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THE UNIVERSITY OF ALBERTA

Speciation, Kin identification, and DNA polymorphisms in *Macaca fuscata*

BY

CAROLINE MAE SEKULICH LANIGAN

A THESIS

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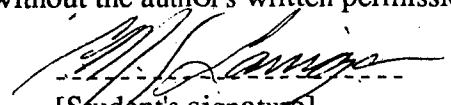

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled

Speciation, Kin identification, and DNA polymorphisms in *Macaca fuscata*

submitted by CAROLINE MAE SEKULICH LANIGAN in partial fulfilment of the requirements for the degree of
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This Thesis is dedicated to

my husband, Gordon A. Lanigan,

in appreciation of his support and understanding;

and to

Linda M. Fedigan,

without whose gentle prodding, this thesis would not have been completed.

ABSTRACT

The genus *Macaca* is one of the largest of the primate genera, being composed of at least 11 species. *Macaca mulatta* is indigenous to Asia, and on the basis of genetic similarity it is supposed that a mulatta-like colonizing species migrated to the Japanese islands. The general consensus is that *Macaca fuscata* diverged from the founding population approximately 500,000 ybp [based on estimated rates of gene substitution at protein and blood group loci]. Hybrids of *M. mulatta* and *M. fuscata* are not only viable but very fertile, having a fecundity rate exceeding that of *M. mulatta*. It is more reasonable to consider these groups as races than as separate species.

Genetic analysis of Japanese macaques (*Macaca fuscata*) has revealed a level of protein polymorphism similar to extremely isolated species (cave dwelling fishes and subterranean rodents), bradytelic lineages (the horseshoe crab); or species which recently experienced a severe population reduction (cheetahs). The level is low enough to preclude individual differentiation. This paper reports the results of a preliminary survey describing the use of recombinant DNA techniques to detect genetic variation in *Macaca fuscata*. DNA was extracted from whole blood samples of Japanese macaques from the Arashiyama West population, and digested with restriction endonucleases. A clone of a unique human DNA site (pAW101/D14S1) was used to probe these genomes. This probe reveals at least 6 different restriction fragment lengths at the sites identified by HindIII digestion, and at least 9 at the HaeIII sites. A method for reconstructing genealogical relationships using a unique DNA probe (such as pAW101) is also described.

Recombinant DNA analysis reveals that a relatively monomorphic species, with respect to electrophoretic variation of serum proteins and enzymes, may be highly variable at the DNA level. Correlation of genetic variation with phenotypic parameters, behavior or morphology, permits the testing of evolutionary hypotheses. A genetic analysis of the genus *Macaca* which correlated genetic change with morphological change may provide insights to hominid evolution as a consequence of the similarity of this genus to early hominid taxa [for example, a wide range, flexible behavior, and adaptability].

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TABLE OF CONTENTS

INTRODUCTION 1

BIBLIOGRAPHY 4

I. SPECIATION IN *MACACA* 5

INTRODUCTION 5

THE GENUS MACACA 5

MACACA FUSCATA AND *MACACA MULATTA* 8

SPECIES AND SPECIATION 9

CONCLUSION 14

BIBLIOGRAPHY 17

II. KIN IDENTIFICATION AND DNA POLYMORPHISMS IN JAPANESE MACAQUES, *MACACA FUSCATA* 49

MATERIALS AND METHOD 51

RESULTS 53

DISCUSSION 55

CONCLUSION 56

LITERATURE CITED 58

APPENDICES 66

APPENDIX 1: NEI'S D WITH RESPECT TO SPECIATION LEVELS IN DROSOPHILA WILLISTONI SPECIES GROUP, ADAPTED FROM AYALA, 1975

APPENDIX 2: REPORTED VALUES FOR NEI'S D, BETWEEN SELECTED MACACA SPECIES

GENERAL DISCUSSION 67

BIBLIOGRAPHY 69

List of Tables

Chapter 1: Speciation in *Macaca*

TABLE 1:	Geological time scale and the emergence of *Macaca*	22
TABLE 2:	Ecology of *Macaca mulatta* and *Macaca fuscata*	23
TABLE 3:	Taxonomic categories for distinguishing the species of *Macaca*	24
TABLE 4:	Species in the genus *Macaca*	25
TABLE 5:	Sexual signaling in *Macaca*	26
TABLE 6a:	Intrageneric hybrids of the genus *Macaca*	27
TABLE 6b:	Intergeneric hybrids of the genus *Macaca*	29
TABLE 7a:	Genetic distance between different populations of a macaque species	30
TABLE 7b:	Genetic distance between macaque species by source	31
TABLE 8:	Genetic variation in macaques	32
TABLE 8a:	Electrophoretic phenotypes observed in macaques	32
TABLE 8b:	Nonvariable loci in macaques	34
TABLE 8c:	Number of shared alleles among macaque species	35
TABLE 9:	Morphology of *Macaca mulatta* and *Macaca fuscata*	37
TABLE 10:	Social organization of *Macaca mulatta* and *Macaca fuscata*	38
TABLE 11:	Comparison of sexual signalling in sympatric macaque species and *M. fuscata*	39
TABLE 12a:	Pattern of sexual signaling in *Macaca fuscata*	40
TABLE 12b:	Female pattern of sexual signaling in *Macaca mulatta*	40
TABLE 13a:	Genetic identity and genetic distance between speciation products of the *Drosophila willistoni* group	41
TABLE 13b:	Range of genetic distance values between speciation products of the *Drosophila willistoni* group	42
TABLE 13c:	Willistoni *Drosophila* species and their speciation categories	43

List of Figures

CHAPTER 1: Speciation in *Macaca*

FIGURE 1: Distribution of *Macaca* 44

FIGURE 1a: Distribution of Fooden's groups 44,45

FIGURE 1b: Distribution of *Macaca mulatta* and *Macaca fuscata* 46

FIGURE 2: Morphology of macaque genitalia 47

FIGURE 3: Partial hybrid sterility: observed patterns of hybrid sterility in the subspecies of *Drosophila willistoni* 48

CHAPTER 2: Kin identification and DNA polymorphisms in Japanese macaques, *Macaca fuscata*

FIGURE 1: Genealogical relationships of subject animals traced through maternal lineages 60

FIGURE 2: Diagrammatic representation of hybridization patterns 61

List of Plates

CHAPTER 2: Kin Identification and DNA polymorphisms in Japanese macaques, *Macaca fuscata*

Plate 1:	Survey subjects - HindIII digestion	62
Plate 2:	Survey subjects - HaeIII digestion	63
Plate 3:	Matrifamily subjects - HindIII digestion	64
Plate 4:	Matrifamily subjects - SstI digestion	65

INTRODUCTION

A persistent problem in paleoanthropology is to determine the amount of morphological divergence necessary to designate fossil hominids as representing different species. One means of addressing this problem is to test fertility levels in the hybrid offspring of analogous species and to measure levels of morphological divergence observed in different speciation products. It should be possible to construct criteria, based on morphological divergence correlated with genetic divergence measures in extant primates, to assist in the taxonomic designation of fossil forms. This thesis proposes that the genus *Macaca* represents an appropriate model. Chapter 1 discusses the genetics, reproductive behaviors, and hybridization ability of *Macaca*, and addresses in particular the species *Macaca fuscata* and *Macaca mulatta*. Chapter 2 reports on a study describing a method of detecting genetic variation in *M. fuscata*. and proposes a methodology for constructing geneological relationships.

Macaca is one of the largest of the primate genera, being composed of at least eleven species [11 - 22 different species are recognized by various authors]. *Macaca mulatta* is indigenous to Asia; and, on the basis of genetic similarity, it is supposed that a mulatta-like colonizing species migrated to the Japanese islands. The general consenus is that *Macaca fuscata* diverged from the founding population approximately 500,000 ybp based on estimated rates of gene substitution at protein and blood group loci, using various calibrations of the molecular clock. Hybrids of *M. mulatta* and *M. fuscata* crosses are not only viable but very fertile, one known hybrid having a fecundity rate exeeding that of either parent species [Wolfe, 1986]. From the perspective presented in this thesis, it is more reasonable to consider these groups as geographic variants rather than as separate species. The general position of this paper is that designating groups which can produce viable and fertile hybrid progeny as separate species is inappropriate. Such application of species designations creates cognitive categories that increase, rather than decrease, the difficulty of understanding evolutionary relationships.

A second issue of importance to anthropology is the utility of certain evolutionary hypotheses in the understanding of primate evolution. In particular, the hypotheses subsumed under the title Sociobiology are of interest to primatologists. The underlying assumption of sociobiological ideas is that behavior has an evolutionary effect; in particular, that behavior can be linked with genetic change in a population. The critical assumption is that behavior has a genetic component that is manifested in differential replication of certain genes; whether it be directly, through an individual's personal fecundity, or indirectly, through the differential replication of a relative's genes. The first step in testing these hypotheses is to measure

individual reproductive success. A commonly used procedure is to describe a species with respect to genetic variation, and then to use these variants either for determining genetic distance or for paternity testing.

In recent years, data on levels of genetic variation have become available in an increasing number of species. Although protein electrophoretic variation has generally been high, some studies have revealed extremely low levels of variation. An example of a relatively non-variable species is the Japanese monkey, *Macaca fuscata*. Nozawa and collaborators surveyed 33 groups of Japanese macaques, representing 1646 individuals for 32 serum protein loci. The proportion of polymorphic sites present in a population and the average heterozygosity per individual of this species [P_{poly}=9.2%, H_{ind}=1.3%, Nozawa *et al.*, 1982] is comparable to that of extremely isolated species, or of stable evolutionary lineages [bradytelic species], and species that are speculated to have experienced a recent, extreme, population reduction [Avise and Selander, 1972; Selander *et al.*, 1970; Nevo *et al.*, 1974; O'Brien *et al.*, 1985]. In addition, only 21.8% of the loci examined in Japanese macaques had three or more alleles. None of the groups tested had more than three alleles present at a single locus. In fact, when a locus revealed a polymorphism, the variant was often present at a low level [the frequency range observed in the alternative allele on average was 2.11-8.25%]. These figures are far lower than that observed in other animals. For humans and the drosopholids, the proportion of polymorphic loci and the average heterozygosity per individual has been estimated as P_{poly}=50%, H_{ind}=12%, where 30% of loci tested had 3 or more alleles [Speiss 1977, p84; see also Nei and Roychoudhoury, 1974].

However, species considered to be relatively nonvariable with respect to soluble proteins, may be highly polymorphic at the DNA [deoxyribonucleic acid] level. Recent sequence analyses of genetic loci have revealed that in spite of a lack of variation in electrophoretic mobility, many alleles may exist at a single locus. For example, the alcohol dehydrogenase locus [ADH] in Drosophila species has only two major electrophoretic variants. At least 29 DNA variants out of 49 chromosomes examined have been identified for a 13 kilobase [kb] region which includes the ADH locus [Aquadro *et al.*, 1986]. Similarily, Kreitman [1983] describes eleven different nucleotide sequences at this locus. Both authors speculate that every individual may be unique in terms of their DNA sequence for this region, variation which is only partially addressed by restriction enzyme analysis. Such data may force a re-evaluation of current evolutionary theory regarding the significance and maintenance of genetic variation in natural populations.

The population of interest to this project consists of the Japanese macaques of Arashiyama, Japan. Ethological observations of these animals began in 1954. In 1966, the group fissioned along maternal lineages into two subgroups (designated A and B). These matrilines represent the descendants of female animals whose biological relationships could not be determined at the time observation of the group began. In 1972, group A, consisting of 147 animals and representing eight maternal lineages, was relocated as an intact social unit from Japan to a ranch in south Texas, USA. No new animals have been introduced to the group since their relocation. The Texas group, Arashiyama West, is presently composed of approximately 400 animals living as a provisioned, semi-free ranging group [Fedigan, personal communication].

Clearly, the Japanese macaque exhibits a far more restricted reservoir of electrophoretic genetic variation than the animals previously discussed (see Smith *et al.*, 1984 for a discussion). Specifically, the levels of genetic variation observed preclude paternity exclusion studies in this species. In contrast, the closely related species, *Macaca mulatta*, is polymorphic enough to permit a combined paternity exclusion probability, in terms of "polymorphic red cell surface antigens, electrophoretic markers, and leukocyte antigen loci ... as high as 0.995" (Smith *et al.*, 1984).

The Arashiyama population provides the rare opportunity to link behavioral data with population genetics. Given the recent history of this group, should a suitable genetic locus be identified, the population provides the opportunity to test evolutionary hypotheses of male reproductive success. Other studies to which this population is particularly suited for research are: [1] the effects of inbreeding in a free-ranging population, [2] the effects of breeding structure on subsequent gene frequency change [in particular the effect of the number of breeding males], [3] measurements of mutation rates in a long lived species, and [4] the effects of selection given the group's adaptation to a dramatically different environment. It is also possible to compare these measures with that of the parent population.

The primary objective of the research reported here was to identify a genetic locus sufficiently variable for paternity exclusion studies. Preliminary results of recombinant DNA analysis of a highly variable, genetic locus in *Macaca fuscata* are reported [Lanigan, *et al.*, in review]. A claim is made that if suitably detailed genetic studies can be made, correlating genetic variation between populations with reproductive isolation, morphological divergence can be correlated with genetic change. Once this is accomplished, the path of speciation observed in the fossil record may be more fully elucidated.

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INTRODUCTION

Taxonomy and phylogeny are closely related in that a good taxonomic classification strives to reflect evolutionary relationships. Morphological, behavioral, and biogeographic information are most often used to construct a classification; rarely is genetic data utilized or available. In fact, hypotheses on the speciation process itself are often based on morphology and biogeography, not on genetic change [Coyne and Orr, unpublished].

Correct interpretation of the genetic structure of a population requires an understanding of the evolutionary history of the species. Genetic data, once collected, may prescribe a re-evaluation of the taxonomic relationship of a population relative to other closely related populations. This appears to be the case for the genus *Macaca*. Hybridization and gene frequency data suggest that the Japanese [*Macaca fuscata*, Blyth 1875] and rhesus [*Macaca mulatta*, Zimmerman 1780] macaques, in particular, do not appear to represent 'good species', in that hybridization produces viable and fertile progeny. This chapter argues that genetic differences among members of the genus *Macaca* render the entire genus problematic, and that the genetic relationship of *Macaca mulatta* and *Macaca fuscata* does not warrant making categorical taxonomic distinctions beyond the level of the Mendelian population.

THE GENUS *MACACA*

Simons [1974] suggests that all extant macaques are descended from one early form. This species spread through the European and Asian continents, and by 1 to 3 million years ago, had reached the extremes of its range [as quoted in Fittinghoff 1978]. The earliest macaque is *Macaca libyca*, dated at 6 million years BP, a late Miocene form found in Egypt. The European and North African fossil forms [very similar to modern *M. sylvanus*] represent the most common cercopithecid during the Villafranchian [late Pliocene] and Pleistocene epochs. The macaque evolutionary picture seems to be one where these early forms migrated out of Africa, and inhabited a monsoon or seasonal forest niche. A type of speciation explosion occurred during the late Pliocene and lower Pleistocene, when macaques occupied and adapted to many new ecological niches, demonstrating extreme genetic and phenotypic plasticity [Table 1]. During these periods macaques were associated with a warm climate and deciduous forests, alternating with open parkland and steppe regions [Eudey 1980].

At present the genus *Macaca* is the most widespread primate genus after *Homo*. An example of their adaptability is in their diet - as omnivorous animals, macaques have been observed to consume foliage, fruit, seeds, and to capture and consume insects [Jolly 1985, Napier and Napier 1985, Bramblett 1976]. However, in addition to these 'traditional' food categories [for primates], macaques have also been observed to exhibit what Fittinghoff succinctly calls 'opportunistic omnivory' [Fittinghoff 1978]. For example, during particularily severe winters, the Japanese macaque consumes bark [Table 2]. This flexibility is also observed in the ability of macaques to learn to exploit radically new flora: again the Japanese macaque, when relocated from the temperate ecozone of Japan to a desert environment in Texas, USA., exploited foodstuffs indigenous to this new environment [Fedigan 1982]. At present, members of the genus inhabit climates where the ambient temperature ranges from -15°C to +30°C; and hence, from temperate to tropical ecologies, between latitudes 45°N and 15°S [Figure 1].

