GEOGRAPHICAL DISTRIBUTION OF TWELVE TRANSFERRIN ALLELES IN BLACK RATS OF ASIA AND OCEANIA¹

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ABSTRACT

About 450 black rats (*Rattus rattus*) were collected from 25 localities in Asia and Oceania. Their serum transferrins were analyzed by a newly developed thin layer acrylamide gel electrophoresis accompanied with acrinol pretreatment, exhibiting 12 transferrin bands. Generally, Asian type rats ($2N=42$) had fast-moving transferrins (R-series), Ceylon type ($2N=40$) moderately moving ones and Oceanian type ($2N=38$) slowly moving ones (C-series). Exceptionally, in northern India and Pakistan all Asian-type rats had C-series Tf. The possibility that divergence of R-series Tf and C-series Tf had preceded the karyotypic differentiation from 42 to 38 is proposed. In combination with the previous molecular data, the time of the divergence is roughly estimated between the order of a million years and ten thousand years.

GENETIC polymorphism of serum transferrin in black rat (*Rattus rattus*), one of the most common feral rats, has already been revealed by the present authors (MORIWAKI, TSUCHIYA and YOSIDA 1969). Fast-moving transferrin band, Tf-R, and slow-moving one, Tf-N, were demonstrated in the Japanese population using starch-gel electrophoresis. In addition to them, four bands, Tf-C, -D, -E and -F, all of which are slower than Tf-N have been observed in the rats collected from Pacific islands (MALECHA and TAMARIN 1969; MALECHA, personal communication, 1970; TAMARIN *et al.* 1972). In order to survey the geographical distribution of these transferrins in the broader area, we have made two expeditions to Asia and Oceania, in 1968 and 1972, and collected several hundred rats from 25 localities. From those expeditions karyotypes of these animals have also been surveyed extensively (YOSIDA, TSUCHIYA and MORIWAKI 1971a, b; YOSIDA *et al.* 1971; YOSIDA and SAGAI 1972; YOSIDA 1973; YOSIDA and SAGAI 1973; YOSIDA *et al.* 1974). In brief, Asian-type rats ($2N=42$) are distributed in East and Southeast Asia, and Oceania-type ones ($2N=38$) in Oceania, Southwest Asia and Central Asia. The third type, rats having 40 chromosomes, were found in Ceylon and are considered to be a transient type from the Asian to the Oceanian (YOSIDA *et al.* 1972).

In the present study, geographical distribution of 12 transferrin alleles of the black rats in Asia and Oceania is investigated with special reference to the three

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cytotaxonomical types mentioned above. A possible differentiation process of those types will be discussed based on the geographical distribution of each allele.

MATERIALS AND METHODS

A total of 445 black rats, *Rattus rattus*, including various subspecies were collected from the following localities in 1968 and 1972. In 1968: Okinawa, Japan (*R. rattus tanezumi*). Hong Kong (*R. rattus flavipectus* and *R. rattus sladeni*). Tainan, Taiwan (*R. rattus* subsp.). Bangkok, Thailand (*R. rattus* subsp.). Kuala Lumpur, Malaysia (*R. rattus diardi*, *R. rattus jalorensis* and *R. rattus argentiventer*). Bogor, Indonesia (*R. rattus argentiventer*). Makassar, Indonesia (*R. rattus argentiventer*). Central Luzon, the Philippines (*R. rattus mindanensis*). Mindanao Island, the Philippines (*R. rattus mindanensis*). Brisbane, Australia (*R. rattus rattus*). Cairns, Australia (*R. rattus rattus*). Wellington, New Zealand (*R. rattus rattus*). Port Moresby, New Guinea (*R. rattus rattus*). In 1972: Hong Kong (*R. rattus flavipectus*). Kuala Lumpur, Malaysia (*R. rattus diardi*, *R. rattus jalorensis* and *R. rattus argentiventer*). Kalyani, India (*R. rattus* subsp.). Kanpur, India (*R. rattus rufescens*). West Ghats Mountains, India (*R. rattus wroughtoni*). Lahore, Pakistan (*R. rattus rufescens*). Islamabad, Pakistan (*R. rattus rufescens*). Kandy, Sri Lanka (*R. rattus kandianus*). Madras, India (*R. rattus* subsp.). Deccan Plateau, India (*R. rattus* subsp.). Poona, India (*R. rattus* subsp.). Karachi, Pakistan (*R. rattus rattus*). Chalus, Iran (*R. rattus rattus*). The taxonomical details of these materials were described elsewhere (YOSIDA, TSUCHIYA and MORIWAKI 1971b; YOSIDA *et al.* 1974).

