAUP version 4.0d61, written by D. Swofford. Support for relationships recovered from cladistic analysis were estimated by nonparametric bootstrap analysis with 2000 replications, each with a single replication of random addition of taxa. Branch support values for unweighted parsimony trees (Bremer, 1994), which represent the difference in tree lengths between the shortest trees that contain versus lack each group (Swofford *et al.*, 1996), were obtained with the Autodecay program, version 3.0 (Eriksson & Wikstrom, 1995) and PAUP 3.1.1 (Swofford, 1991).

Maximum likelihood analysis was performed on the nucleotide data using the general time reversible model, which allows for transition bias and does not assume equal base frequencies (Hasegawa, Kishino & Yano, 1985), with a proportion of the nucleotide sites assumed invariable, and rates at variable sites assumed to follow

a gamma distribution (GTR, $I + \Gamma$ in Sullivan & Swofford, 1997). In order to expedite likelihood analysis, we used a heuristic method suggested by Swofford *et al.* (1996). We first estimated the gamma shape parameter, the proportion of invariable sites, and the substitution rate matrix from the four most parsimonious tree topologies with PAUP. The parameters of the most likely tree (lowest-ln L value) were then used to perform likelihood analysis with nucleotide frequencies estimated from the data, yielding a novel, more likely tree. We then estimated the parameters again from this tree and repeated a second likelihood analysis with these new parameters. This procedure was repeated until additional iterations of parameter estimation and likelihood analysis did not further optimize the tree likelihood.

To investigate whether Sulawesi was colonized by macaques once or twice, unweighted parsimony and likelihood searches were conducted with constraints for Sulawesi monophyly, which would be consistent with a single colonization. Constrained and unconstrained analyses were compared using parsimony criteria (number of steps) and likelihood criteria (-ln L). Significant differences in likelihood of constrained and unconstrained maximum likelihood topologies were evaluated with the Kishino-Hasegawa test (Kishino & Hasegawa, 1989), using PAUP. Additionally, pairwise uncorrected 'p-distances' among mtDNA haplotypes were calculated with PAUP.

RESULTS

Linear relationships between number of transitional changes in each codon position and distance suggest that these characters are not saturated (Fig. 2). Transversional changes of first (slope = 41.3, $r^2 = 0.20$), second (slope = 17.8, $r^2 = 0.14$), and third (slope = 56.77, $r^2 = 0.22$) codon positions and of tRNA (slope = 2.7, $r^2 = 0.02$) also show no evidence of saturation (data not shown). Unweighted parsimony analysis identified four most parsimonious trees of 1117 steps that differ in mtDNA haplotype relationships among a few *M. tonkeana* haplotypes from central Sulawesi, and among haplotypes of *M. maura* and *M. ochreata/brunnescens* with respect to east Borneo *M. nemestrina* (Fig. 3). The number of unambiguous transitions in the four most parsimonious trees ranged from 706 to 720; unambiguous transversions ranged from 81 to 83. Iterative likelihood analyses at the last iteration recovered a tree with a -ln L = 7005.22 (Fig. 4). This tree was estimated with the following parameters: proportion of invariable sites = 0.442294, gamma shape parameter = 0.660586, user-specified substitution rate matrix = 1.81 (A-C), 66.23 (A-G), 1.56 (A-T), 4.38 (C-G), 44.85 (C-T).

M. fascicularis and *M. nemestrina*

Parsimony and likelihood analyses recovered trees with different topologies but with major consistencies: (1) mtDNA haplotypes in the Sunda region fall into two major clades, one with *M. fascicularis* and a second with *M. nemestrina* and the Sulawesi macaques; (2) haplotypes of *M. nemestrina* and the Sulawesi macaques are divided into at least two major groups: one group includes macaques from north and central (north/central) Sulawesi; the second group contains *M. nemestrina* haplotypes from east Borneo, Sumatra, and the Mentawai Islands and haplotypes from southwest

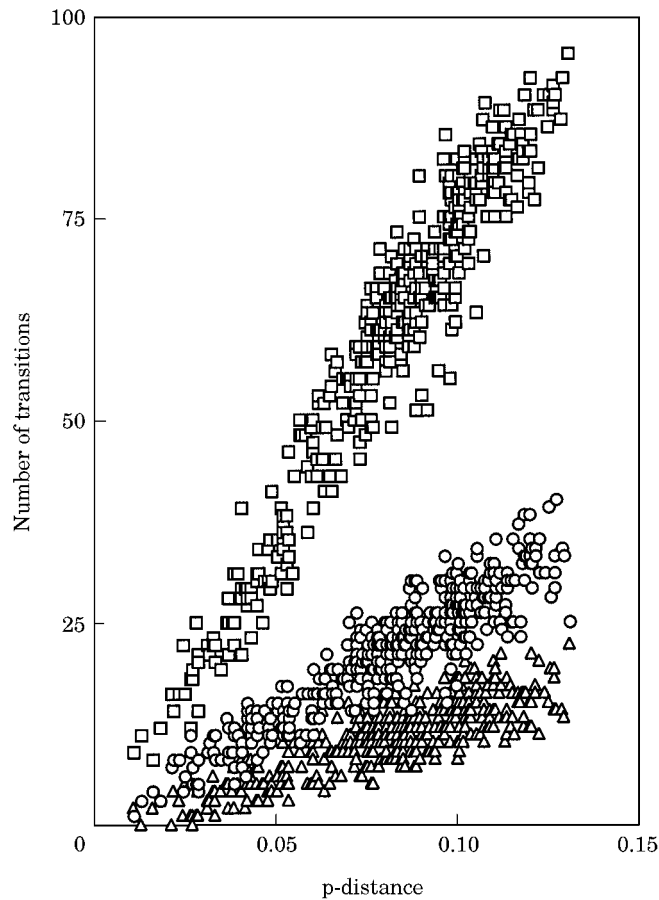


Figure 2. Plot of number of transitions in first (○; slope = 261.8, $r^2 = 0.838$), second (△; slope = 141.6, $r^2 = 0.689$), and third (□; slope = 735.4, $r^2 = 0.934$) codon position *vs.* pairwise uncorrected p-distance. For clarity, transitions of tRNA (slope = 67.5, $r^2 = 0.361$), are not shown.