Macaque species are differentiated by a number of morphological and geographic criteria [Table 3]. In general macaques are medium sized [head and body length averages about 50 cm], relatively stocky animals, bearing a yellowish-brown, undistinguished coat [Bramblett 1976, Napier and Napier 1985, Osman-Hill 1972]. Nonmetric traits vary widely within the genus; e.g. presence of tail, ischial callosities, coat color and hair length patterning [Napier and Napier 1985, Osman-Hill 1972]. Sympatric species tend to be differentiated by morphological criteria; and allopatric groups are assigned separate species names by area, especially those that are the only indigenous species to an area [for example, *Macaca cyclopsis* and *M. fuscata* are the only indigenous macaques on their respective islands]. Table 4 lists 38 differently named *Macaca* species [including fossil forms]. Some disagreement exists as to the actual number of species included in this genus, as well as which populations represent separate species. This disagreement extends to the fossil forms as well as extant populations, as the fossil evidence is fragmentary and the early forms are very similar to colobines [Table 4].

The social organization of macaques is very consistent. All species live in multimale-multifemale groups in which female behavioral hierarchies are stable and males emigrate from the natal group [Jolly 1985]. Some macaque species are noted as being seasonal breeders [for example *M. fuscata* and *M. mulatta*], some exhibit pronounced sexual swelling and skin reddening [*M. nemestrina*], and others show few physical signs of sexual receptivity [*M. sinica* and *M. radiata*] [Table 5]. Gestation length is consistent within the genus, lasting approximately 165 days [Napier and Napier 1985]. These are

long lived species, in which sexual maturity occurs at between three and five years of age in females and at four and five years in males, with a lifespan of approximately 30 years.

All macaque species bear 2n=42 chromosomes. No significant interspecific banding pattern variation has been observed; that is, all homologous chromosomes between macaque species bear cytogenetic differences at a level that does not preclude successful mating [in which successful matings are those that produce progeny]. In some species, notably *M. mulatta* and *M. fascicularis*, the banding pattern of chromosomes are identical [DeVries, *et al.* 1975]. Any variants can be explained as minor readjustments of the chromosomes, probably of the order seen in humans in which inversions [both paracentric and pericentric] occur, but individuals bearing such chromosomes are phenotypically normal [Soudek 1973, Chiarelli 1962]. Although Soudek states that the karytyped individuals were 'normal' phenotypically, she means normal in the clinical sense - at the time the sample was obtained the individual was not clinically diseased. Therefore, the occurrence of such chromosomal rearrangements may have evolutionary ramifications in that such individuals may experience reduced fertility or be sterile.

Striking morphological differences occur in the genitalia of the genus, as shown in Figure 2; and Fooden (1980) differentiates members of his four subgeneric groupings primarily on the basis of genitalia morphology, and then uses other morphological, geographical, and behavioral characteristics to distinguish species [Table 4]. The ancestral type is taken to be of the silenus-sylvanus group, with the fascicularis group diverging first, and sinica and arctoides groups diverging subsequently [Fooden, 1980]. Given the morphological variation in genitalia, it would seem that viable fertile hybrids should occur only within Fooden's subgeneric groups. Within Fooden's groups successful hybridization occurs, but hybridization also occurs across Fooden's groups [Table 6a]. For example, note the following crosses, from which viable progeny resulted

SPECIES NAMES		FOODEN'S GROUP	
M. mulatta	x *M. nemestrina*	fascicularis	x silenus-sylvanus
M. fascicularis	x *M. radiata*	fascicularis	x silenus-sylvanus
M. mulatta	x *M. radiata*	fascicularis	x sinica
M. mulatta	x *M. arctoides*	fascicularis	x arctoides
M. nemestrina	x *M. fascicularis*	silenus-sylvanus	x fascicularis

It is clear that species boundaries are not distinct within this genus. Backcrosses and F2 crosses result in viable progeny [Table 6a-1c]. Some of these intra-subgeneric hybrids are known to be fertile. Even more confounding is the production of intergeneric

hybrids [Table 6b]. Although viability and fertility data are incomplete, it appears that mating is not completely inhibited between genera. In fact, viable progeny are produced from some of these matings, suggesting that speciation, in terms of postzygotic reproductive isolation, is not complete between these populations. Since intergeneric hybridization occurs, it is difficult to justify the classification of these animals into different genera let *al.* one support the separate species designation of various *Macaca* populations.

A number of workers have computed genetic distances between different populations of one macaque species and between the different species of *Macaca* [Tables 7a and 7b]. In fact, from his data, Nozawa *et al.* [1977] conclude:

> 'the classification system of the Asian macaques would be more acceptable biologically by lowering the so-called "genus" to the rank of species and the so-called "species" to the rank of subspecies' [p.27].

MACACA FUSCATA AND *MACACA MULATTA*

Nozawa *et al.* [1982] postulates that a small founding population of rhesus-like macaques entered Japan across a land bridge from Korea approximately 700,000 yBP. This hypothesis requires that the Japanese Islands be connected to Korea as a result of the drop in sea level during a Pleistocene glaciation period. The most likely time period would be during the Riss or Mindel glaciations [Table 1]. Delson [1980] and Eudey [1980] give a Pleistocene date for the divergence of *Macaca fuscata* from an ancestral population which corresponds to Nozawa's divergence date of 530,000 YBP based on electrophoretic data [Nozawa *et al* .1977].

Japanese macaques are characterized by a paucity of genetic variation. The species exhibits clinal distribution of genetic variation in which groups within 100 km of each other on the same island show a positive correlation of the presence of an electrophoretic variant with distance. Nozawa *et al.* [1982] state that almost every variant in Japanese macaques is present in the rhesus monkey, making a rhesus-like ancestor all the more appealing [Table 8]. An examination of the alleles shared between macaque species reveals that *Macaca mulatta* and *M. fuscata* share the greatest proportion of alleles with each other; and that in all comparisons, *M. mulatta* shares more alleles with all other species [for which data is available] than any other species [Table 8c]. Although various phylogenies place *M. fuscata* as closely related to such species as *M. fascicularis* and *M. cyclopsis*, the general consensus is that *M. mulatta* seems the most generally adapted animal; and probably resembles closely the ancestral population to all these species [Melnick and Kidd 1985, Nozawa *et al* .1977, Cronin *et al* .1980, Avise and Duvall 1977].

Morphologically both rhesus and Japanese macaques are described as yellowish-brown, medium sized, quadrupedal, terrestrial animals. Coat color and hair length patterning differs little between these species. In fact, Japanese and rhesus macaques are often described in terms of each other [Bramblett 1976, Napier and Napier 1985]. The main difference between the species is in weight and tail length; Japanese macaques are somewhat larger and have very short tails as compared with rhesus monkeys [Table 9].The rhesus macaque inhabits much of Asia, China, Korea, and Indochina; whereas Japanese macaques are the only macaque indigenous to the islands of Japan. From this information one would presume that the Japanese monkey is adapted to a temperate zone whereas the rhesus monkey is a more general animal in that it is indigenous to both temperate and tropical climatic zones.

Very few differences occur between these species in terms of social organization and ecology [Tables 2 and 10]. Japanese and rhesus monkeys conform to the general macaque pattern of social organization. Both live in multimale-multifemale groups, exhibit male emigration and stable female behavioral hierarchies, and live in groups ranging in size from eleven to 100 individuals. One would expect that, since the rhesus monkey occurs sympatrically with a number of other macaque species, *M. nemestrina, M. assamensis, M. fascicularis, M. arctoides, and M. thibetana.*, behavioral differences between the sympatric species, especially with respect to mating, would be significant [Table 11]. Japanese and rhesus macaques have mated naturally in a free ranging environment, suggesting that mating behavior which discriminates extraspecific macaques as inappropriate mating partners is not manifested in these species [Table 12a and 12b]. These matings have produced viable, fertile hybrid offspring [Wolfe 1986]. In fact, a female hybrid born in 1968 in the Arashiyama East [Japan] group had borne 6 fertile offspring by the age of 10 years. She first gave birth at four years of age, and her relative reproductive success exeeded that of three of four of her birth cohort of two rhesus and two Japanese monkeys [Wolfe 1985]. Clearly no reduction in evolutionary fitness is evident in this individual.

SPECIES AND SPECIATION

Modern evolutionary theory asserts that over time, a population will change in terms of the proportions of observed phenotypes. Characteristics that increase the adaptive value of the phenotype will tend to confer differential reproductive success on the members of an interbreeding population, resulting in, over time, a change in gene frequencies. Both races and species are defined in terms of the frequency of specific genes. The problem now

becomes obvious - to determine when a level of genetic divergence warrants designating groups as belonging to different species or to different races.

Genetic differences refer to heritable variants, more specifically to the occurrence of different homologous genes [alleles] in one or more individuals. Genetic differences can be described between genes, between individuals, [in terms of their phenotype or of allelic differences], or in terms of the frequency of heritable phenotypic differences in groups of individuals. Groups can then be compared with respect to the frequencies of particular alleles. When taxa can be subdivided into groups, on the basis of the frequency of heritable differences, these subdivisions are called races, ecotypes, or local populations. Variation within a species which results in demic differentiation [into races, ecotypes, or local populations] includes not only differences in gene frequency, but also chromosomal [or karyotypic] variants. In other words, genetic rearrangements [translocations, inversions, etc.] can in some cases have no evolutionary impact.

Ultimately, diverging populations manifest different gene frequencies, requiring a more precise taxonomic designation reflecting their divergence from the ancestral group. The important feature of this divergence is its effect upon the reproductive capacity of the diverging populations: Reproductive isolating mechanisms are defined by Dobzhansky (1962) as: "any genetic agencies that decrease or prevent gene exchange between species" [p. 184]. The mechanisms of reproductive isolation pertinent to the current discussion are classified as follows [after Dobzhansky 1970, p. 314]:

1. Premating or prezygotic mechanisms preventing the formation of hybrid zygotes
    a. Ecological or habitat isolation. The populations concerned occur in different habitats in the same general region.
    b. Seasonal or temporal isolation. Mating periods occur in different seasons.
    c. Sexual or ethological isolation. Members of different groups either do not or very rarely attempt to mate.
    d. Mechanical isolation. Physical noncorrespondence of the genitalia prevents copulation.

2. Postmating or zygotic isolating mechanisms affect the fertility and viability of hybrid zygotes.
    g. Hybrid inviability. Hybrid zygotes have reduced viability or are inviable [i.e. are not formed].
    h. Hybrid sterility. The F1 hybrids of one sex or of both sexes fail to produce functional gametes.
    i. Hybrid breakdown. The F2 or backcross hybrids have reduced viability or fertility.

It is important to note that these mechanisms operate in speciation, but they are not definitions of species. Populations which exhibit ethological reproductive isolation are not necessarily genetically different [e.g. assortative mating; humans assortatively mate on the basis of height and skin color], and genetically different populations are not necessarily reproductively isolated [e.g. human races are genetically different groups but members of different racial groups can freely hybridize]. To determine if different populations represent different species, it is necessary to know the fate of hybrids. In the long run, if the populations are not completely reproductively isolated [postmating mechanisms are not present], the demes ultimately share a common gene pool. In the short run, morphological differences may result in assortative mating which mimics a speciation event.

Ayala (1975) in a very cogent analysis of the speciation process in Drosophila species, defines subspecies as "allopatric populations which exhibit partial hybrid sterility". That is, hybrids produced are sterile depending on their sex or on which parent was a member of a particular species [see Figure 3]. Such groups are, according to Ayala, in the first stage of the speciation process. A semispecies is a subpopulation of a single species in which complete hybrid sterility occurs. These subpopulations may mate and produce viable offspring, but these hybrids are completely sterile. Sibling species are morphologically indistinguishable groups, which have attained complete reproductive isolation. In other words, matings between members of sibling species does not result in offspring. Finally is the occurrence of morphologically distinguishable species. The populations are completely reproductively isolated; and can be classified, on the basis of morphological characteristics, as separate species. These speciation products represent both morphologically identical [within a range of variation] populations, and morphologically distinct populations, which cannot successfully [in any sense] interbreed. Ayala computed values for genetic identity [I] and genetic distance [D] between these levels of reproductive isolation [see Tables 13a and 13b]. [These values are based on electrophoretic variation of Drosopholid proteins. Ayala tested 36 enzyme loci, and the genotypes of several hundred to several thousand individuals in each of six sibling species plus four subspecies and six semispecies as listed in Table 13c.]

Ayala also reviewed genetic distance data in other invertebrates, fishes, salamanders, lizards, and mammals; and found a surprising correlation between his Drosophila data and these other taxa. In general, ten to 25 allelic substitutions occur in the evolution of subspecies. He expects that semispecies will show comparable levels of allelic substitution, and that the evolution of reproductive isolation does not require a large change

in alleles present. This result also suggests that reproductive and perhaps morphological evolution may not require a large genetic change, but may be more dependent on the kind of genetic change; for example, in gene regulation rather than in gene structure.

If the values of Nei's D [genetic distance calculated from electophoretic data] for various macaque species are compared with Ayala's values for various speciation products of the *Drosophila willistoni* group, one finds that the groups that have been surveyed by population show a genetic distance range within Ayala's local population level [Tables 13b and 7b]. At the 'species' level, only Cronin *et al* . [1980] compute values in which all macaque species tested are differentiated at the species level [Table 7b]. Every value reported by Cronin *et al* . exceeds 0.300, which clearly marks the sibling/morphologically distinct species class [species this distant are zygotically, reproductively isolated]. Avise and Duvall [1977] and Nozawa *et al* .[1977] compute more ambiguous figures in which, fitting the hybridization pattern of the genus, various species comparisons range from the local population through to the sibling species level of differentiation [Table 7b]. Cronin *et al*.'s method differs from the other workers in that they makes two important assumptions: [1] that a single animal is sufficient to represent a species; and [2] that protein mobility differences detectable with a general protein stain, which would generate multiple bands representing many genetic loci, are significant at the phylogenetic level.

The argument of Cronin *et al* . is that any phyogenetically significant differences will be present in any animal of a particular species, and that this individual will exhibit enough species specific bands to ensure that it will be sorted into its own category based on this variation. The problem with this approach is that most of the proteins detectable by this method are of unknown etiology. In addition, Cronin *et al*. give no information on their sample animals. Immature individuals are known to exhibit age-related proteins. This problem alone would make classification of individuals spurious, not to even address the problems of sample size. If the purpose of phylogeny construction is to determine evolutionary relationships between organisms, it is incorrect to assume a priori that differences between groups designated as different species will be significant. The purpose of the test is to determine if the differences are phylogenetically significant.

Ayala [1975] has clearly demonstrated that relatively little genetic change occurs between various speciation categories; therefore Cronin *et al*.'s results seem unreliable [see also Melnick and Kidd 1985]. Nozawa *et al* .[1977] and Avise and Duvall [1977] compute values that fit the observed genetic fluidity of the genus.

For species to diverge, mating between groups must result in a reduction of either viability or reproductive ability in the hybrids produced. Selection will then operate to eliminate mating between the groups as a consequence of the hybrid depression. The hybrids then must either die before reproducing, or be unable to reproduce at all. The determining feature of the completion of the speciation process is that mating between members of reproductively isolated populations does not result in progeny. Only if this feature is present is it appropriate to designate the populations as separate species. Other manifestations of reproductive isolation should be labelled as one of the previously discussed speciation products. This position leads us to the crux of this discussion: is the criteria for designating populations as separate species one of degree of differentiation, or is it in kind of differentiation?