For the separation and identification of serum transferrins, we have developed a successful method consisting of acrinol-fractionation of serum proteins and of thin layer acrylamide-gel electrophoresis. A small amount of serum, approximately 100 μ l, was diluted 4 times by pH 8.8 Tris buffer and mixed with the equal volume of 0.6% acrinol solution. Most of the serum proteins could be removed by this treatment and the residual transferrin fraction was precipitated

FIGURE 1.—Thin layer acrylamide-gel electrophoretic patterns of black rat sera obtained from Asia and Oceania. White dots indicate major bands of different transferrins whose designations are shown on the right margin. The upper 7 samples were pretreated with acrinol and cold ethanol, but the lower 4 were not.

FIGURE 2.—Schematic illustration of 12 transferrin bands separated by the thin layer acrylamide-gel electrophoresis.

by the further addition of cold ethanol. The partially purified transferrin was subjected to thin layer acrylamide-gel electrophoresis. The details of this procedure are published elsewhere (MORIWAKE, SADAIE and HIRASAWA 1974). As shown in Figure 1, 12 transferrins, *R*, *R*₁, *R*₂, *N*, *C*₁, *C*₂, *C*, *D*, *D*₁, *E*, *F* and *G*, in the black rats obtained from Asia and Oceania were able to be clearly separated on the gel and accurately identified without any use of the hazardous gamma emitter, Fe⁵⁹. Figure 2 summarizes schematically the electrophoretic patterns of the 12 transferrins.

RESULTS

The localities of collection, the number of individuals collected, their transferrin types and the frequencies of each transferrin allele are summarized in Table 1.

Asian-type rats (2N=42):

Major transferrin alleles in Asian-type black rats are *R* and *N* as observed in the several subspecies collected from Japan (*R. rattus tanezumi*), Taiwan (*R. rattus* subsp.), Hong Kong (*R. rattus flavipectus*), Thailand (*R. rattus* subsp.) and Malaysia (*R. rattus diardi* and *R. rattus jalorensis*). Exceptionally, the rats in Philippine (*R. rattus mindanensis*) have *R*₁ allele predominantly and those in Indonesia (*R. rattus argentiventer*) have *R*₂ as a monomorphic allele, which is observed in Malaysian *R. rattus argentiventer* as well. *R. rattus diardi* in Malaysia has a characteristic *D*₁ allele besides *R* and *N*. Asian-type rats in Thailand are occasionally possessed of the Oceanian-type transferrin alleles such as *C*, *D* and *E*, though they are in the minority. Concerning those major alleles in the Asian-type rats, it is noteworthy that the frequency of *N* allele in southern Asia, Thailand and Malaysia is considerably higher than that in northern Asia, Japan, Hong Kong and the Philippines.

In spite of their Asian-type chromosomes (2N=42), the rats in northern India (Kanpur) and in northern Pakistan (Islamabad and Lahore) exhibited either *C*

TABLE 1
*Distribution of transferrin phenotypes and allelic frequencies in Rattus rattus
collected from various localities in Asia and Oceania*

Locality of collection	Name of subspecies	Number of samples	Number of individuals with each transferrin phenotype	Allelic frequencies of transferrins											
				R	R ₁	R ₂	N	C ₁	C ₂	C	D	D ₁	E	F	G
Asian type (2n = 42)															
Mainland, Japan	<i>R. rattus tanezumi</i>	168*	R (124), RN (36), N (8)	.85			.16								
Okinawa, Japan	<i>R. rattus tanezumi</i>	31	R (25), RN (4), N (2)	.87			.13								
Hong Kong	<i>R. rattus flavipectus</i>	9	R (8), RN (1)	.94			.06								
	<i>R. rattus sladeni</i>	8	C ₁ (8)					1.0							
Tainan, Taiwan	<i>R. rattus subsp.</i>	3	R (3)	1.0											
Bankok, Thailand	<i>R. rattus subsp.</i>	40	R (5), RN (8), N (20), NE (2), C ₁ (2), CN (3)	.23			.66	.05		.04			.03		
Kuala Lumpur, Malaysia	<i>R. rattus diardi</i>	10	R (3), RN (2), RD ₁ (2), N (1), ND ₁ (3)	.42			.29						.29		
	<i>R. rattus jalorensis</i>	15	R (5), RN (8), N (2)	.60			.40								
	<i>R. rattus argenbiventer</i>	8	R ₂ (8)			1.0									
Bogor, Indonesia	<i>R. rattus argenbiventer</i>	12	R ₂ (12)			1.0									
Makassar, Indonesia	<i>R. rattus argenbiventer</i>	47	R ₂ (47)			1.0									
Central Luzon, Philippines	<i>R. rattus mindanensis</i>	43	R ₁ (29), R ₁ N (9), N (5)	.78			.22								
Mindanao Is., Philippines	<i>R. rattus mindanensis</i>	6	R ₁ (2), R ₁ N (3), N (1)	.58			.42								
Kalyani, India	<i>R. rattus subsp.</i>	7	RC ₁ (2), C ₁ (5)	.14				.86							