and southeast Sulawesi (Figs 3, 4). Within the monophyletic mtDNA haplotypes of *M. fascicularis*, that of *M. f. philippinensis* is more closely related to the *M. f. fascicularis* haplotype from Borneo (#2) than the haplotype from Java (#3) (Figs 3, 4). Both analyses suggest mitochondrial DNA haplotypes of *M. nemestrina* are paraphyletic with respect to the Sulawesi macaques, though these relationships are not strongly supported (Figs 3,4). Parsimony analysis suggests monophyly of haplotypes of *M. nemestrina* and Sulawesi macaques on the northern Sunda shelf and Sulawesi (mainland Asia, north and northeast Borneo, and north/central Sulawesi) and monophyly of mtDNA haplotypes on the southern Sunda shelf and Sulawesi (Sumatra, Mentawai Islands, east Borneo, and south Sulawesi) (Fig. 3). In likelihood analysis, the mtDNA haplotype of *M. nemestrina* from northeast Borneo (#11) falls basal to other haplotypes of *M. nemestrina* and the Sulawesi macaques; haplotypes from north/central Sulawesi are a monophyletic group, as are the haplotypes of other *M. nemestrina* and the macaques from south Sulawesi (Fig. 4). Cladistic analysis suggests mtDNA haplotypes of *M. n. leonina* (#5, 6) are derived from *M. n. nemestrina* haplotypes from north and northeast Borneo (#10, 11) (Fig. 3). However, likelihood analysis suggests that the

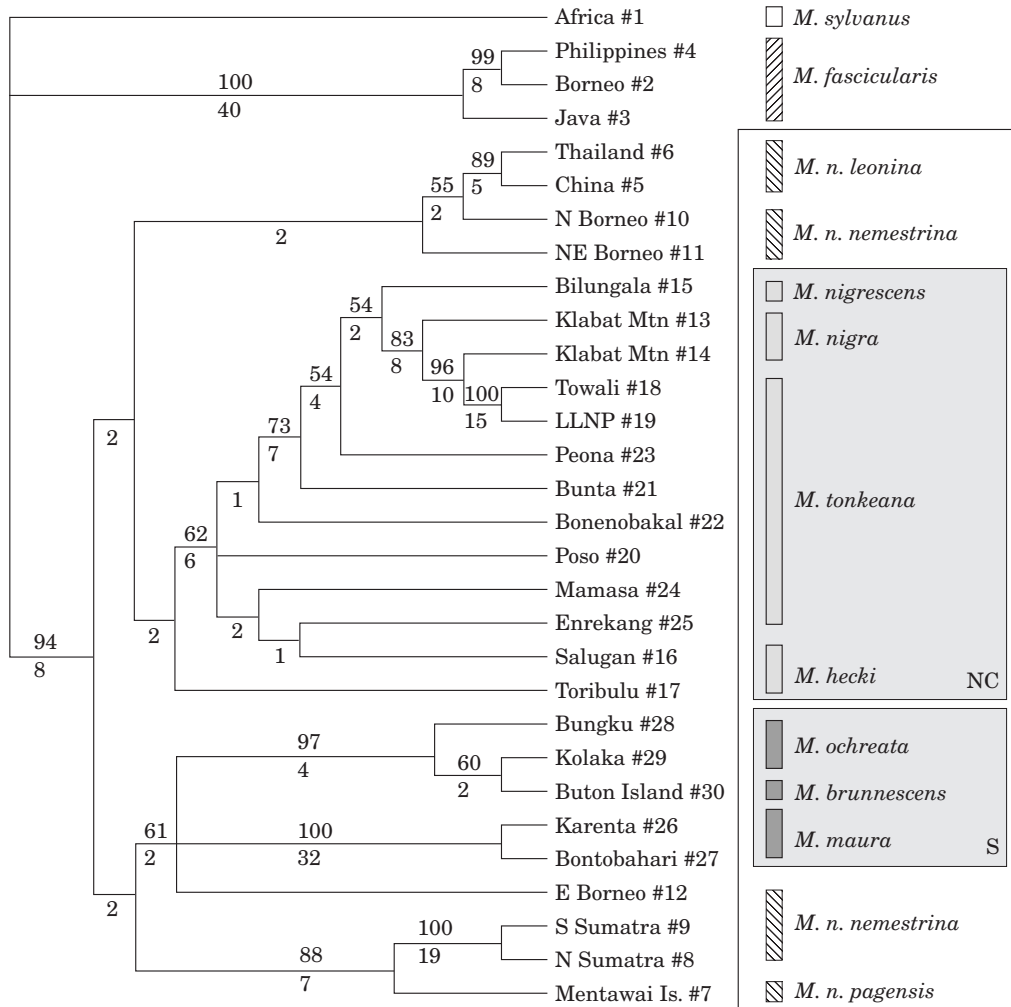


Figure 3. Strict consensus tree of four most parsimonious trees (CI=0.427, RI=0.612, RC=0.261, HI=0.573). Bootstrap values over 50 are indicated above branches, branch support values are indicated below branches. Localities precede numbers in parentheses, which refer to sample numbers in Table 1. *M. nemestrina* is in a box, central/north Sulawesi and south Sulawesi are also boxed with labels NC and S, respectively.

