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Species and Geographic Differences in the Transferrin Polymorphism of Macaques

Abstract. *Eleven molecular forms and 34 phenotypes of transferrin have been detected in 372 serums from six macaque species. The tendency to polymorphism varies from species to species and from one local population to another. The most extensive polymorphism was observed in* Macaca mulatta, *which showed at least ten transferrins and 24 phenotypes. Transferrins present in stump-tail macaques* (M. speciosa) *from Thailand were also found in crab-eating macaques* (M. irus) *from Thailand, but not in crab-eating macaques from the Philippines. The results suggest that macaque species are semispecies rather than complete species.*

The question of whether the "species" of macaques cross-breed with each other remains open (*1, 2*). Our analysis of similarities and differences in transferrins between populations of macaques suggests a resolution of this question. Transferrin is the serum protein which picks up iron from the iron-storage protein ferritin, transports the iron throughout the body, and delivers it to tissue sites where hemoglobin and the different iron-containing enzymes are synthesized. Since distinguishable genetic variants of transferrin exist within and between various mammalian populations (*3*), knowledge of these transferrins can be applied to questions of the genetic origins of natural populations. Rhesus monkeys and certain other groups of macaques show an extensive polymorphism of transferrin (*4, 5*). We have already described nine molecular forms of transferrin and 19 transferrin phenotypes among 199 serums from four species of macaques. Subsequent work (*6*) on serums from the Wisconsin colony of rhesus monkeys furnished genetic evidence that a series of codominant alleles controlled the observed transferrin variations. Our analysis of 173 more macaque serums revealed two additional molecular forms of transferrin and 15 additional phenotypes. Altogether, 11 molecular forms and at least 34 phenotypes have been observed by starch-gel electrophoresis in 372 serums from six species. Not only are there sharp differences among macaque species in the distribution of transferrin phenotypes, but also marked geographic differences within a macaque species in the degree of the transferrin polymorphism and in the frequencies of the particular transferrin alleles.

The principal method employed was starch-gel electrophoresis (*7*) in the borate–tris buffer system (*8*), gels 14 cm wide, 16 cm long, and 1.4 cm deep being used. In this method up to 15 samples inserted into a starch gel on small squares of filter paper can be run side by side and compared. The transferrins of macaque serums, unlike those of human and chimpanzee serums, stand out after electrophoresis in an area of gel devoid of other serum proteins. Thus typing of macaque transferrins can readily be done simply by staining the starch gels with amido black.

The photograph of a starch gel in Fig. 1*A* shows the mobility differences among the 11 macaque transferrins, and the developed (*9*) autoradiograph in Fig. 1*B* demonstrates iron binding in the transferrins (*10*). Going from slowest to fastest electrophoretic mobility, the 11 macaque transferrins are