Are we concerned with quantitative or qualitative change in the speciation process? From the above evidence it is insufficient to designate populations as representing separate species unless it can be demonstrated that genetic differences are what maintain the gene frequencies present. If species are separated simply by the criteria of behavioral, temporal, or geographic differences separate species designation is difficult if not impossible to justify. Sufficient morphological divergence must be documented and correlated with genetic divergence at a level that ensures complete reproductive isolation.

Consider the phenomenon of phenotypic plasticity. Phenotypic plasticity refers to morphological differences in phenotype due to the developmental environment. One genotype in two environments can result in two dramatically different phenotypes or phenocopies [Suzuki, *et al.* 1986, p. 458]. Such developmental effects can produce developmental 'morphs': nongenetic in origin, but which mimic, in morphology, known genetic effects. The lesson of this observation is that if the relationship between a morphological character and the genes is not known, it is not possible to interpret a consistent morphological variant as being genetic in origin. It could be due to some environmental stimulus which redirects the course of development.

At this point, the only available correlation of morphological change with genetic divergence is that of Ayala's drosophila data. Ayala has linked Nei's D with fertility information, permiting a comparison with other taxa. The current task of evolutionary biology is to determine if comparisons of this sort are meaningful, or to document other ways to correlate phenotypic data [morphological, biochemical, etc.] with genetic data. Although it has been suggested that speciation in drosophila may be different from that in other animals, I would suggest that the difference is that drosphila species have been far

better studied both in the wild and in the lab. Critical matings can be induced in these animals, whereas, in other animal taxa, manipulation of matings is far more difficult.

I would suggest that quantitative change characterizes races, but that qualitative change defines the species. The only evolutionarily significant kind of change is that which affects populations' ability to hybridize. For the two concepts to be useful, the difference between a race and a species must be a difference in kind, not simply one of degree. In my opinion, for populations designated as separate species postmating mechanisms of reproductive isolation must be present. Therefore, it is only appropriate to designate populations as belonging to separate species, when postmating reproductive isolation occurs. Any other case allows for potential successful hybridization.

The point to be made with respect to taxonomy is that taxonomists create cognitive categories which imply certain relationships between the organisms so classified. Hence, when two populations are designated as separate species, persons studying those organisms will treat their data on these populations differently than they would if the populations were classified as some subdivision of a single species.

The biological species concept is widely accepted by biologists [Futuyma 1986; p. 111] but it is apparent from the above analysis that it is not applied by all biologists to natural populations. The designation of separate species status ascribes significance to the differences between the groups, which are imputed to be primarily inheritable. The danger is of inaccurately reflecting the evolutionary relationships between groups, and imputing an unrealistic significance to the characters used by the taxonomists to assign separate species status to groups under analysis.

CONCLUSION

The criteria for designating groups as separate species must be a difference in kind of differentiation. In classifying groups as separate species, qualitative criteria must be used - the groups must be genetically different enough so that when mating occurs, no progeny result. Species categories as analyzed by Ayala [1975] are an informative means of naming species:

Local population, geographic variant	interfertile
subspecies	freely intermating, partial hybrid sterility
semispecies	intermating inhibited, partial hybrid sterility
sibling species	intersterile, morphologically indistinguishable
nonsibling species	intersterile, morphologically distinguishable

Clearly rhesus and Japanese macaques can and do mate and produce viable, fertile, hybrid progeny. The only justification for their classification is that since the two groups' ranges are not overlapping, and morphological differences do exist, the populations were placed into separate species categories.

The question now becomes: "Is geographic isolation sufficient cause for assigning separate species designations?" Clearly the answer is no. Some human populations are geographically isolated, yet there is no question that morphologically distinct and geographically isolated human groups, for example the !Kung- San of Africa, Ainu of the Japanese islands, Australian aborigines, and Hutterites of western Canada would be able to interbreed successfully should mating occur.

The Popperian philosophy of falsification [sophisticated methodological falsificationism] claims that no amount of observation can verify a theory logically; one single observation, however, can falsify it, requiring construction of an alternative theory [Lakatos 1978]. I would venture that the behavioral and fertility data resulting from experiments attempting to mate different macaque species and to measure fertility in the resulting progeny can only prove the positive case - produce progeny and one has demonstrated that these are of the same species. Aggression, lack of mating, and lack of progeny production do not prove the negative case. Clearly the entire genus *Macaca* is problematic. The case of Japanese and rhesus monkeys is less ambiguous than other macaques because of their overall homology. A single positive case, especially in these groups, can provide enough evidence to warrant their reassignment. It provides enough information to designate unambiguously these groups as sharing a common gene pool. Japanese and rhesus macaques warrant different common names, but should be considered members of a single species, i.e. macaque races. I would suggest using an inclusive name according to taxonomic convention of priority, in which these taxa are discriminated at the subspecies level; e.g. *Macaca mulatta mulatta*, and *Macaca mulatta fuscata*.[Napier and Napier 1967].

One could assert that it is impossible to reanalyze taxonomic designations of living creatures to align the names with the biological species definition using the rationale that because of the difficulty of the task we must simply live with the names as they have been assigned. If we don't apply the same definitions to the names we can't communicate effectively. If the biological species concept is accepted by biologists then it must be applied by biologists to the naming of taxa. At present, paleontologists use terms like

morphospecies, or chronospecies to qualify their names; biologists can apply the terms semispecies, subspecies, or sibling species to problematic taxa, for which Ayala [1975] has provided genetic correlations.

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Table 1: Geological time scale and the emergence of *Macaca*

ERA	PER	TIME mya	EPOCHS	GLACIATION Alpine	GLACIATION Scand.	Macaca species
C	Q	0.010	Holocene	inter	inter	
E	U					
N	A	0.040	upper	Wurm	Weischel	
O	T	0.075	Pleisto-			
Z	E		cene			
O	R	0.100		inter	Eemian	M. fascicularis
I	N	0.125				
C	A	0.150	M P			
	R	0.175	I L	Riss	cold	
	Y	0.225	D E		warm	
		0.265	D I		Saale	
C	Q	0.275	L S	Inter	inter	
E	U	0.300	T			
N	A	0.350	O		Elster	
O	T	0.380	C	Mindel	Holstein	M. speciosa subfossilis
Z	E	0.400	E		Elster	
O	R	0.430	N			
I	N	0.450	E	Inter	Cromerian	M. sylvanus, M. suevica
C	A	0.500		Gunz	Menapian	M. tolosana
	R	0.700				
C	Y	1.000	L P			M. majori, M. robusta,
E			O L			M.anderssoni, Procynocephalus
N			W E			wimani, M. speciosa subfossilis
O		1.250	E I			
Z		1.500	R S			
O	-----	2 MYA	-------VILLAFRANCHIAN----------			
I				Pliocene		M.pliocena, M.flandrini, M.prisca,
C	T					M.florentina, M.paleindicus,
	E					M.anderssoni, M.falconeri
	R	5 mya	------- RUSCINIAN [4-5 MYA]			M.sylvanus prisca
	T		Miocene			M.libyca [6 mya], M.flandrini[7mya]
	I	25 mya	------- TUROLIAN [7 MYA]			
	A		Oligocene			
	R	35 mya				
	Y		Eocene			

SOURCES: Delson, Eric (1980); Eudey, Ardith A. (1980); Fittinghoff, Nicolas A. [1978]; Hill, W.C. Osman (1972).

Legend: mya, MYA = millions of years ago
inter = inter glacial period
PER = geological period

Table 2: Ecology of *Macaca mulatta* and *Macaca fuscata*

CHARACTERISTIC		*M. mulatta*	*M. fuscata*
1. temperature tolerance:		tropical-temperate	-5 C - +37 C [2]
2. foraging group size :		11-100 [1]; 18-70 [3]	11-100 [1]
3. range size: home (km)		8.0 [3]	0.800-26.7 [1]
	per individual (km)	0.023 [5]	0.015 - 0.607 [1]
4. diet:	foliage	19% [1]; 19% [3]	yes [2,4]
	fruit, seeds, nuts	72% [1]; 9% [3]	yes [2,4]
	flowers	4% [1]; 4% [3]	yes [4]
	prey	2% [1]; 1% [3] (eggs, birds, insects)	yes [2,4]
	herbs	66% [3]	no data
	bark	no data	yes [6]
5. habitat:	mixed deciduous & evergreen forest [3]	mountainous montaine forest [4]	

SOURCES:

[1] Jolly 1985: pp. 47, 93, 117, 125, 128, 149, 226, 239
[2] Fedigan 1982: p.218
[3] Richards 1985: pp. 109, 182, 244, 308, 371
[4] Kurland 1977: p. 26
[5] Clutton-Brock & Harvey 1979; p. 205 report 44 animals/km
1km/44 animals = 0.023 km/animal
[6] Napier & Napier 1985: p.129

Table 3: Taxonomic categories for distinguishing the species of Macaca

TRAIT TYPE	CHARACTERISTIC	DETAILS
A. MORPHOLOGICAL		
	I. METRIC [Continuous traits]	
	rump patch	form
		color
	head shape	
	coat color	cheeks
		ventral surface
		body
		forelimb
		hind limb
	ischial callosities	form
		color
	tail	length
		color
	II. NONMETRIC [PRESENCE OR ABSENCE]	
	tail	
	ischial callosity	
	gluteal fields	
	crest on rump patch	
	age greying of coat	
B. BIOGEOGRAPHICAL		
	HABITAT	tropical
		temperate
	DIET	
C. BEHAVIORAL		
	BREEDING PATTERN	seasonal / nonseasonal
	FACIAL EXPRESSIONS	
	HIERARCHICAL RELATIONSHIPS	female stability
		male stability
	EMIGRATION PATTERN	female/male

Table 4: Species in the genus *Macaca* †

MACACA SPECIES NAME	Jolly 1985	Fooden 1980*	Napier 1985*	Hill 1973	COMMON NAME[s], other sources
1. anderssonni	-	-	-	yes	fossil form; Osman-Hill [1972]
2. arctoides [2]	yes	ac	ac	yes	stumptailed, bear, redfaced
3. assamensis	yes	si	si	yes	Assamese
4. brunnescens	-	ss	-	-	Muna-Butung; Groves [1980]
5. cyclopsis	yes	fa	fa	yes	Formosan rock,Taiwan
6. falconeri	-	-	-	-	fossil form; Fittinghoff [1978]
7. fascicularis [1]	yes	fa	fa	-	crabeating, longtailed, kra
8. flandrini	-	-	-	yes	fossil form; Osman-Hill [1972]
9. florentina	-	-	-	yes	fossil form; Osman-Hill [1972]
10. fuscata	yes	fa	fa	yes	Japanese
11. irus [1]	-	-	-	yes	longtailed
12. hecki	-	ss	-	-	Heck's; Groves [1980]
13. lacepede	-	-	-	-	fossil form; Osman-Hill [1972]
14. lapunder [3]	-	yes	-	-	
15. libyca	-	-	-	yes	fossil form; Osman-Hill [1972]
16. majori	-	-	-	yes	fossil form; Osman-Hill [1972]
17. maurus	yes	ss	ss	yes	moor Groves [1980]
18. mulatta	yes	fa	fa	yes	rhesus
19. nemestrina [3]	yes	ss	ss	yes	pigtailed,Paigai I, Mentawai
20. nigra	yes	ss	ss	-	Sulawesi black ape,Sulawesi crested, niger; Groves [1980]
21. nigrescens	-	ss	-	-	Gorontalo; Groves [1980]
22. ochreata	yes	ss	ss	-	ochre,booted; Groves [1980]
23. paleindicus	-	-	-	-	fossil form; Fittinghoff [1978]
24. pliocaena	-	-	-	yes	fossil form; Osman-Hill [1972]
25. praeinus	-	-	-	-	fossil form; Fittinghoff [1978]
26. prisca	-	-	-	yes	fossil form; Osman-Hill [1972]
27. radiata	yes	si	si	yes	bonnet
28. robusta	-	-	-	yes	fossil form; Fittinghoff [1978]
29. silenus	yes	ss	ss	yes	liontailed
30. sinica	yes	si	si	yes	toque
31. sivalensis	-	-	-	yes	fossil form; Osman-Hill [1972]
32. speciosa [2]	-	-	-	-	fossil form; Fittinghoff [1978]
33. suevica	-	-	-	yes	fossil form; Osman-Hill [1972]
34. sylvanus	yes	ss	ss	yes	Barbary ape
35. thibetana	yes	si	si	yes	Thibetan
36. togeana	yes	-	-	-	togian
37. tolosana	-	-	-	yes	fossil form; Osman-Hill [1972] = sylvanus
38. tonkeana	-	ss	ss	-	Tonkean,Sulawesi stumptailed; Groves [1980]
total	16	19	16	24	

LEGEND: † Jolly and Plog [1986] recognized 11 unnamed species
* Species recognized by Fooden and Napier are classified into 4 groups:
fa = fascicularis; ss = silenus-sylvanus; ar = arctoides group; si = sinica
[1], [2], [3] synonyms for the same species according to Fooden [1980]

Table 5: Sexual signaling in Macaca*

Table 5a: Female Signals

SPECIES	CYCLE LENGTH	RECEPTIVITY	PROCEPTIVE BEHAVIORS	PERINEAL INDICATOR	VAGINAL DISCHARGE
M. arctoides	31 days	noncyclic	present hind	rare	yes
M. assamensis	-	-	-	none	-
M. cyclopsis	30 days	-	-	swell	-
M. fascicularis	31 days	periodical	present hind	swell	yes
M. fuscata [2]	28 days	1-92 days	sit near,pres	reddening	yes
M. mulatta	29 days	8-11 days	present hind	redd+swell	yes
M. nemestrina	32 days	noncyclic	present hind	swell	yes
M. nigra	34 days	-	-	swell	-
M. radiata	25-36 days	-	present hind	mild swell	yes
M. silenus	40 days	noncyclic	-	swell	-
M. sinica	29 days	-	-	none	yes
M. sylvana	31 days	-	grab male	swell	-

Table 5b: Male Signals

SPECIES	SOLICIT	MOUNT PATTERN	MATING FREQUENCY	BREEDING SYSTEM
M. arctoides	yes	single	-	multimale
M. assamensis	-	-	-	-
M. cyclopsis	-	-	-	-
M. fascicularis	-	single	-	multimale, consort
M. fuscata [2]	yes	serial	2-30/hour	multimale, consort
M. mulatta [1]	-	serial	18/hour	multimale
M. nemestrina	yes	multiple	7.5/hour	multimale, consort
M. nigra	-	serial	-	-
M. radiata	tongue flicks	single	3.5/hour	multimale, consort
M. silenus	-	serial	-	unimale, multimale
M. sinica	yes	single	-	multimale
M. sylvana	-	single	-	multimale, consort

* data modified and based on Table 31-1 in Blaffer-Hrdy and Whitten [1987]
[1] Carpenter [1942]; [2] Enomoto [1974]