M. nemestrina haplotypes from north and northeast Borneo are more closely related to haplotypes in south Sulawesi and that *M. n. leonina* haplotypes are more closely related to *M. n. nemestrina* from Sumatra and the Mentawai Islands. Both cladistic and likelihood analyses suggest *M. n. nemestrina* from Sumatra is monophyletic and sister to *M. n. pagensis* (Figs 3, 4).

Sulawesi macaques

In Sulawesi, relationships among mtDNA haplotypes from adjacent regions of north/central Sulawesi or southern Sulawesi are generally close, though there are

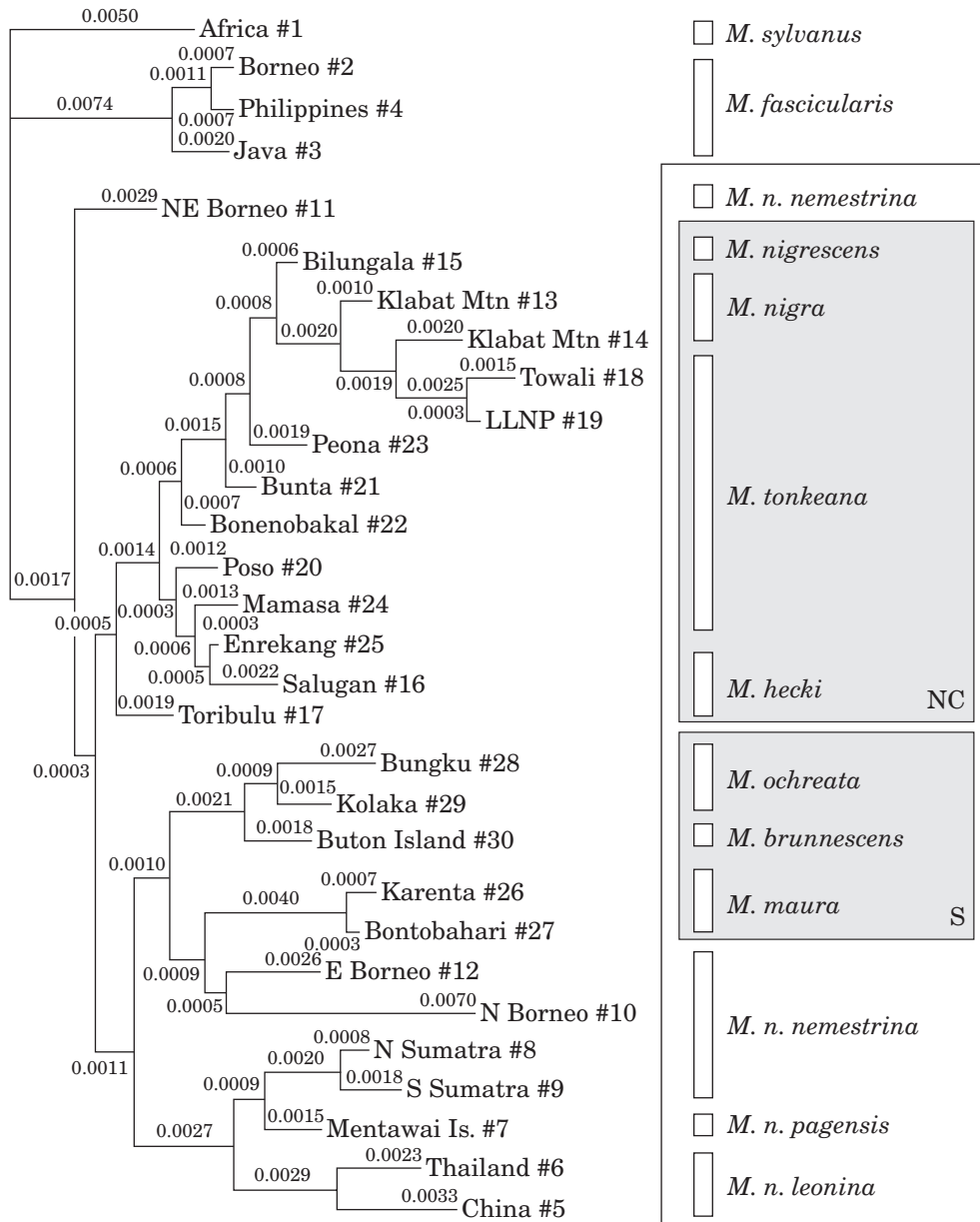


Figure 4. Maximum likelihood tree obtained from iterations of analysis; boxes and labelling are as in Fig. 3. Numbers above branches represent maximum likelihood estimates of branch lengths (substitutions/site).

a few exceptions. The relationship among *M. maura*, *M. ochreata/brunnescens*, and east Borneo *M. nemestrina* is unresolved in cladistic analysis (Fig. 3); likelihood analysis suggests monophyly of east and north Borneo *M. nemestrina* (#10, 12) and *M. maura* (#26, 27) (Fig. 4). Within north/central Sulawesi, a southern *M. hecki* haplotype is basal to other haplotypes. *M. tonkeana* haplotypes, (except those from Towali and Lore Lindu National Park) form a basal, paraphyletic assemblage derived from

southern *M. hecki*, but also related to a northern haplotype of *M. hecki* (Figs 3, 4). Haplotypes of the parapatric species *M. nigra* and *M. nigrescens* in the northern peninsula are derived from these haplotypes of *M. tonkeana* from the eastern and southern parts of central Sulawesi. Mitochondrial DNA haplotypes of *M. tonkeana* from northwest central Sulawesi, near Towali and in Lore Lindu National Park (#18, 19) are derived from *M. nigra* and *M. nigrescens* (Figs 3, 4). This relationship constitutes a significant geographical inconsistency as it exists in the absence of haplotypes from the geographically intervening species *M. hecki*.