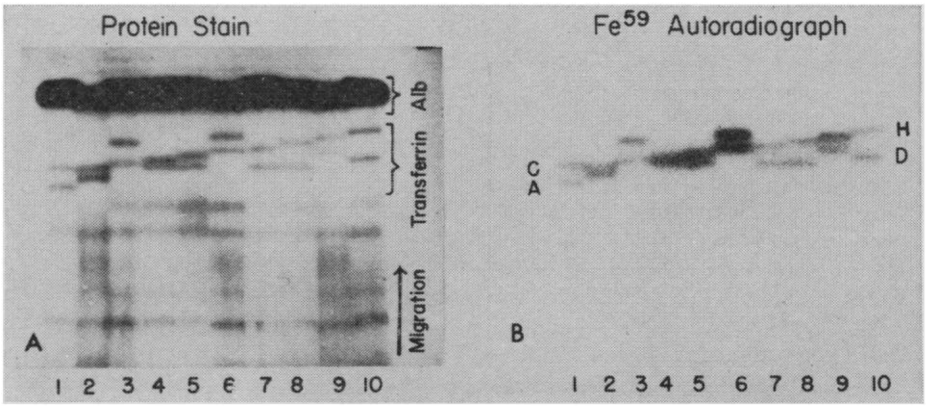


Fig. 1. Horizontal starch-gel electrophoresis of ten macaque serums having transferrin phenotypes AC, BC, D′G, CD, CE, F′H′, CF, CG, F′H′, and DH. Sample 2 was from a crab-eating macaque; samples 6 and 9 were from stump-tail macaques; and all other samples were from rhesus monkeys. Fe^{59} was added prior to electrophoresis according to Giblett *et al.* (*9*). The electrophoresis, however, was by the horizontal method at 6 cm for 4 hours. The gel half shown on the left (*A*) was stained with amido black. The other half was applied to x-ray film to make the autoradiograph shown on the right (*B*). (A transferrin such as F′ in sample 9 might not be typed accurately from the kind of results shown here, since in this experiment sample 9 was not placed adjacent to a serum containing known E, F′, or F transferrin. In the original typing suspected F′s were run several times between known E and known F and several times alongside of known F′ to confirm their classification. By similar procedures all the transferrins in samples 1 to 10 had previously been typed.)

labeled: A, B, C, D′, D, E, F′, F, G, H′, and H. D′ and F′ were discovered in the present study. The transferrin phenotypes (Table 1) are considered to be genotypes, in which types CC, DD, EE, F′F′, FF, GG, H′H′, and HH are controlled by homozygous alleles and all other types from AC to FG by heterozygous alleles. While the anomalous phenotype, B(D)G, consisting of three molecular transferrins (*5*), is considered a heterozygous one, the genetic basis for such a type has not been determined.

It would be extremely difficult to distinguish certain possible heterozygous phenotypes from known homozygous types such as D′D from DD and DE from EE. Thus there may actually be more than 34 phenotypes represented in our collection of macaque serums. Indeed, we have indirect evidence that this is the case. On retyping, we found that a male parent (R-64) in the Wisconsin colony was CD′ rather than CD as originally typed (*6*). Its mate (381) was still CD, but their offspring (A-75) which had originally been typed DD could just as readily be typed D′D from the location in the starch gel of its transferrin fraction. The phenotype D′D conforms to the genetic hypothesis, whereas DD does not.

The high degree of the transferrin polymorphism in rhesus monkeys (*Macaca mulatta*) is demonstrated by the data recorded in Tables 1 and 2 and Fig. 2. Ten transferrins and at least 24 phenotypes were recognized in the serums of these monkeys. Over half of the monkeys (that is, 97) came originally from the Nepal border area of India and this geographically homogeneous group was as polymorphic as the total group. It showed 21 phenotypes, of which 5 were homozygous and 16 were heterozygous types. All ten transferrins were represented. The transferrin of greatest frequency in this group as well as in the miscellaneous group was C: of the Nepal border monkeys 23 percent belonged to the homozygous CC phenotype and 45 percent belonged to eight heterozygous types containing the C transferrin.

In no other macaque species was the C transferrin so common. However, a high frequency of C transferrin did occur in certain groups of *M. irus* (cynomolgus or crab-eating macaque). The A, D′, and H transferrins were found only in *M. mulatta*. On the other hand, the H′ transferrin was not found

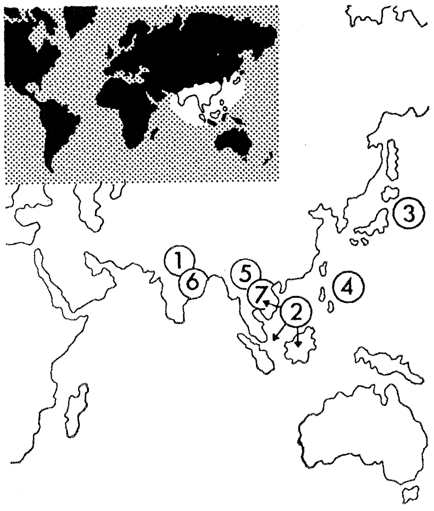
Table 1. Frequencies of transferrin alleles in various populations of macaques. The anomalous class B(D)G in Table 2 was arbitrarily counted as BG.