Table 6a: Intrageneric hybrids of the genus *Macaca*

FG	MATER	FG	PATER	SEX	VIA	FERT	NUM	SOURCE
fa	fascicularis	ss	nemestrina	mf	y	?	>1	Chiarelli 1973, Bernstein 1966
fa	fascicularis	si	radiata	f	y	?	1	Chiarelli 1973
fa	fascicularis	fa	mulatta	?	?	?	?	Chiarelli 1973
ss	hecki	ss	nemestrina	?	n	n	1	Bernstein & Gordon 1980
ss	maura	ss	nigra	?	?	?	1	Chiarelli 1973
ss	maura	ss	nemestrina	?	y	?	1	Chiarelli 1973
ss	maura	fa	fascicularis	f	y	?	1	Bernstein & Gordon 1980
fa	fuscata	fa	mulatta	f	y	y	1	Wolfe 1986
fa	mulatta	fa	fascicularis	mf	y	?	>1	Chiarelli 1973, Bernstein 1974 Bernstein & Gordon 1980
fa	mulatta	ac	arctoides	?	y	?	>1	Chiarelli 1973
fa	mulatta	ss	nemestrina	m?	?	y	>1	Chiarelli 1973, Markajaran *et al.*, 1974
fa	mulatta	si	radiata	m	y	?	1	Chiarelli 1973
ss	nemestrina	ss	silenus	mf	y	y	>2	Chiarelli 1973, Bernstein 1974 Bernstein & Gordon 1980
ss	nemestrina	fa	mulatta	f	y	y	>1	Chiarelli 1973, Bernstein 1974 Bernstein & Gordon 1980,
ss	nemestrina	fa	fascicularis	mf	y	prob	>7	Chiarelli 1973, Bernstein 1974 Bernstein & Gordon 1980, Bernstein 1966,
ss	nemestrina	ss	nigra	fm	p	y	5	Bernstein & Gordon 1980, Bernstein 1977
ss	nemestrina	si	assamensis	?	n	n	1	Bernstein & Gordon 1980
ss	nemestrina	si	irus	fm	y	?	2	Bernstein 1966
ss	nigra	fa	fascicularis	f	7h	n	1	Chiarelli 1973
ss	nigra	ss	nemestrina	mf	y	f	>3	Chiarelli 1973, Bernstein 1974, Bernstein & Gordon 1980
ss	nigra	ss	maura	m	?	?	1	Chiarelli 1973
ss	nigra	ss	silenus	fm	y	?	2	Bernstein 1974, Bernstein & Gordon 1980
ss	nigra	ss	tonkeanna	m	y	?	1	Bernstein 1974, Bernstein & Gordon 1980
si	sinica	si	radiata	f	y	?	1	Chiarelli 1973
ss	silenus	ss	nemestrina	mf	y	y	>1	Chiarelli 1973
ss	tonkeanna	ss	maura	mf	y	?	3	Bernstein & Gordon 1980
ss	tonkeanna	ss	nemestrina	m	1d	?	1	Bernstein & Gordon 1980
ss	tonkeanna	ss	nigra	?	n	n	1	Bernstein & Gordon 1980

Table 6a [continued] : Intrageneric hybrids of the genus *Macaca*

1b. NATURALLY OCCURRING HYBRIDS

FG	MATER	FG	PATER	SEX	VIA	FERT	NUM	SOURCE
ss	hecki	ss	tonkeana	?	yes	?	>1	Groves 1980
ss	maura	ss	tonkeana	?	no		>1	Groves 1980
ss	nigra	ss	nigrescens	?	yes	?	>1	Groves 1980
ss	nigrescens	ss	hecki	?	no?	?	>1	Groves 1980
ss	ochreata	ss	brunnescens	?	yes	?	>1	Groves 1980
ss	ochreata	ss	maura	?	yes	?	>1	Groves 1980
ss	ochreata	ss	tonkeana	?	?	?	>1	Groves 1980
fa	irus [fascic.]	fa	nemestrina	?	?	?	1	Bernstein 1966

1c. SECOND GENERATION HYBRIDS

FG MATER	FG PATER	SEX	VIA	FERT	NUM	SOURCE
[mul x nem]	[fasc x nem]	m	y	?	1	Bernstein 1974
[nem x nigra]	[fasc x nem]	f	y	?	1	Bernstein 1974
[nem x fasc]	fascicularis *	?	part	part	2	Bernstein & Gordon 1980
nemestrina	[nem x silenus]	?	yes	?	1	Bernstein & Gordon 1980
[nem x mul]	unspecified	?	yes	yes	2	Bernstein & Gordon 1980
unspec. hybrid	[nem x fasc]	?	yes	?	2	Bernstein & Gordon 1980
[nem x mul]	mulatta	?	yes	?	1	Bernstein & Gordon 1980
[nem x nigra]	not stated	?	1/2	yes	2	Bernstein & Gordon 1980
[nigra x nem]	not stated	?	1/6	1/6	6	Bernstein & Gordon 1980
[fus x mul]	fuscata	mf	y	y	6	Wolfe 1986
[[fus x mul] x fus]	fuscata	mf	y	y	2	Wolfe 1986

Legend:

FG = Fooden's subgeneric group: ar = arctoides group si = sinica group
fa = fascicularis group ss = silenus-sylvanus group

fasc = fascicularis, fus = fuscata, mul = mulatta, nem = nemestrina

? = not known or not specified by source.

VIA: viability, length of hybrid's life, where'd'= days, 'h'= hours, 'm'= months, 'y'= years

FERT: fertility 'y'= yes, 'n'= no, '?' = unknown

NUM: number produced, '>' = greater than following number

* sex of parent not known

Table 6b : Intergeneric hybrids of the genus Macaca

FG	MATER	FG	PATER	SEX	VIA	FERT	NUM	SOURCE
a. Macaca x Papio								
	P. leucophaeus	ss	M. nemestrina	?	2d	n	1	Chiarelli 1973
	P. anubis	ss	M. nemestrina	?	4m	n	1	Chiarelli 1973
	P. ursinus	ss	M. nemestrina	?	?	?	>1	Chiarelli 1973
	P. hamadryas	ss	M. nemestrina	f	4d	n	1	Chiarelli 1973
	P. sphynx	fa	M. fascicularis	?	y	?	>1	Chiarelli 1973
	P. leucophaeus	fa	M. fascicularis	m	2h	n	1	Chiarelli 1973
	P. cynocephalus	fa	M. fascicularis	?	?	?	>1	Chiarelli 1973
fa	M. fascicularis		P. cyanocephalus	?	?	?	>1	Chiarelli 1973
[mulatta x nemestrina]			x P. hamadryas	f	y	no	2	Markajaran *et al.* 1974
b. Macaca x Cercocebus								
	Cercocebus sp.	ss	M. sylvana	m	?	?	1	Chiarelli 1973
	C. torquatus	fa	M. fascicularis	?	3m	?	>1	Bernstein & Gordon 1980
ss	M. nemestrina		C. torquatus	?	y	?	1	Chiarelli 1973
fa	M. fascicularis		C. torquatus	?	?	?	1	Chiarelli 1973
c. Macaca x Cercopithecus								
	C. aethiops	si	M. sinica	m	y	?	1	Chiarelli 1973
	C. aethiops	si	M. radiata	?	?	?	1	Chiarelli 1973

Legend:

FG = Foodens subgeneric group:

fa = Fascicularis group — ss = silenus-sylvanus group

si = sinica group — ar = arctoides group

? = not known or not specified by source.

VIA: viability, length of hybrid's life, where 'm'= months, 'd'= days, 'y'= years, 'h'= hours

FERT: fertility 'y'= yes, 'n'= no, '?' = unknown

NUM: number produced, '>' = greater than following number

Table 7a: Genetic distance between different populations of a macaque species

SPECIES	Nozawa *et al.*, 1977 Nei's D
1. fascicularis	0.0131-0.0179
2. fuscata	0.0002-0.0075
3. mulatta	0.0036-0.1637

Table 7b: Genetic distance between macaque species by source.

SPECIES COMPARED		* Cronin *et al.* 1980	* Avise & Duvall 1977	* Nozawa *et al.* 1977
arctoides	radiata	C 0.69	-	B 0.1091
arctoides	nigra	C 0.82	-	-
arctoides	maurus	C 0.89	-	-
arctoides	silenus	C 0.92	-	-
cyclopsis	mulatta	-	-	A 0.0092-0.0154
fascicularis	nemestrina	C 0.69	A 0.080	A 0.1100-0.1404
fascicularis	arctoides	C 0.69	-	B 0.1413-0.1941
fascicularis	radiata	C 0.82	-	B 0.1266-0.1782
fascicularis	tonkeana	-	A 0.126	-
fascicularis	nigra	C 0.84	B 0.205	-
fascicularis	maurus	C 1.02	-	-
fuscata	cyclopsis	-	-	B 0.1266-0.1687
fuscata	mulatta	C 0.63	-	B 0.0985-0.1637
fuscata	fascicularis	C 0.69	-	C 0.1660-0.2804
fuscata	nemestrina	C 0.76	-	C 0.2802-0.3347
fuscata	arctoides	C 0.64	-	B 0.1199-0.1650
fuscata	nigra	C 0.76	-	-
fuscata	maurus	C 0.80	-	-
fuscata	silenus	C 0.60	-	-
fuscata	radiata	C 1.05	-	B 0.1254-0.1710
mulatta	fascicularis	C 1.05	A 0.102	A 0.0472-0.1008
mulatta	nemestrina	C 0.87	B 0.201	A 0.0801-0.0946
mulatta	arctoides	C 0.40	-	A 0.0937-0.1379
mulatta	radiata	C 0.80	-	
mulatta	nigra	C 0.80	C 0.250	-
mulatta	silenus	-	C 0.236	-
mulatta	tonkeana	-	B 0.186	-
mulatta	maurus	C 0.94	-	-
nemestrina	arctoides	C 0.51	-	B 0.1929
nemestrina	radiata	C 0.97	-	B 0.1830
nemestrina	silenus	C 0.43	A 0.124	-
nemestrina	tonkeana	-	A 0.106	-
nemestrina	nigra	C 0.60	B 0.202	-
nemestrina	maurus	C 0.55	-	-
nigra	tonkeana	-	B 0.161	-
nigra	radiata	C 0.89	-	-
nigra	maurus	C 0.48	-	-
radiata	maurus	C 0.97	-	-
silenus	fascicularis	C 0.87	B 0.161	-
silenus	maurus	C 0.40	-	-
silenus	tonkeana	-	A 0.084	-
silenus	nigra	C 0.40	C 0.232	-

* = level of differentiation, Nei's D with respect to Ayala [1975]:
A = local population, D = 0.019 - 0.129
B = local population to subspecies interface, D = 0.130 - 0.213
C = subspecies to morphologically distinct species, D = 0.214 - 1.325

Table 8a: Electrophoretic phenotypes observed in macaques

Locus	allele	*Macaca* species						
		fuscata	cyclopsis	mulatta	fascicularis	nemestrina	arctoides	radiata
1. Acp	A	x	x	x	x	x	x	x
	C	x	-	x	x	-	-	-
2. ADA	1	x	-	x	-	-	x	-
	2	-	x	x	x	x	x	-
	3	-	-	x	x	-	-	-
3. CA-I	a	x	x	x	x	x	x	x
	b	-	-	-	-	x	-	-
	c	x	-	x	x	-	x	x
	d2	x	x	x	-	-	-	-
4. Cell Es	1	x	x	x	x	nd	x	x
	2	x	-	-	-	nd	-	-
	3	x	-	-	x	nd	x	-
5. ChEs	1	x	x	x	x	x	x	x
	4	-	-	-	-	-	-	x
6. Dia	A	x	x	x	x	x	x	x
	C	-	x	x	x	x	-	-
	D	-	x	x	-	-	-	-
7. Hb	S	x	x	x	x	-	x	x
	F	-	-	-	x	x	x	-
	X	x	-	-	-	-	-	-
8. IDH	1	-	x	x	x	x	-	x
	2	x	x	x	x	-	x	x
	3	-	-	x	-	-	-	-
9. LDH-A	1	x	x	x	x	x	x	x
	2	x	-	x	-	-	-	-
	3	x	-	-	x	-	-	-
10. LDH-B	1	x	x	x	x	x	x	x
	2	x	-	-	-	-	-	-
11. MDH	1	x	x	x	x	x	x	x
	2	x	-	-	-	-	-	-
	3	x	-	-	-	-	-	-
	2'	-	-	x	-	-	-	-
12. PGD	A	x	x	x	x	x	x	x
	B	-	-	x	-	-	-	-
	C	-	-	-	x	-	-	-
	D	-	-	-	-	-	x	-
13. PGM-I	1	x	x	x	x	x	x	x
	2	x	-	x	-	x	-	-
	3	x	-	x	-	-	-	-
	4	-	-	-	x	-	-	-
	5	-	-	-	-	-	-	x

Table 8a continued: Electrophoretic phenotypes observed in macaques

Locus	allele	*Macaca* species						
		fuscata	cyclopsis	mulatta	fascicularis	nemestrina	arctoides	radiata
14. PGM-II	1	x	x	x	x	x	x	x
	2	x	-	x	-	-	-	-
15. PHI	1	x	x	x	x	x	x	x
	2	x	x	x	x	-	-	-
	4	x	x	-	-	-	-	-
	5	-	-	x	x	x	-	-
	7	x	-	-	-	-	-	-
	8	x	-	-	-	-	-	-
	9	-	-	x	-	-	-	-
	11	-	-	x	-	-	-	-
	12	-	x	-	-	-	-	-
16. Pi	B	x	x	-	x	-	-	-
	C	x	x	x	x	x	x	x
	D	x	-	-	-	-	-	-
17. Tf	C	-	x	x	x	-	-	x
	D'	-	-	x	-	-	-	-
	D	-	x	x	x	x	-	-
	E	x	x	x	-	x	-	x
	F'	-	-	-	-	-	x	-
	F	x	x	x	x	x	-	-
	G-	x	-	-	-	-	-	-
	G	x	x	x	x	x	-	-
	H'	x	-	x	-	-	x	-
	H	-	-	-	x	-	-	-
18. TBPA	S	x	x	x	x	x	-	x
	F	-	x	x	x	x	x	x

Source: Nozawa *et al.* 1977, Nozawa *et al.* 1982, Smith 1975, Avise and Duvall 1975
note: Nozawa *et al.* use the name speciosa rather than arctoides

Abbreviations for Table 8:

1.	Acp	acid phosphatase	10.	LDH-B	lactate dehydrogenase B
2.	ADA	adenosine deaminase	11.	MDH	malate dehydrogenase
3.	CA-1	carbonate anhydrase	12.	PGD	6-phosphogluconate dehydrogenase
4.	Cell Es	cell esterase	13.	PGM-I	phosphoglucomutase I
5.	ChEs	cholinesterase	14.	PGM-II	phosphoglucomutase I
6.	Dia	NADH diaphorase	15.	PHI	phosphohexose isomerase
7.	Hb	hemoglobin	16.	Pi	protease inhibitor
8.	IDH	isocitrate dehydrogenase	17.	Tf	transferrin
9.	LDH-A	lactate dehydrogenase A	18.	TBPA	thyroxin binding prealbumin

Legend: x = variant present, - = variant absent, nd = no data

Table 8b. Nonvariable loci in macaques

abbreviation	name	abbreviation	name
1. PA	prealbumin	6. Hp	haptoglobin
2. Amy	amylase	7. Cat	catalase
3. Alp	alkaline phosphatase	8. LAP	leucine aminopeptidase
4. TBPA	thyroxin-binding prealbumin	9. TO	tetrazolium oxidase
5. G6PD	glucose-6-phosphate dehydrogenase	10. IDH	isocitrate dehydrogenase

Table 8c. Number of shared alleles among macaque species

species	total loci examined	total alleles examined	number observed in species
fuscata	18	67	42
cyclopsis	18	67	31
mulatta	18	67	44
fascicularis	18	67	36
nemestrina	17	64	22
arctoides	18	67	24
radiata	18	67	22

species compared	number of shared alleles			percent shared	
	maximum possible	observed [x] number		x/max	x/total [1]
fuscata comparisons					
1. fuscata-mulatta	42	29	*	69.1	43.3
2. fuscata-cyclopsis	31	23		74.2	34.3
3. fuscata-fascicularis	36	24		66.7	35.8
4. fuscata-nemestrina	25	17		68.0	26.6
5. fuscata-arctoides	24	19		79.2	28.3
6. fuscata-radiata	22	16		72.7	23.9
cyclopsis comparisons					
1. cyclopsis-mulatta	31	28	*	90.3	41.8
2. cyclopsis-fascicularis	31	25		80.7	37.3
3. cyclopsis-nemestrina	25	21		84.0	32.8
4. cyclopsis-arctoides	24	17		70.8	25.3
5. cyclopsis-radiata	22	19		86.4	28.3
6. fuscata-cyclopsis	31	23		74.2	34.3
mulatta comparisons					
1. mulatta-fascicularis	36	29		80.6	43.3
2. mulatta-nemestrina	25	23		92.0	35.9 [2]
3. mulatta-arctoides	24	20		83.4	29.8
4. mulatta-radiata	22	20		90.9	29.8
5. fuscata-mulatta	42	29	*	69.1	43.3
6. cyclopsis-mulatta	31	28		90.3	41.8
fascicularis comparisons					
1. fascicularis-nemestrina	25	22		88.0	34.4 [2]
2. fascicularis-arctoides	24	20		83.4	29.8
3. fascicularis-radiata	22	18		81.8	26.9
4. fuscata-fascicularis	36	24		66.7	35.8
5. mulatta-fascicularis	36	29	*	80.6	43.3
6. cyclopsis-fascicularis	31	25		80.7	37.3