Constraining for Sulawesi monophyly

When Sulawesi is constrained to be monophyletic, two most parsimonious topologies are produced from cladistic analysis, each with 1136 steps. One constrained parsimony topology has haplotypes of *M. n. leonina* (#5, 6) and *M. n. nemestrina* from north and northeast Borneo (#10, 11) in a basal position and haplotypes of *M. n. nemestrina* from Sumatra (#7, 8, 9) and SE Borneo (#12) closely related to the constrained Sulawesi haplotypes. The other constrained topology has the latter group of *M. nemestrina* haplotypes basal and the former group sister to the constrained Sulawesi haplotypes. Both constrained topologies have 19 more steps than the four most parsimonious trees from unconstrained analysis and over 50 000 more parsimonious trees exist that violate the constraint for Sulawesi monophyly. Maximum likelihood analysis constrained for Sulawesi monophyly produced a tree (1143 steps, $-\ln L = 7021.87$) that was not significantly less likely than the unconstrained likelihood tree, according to the Kishino-Hasegawa test ($P = 0.1549$). In this topology, *M. n. nemestrina* from NE Borneo (#11) is basal to Sulawesi and other *M. nemestrina* samples (as in unconstrained likelihood analysis), and the other *M. nemestrina* samples are monophyletic with respect to the constrained Sulawesi haplotypes.

DISCUSSION

Molecular phylogenies of a gene offer insight into the hierarchy of splitting events in the evolution of a locus, but do not necessarily reflect current genetic relationships among the operational taxonomic units (individuals, species, genera, etc.), which are the evolutionary manifestations of many loci. In the case of macaques, female philopatry and male migration can reduce the effective population size of the nuclear genome but raise the effective population size of the mitochondrial genome, potentially generating longer coalescence times in the mitochondrial genome than the nuclear genome (Hoelzer, 1997). Fluctuations in sea level and ecology have affected faunal composition and distribution in this region (Verstappen, 1975; Eudey, 1980; Morley & Flenley, 1987; Brandon-Jones, 1996). Population structure associated with these fluctuations could also influence the relative effective population sizes of mtDNA and nuclear DNA of macaques. For these reasons, paraphyletic mitochondrial DNA haplotypes of many taxa in this study (*M. nemestrina*, *M. hecki*, *M. tonkeana*, *M. ochreata*, *M. nigra*) do not necessarily conflict with other research that suggests these groups are morphologically distinct species (Fooden, 1969, 1975; Albrecht, 1978). Clearly, continued investigation of variation in morphology and

the nuclear genomes of these species could offer additional insight into their phylogeny, and the evolutionary mechanisms of macaque speciation.

M. fascicularis

Macaca fascicularis, unlike *M. nemestrina*, is absent from the Mentawai Islands but present in Java, the Lesser Sunda Islands, and the Philippines. *M. fascicularis* may have dispersed through the Sunda shelf after *M. nemestrina* and encountered different biogeographical opportunities (Fooden, 1969, 1976; Eudey, 1980; Brandon-Jones, 1996). If mtDNA has evolved at a similar rate in *M. fascicularis* and *M. nemestrina*, the small phenetic distances among *M. fascicularis* in Java, Borneo, and the Philippines (range = 0.01283–0.03170) relative to those among *M. nemestrina* in Borneo (range = 0.07245–0.08906) are congruent with a more recent diversification of *M. fascicularis* in the eastern Sunda region (Table 2). Well supported monophyly of *M. fascicularis* relative to other macaques in the study area, and the derived relationships of haplotypes in distal portions of northern Sulawesi (*M. nigra* and *M. nigrescens*) relative to most other haplotypes from central Sulawesi, conflict with the postulated *M. fascicularis* ancestry and/or Philippine origin for any of the Sulawesi macaques (Beaufort, 1951; Hooijer, 1958). Monophyly of Borneo *M. f. fascicularis* and *M. f. philippinensis* relative to Javan *M. f. fascicularis* supports a Bornean ancestry for the Philippine macaques, as expected for macaques and most other non-volant land mammals in the Philippines (Heaney, 1985; Fooden, 1991). Much of the Philippines' terrestrial fauna probably reached the archipelago from Borneo during Pleistocene glacial periods (Fooden, 1991). Even species in the rodent genus *Crunomys*, which is shared by Sulawesi and the Philippines, may be of independent origin to each region (Musser, 1987).