No. of samples	Alleles										
	Tf^{A}	Tf^{B}	Tf^{C}	$Tf^{D'}$	Tf^{D}	Tf^{E}	$Tf^{F'}$	Tf^{F}	Tf^{G}	$Tf^{H'}$	Tf^{H}
a. *M. mulatta* (Nepal border)											
97	0.010	0.036	0.454	0.010	0.217	0.072	0.005	0.046	0.119		0.031
b. *M. mulatta* (miscellaneous)											
78	0.013		0.654	0.026	0.115	0.019		0.032	0.135		0.006
c. *M. nemestrina*											
18			0.056		0.444			0.167	0.333		
d. *M. fuscata fuscata* (Japan)											
5								1.000			
e. *M. fuscata yakui* (Japan)											
5								1.000			
f. *M. radiata*											
2								1.000			
g. *M. irus* (Philippines)											
96			0.005		0.995						
h. *M. irus* (Thailand)											
20		0.075	0.275		0.400	0.025	0.075	0.025	0.050	0.075	
i. *M. irus* (Malaya)											
6			0.417		0.333		0.167			0.083	
j. *M. irus* (Viet Nam)											
2		0.250	0.250		0.500						
k. *M. irus* (miscellaneous)											
23			0.196		0.674	0.022	0.108				
l. *M. speciosa* (India)											
21										1.000	
m. *M. speciosa* (Thailand)											
16							0.469			0.531	
n. *M. speciosa* (miscellaneous)											
1										1.000	

Table 2. Distribution of transferrin phenotypes in macaque populations.

Phenotypes	Populations*													
	a	b	c	d	e	f	g	h	i	j	k	l	m	n
AC		1												
AD	2	1												
BC	4									1				
BD	1							1						
B(D)G	1													
BE								1						
BG								1						
BH	1													
CC	22	35						1	1		1			
CD′	1	2												
CD	14	8	1				1	6	2		6			
CE	8	3									1			
CF′	1							2	1					
CF	4	2												
CG	10	15	1											
CH′								1						
CH	2	1												
D′F	1													
D′G		2												
DD	7	3	4				95	3		1	11			
DF′									1		3			
DF	4	1	3					1						
DG	6	2	4											
DH′								2	1					
DH	1													
EE	3													
F′F′											1		3	
F′G								1						
F′H′													9	
FF		1	1	5	5	2								
FG			1											
GG	3	1	3											
H′H′												21	4	1
HH	1													

* Species names and the original geographic locations of these populations are listed in Table 1.



Group 1, *M. mulatta*, Nepal Border: A, 2.1; B, 7.2; C, 68.0; D′, 2.1; D, 37.1; E, 11.3; F′, 1.0; F, 9.3; G, 20.6; H, 5.2. Group 2, *M. nemestrina*: C, 11.1; D, 66.7; F, 27.8; G, 50.0. Group 3, *M. fascata*, Japan; F, 100. Group 4, *M. irus*, Philippines: C, 1.1; D, 100.0. Group 5, *M. irus*, Mainland: B, 14.3; C, 53.6; D, 64.3; E, 3.6; F′, 17.8; F, 3.6; G, 7.2; H′, 14.3. Group 6, *M. speciosa*, India: H′, 100.0. Group 7, *M. speciosa*, Thailand: F′, 75.0; H′, 81.3.