Table 8c continued. Number of shared alleles among macaque species

species compared	number of shared alleles			percent shared	
	maximum possible	observed [x] number		x/max	x/total [1]
nemestrina comparisons [2]					
1. nemestrina-arctoides	24	15		62.5	23.4
2. nemestrina-radiata	22	16		72.7	23.0
3. fascicularis-nemestrina	25	22		88.0	34.4
4. mulatta-nemestrina	25	23	*	92.0	35.9
5. cyclopsis-nemestrina	25	21		84.0	32.8
6. fuscata-nemestrina	25	17		68.0	26.6
arctoides comparisons					
1. arctoides-radiata	22	16		72.7	23.9
2. nemestrina-arctoides	24	15		62.5	23.4 [2]
3. fascicularis-arctoides	24	20	*	83.4	29.8
4. mulatta-arctoides	24	20	*	83.4	29.8
5. cyclopsis-arctoides	24	17		70.8	25.3
6. fuscata-arctoides	24	19		79.2	28.3
radiata comparisons					
1. cyclopsis-radiata	22	19		86.4	28.3
2. arctoides-radiata	22	16		72.7	23.9
3. fuscata-radiata	22	16		72.7	23.9
4. nemestrina-radiata	22	16		72.7	23.0 [2]
5. fascicularis-radiata	22	18		81.8	26.9
6. mulatta-radiata	22	20	*	90.9	29.8

legend: [1] = total equals 67
[2] = total equals 64
* = largest number of shared alleles

Table 9: Morphology of *Macaca mulatta* and *Macaca fuscata*

Characteristic		*Macaca mulatta*	*Macaca fuscata*
1. length [adult average]		53.5 cm [9]	57.1 cm [9]
2. weight [kg.]	male	9.0 [4], >8 [5]	14.6 kg [2]
	female	6.5 [4], 3-8 [5]	12.3 kg [2]
[adult average]		9-18 kg [6]	
3. tail length		25.5 cm [7]	very short [6]
[% head-body length]		50 [1]	25 [1], 18 [9]
4. coat	color	yellowish brown	yellowish brown
	winter	yes [8]	yes [7]
	natal	yes dark [10]	yes dark [10]
	eye	dark [10]	dark [10]
	face	pink [6]	pink [6]
5. sex signs			
a. female [6]	reddening	face, hind, thighs, buttocks, hips, arms	face, hind
	swelling	sex skin	sex skin
b. male [6]	brightening	scrotum, ischial	scrotum
	reddening	face	face

SOURCES:
[1] Napier & Napier 1985: p. 129
[2] Fedigan 1982: p. 218
[3] Osman-Hill 1972
[4] Clutton-Brock & Harvey 1979: p 205
[5] Jolly 1985
[6] Bramblett 1976: p.156,166, 175, 177
[7] Kurland 1977: p.27
[8] Bourne, 1975 p. 4,5
[9] Fooden 1980
[10] Lanigan, personal observation, California Primate Rresearch Centre, Davis CA.

Table 10: Social Organization of *Macaca mulatta* and *Macaca fuscata*

CHARACTERISTIC	*M. mulatta*	*M. fuscata*
1. Territoriality	no [1]	no [2]
2. Social structure		
male transfer	yes [1]	yes [1]
females breed in natal grp	yes [1]	yes [1]
female hierarchy	stable [1]	stable [1]
multimale, multifemale grp	yes [1,2]	yes [1,2]
3. Adult sex ratio [m/f]		
provisioned	0.6 [1]; 2.7 [3]	1.0-0.9 [1]; 2.5 [3]
nonprovisioned	0.5 [1]; 2.0 [3]	
4. Provisioned group: reproduction statistics		
% females give birth	72-100% [3]	39-80%[1],40-88%[3]
% infant mortality	0-44% [3]	10-33%[1], 13% [3]
% two year survival		26-80%[1]
age at first birth [years]		5.21% [3]
Nonprovisioned group: reproduction statistics		
% females give birth	38% [3]	20-39% [1]
% infant mortality	46% [3]	20-40% [1]
% 2 year survival		15-39% [1]
age at first birth [years]		6.74 yrs [3]
5. Mating season	Sept.-Oct	Oct.-April esp Jan-Feb[7]
6. Birth season	yes [5]	yes [5]
		Jan-July [6] May-Sept [6]
7. Sexual behaviors - consorts	serial	serial mating
	extraseasonal	only seasonal

SOURCES:

[1] Jolly 1985: pp. 47, 93, 117, 125, 128, 149, 226, 239
[2] Fedigan 1982: p. 218, plus personal communication
[3] Richards 1985: pp. 109, 182, 244, 308, 371
[4] Kurland 1977: p. 26
[5] Napier & Napier 1985: p. 130
[6] Bramblett, 1976; p.166, 167, 177
[7] Osman-Hill, 1972; p129

Table 11: Comparison of sexual signaling in sympatric macaque species and *Macaca fuscata*

Table 11a: Female Signals

SPECIES	CYCLE LENGTH	RECEPTIVITY	PROCEPTIVE BEHAVIORS	PERINEAL INDICATOR	VAGINAL DISCHARGE
M. arctoides	31 days	noncyclic	present hind	rare	yes
M. assamensis	-	-	-	none	-
M. fascicularis	31 days	periodical	present hind	swell	yes
M. mulatta	29 days	8-11 days	present hind	redd+swell	yes
M. nemestrina	32 days	noncyclic	present hind	swell	yes
M. fuscata [2]	28 days	1-92 days	sit near,pres	reddening	yes

Table 11b: Male Signals

SPECIES	SOLICIT	MOUNT	MATING PATTERN	BREEDING FREQUENCY	SYSTEM
M. arctoides	yes		single	-	multimale
M. assamensis	-		-	-	-
M. fascicularis	-		single	-	multimale, consor
M. mulatta [1]	-		serial	18/hour	multimale
M. nemestrina	yes		multiple	7.5/hour	multimale, consort
M. fuscata [2]	yes		serial	2-30/hour	multimale, consort

* data modified and based on Table 31-1 in Blaffer-Hrdy and Whitten [1987]
[1] Carpenter [1942]; [2] Enomoto [1974]
legend: redd =reddening of face, hindquarters
pres = present hindquarters
redd+swell = reddening and swelling

Table 12a: Pattern of Sexual signaling in *Macaca fuscata*

i. FEMALE BEHAVIORS

McDonald [1985]	Enomoto [1974]
Stage: advertising/monitering	
excitement level high	crouch
vocalizations	kick male
estrus screams and hacks	lead and follow
solitary behavior	head bob, nod, duck
sit, walk, run,	look back
forage, self groom	attack third monkey
interactive behavior	scratch ground, beat ground
chase	scan area
Stage: proximity	
female-male inch closer	present hindquarters
startled jumps	hitting
fear grimaces	self grooming
vocalizations	
chasing male-female	
coy behavior	
walk in front	
present, lip quiver	
Stage: attempt to establish mount	
Vocalizations	
hacks and contact calls	
body jerks, present	
manipulate objects; slap ground	
threaten others, chase	
hip touch: female-male	
ii. MALE BEHAVIORS	approach: run trot, chase, attack
	lead and follow; look back
	attack third monkey
	head bob, nod, duck
	lip smack; walk by
	hindquarters display, genital display
	walk over, hands on back
	sitting with, muzzeling

Table 12b: Female pattern of sexual signaling in *Macaca mulatta*

Carpenter [1942]	Zumpe and Michael [1970]
hyperactive, explorative	anogenital display
aggressive	lookback
attack male	walkback
attack othe females	pull male into mount
affiliative gestures	hand reach
rhythmic lip movements	head duck
estrus facial expressions	head bob
anogenital display	
once establish consortship	
groom, follow	

Table 13a: Genetic identity and genetic distance between speciation products of the Drosophila willistoni group [data of Ayala (1975)]

LEVEL	I*	D*	# ALLELE SUBSTITUTIONS
Local population	0.970 +/- 0.006	0.031 +/- 0.007	3
Subspecies	0.795 +/- 0.013	0.230 +/- 0.016	23
Semispecies	0.798 +/- 0.026	0.226 +/- 0.033	23
Sibling species	0.563 +/- 0.023	0.581 +/- 0.039	58
Nonsibling species	0.352 +/- 0.023	1.056 +/- 0.068	100

* I and D are those of Nei (1972)

Table 13b: Range of genetic distance values between speciation products of the Drosophila willistoni group *

LEVEL	range of D	average D
Local population	0.019-0.129	0.031+/-0.007
Local Population/subspecies interface	0.130-0.213: diff=0.120	
Subspecies	0.214-0.246	0.230 +/- 0.016
subspecies/semispecies interface	completely overlapping	
Semispecies		0.226 +/- 0.033
Sibling species	0.232-1.208	0.581 +/- 0.039
sibling species/morph. species interface	none	
morphologically distinguishable species	0.854-1.325	1.056 +/- 0.068

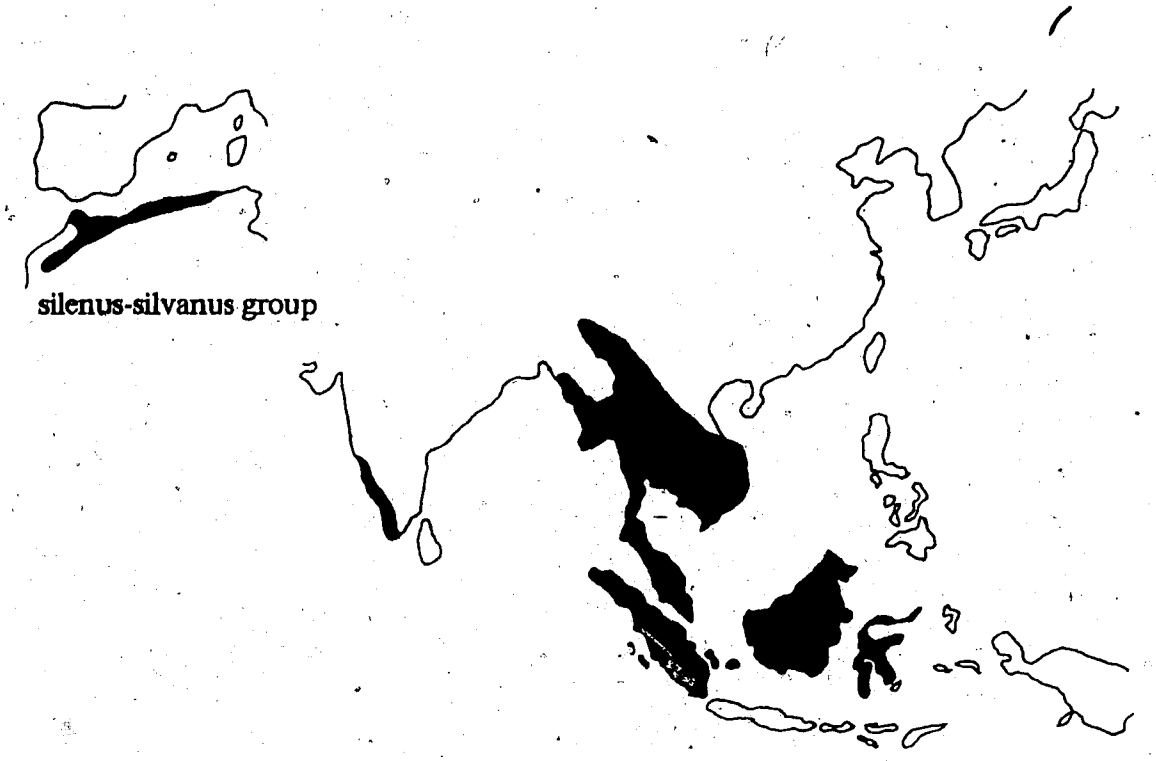
* recalculated from the data of Ayala [1975]

Table 13c Willistoni Drosophila Species and their Speciation Categories [Data of Ayala 1975].

SIBLING SPECIES	SUBSPECIES	SEMISPECIES
D. insularis		
D. pavlovskiana		
D. willistoni	D.w. willistoni	
	D.w. quechua	
D. equinoxialis	D.e. caribbensis	
	D.e. equinoxialis	
D. tropicalis		
D. paulistorum		transitional
		Andean-Brazilian
		interior
		Amazonian
		Centro-American
		Orinocan