M. nemestrina

In *M. nemestrina*, cladistic analysis suggests mtDNA relationships correspond with Pleistocene drainage basins in the northern Sunda region (between Borneo and mainland Asia), and the southern Sunda region (between south Sumatra, Java, and Borneo) (Verstappen, 1975; Eudey, 1980; Morley & Flenley, 1987) (Fig. 3). Major faunal divisions of fish are also associated with these regions (Beaufort, 1951). Cranial size of *M. nemestrina* is abruptly discontinuous between *M. n. leonina* and *M. n. nemestrina* at about 8°N latitude (Fooden, 1975: 86). Additionally, skull length of *M. n. nemestrina* in the Malay Peninsula, Sumatra, and Borneo north of 3°N averages 10 mm less than that of *M. n. nemestrina* south of this latitude (Fooden, 1975: 100). This latter morphological difference is roughly consistent with separate *M. nemestrina* mtDNA haplotype clades associated with the northern and southern Sunda shelf in cladistic analysis (Fig. 3).

In contrast, eastern and western regions (and not necessarily northern and southern regions) of the Sunda shelf have major genetic and morphological differences in other primates (Brandon-Jones, 1996; Xu & Arnason, 1996) and other mammals such as rhinos (Morales *et al.*, 1997) and rodents (Musser, 1979). Outside of the haplotype of *M. nemestrina* from northeast Borneo, mtDNA relationships as discerned from likelihood analysis suggest separate mtDNA haplotype diversification in *M.*

TABLE 2. Pairwise uncorrected 'p-distances' of region sequenced in mtDNA; numbers (1-30) refer to taxa in Table 1

| # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1 | 0.00000 | | | | | | | | | | | | | | |
| 2 | 0.09736 | 0.00000 | | | | | | | | | | | | | |
| 3 | 0.09887 | 0.03170 | 0.00000 | | | | | | | | | | | | |
| 4 | 0.09660 | 0.01283 | 0.03094 | 0.00000 | | | | | | | | | | | |
| 5 | 0.12302 | 0.12000 | 0.12755 | 0.12528 | 0.00000 | | | | | | | | | | |
| 6 | 0.12084 | 0.11785 | 0.12087 | 0.12162 | 0.05437 | 0.00000 | | | | | | | | | |
| 7 | 0.10038 | 0.09434 | 0.09887 | 0.09811 | 0.07472 | 0.06722 | 0.00000 | | | | | | | | |
| 8 | 0.10113 | 0.09736 | 0.10264 | 0.10264 | 0.08377 | 0.07555 | 0.04075 | 0.00000 | | | | | | | |
| 9 | 0.10868 | 0.10415 | 0.10943 | 0.10943 | 0.09057 | 0.08385 | 0.04906 | 0.02491 | 0.00000 | | | | | | |
| 10 | 0.13132 | 0.12604 | 0.12679 | 0.12679 | 0.09962 | 0.08151 | 0.11094 | 0.11472 | 0.12453 | 0.00000 | | | | | |
| 11 | 0.08151 | 0.08377 | 0.08528 | 0.08604 | 0.07623 | 0.07250 | 0.07321 | 0.07547 | 0.08377 | 0.07698 | 0.00000 | | | | |
| 12 | 0.09811 | 0.09585 | 0.10340 | 0.09811 | 0.11094 | 0.11029 | 0.07019 | 0.08075 | 0.08906 | 0.08906 | 0.07245 | 0.00000 | | | |
| 13 | 0.09962 | 0.10189 | 0.10792 | 0.10415 | 0.08604 | 0.08082 | 0.10113 | 0.09660 | 0.10189 | 0.08906 | 0.04226 | 0.09811 | 0.00000 | | |
| 14 | 0.11547 | 0.11396 | 0.11321 | 0.11623 | 0.08981 | 0.08686 | 0.10792 | 0.10171 | 0.10943 | 0.08830 | 0.05962 | 0.10792 | 0.03849 | 0.00000 | |
| 15 | 0.08755 | 0.09358 | 0.10038 | 0.09434 | 0.09660 | 0.09895 | 0.09283 | 0.09057 | 0.09585 | 0.11094 | 0.05132 | 0.09057 | 0.02868 | 0.04906 | 0.00000 |
| 16 | 0.08075 | 0.07623 | 0.08453 | 0.07849 | 0.10340 | 0.10574 | 0.07849 | 0.08151 | 0.08377 | 0.11094 | 0.05208 | 0.07321 | 0.05208 | 0.06642 | 0.03849 |
| 17 | 0.08000 | 0.08377 | 0.08528 | 0.08604 | 0.09660 | 0.09368 | 0.07396 | 0.07774 | 0.08604 | 0.09132 | 0.04226 | 0.06189 | 0.05283 | 0.07170 | 0.04453 |
| 18 | 0.11774 | 0.12679 | 0.12981 | 0.12906 | 0.10566 | 0.08912 | 0.12000 | 0.11396 | 0.12000 | 0.09509 | 0.07321 | 0.11849 | 0.05283 | 0.04302 | 0.05887 |
| 19 | 0.11396 | 0.11849 | 0.12302 | 0.12075 | 0.09887 | 0.09215 | 0.11396 | 0.10868 | 0.11472 | 0.09811 | 0.06792 | 0.11396 | 0.04302 | 0.04075 | 0.04755 |
| 20 | 0.07849 | 0.08830 | 0.08981 | 0.08906 | 0.08755 | 0.08837 | 0.07245 | 0.07623 | 0.08000 | 0.09358 | 0.04226 | 0.07245 | 0.04075 | 0.04830 | 0.03472 |
| 21 | 0.08604 | 0.09208 | 0.09434 | 0.09283 | 0.08755 | 0.08990 | 0.08755 | 0.08151 | 0.08679 | 0.09434 | 0.04679 | 0.07774 | 0.03774 | 0.05057 | 0.02717 |
| 22 | 0.08377 | 0.08151 | 0.08604 | 0.08226 | 0.08830 | 0.09443 | 0.07774 | 0.07849 | 0.08377 | 0.09509 | 0.04302 | 0.07774 | 0.04453 | 0.05358 | 0.03321 |
| 23 | 0.08528 | 0.10038 | 0.10113 | 0.10113 | 0.09509 | 0.09441 | 0.08755 | 0.08453 | 0.09132 | 0.10340 | 0.05057 | 0.08755 | 0.04302 | 0.05283 | 0.02717 |
| 24 | 0.07698 | 0.08000 | 0.08755 | 0.08075 | 0.09962 | 0.10272 | 0.07245 | 0.07547 | 0.08226 | 0.10340 | 0.05208 | 0.07019 | 0.05358 | 0.06566 | 0.04604 |
| 25 | 0.07472 | 0.07472 | 0.08302 | 0.07547 | 0.09585 | 0.10121 | 0.06943 | 0.07094 | 0.07774 | 0.09887 | 0.04453 | 0.06415 | 0.05208 | 0.06113 | 0.04151 |
| 26 | 0.11019 | 0.10113 | 0.10566 | 0.10038 | 0.11698 | 0.11104 | 0.08075 | 0.08906 | 0.09887 | 0.10113 | 0.08453 | 0.06868 | 0.10415 | 0.11170 | 0.09962 |
| 27 | 0.11019 | 0.09811 | 0.10566 | 0.09887 | 0.11849 | 0.11104 | 0.07925 | 0.08755 | 0.09887 | 0.09811 | 0.08151 | 0.06566 | 0.10113 | 0.11019 | 0.09660 |
| 28 | 0.10264 | 0.10491 | 0.10717 | 0.10415 | 0.11396 | 0.11255 | 0.08377 | 0.08830 | 0.09660 | 0.09962 | 0.08151 | 0.06038 | 0.09057 | 0.10415 | 0.08377 |
| 29 | 0.10113 | 0.10189 | 0.10642 | 0.10113 | 0.11925 | 0.11254 | 0.08377 | 0.08981 | 0.09660 | 0.10264 | 0.07245 | 0.06340 | 0.08453 | 0.09660 | 0.07623 |
| 30 | 0.09811 | 0.10038 | 0.10792 | 0.10264 | 0.10717 | 0.10801 | 0.08226 | 0.08377 | 0.08755 | 0.10566 | 0.07245 | 0.06491 | 0.08302 | 0.09208 | 0.07170 |