Fig. 2. Under each macaque group the molecular transferrins are listed. The number opposite each designated molecular transferrin is the percentage of serums in the group possessing the transferrin. Information on the original geographic location of the macaques was available for all the groups listed in this figure (see Table 1) except for group 2, *Macaca nemestrina*. The distribution of this species, indicated on the map, is taken from Fiedler (*11*).

in this species, but was present at moderate frequency in certain groups of *M. irus* and at very high frequency in *M. speciosa* (stump-tail macaque). The D transferrin, which had a moderate frequency in *M. mulatta*, had very high frequencies in *M. irus* and *M. nemestrina* (pig-tail macaque). The F′ transferrin, which had a very low frequency in *M. mulatta*, had moderate frequencies in certain groups of *M. irus* and a very high frequency in a *M. speciosa* group.

Not all groups in the genus *Macaca* are polymorphic for transferrin, and some are polymorphic in one part of their range but not in another. Our limited data suggest that island populations of macaques tend to be homozygous for one or another transferrin allele. Thus Japanese macaques were Tf^{F} homozygotes and crab-eating macaques from the Philippines were Tf^{D} homozygotes. In contrast to the state of homozygosity in the Philippines, heterozygosity was the rule among the crab-eating macaques from Thailand, Malaya, and Viet Nam. Twenty-eight of these mainland crab-eating macaques were examined, and they showed 12 different transferrin phenotypes, of which ten were heterozygous types and two were homozygous types.

Transferrin monomorphism in one geographical area and polymorphism in another were also found among stump-tail macaques (*M. speciosa*). A group of 21 of these monkeys from India were all typed as H′H′, a homozygous type not detected in any other macaque species. But in a group of 16 of these monkeys from Thailand only four were typed as H′H′, three were typed as F′F′, and nine were typed as F′H′. Thus, as in the transferrin polymorphisms of rhesus monkeys, pig-tail macaques, and Thailand crab-eating macaques, heterozygosity of transferrin genes was more common in the Thailand stump-tail macaques than was homozygosity. However, the heterozygosity of the Thailand stump-tail group involved only two alleles, whereas the heterozygosity of the other polymorphic macaque groups involved from four to ten alleles.

In exploring the possible significance of these findings concerning the distribution of transferrins in the genus *Macaca*, it may be helpful to look upon *Macaca* as a monophyletic assemblage of "semispecies," Mayr's (*12*) term for populations among which gene exchange is possible, but not as freely as among conspecific populations or races of a single species. The view that gene exchange between different macaque populations occurs is supported by the close similarity of the chromosome karyotypes of various macaque species (*13*) and by the fact that, in captivity, different macaques (such as rhesus and crab-eating macaques) produce fertile hybrids. Fooden (*1*) has presented evidence of morphological intergradation in Thailand between rhesus and crab-eating macaques and believes that these two groups should be considered races of a single species. He says that his studies also indicate that other currently recognized macaque species intergrade to form an enlarged species. Even if complete conspecificity does not exist, a small amount of gene exchange could have allowed a polymorphism in one macaque group to spread to another group. Thus the gene $Tf^{F'}$ could have been introduced into the Thailand stump-tail macaques from a contiguous population of crab-eating macaques. Once introduced into the stump-tail population its frequency in this population would increase tremendously with time if the selective conditions in Thailand favored its spread. However, nothing is yet known concerning the selective pressures maintaining the transferrin polymorphism in macaques.

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10. That these proteins were transferrins was also shown by immunological examination of the beta proteins of representative macaque serums with antiserums to human transferrin. We thank M. D. Poulik for further identifying the transferrin bands in several serums by noting the effect of diphtheria toxin on these serums. This toxin, owing to its content of neuraminidase, splits off sialic acid from transferrin, causing more slowly migrating bands of transferrin to appear during starch-gel electrophoresis: M. D. Poulik, *Clin. Chim. Acta* **6**, 493 (1961); W. C. Parker and A. G. Bearn, *J. Exptl. Med.* **115**, 83 (1962). Thus a stump-tail macaque serum which originally showed two bands in the transferrin zone showed four bands after the toxin treatment. Since untreated macaque serums never showed this many bands and since these untreated serums were always stored in the frozen state, the variation of transferrins observed among the macaque serums was not an artifactual one caused by bacterial toxin.
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