Figure 1: Distribution of *Macaca*

Figure 1a: Distribution of Fooden's Groups



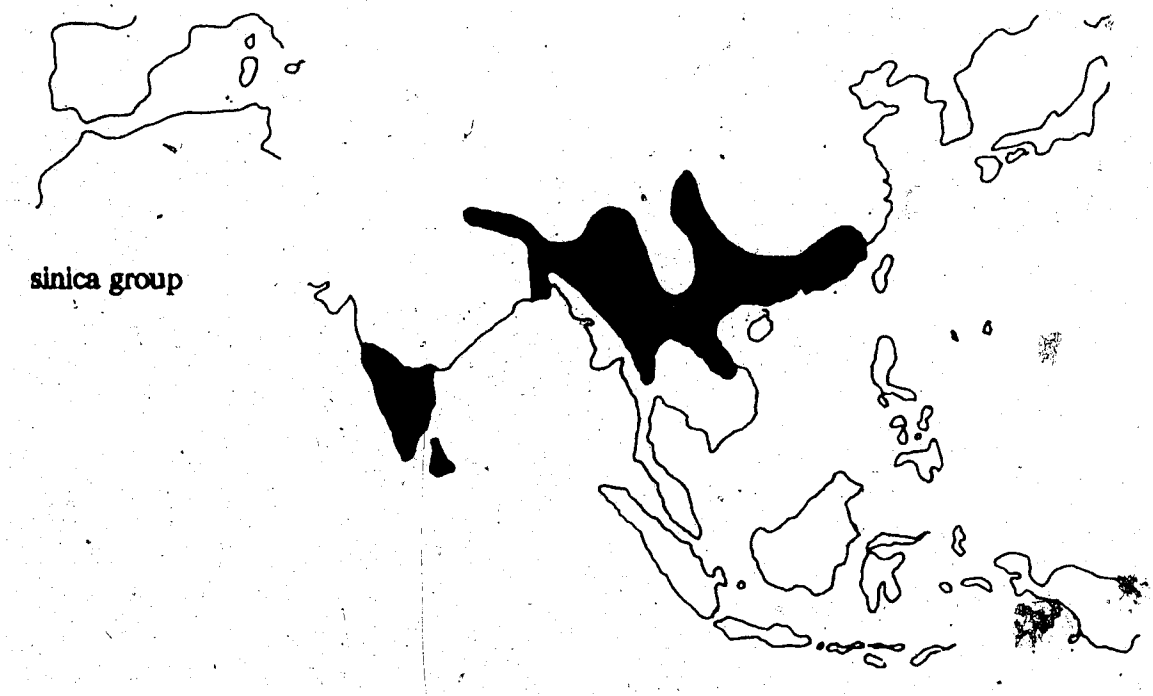


Figure 1: Distribution of *Macaca*

Figure 1a: Distribution of Fooden's Groups

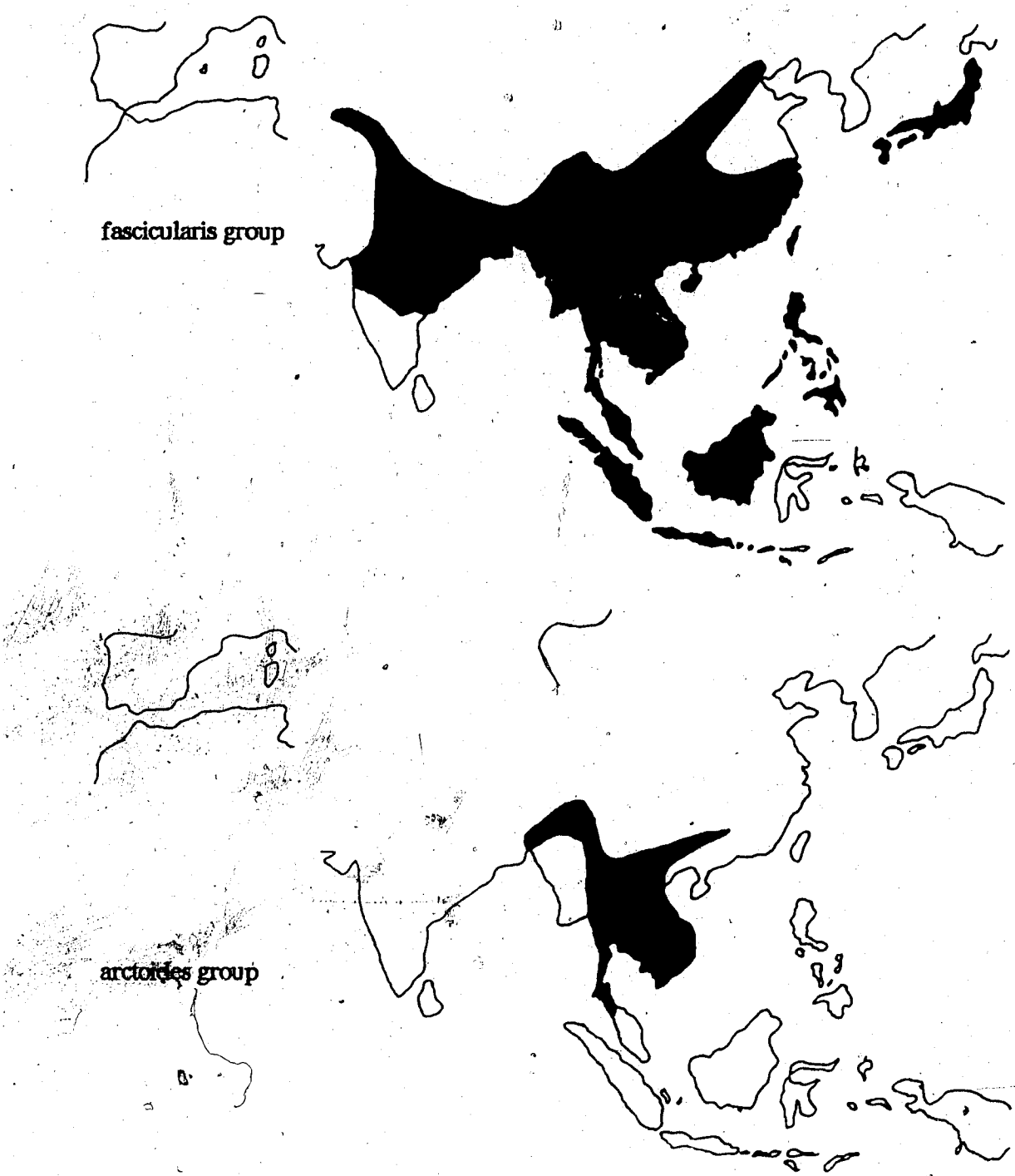


Figure 1: Distribution of *Macaca*

Figure 1b: Distribution of *Macaca mulatta* and *Macaca fuscata*

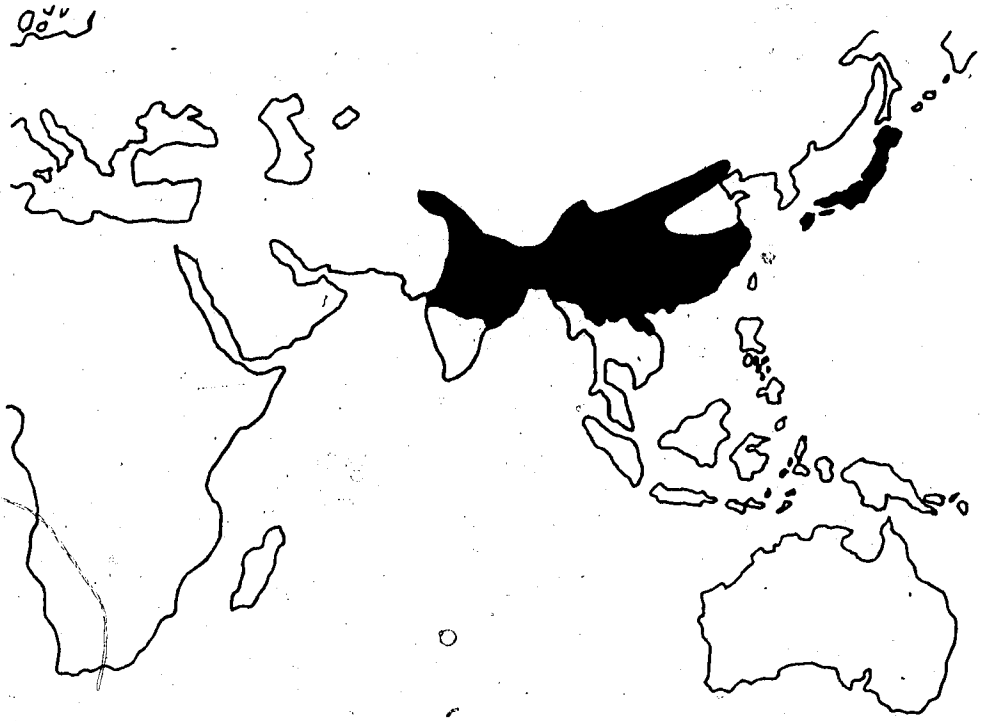
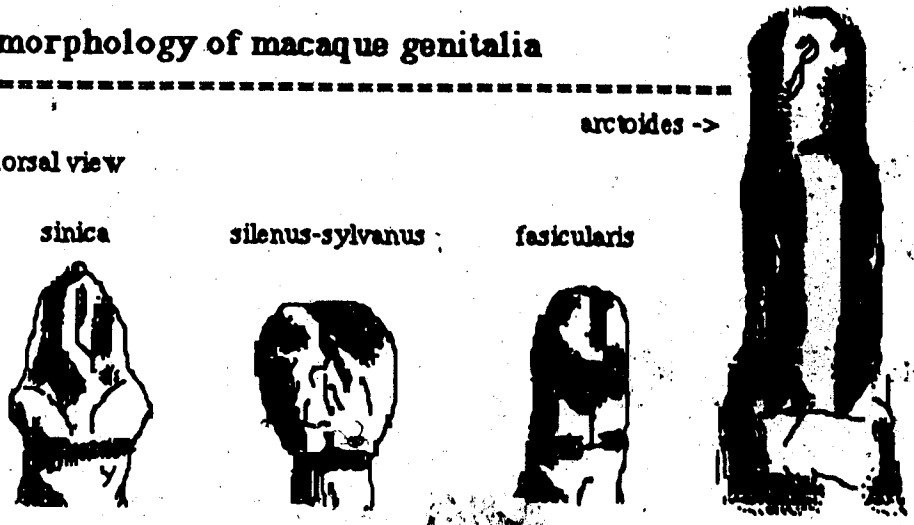


Figure 2: morphology of macaque genitalia

2a. Penis - dorsal view



2b. Female reproductive tract morphology - sagittal view

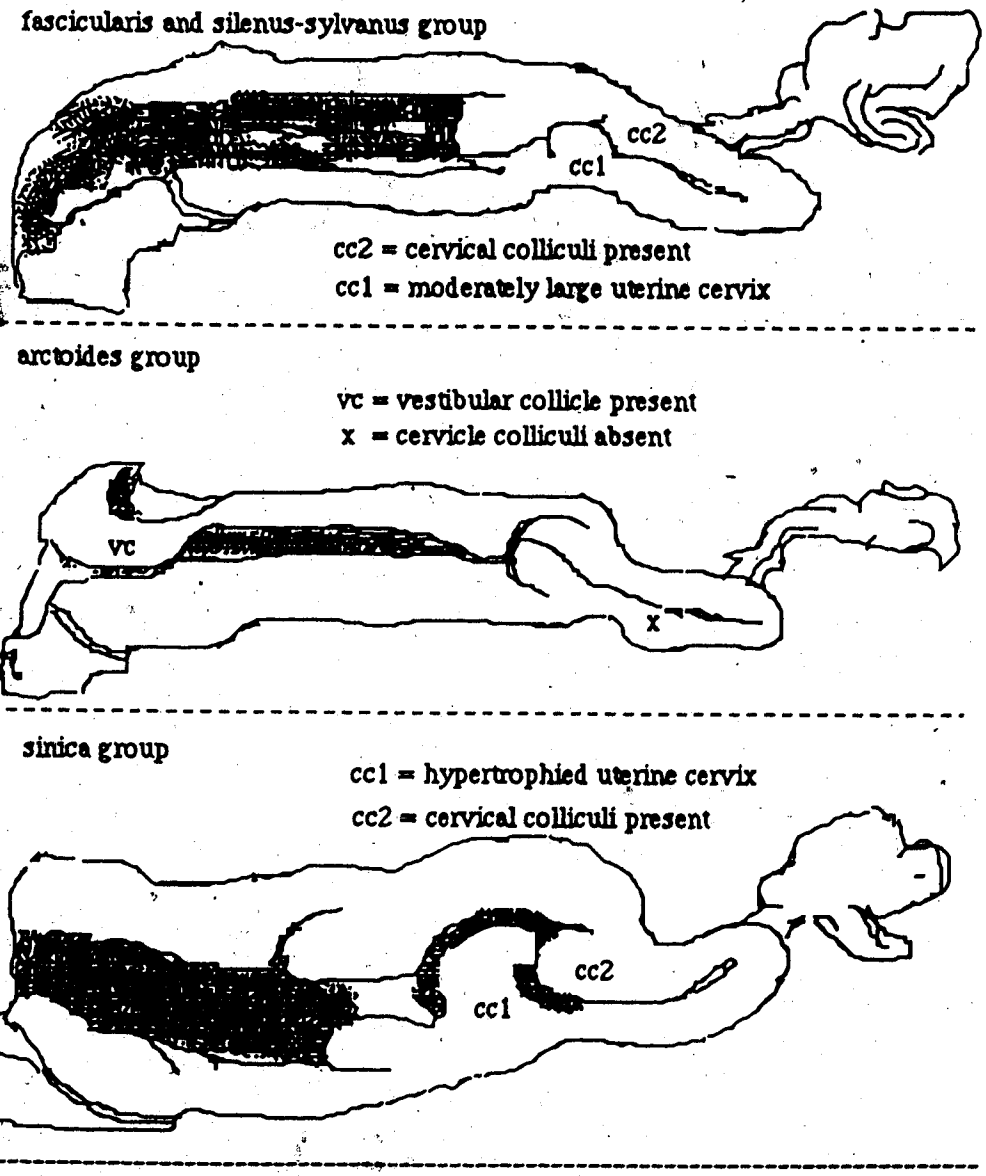


Figure 3: Partial Hybrid Sterility: Observed patterns of hybrid sterility in the subspecies of Drosophila willistoni

Case 1:	cross: female	*D.w.quecha*
	male	*D.w.willistoni*
	progeny fertility pattern = sterile males, fertile females	
Case 2:	cross: female	*D.w.willistoni*
	male	*D.w.quecha*
	progeny fertility pattern = fertile males, fertile females	
Case 3:	cross: female	*D.e.equinoxialis*
	male	*D.e.caribensis*
	progeny fertility pattern = sterile males, fertile females	
Case 4:	cross: female	*D.e. caribensis*
	male	*D.e.equinoxialis*
	progeny fertility pattern = sterile males, fertile females	

Data of Ayala 1975

Chapter 2: KIN IDENTIFICATION AND DNA POLYMORPHISMS IN JAPANESE MACAQUES [*Macaca fuscata*] [1]

In recent years, data on levels of genetic variation have become available in an increasing number of species. Although protein electrophoretic variation has generally been high, some studies have revealed extremely low levels of variation. However, species considered to be relatively nonvariable with respect to soluble proteins may be highly polymorphic at the DNA level. Data on such species may force a reevaluation of current evolutionary theory regarding the significance and maintenance of genetic variation in natural populations.

Recent sequence analyses of genetic loci have revealed that in spite of a lack of variation in electrophoretic mobility, many alleles may exist at a single locus. For example, the alcohol dehydrogenase locus (ADH) in *Drosophila* species has only two major electrophoretic variants, but at least 29 DNA variants out of 49 chromosomes examined (restriction fragment length polymorphisms), have been identified for a 13 kilobase [kb] region which includes the ADH locus [Aquadro *et al.*, 1986]. Similarily, Kreitman (1983) describes eleven different nucleotide sequences at this locus. Both authors speculate that every individual may be unique in terms of their DNA sequence for this region, variation which is only partially addressed by restriction enzyme analysis. Clearly then, electrophoretic mobility of proteins is a crude measure of the amount of variation present at a single genetic site.

An example of a relatively non-variable species is the Japanese monkey, *Macaca fuscata*. The proportion of polymorphic sites present in a population and the average heterozygosity per individual of this species [P_{poly}=9.2%, H_{ind}=1.4%, Nozawa *et al.*, 1982] is comparable to that of extremely isolated species, or of stable evolutionary lineages [bradytelic species], and species that are speculated to have experienced a recent, extreme, population reduction [Avise and Selander, 1972; Selander *et al.*, 1970; Nevo *et al.*, 1974; O'Brien *et al.*, 1985].

[1] A version of this chapter has been submitted for publication: Lanigan, C.M.S., L.M. Fedigan, S.M. Williams and C.Strobeck 'Kin identification and DNA polymorphisms in Japanese macaques [*Macaca fuscata*]'.

Nozawa and collaborators surveyed 33 groups of Japanese macaques, representing 1646 individuals for 32 serum protein loci. None of the groups tested had more than 3 alleles present at a single locus. In fact, when a locus revealed a polymorphism, the variant was often present at a low level [the frequency range observed in the alternative allele on average was 2.11-8.25%]. In addition, only 21.8% of the loci examined had 3 or more alleles [Nozawa *et al* . 1982].

These figures are far lower than that observed in other animals. For example, in other members of the genus the following average values are observed: *M. cyclopsis* P_{poly}=24.1%, H_{ind}=4.0%; *M. mulatta* P_{poly}=29.1%, H_{ind}=7.5%; *M. fascicularis* P_{poly}=30%, H_{ind}=7.0%; *M. nemestrina* P_{poly}=21%, H_{ind}=7.0%; *M. silenus* P_{poly}=21%, H_{ind}=8.0%; *M. radiata* P_{poly}=14%, H_{ind}=6% (Nozawa, *et al* . 1977). Although the range for the proportion of polymorphic loci includes the values for *M. fuscata.* [P_{poly}=14-30%], the heterozygosity is far higher in other macaques [range of H_{ind}=6.0-8.0%] than observed in *M. fuscata* ; H_{ind}=1.3%. The Class Insecta [excluding *Drosophila*] reveals a range of P_{poly}=58% as a high in *Gryllus* species to a low of 7% observed in *Gryllotalpa* species, and from a high of H_{ind}=17.4% in *Magicada* to 0.3% in *Gryllotalpa* species [Nevo, 1974]. Drosopholids reveal somewhat higher levels of genetic variation P_{poly}=13-69%, H_{ind}=2.5-24.2%, but this result may be partially an artifact of the depth of study of drosophila species. The figures for the Class Insecta are based on 24 populations representing 23 species for a total of approximately 2,025 individuals, in which the population size ranged from thirty to 548 individuals for thirteen to 26 loci; whereas the *Drosphila* data are based on 51 populations representing 32 species for a total of 19,954 individuals, in which the population size ranges from seven to 1465 individuals for twelve to 40 loci [Nevo, 1978]. In humans the proportion of polymorphic loci and the average heterozygosity per individual has been estimated as P_{poly}=50%, H_{ind}=12%, in which 30% of loci tested had three or more alleles [Speiss 1977, p84; see also Nei and Roychoudhoury, 1974].