TABLE 2. (continued)

| # | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 17 | 0.04528 | 0.00000 | | | | | | | | | | | | | |
| 18 | 0.08830 | 0.08528 | 0.00000 | | | | | | | | | | | | |
| 19 | 0.07698 | 0.07396 | 0.01585 | 0.00000 | | | | | | | | | | | |
| 20 | 0.03245 | 0.03623 | 0.07623 | 0.06491 | 0.00000 | | | | | | | | | | |
| 21 | 0.03698 | 0.04226 | 0.06038 | 0.05358 | 0.03321 | 0.00000 | | | | | | | | | |
| 22 | 0.02792 | 0.03245 | 0.07321 | 0.06340 | 0.02340 | 0.02642 | 0.00000 | | | | | | | | |
| 23 | 0.04453 | 0.05208 | 0.05283 | 0.04755 | 0.03698 | 0.02868 | 0.03698 | 0.00000 | | | | | | | |
| 24 | 0.03321 | 0.04302 | 0.09057 | 0.08226 | 0.02642 | 0.03849 | 0.02868 | 0.04830 | 0.00000 | | | | | | |
| 25 | 0.02113 | 0.03849 | 0.08453 | 0.07623 | 0.02189 | 0.03094 | 0.02415 | 0.04528 | 0.01811 | 0.00000 | | | | | |
| 26 | 0.08906 | 0.07698 | 0.12151 | 0.11698 | 0.08453 | 0.08830 | 0.08226 | 0.09962 | 0.08302 | 0.07849 | 0.00000 | | | | |
| 27 | 0.08755 | 0.07547 | 0.12000 | 0.11547 | 0.08302 | 0.08679 | 0.08075 | 0.09962 | 0.08151 | 0.07547 | 0.01057 | 0.00000 | | | |
| 28 | 0.07321 | 0.06642 | 0.11774 | 0.11170 | 0.06943 | 0.07698 | 0.07321 | 0.08981 | 0.07019 | 0.06264 | 0.06717 | 0.06566 | 0.00000 | | |
| 29 | 0.03887 | 0.06038 | 0.11321 | 0.10642 | 0.06038 | 0.06868 | 0.06264 | 0.07547 | 0.06113 | 0.05509 | 0.07774 | 0.07623 | 0.04075 | 0.00000 | |
| 30 | 0.03660 | 0.06189 | 0.11019 | 0.10189 | 0.05736 | 0.05962 | 0.05660 | 0.07094 | 0.05811 | 0.05358 | 0.07925 | 0.07925 | 0.05132 | 0.03925 | 0.00000 |

nemestrina on the eastern Sunda region (Sumatra, Mentawai Islands, and continental Asia) and on Borneo. In the western Sunda shelf, a close relationship among *M. n. pagensis* and Sumatra *M. n. nemestrina* supports a more recent common ancestry for these taxa (Figs 3, 4), and not *M. n. pagensis* and *M. fascicularis*, as proposed by others (Crocket-Wilson & Wilson, 1977).

Dispersal to Sulawesi

The mtDNA haplotypes of Sulawesi macaques generally have been considered monophyletic with respect to those of *M. nemestrina* (Hayasaka *et al.*, 1996; Hoelzer & Melnick, 1996; Morales & Melnick, 1998). If one assumes that dispersal of macaque mtDNA haplotypes occurred exclusively from west to east across the Makassar Strait between Borneo and Sulawesi, the mtDNA phylogenies suggest that *M. nemestrina* dispersed to Sulawesi at least twice. One colonization founded the macaques that now occupy the southern peninsulas of Sulawesi, *M. maura*, *M. ochreata*, and *M. brunnescens*. A second dispersal reached north/central Sulawesi, perhaps in the southern extent of the range of *M. hecki* and founded *M. hecki*, *M. tonkeana*, *M. nigrescens*, and *M. nigra*. Analyses constraining for Sulawesi monophyly, which is consistent with a single dispersal of macaques to Sulawesi, produced evolutionary hypotheses that are less parsimonious (more steps) or less likely (higher $-\ln L$) than unconstrained analyses. Morphologically, *M. maura*, *M. hecki*, and *M. ochreata* have more primitive craniofacial characteristics than do the other Sulawesi species (Albrecht, 1978: 89). An alternative hypothesis to a double dispersal postulates a single colonization of a polymorphic ancestor followed by haplotype fixation in different regions of Sulawesi. However, since mtDNA relationships span the Makassar Strait, this alternative explanation implies that independent and symmetrical fixation of ancestral haplotypes occurred in Borneo and in Sulawesi after Sulawesi was colonized. This second scenario is possible though it requires more independent events to explain the observed molecular phylogeography of mtDNA haplotypes. Two separate colonizations of Sulawesi by macaques at different times have been proposed by Takenaka *et al.* (1987) to account for variation in hemoglobin beta chain amino acid sequence, though their hypothesis relies on temporally (not necessarily spatially) separated dispersal events to Sulawesi. Froehlich *et al.* (1996) also proposed a double dispersal of macaques to Sulawesi to explain dermatoglyphic patterns of Sulawesi macaques. Their hypotheses agree almost exactly with the results of this study, with the exception that they propose a southern dispersal of *M. nemestrina* from Java, not Borneo. Relationships among extant mtDNA haplotypes favour the hypothesis that a *M. nemestrina*-like ancestor dispersed to the southern peninsulas of Sulawesi from east Borneo possibly by island hopping over the Doangdoang shoals region of the southern Makassar Strait. However the possibility that a more recent common ancestor of the southern Sulawesi macaques inhabited Java at one time but is now extinct (Groves, 1976; 1980) cannot be ruled out. Additional fossil evidence is needed to resolve whether *M. nemestrina* ever inhabited Java. A second colonization of north/central Sulawesi probably also occurred by rafting over water and may have arrived in habitat now occupied by *M. hecki*. The colonization of macaques into Sulawesi has been estimated to have occurred during the middle Pleistocene (Fooden, 1969; Delson, 1980), though large molecular distances observed among Sulawesi macaques in the ND3, ND4L, and ND4 genes

in this study (as much as 11%) and mtDNA ribosomal genes (4.5%, Morales & Melnick, 1998) suggest this colonization may have been earlier.

Evidence is accumulating for multiple origins of other Sulawesi taxa. A double dispersal hypothesis is proposed to explain the diversification of the lowland and mountain anoa, a dwarf buffalo, based on amino acid sequences of hemoglobin beta chains (Kako *et al.*, 1994). Molecular analysis of Asian shrew phylogeny also supports two colonizations of shrews into Sulawesi at different times (Ruedi, 1996) and a double dispersal is hypothesized to explain morphological variation in crested tree swifts on Sulawesi (Somadikarta, 1975). However, the proposed times and places of colonization and subsequent dispersal patterns of these taxa are not entirely equivalent to the macaque colonizations proposed in this study.

Dispersal within Sulawesi

Common ancestry of mtDNA haplotypes in the southern peninsulas is mirrored by similarities in cranial and external morphology (Albrecht, 1978). Other studies have revealed similarities between *M. ochreata* and *M. nemestrina* morphology (Watanabe *et al.*, 1985) and blood protein allele frequencies (Fooden & Lanyon, 1989) that also underscore this close relationship. Oceanic intrusion into the Tempe Depression at the northern extent of the distribution of *M. maura* is likely to have periodically isolated the southwest peninsula from central Sulawesi (Fooden, 1969; Whitten *et al.*, 1987). Portions of the region between the southwest and southeast peninsulas were dry during periods of low sea level associated with Pleistocene glaciations (Fooden, 1969). Additionally, land under the Sea of Bone between the southwest and southeast peninsulas of Sulawesi has been subsiding; coral reefs have been found in its southern region (Sartono, 1973; Audley-Charles, 1981; Bergman *et al.*, 1996). These factors may have offered a dry route for dispersal of macaques between these southern peninsulas of Sulawesi that would account for the close molecular relationships in macaques from these areas.

Haplotypes in north/central Sulawesi appear distinct from those in the southern peninsulas of Sulawesi. Within north/central Sulawesi, the basal location of a southern *M. hecki* (#17) haplotype suggests that some mtDNA haplotypes of this taxon are primitive with respect to the other species in this region. Other studies have identified *M. hecki* as distinct from or primitive with respect to other Sulawesi macaques, including dermatoglyphic data (Froehlich *et al.*, 1996), the frequency of hemoglobin beta chain type 1 (Takenaka *et al.*, 1987), body colour (Hamada *et al.*, 1988), and morphology (Albrecht, 1978: 89). Restriction enzyme analysis of mtDNA suggests a close relationship between *M. hecki* and *M. nemestrina* from Sabah in northeast Borneo (Harihara *et al.*, 1996).