Clearly the Japanese macaque exhibits a far more restricted reservoir of genetic variation than humans or fruit flies, and other macaques (see Smith *et al.*, 1984 for a discussion). Specifically the levels of genetic variation observed preclude paternity exclusion studies in this species. In contrast, the closely related species, *Macaca mulatta,* is polymorphic enough to permit a combined paternity exclusion probability, in terms of

"polymorphic red cell surface antigens, electrophoretic markers, and leukocyte antigen loci ... as high as 0.995" (Smith *et al.*, 1984).

The species *Macaca fuscata* is believed to have descended from a small group of *Macaca mulatta* -like macaques. The ancestral macaques colonized the Japanese islands when these islands and the Korean peninsula were connected, probably during a recent glacial period. At present the species is estimated to consist of 20,000-70,000 animals on the Japanese islands. They are organized in hundreds of maternally related groups composed of twenty to 200 animals (Nozawa *et al.*, 1982).

Ethological observations of the Arashiyama group of Japanese macaques began in 1954. In 1968, the group fissioned along maternal lineages into two subgroups (designated A and B). These matrilines represent the descendants of female animals whose biological relationships could not be determined at the time observation of the group began [Fedigan, personal communication]. In 1972, group A, consisting of 147 animals and representing eight maternal lineages, was relocated as an intact social unit from Japan to a ranch in south Texas, USA. No new animals have been introduced to the group since their relocation. The Texas group, Arashiyama West, is presently composed of approximately 400 animals living as a provisioned, semi-free ranging group [Fedigan, 1982].

This population provides the rare opportunity to link behavioral data with population genetics. Given the recent history of this group, should a suitable genetic locus be identified, the population provides the opportunity to test the effects of inbreeding in a free-ranging population, and the effects of breeding structure on subsequent gene frequency change [in particular the effect of the number of breeding males]; and the opportunity to measure mutation rates in a long lived species, to measure the effects of selection [given the groups adaptation to a dramatically different environment], and to compare these measures with that of the parent population. Reported here are preliminary results of recombinant DNA [RDNA] analysis of a highly variable, genetic locus in *Macaca fuscata*. The primary objective of this project was to identify a genetic locus sufficiently variable for paternity exclusion studies.

MATERIALS AND METHOD

Two sets of study animals were selected for this project. The first set, the survey animals, was composed of twelve macaques [tattoo numbers: 276, 459, 455, 4, 607, 266, 40, 329, 88, 645, 128, 378]. Since maternal relationships were known, individuals were

selected to represent the greatest possible amount of genetic variation present in this population. Four founding individuals and six of the eight matrilineages were represented in the study sample (blood samples were not available for two of the matrilines). Sex differences and differences between related individuals were of interest; therefore four females from four different matrilines and eight male animals were selected. The second set was composed of eight additional animals, four females and four males, representing two 'matrifamilies' [tattoo numbers 68, 325, 354, 446, 157, 277, 367, 457]. Each matrifamily consisted of a mother and three of her offspring. Figure 1 displays the genealogical relationships of the subject animals and the matrilines.

Blood samples of up to 5 ml were drawn into ethyl diamine tetraethyl amine [EDTA] containing Vaccutubes, centrifuged, and the plasma and cell fractions separated. The cells were suspended in an equal volume of cryoprotective agent (60g trisodium citrate/40% ethylene glycol/liter) and stored frozen at -35°C or lower until needed. This cryoprotective appears to lyse selectively red blood cells but is protective of DNA. The frozen cell fractions were thawed slowly (on ice or at 4°C), centrifuged at 4000 rpm in a Sorval SS34 rotor (2000 g) for five minutes, the cryoprotective removed, and the cells resuspended in 2 ml of a saline solution (130 mM NaCl/5 mM KCl/7.5 mM MgCl2). The cells were then lysed in 8 ml of 0.017 M Tris/0.139 M Ammonium Chloride (pH 7.6), 1 mg proteinase K, 1 ml 20% SDS and 0.5 ml of 0.5 M EDTA (pH 8). The mixture was incubated for one hr at 44°C. DNA was isolated and precipitated with ammonium acetate in 95% ethanol after mixing gently at 4°C overnight on a hematology mixer. The pellet was dried briefly in a vacuum, and resuspended in 2 ml TE (10 mM Tris, pH 7.2/1 mM EDTA pH 8.0) by mixing eighteen to 48 hrs on a hematology mixer at 4°C. Up to 1 mg of DNA was obtained from a 5 ml sample of whole blood.

Six to ten micrograms of DNA were digested in a total restriction volume of no more than 65 microliters, using four units of enzyme per microgram of DNA. Restriction enzymes were obtained from Gibco/BRL, and used according to manufacturers directions. Digestion progressed over six to eighteen hours until complete. Digestion was terminated by the addition of 1/10th volume sucrose loading/tracking dye (50% sucrose/20 mM EDTA, pH 8.0/2.5% SDS/0.2% Bromophenol Blue). The digested DNAs were electrophoresed in agarose gels of varying concentrations [0.6-1.0%] in a Tris-Boric acid-EDTA [TBE] buffer [Maniatis *et al.*, 1982]. DNA was transferred from the agarose gel to Biodyne™ membranes, according to manufacturer's directions. Transfer progressed for a minimum of twelve hours.

The plasmid pAW101 was provided as a gift by Dr. Arlene Wyman. This plasmid contains a unique [single copy] human DNA fragment, 3.4 kb in length, which had been shown to be highly polymorphic in humans (Wyman and White, 1980). This fragment, initially cloned as an anonymous fragment, has been mapped to human chromosome 14, at position q32.1-32.2 (Willard *et al.*, 1985). Clones were obtained as either plasmid DNA or stab cultures, and the plasmids isolated from 200 ml cultures amplified with chloramphenicol [Maniatis *et al.*, 1983]. The inserts were radiolabelled with ^{32}P-dCTP (NEN-Dupont) as per the method of Feinberg and Vogelstein (1983). The membrane bound DNA was then hybridized to the cloned fragments as described by Klessig and Berry (1983). Two blots were hybridized per plastic bag, in 3-5 ml of hybridization buffer, with a minimum of 20 ng of radiolabelled probe. Hybridization proceeded for 24 to 48 hours. Autoradiography was for 48 hours to thirty days at -70°C, with 8"x10" Kodak XAR-5 film in metal x-ray cassettes backed by intensifying screens.

Thirteen enzymes were tested on specific test membranes (BamHI, BglII, BstEII, CfoI, EcoRI, EcoRV, HaeIII, HindIII, PstI, SalI, SstI, TaqI, XbaI). These test membranes were constructed of four sets of three digested DNA samples. A test set consisted of two macaques (one male and one maternally unrelated female), and one human sample, digested with the same restriction endonuclease. Up to four different enzymes were represented on one gel and transferred to a Biodyne™ membrane. If a variable hybridization banding pattern occurred between the 2 macaques, a survey membrane was constructed using that enzyme. Survey membranes were composed of twelve macaque and two human samples (a male and a female) digested with the enzyme of interest, and one Lambda fragment size standard. If the survey membrane revealed a polymorphic hybridization pattern an analysis of the inheritance of the site was done. The probe was tested on a membrane constructed of the matrifamily subject set digested with that particular enzyme. The results of hybridization experiments of plasmid pAW101 on the survey subjects with two enzymes, HaeIII and HindIII, and the matrifamily subjects with HindIII and SstI, are reported here.

RESULTS

In *Macaca fuscata* a highly variable hybridization pattern was observed with the clone pAW101. An analysis of the hybridization pattern revealed by HindIII shows eleven different genotypes representing at least six fragment sizes (Plate 1). The hybridization

pattern revealed by the HaeIII digest was even more complex. At least 9 different fragment sizes were observed, as shown in Plate 2. This result follows from the HindIII pattern as it is expected that a restriction endonuclease that recognizes a four base pair sequence [HaeIII] will detect more sites, strictly by chance, than will a restriction enzyme that recognizes a six base pair sequence [HindIII]. The data do not allow an estimate of the frequency of the alleles in the population, however, given the number of different genotypes observed in a sample of twelve individuals, it would appear that representatives of each size class are frequent. These preliminary data reveal a level of variation not previously observed in this species. It is interesting to note that the human samples show a HindIII band [at the 10 kb position, as expected from the results of Wyman and White 1980], which does not appear in this set of macaques.

An examination of the HindIII matrifamily data set [Figure 2, Plate 3] revealed that in matrifamily Betta596671, none of the siblings [325, 354, and 446] share identical genotypes, 325 being heterozygous for bands #2 and #4, 354 heterozygous for bands #2 and #3, and 446 being heterozygous for bands #3 and #4 . Siblings then appear to share a single band.

For matrifamily Nose6270, the mother, 157, and siblings 277 and 457 share apparently identical genotypes, having both bands #1 and #2, and 367 being homozygous for #1 band. Since the mother's genotyope is known, we can deduce that the paternal contribution for subject 367 was band #1. The siblings cannot be discriminated with respect to possible fathers as in all 3 progeny, a single male could potentially have inseminated the mother; nor can the father's genotype be determined [for example the paternal genotype could be homozygous for band #1, homozygous for band #2, heterozygous for bands #1 and #2, or bands #2 and #3, etc.].

An examination of the SstI data [Figure 2, Plate 4], reveals that matrifamily Nose6270 shows identical results as with the HindIII digest in that 157, 277, and 457 have identical heterozygous genotypes and 367 is homozygous. In matrifamily Betta596671, the maternal genotype is bands #2 and #4, and each of her offspring share exactly one of these bands. The maternal contribution to siblings 325 and 446 is band #4, but for 354 her contribution is band #2. From these data it is clear that the mother is of a different genotype than any of her offspring and that she was inseminated by at least two males with possible genotypes [1,2] and [1,3]. It appears then from this data set that these bands are inherited in a Mendelian fashion.

DISCUSSION

The elucidation of a complex, combined hybridization pattern, for a single genetic locus with a known pattern of inheritance, a haplotype, could be a means of reconstructing the genealogy of members of a group. Ideally the site selected for analysis will be so polymorphic that virtually every individual will be heterozygous.

The matrifamily data show different alleles segregating, and the possibility of discriminating paternally inherited alleles. The utility of this kind of data is demonstrated by the following analysis. Heterozygous individuals, with respect to pAW101, have been identified. The mother-son pair tested in the HindIII survey revealed that the mother, 4, was heterozygous for 2 fragment lengths at this site [Figure 2 shows 2 bands at approximately 9 and 8kb, bands #1 and #4], as her son, 607, appears to be homozygous for a single band at the 9kb position [band #4, Figure 2, Plate 1]. In addition, this matriline, Matsu, shows that the more distantly related member 266, with respect to his relationship with 4, is heterozygous for a third allele [266 shows 2 bands at approximately 8 and 7 kb].

A second example is represented by subjects 276 and 128. From the HindIII data it appears that 276 and 128 have identical genotypes [bands #1 and #3]. In the HaeIII survey data it is clear that 276 and 128 are different: 276 is heterozygous for bands #1 and #7, whereas 128 is heterozygous for bands #6, and #4. Such a result was not obtained for subjects 645 and 378: both digests indicate that these individuals are genetically identical at the site identified by pAW101. We expect that continued analysis will eliminate such ambiguity, as in the case of subjects 276 and 128; and each subject can be described uniquely with respect to a combined hybridization pattern.

Restriction fragment length polymorphisms [RFLP] can result from the creation or elimination of restriction sites, by base pair substitutions or by rearrangement of DNA segments through deletions, insertions, or inversions. If the variation is due to the loss of a restriction site, changes in the number of bands observed for an individual would be expected. Although the data reported here does not completely rule out this observation, they do not incontrovertably support it. A rearrangement of the genetic material would be expected to result in a great difference in fragment sizes observed between individuals [as in the HaeIII data], but the number of sites per individual would remain constant [as observed in the HaeIII and HindIII survey data sets]. Relatively rarely, i.e in the case of a deletion, would a restriction site be lost, especially in a case where the fragment sizes

observed are quite large, as observed here. Clearly these data support this notion. From the number of variants detectable by each enzyme, and the fact that similar patterns are revealed by other enzymes [not reported here] in terms of consistent numbers of bands within an individual between enzymes, it would appear that the variation is due to rearrangement of the DNA sequence, rather than base pair substitutions. That is, the macaque RFLP observed in this project appears to be analagous to that reported in humans by Wyman and White [1980].

CONCLUSION

The genus *Macaca* is particularily problematic from an evolutionary perspective. Measures of genetic distance between members of the genus reveal overall low levels of genetic variation and hence low levels of genetic distance. In fact, reported levels of Nei's D [see Nozawa, *et al.*, 1977, and Avise and Duvall, 1977] suggest speciation in the range of subspecies or semispecies, as delineated by Ayala [1975]. Only three reported comparisons [of at least 20] show a level of differentiation greater than Ayala's semispecies level - Nei's D for *M. fuscata* vs *M. fascicularis* ranged between 0.1660-0.2804, for *M. fuscata* vs *M. nemestrina* ranged between 0.2802-0.3347, [Nozawa *et al.*, 1977] and for *M. mulatta* vs *M. nigra* was computed as 0.250 [Avise and Duvall, 1977]. Recombinant DNA technology may permit a resolution of such phylogenetic impasses. As suggested by Melnick [1985], the use of clones like pAW101 will be invaluable for testing evolutionary theories.

This paper describes the use of RDNA technology to identify genetic variation in a species previously reported to be relatively nonvariable and proposes a means by which genealogical relationships can be reconstructed in free-ranging populations using highly polymorphic single copy clones like pAW101. Species not known to be highly genetically variable can, through the use of RDNA techniques, become amenable for genealogy reconstruction, a result previously considered unlikely. Recently, these techniques have been applied to the genetic analysis of house sparrows [*Passer domesticus*] and snow geese [*Anser caerulescens caerulescens*] revealing a level of polymorphism such that individuals were discriminated sufficiently to identify nest parasitism in which a pair of nest attendants would, from protein analysis, be ascertained to be the parents of the fledglings [Wetton *et al.*, 1987, Burke and Bruford 1987, and Quinn and White 1987]. Clearly the RDNA data permit an extremely detailed level of testing of evolutionary hypotheses

Species like the Japanese macaque, on which extensive ethological data exist, but which previously eluded fine genetic analysis, can now be utilized in the testing of evolutionary hypotheses.

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Legend to Figure1: Animal names are genealogical codes. The first 2 digits are either the birthyear of the animal or the birthyear of the eldest female ancestor of the animal. Some individuals have 2 name designations. The other name is a new name now used for this lineage. Underlined names are animals used in this project. The tattoo number and sex is indicated for the subject animals. Asterisk denotes member of Texas group founding population.