Some haplotypes of *M. tonkeana* are basal with respect to the northern Sulawesi macaques, in agreement with a postulated primitive morphology of *M. tonkeana* (Fooden, 1969; Albrecht, 1978). Close relationships among *M. tonkeana* and *M. nigra* have been demonstrated in dermatoglyphic analyses (Froehlich *et al.*, 1996) and analysis of body color (Hamada *et al.*, 1988), and have been proposed to parsimoniously account for frequencies of different hemoglobin beta chain types in these species (Takenaka *et al.*, 1987). Though most of *M. tonkeana* appears primitive with respect to *M. nigrescens* and *M. nigra*, *M. tonkeana* haplotypes from northwest central Sulawesi near Towali and Lore Lindu National Park have a derived

relationship with respect to these species from the northern peninsula of Sulawesi. Two distinct mtDNA haplotype lineages were also uncovered in *M. tonkeana* by analysis of restriction enzyme sites in the mtDNA (Williams, 1990; Harihara *et al.*, 1996). Interestingly, one of the first studies of Sulawesi macaques tentatively recognized two species in central Sulawesi, *M. tonkeana* and *M. tonsus* (Buttikofer, 1917). Other studies suggest that *M. tonkeana* is not morphologically homogeneous throughout its range; differences within *M. tonkeana* have stimulated the proposal of another Sulawesi species, *M. togeanus*, in the eastern tip of Sulawesi (Froehlich & Supriatna, 1996).

The morphology of *M. nigrescens* and *M. nigra* is different from that of *M. tonkeana* throughout its range, including northwest central Sulawesi (Fooden, 1969; Albrecht, 1978). The morphology of *M. nigra* and *M. nigrescens* is so unique that they have been placed in a separate genus in the past (Hill, 1974, but see Fooden, 1976). The diversification of *M. nigra* and *M. nigrescens* may be a consequence of genetic drift in the north peninsula that occurred while descendants (with derived mtDNA haplotypes) near Towali and Lore Lindu National Park introgressed with primitive *M. tonkeana*-like inhabitants of central Sulawesi.

The Togeian Islands, located between Bunta and the northern peninsula of Sulawesi, offer a possible shortcut for dispersal from central to north Sulawesi that bypasses the primitive *M. hecki* haplotype. These islands were probably connected to the eastern tip of central Sulawesi during Pleistocene oceanic regression but they were not connected to the northern peninsula (Froehlich & Supriatna, 1996). Macaques that currently inhabit Malenge Island (an island within the Togian archipelago) were probably introduced by humans (Babo, 1995). Thus the complex distribution of mtDNA haplotypes of *M. tonkeana* is probably a result of either a polymorphic ancestor or multiple dispersal events to central Sulawesi.

Comparative biogeography and conservation of Sulawesi

Regardless of how Sulawesi's fauna reached this island, Pleistocene climatic and tectonic changes have clearly had a universal impact on local evolution. Patterns of endemism roughly overlap with distributions of macaque species in Sulawesi, though differences exist, possibly due to regional extinction and variation in dispersal and life history (Groves, 1980, 1985; Musser, 1987; Shekelle *et al.*, 1997; Walton, Butlin & Monk, 1997). Each of these seven regions of Sulawesi is thus worthy of separate consideration for conservation management. *M. nigrescens* and *M. brunnescens* are probably best protected by national parks which compose a significant portion of their range; other Sulawesi macaques are less well protected (Supriatna, 1991). In addition to habitat loss, poaching of macaques (even within National Parks) for use as pets or food poses a serious threat to these animals, especially *M. nigra* (Lee, 1996). Based on mtDNA haplotype phylogeny, macaques from north/central Sulawesi and from the southern peninsulas of Sulawesi appear of separate origin and are likely to be evolutionarily distinct. Further conservation resources should be allocated to the endangered Sulawesi macaque species *M. nigra* (tip of the northern peninsula) and *M. maura* (southwestern peninsula), which are among the most divergent pairs of Sulawesi macaques and also those with the most fragmented habitat. In order to preserve Sulawesi's unique fauna, further protection of habitat is particularly urgent, as development and land conversion are occurring at an alarming pace.

SUMMARY OF RESULTS

- (1) *M. fascicularis* is not a sister taxon of any species of Sulawesi macaque.
- (2) *M. nemestrina* mtDNA haplotypes are paraphyletic with respect to the mtDNA haplotypes of the Sulawesi macaques.
- (3) Cladistic and likelihood analyses postulate different relationships among mtDNA haplotypes of *M. nemestrina*. Cladistic analysis suggests that relationships are divided into northern and southern clades that correspond to Pleistocene drainage basins of the Sunda region. Likelihood analysis suggests that relationships are partitioned into eastern and western regions of the Sunda shelf.
- (4) Sulawesi was probably colonized twice by macaques from Borneo. One colonization reached the base of the north peninsula (now occupied by *M. hecki*) and a second colonization reached the base of the southwest peninsula (now occupied by *M. maura*).
- (5) Within north/central and southern Sulawesi, patterns of dispersal are largely consistent with contemporary and past geography of the island, with the exception of a geographically discontinuous relationship between *M. nigra* and a portion of *M. tonkeana* from northwestern central Sulawesi.

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