Figure 1: Genealogical Relationships of Subject Animals Traced Through Maternal Lineages

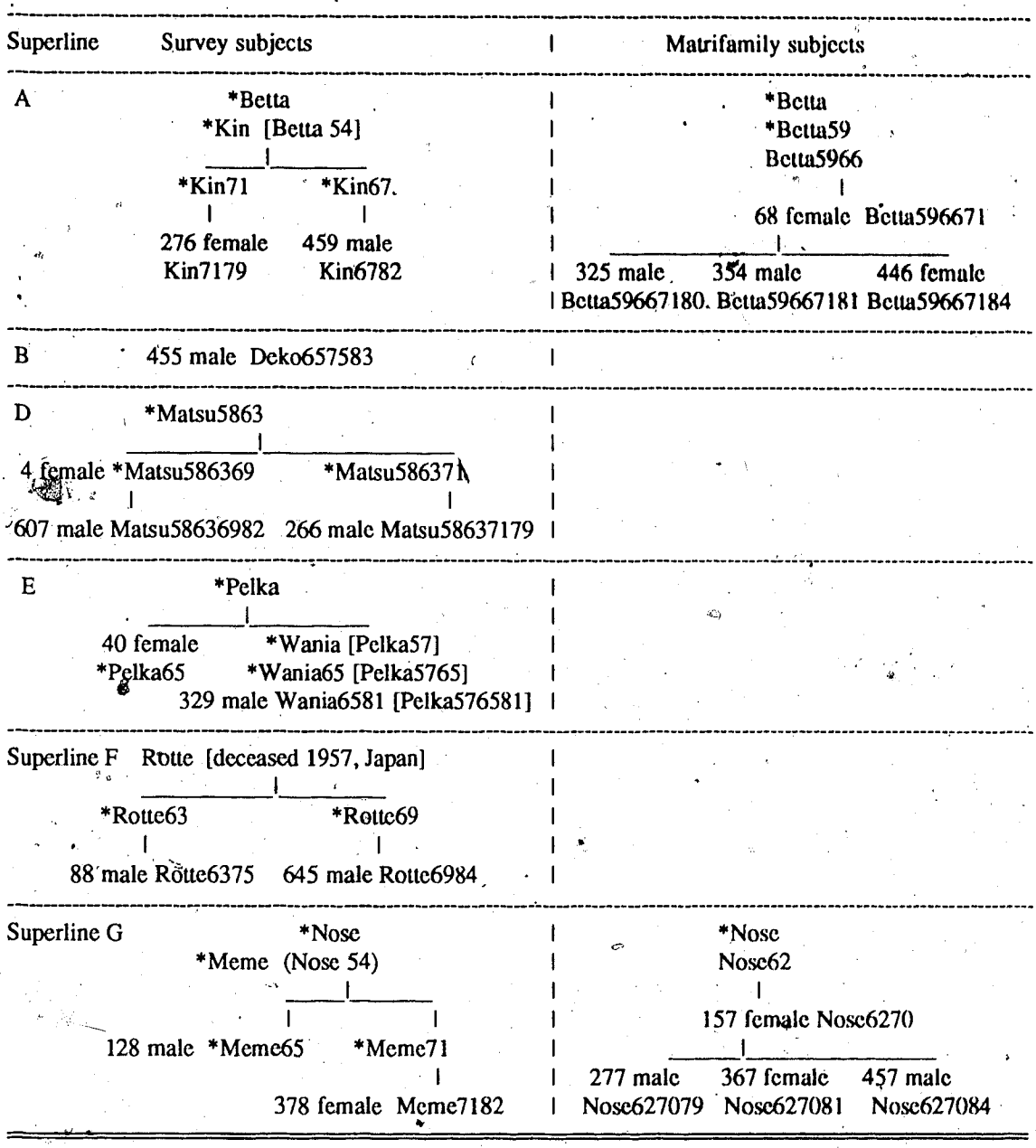

Figure 2. Diagrammatic representation of hybridization patterns

HindIII						Survey subjects								
band	276	459	455	4	607	266	40	329	88	645	128	378	H1	H2
1	--			--		--			--		--			
2		--	--			--		--		--		--		
3	--						--	--			--			
4		--		--	--								--	--
5										--		--		
6														--
geno	1,3	2,4	2	1,4	4	1,2	3	2,3	1	2,5	1,3	2,5	4	4,6

HaeIII				Survey subjects										
band	276	459	455	4	607	266	40	329	88	645	128	378	H1	H2
1	--					--								
2				--										
3								--					--	--
4				--			--				--			
5		--	--				--	--		--		--		
6											--			
7	--													
8		--												
9										--		--		
geno	1,7	5,8	5	2,4	ø	1,4	4,5	3,5	ø	5,9	4,6	5,9	3	3

HindIII: Matrifamilies	Betta596671				Nose6270			
band \ subject	68	325	354	446	157	277	367	457
1					--	--	--	--
2		--	--					
3			--	--	--	--		--
4		--		--				
genotype	ø	2,4	2,3	3,4	1,3	1,3	1	1,3

SstI: Matrifamilies	Betta596671				Nose6270			
band \ subject	68	325	354	446	157	277	367	457
1		--			--	--	--	--
2	--		--					
3				--	--	--		--
4	--	--		--				
genotype	2,4	1,4	2	3,4	1,3	1,3	1	1,3

Plate 1. Survey subjects - HindIII digestion

Legend to Plate 1:

Six fragment lengths [bands] are observed in this data set with the smallest designated #1.

Subject arrangement is as follows: [from left to right] lane 1-macaque tattoo number 276, lane 2-macaque tattoo number 459, lane 3-macaque tattoo number 455, lane 4-macaque tattoo number 4, lane 5-macaque tattoo number 607, lane 6-macaque tattoo number 266, lane 7-macaque tattoo number 40, lane 8-macaque tattoo number 329, lane 9-macaque tattoo number 88, lane 10-macaque tattoo number 645, lane 11-macaque tattoo number 128, lane 12-macaque tattoo number 378, lane 13-human female, lane 14-human male.

Subject genotypes in terms of observed bands are as follows: 276[1,3]; 459 [2,4]; 455 [2]; 4 [1,4]; 607 [4]; 266 [1,2]; 40 [3]; 329 [2,3]; 88 [1]; 645 [2,5]; 128 [1,3]; 378 [2,5]; female [4], male [4,6].

Plate 2. Survey subjects - HaeIII digestion

Legend to Plate 2:

At least 9 fragment lengths [bands] are observed in this data set with the smallest designated #1.

Subject arrangement is as follows: [from left to right] lane 1-macaque tattoo number 276, lane 2-macaque tattoo number 459, lane 3-macaque tattoo number 455, lane 4-macaque tattoo number 4, lane 5-macaque tattoo number 607, lane 6-macaque tattoo number 266, lane 7-macaque tattoo number 40, lane 8-macaque tattoo number 329, lane 9-macaque tattoo number 88, lane 10-macaque tattoo number 645, lane 11-macaque tattoo number 128, lane 12-macaque tattoo number 378, lane 13-human female, lane 14-human male.

Subject genotypes in terms of observed bands are as follows: 276[7,1]; 459[8,5]; 455[5]; 4[9,4,2]; 607[ø]; 266[1,4]; 40[5,4]; 329[3,5]; 88[ø]; 645[5,9]; 128[4,6]; 378[5,9]; female [3], male [3].

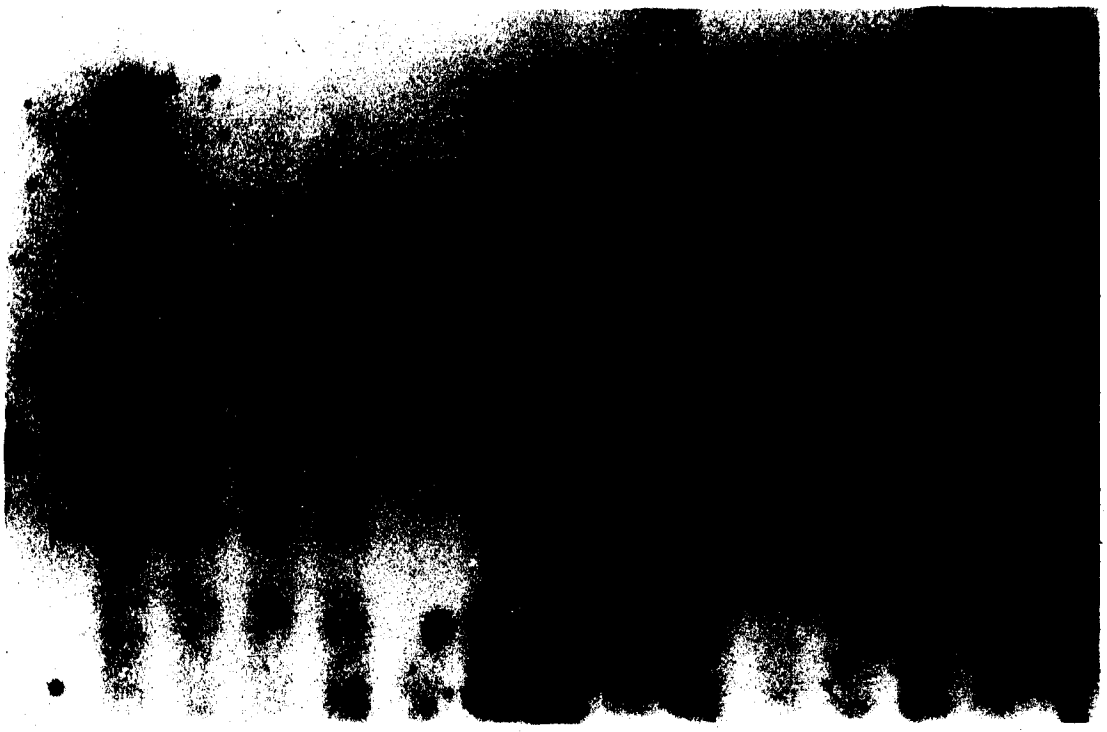

Plate 3. Matrifamily subjects - HindIII digestion

Legend to Plate 3:

Four fragment lengths [bands] are observed in this data set, the smallest is designated #1.

Subject arrangement is as follows: from left to right; lane 1-macaque tattoo number 325, lane 2-macaque tattoo number 354, lane 3-macaque tattoo number 446, lane 4-macaque tattoo number 157 [mother], lane 5-macaque tattoo number 277, lane 6-macaque tattoo number 367, lane 7-macaque tattoo number 457.

Subject genotypes in terms of observed bands are as follows:

Matrifamily Betta596671	325[2,4]; 354[2,3]; 446[3,4];
Matrifamily Nose6270	157[1,3]; 277[1,3]; 367[1]; 457[1,3].

Plate 4. Matrifamily subjects - SstI digestion

Legend to Plate 4:

Six fragment lengths [bands] are observed in this data set, the smallest is designated #1. The 2 largest bands are not easily interpreted from this film but appear to represent nonvariable regions from the data presently available.

Subject arrangement is as follows: from left to right; lane 1-macaque tattoo number 68 [mother], lane 2-macaque tattoo number 325, lane 3-354, lane 4-macaque tattoo number 446, lane 5-macaque tattoo number 157 [mother], lane 6-macaque tattoo number 277, lane 7-macaque tattoo number 367, lane 8-macaque tattoo number 457.

Subject genotypes in terms of observed variable bands are as follows:

Matrifamily Betta596671	68[2,4]; 325[1,4]; 354[2]; 446[3,4];
Matrifamily Nose6270	157[1,3]; 277[1,3]; 367[1]; 457[1,3].

APPENDIX: Genetic distance in Macaca

Appendix 1: Nei's D with respect to speciation levels in *Drosophila willistoni* species group, adapted from Ayala, 1975

Differentiation level	range of Nei's D	Average number of allele substitutions
a. local population	0.019-0.129	3
b. local population-subspecies interface	0.130-0.213	
c. subspecies-semispecies	0.214-0.246	23
d. sibling species	0.232-1.208	58
e. morphologically distinguishable species	0.854-1.325	100

Appendix 2: Reported values for Nei's D, between selected Macaca species

Species compared		Avise & Duvall * 1977		Nozawa, *et al* . 1977 *	
fascicularis	nemestrina	a	0.080	a	0.1100-0.1404
fascicularis	arctoides		Ø	b	0.1413-0.1941
fascicularis	radiata		Ø	b	0.1266-0.1782
fuscata	cyclopsis		Ø	b	0.1266-0.1687
fuscata	mulatta		Ø	b	0.0985-0.1637
fuscata	fascicularis		Ø	c	0.1660-0.2804
fuscata	nemestrina		Ø	d	0.2802-0.3347
fuscata	arctoides		Ø	b	0.1199-0.1650
fuscata	radiata		Ø	b	0.1254-0.1710
cyclopsis	mulatta		Ø	a	0.0092-0.0154
mulatta	fascicularis	a	0.102	a	0.0472-0.1008
mulatta	nemestrina	b	0.201	a	0.0801-0.0946
mulatta	arctoides		Ø	a	0.0937-0.1379
mulatta	nigra	d	0.250		Ø
mulatta	silenus	c	0.236		Ø
mulatta	tonkeana	b	0.186		Ø
nemestrina	arctoides		Ø	b	0.1929
nemestrina	radiata		Ø	b	0.1830
silenus	fascicularis	b	0.161		Ø
silenus	nigra	c	0.232		Ø

* level of differentiation with respect to Nei's D for D. willistoni group as per Table A1.

CONCLUSION

The genus *Macaca* presents evolutionary biologists with a number of serious problems. The genus is widespread geographically; and exhibits behavioral, ecological, and morphological variation. The overall level of genetic variation within and between populations is relatively low, considering other measures of divergence. Application of species names to various groups appears arbitrary and does not conform to the biological species concept.

A number of evolutionary hypotheses have been applied to the genus with varying levels of success. Hypotheses relating biology to behavior have been applied to various well-studied populations; however, because of the low level of genetic variation, many of these are essentially untestable, hence speculative.

This thesis presents a method for identifying highly variable genetic loci within a species of *Macaca*. The level is high enough to permit testing of evolutionary hypotheses such as those of sociobiology.

This method may also permit the correlation of morphological change in a mammalian genus with various levels of speciation in a manner similar to that performed by Ayala on *Drosophila* species [Ayala 1975]. The fossil record of Primates is steadily increasing in number and type of forms. The problem is to evaluate these forms in a manner such that it is possible to name them in a biologically meaningful fashion. If RDNA data can be correlated with skeletal change, the primate fossil record could be evaluated in a more informative manner.

The biological species concept is widely accepted by biologists [Futuyma 1986; p. 111]. It is apparent from the analysis presented in this thesis that it is not applied by all biologists to natural populations. The designation of separate species status ascribes significance to the differences between the groups, which are imputed to be primarily inheritable. The danger is of inaccurately reflecting the evolutionary relationships between groups, and imputing an unrealistic significance to the characters used by the taxonomists to assign separate species status to the groups under analysis. The criteria for designating groups as separate species must be more than one of a simple difference in degree of differentiation. It must be a difference in kind of differentiation. In classifying groups as separate species, qualitative criteria must be used - the groups must be genetically different enough so that when mating occurs, no progeny result.

One could assert that it is impossible to reanalyze taxonomic designations of living creatures to align the names with the biological species definition, using the rationale that

because of the difficulty of the task we must simply live with the names as they have been assigned. I claim if we do not apply the same definitions to the names of taxa, we can not communicate our research results to each other effectively. If the biological species concept is accepted by biologists then it must be applied by biologists to the naming of taxa.

Recombinant DNA technology may permit a resolution of phylogenetic impasses. Species not known to be highly genetically variable can, through the use of recombinant DNA techniques, become amenable to genealogy reconstruction, a result previously considered unlikely. Clearly the RDNA data permits an extremely detailed level of testing of evolutionary hypotheses. Species, like the Japanese macaque, on which extensive ethological data exists, but that previously eluded fine genetic analysis, can now be utilized in the testing of evolutionary hypotheses. This particular species is not useful for the interpretation of the fossil record, given its ambiguous taxonomic status, however, the RDNA method described here provides a means of using the accumulated behavioral data available for this species for the testing of evolutionary hypotheses with a behavoiral component.

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