TABLE 1 (Continued)

Locality of collection	Name of subspecies	Number of samples	Number of individuals with each transferrin phenotype	Allelic frequencies of transferrins									
				R	R ₁	R ₂	N	C ₁	C ₂	C	D	D ₁	G
Kanpur, India	<i>R. rattus rufescens</i>	18	C ₂ F(1), C(11), CD(1), CF(5)						.03	.78	.03		.17
W.Chats Mts., India	<i>R. rattus wroughtoni</i>	8	C ₁ (8)				1.0						
Lahore, Pakistan	<i>R. rattus rufescens</i>	30	C(4), CE(12), E(14)							.33		.67	
Islamabad, Pakistan	<i>R. rattus rufescens</i>	14	C(3), CE(7), E(4)							.46		.54	
Ceylon type (2n = 40)													
Kandy, Sri Lanka	<i>R. rattus kandelianus</i>	19	C ₁ (4), C ₁ C(4), C ₁ D(1), C ₂ G(1), CG(1), D(5), DE(3)				.37			.13	.37	.08	.05
Oceanian type (2n = 38)													
Brisbane, Australia	<i>R. rattus rattus</i>	20	C(5), CD(5), CF(2), D(4), DF(4)							.43	.43		.15
Cairns, Australia	<i>R. rattus rattus</i>	8	RC(1), C(1), CD(3), D(3)	.06						.38	.56		
Wellington, New Zealand	<i>R. rattus rattus</i>	23	RC(1), NC(3), C(17), CE(2)	.04			.07			.87		.04	
Port Moresby, New Guinea	<i>R. rattus rattus</i>	11	NE(1), CE(6), E(4)				.05			.27		.68	
Madras, India	<i>R. rattus subsp.</i>	10	C(1), CD(1), D(5), DE(2), EF(1)							.15	.70	.10	.05
Deccan Plateau, India	<i>R. rattus subsp.</i>	20	ND(1), C(8), CD(2), CE(1), CF(3), D(3), DE(1), DF(1)				.03			.55	.28	.05	.10
Poona, India	<i>R. rattus subsp.</i>	20	C(10), DC(6), D(4)							.65	.35		
Karachi, Pakistan	<i>R. rattus rattus</i>	3	CE(3)							.50		.50	
Chalus, Iran	<i>R. rattus rattus</i>	2	C(2)							1.0			

* Cited from the previous report (MORIYAKI, TSUCHIYA and YOSIDA 1969).

or *E* as the major transferrin alleles which are rather common in Oceanian-type ($2N=38$). A rare allele, *C*₂, was seen only in the Asian-type rats obtained from Kanpur.

Ceylon-type rats ($2N=40$):

*C*₁ allele was observed in the Ceylon-type rats and also in the Asian-type rats inhabiting such restricted areas as Hong Kong, Kalyani (Eastern India) and the West Ghats Mountains (Southern India). The Ceylon-type black rats have *C*, *D* and *E* transferrin alleles to some extent and also *G* allele in a lower frequency, in addition to the major *C*₁ allele.

FIGURE 3.—Geographic distribution in Asia and Oceania of *R*-series transferrins (*Tf-R*, *-R*₁, *-R*₂ and *-N*) and *C*-series transferrins (*Tf-C*₁, *-C*₂, *-C*, *-D*, *-D*₁, *-E*, *-F* and *-G*) of black rat, *Rattus rattus*. The border of the distribution of Asian-type rats ($2N=42$) and Oceanian-type rats ($2N=38$) is indicated by a broken line. Arabic numerals represent the places of collection. 1: Mainland, Japan. 2: Okinawa, Japan. 3: Tainan, Taiwan. 4: Hong Kong. 5: Central Luzon, Philippine. 6: Mindanao, Philippine. 7: Makassar, Indonesia. 8: Bogor, Indonesia. 9: Kuala Lumpur, Malaysia. 10: Bangkok, Thailand. 11: Kalyani, India. 12: Kanpur, India. 13: New Delhi, India. 14: Lahore, Pakistan. 15: Islamabad, Pakistan. 16: Kandy, Sri Lanka. 17: Madras, India. 18: W. Ghats Mts., India. 19: Deccan Plateau, India. 20: Poona, India. 21: Karachi, Pakistan. 22: Chalus, Iran. 23: Port Moresby, New Guinea. 24: Cairns, Australia. 25: Brisbane, Australia. 26: Wellington, New Zealand. 27: Eniwetok Islands.

Oceanian-type rats (2N=38):

In the Oceanian-type rats collected from Australia, New Zealand, New Guinea, Southern India, Southern Pakistan and Iran, the transferrin alleles, *C*, *D* and *E*, are predominant, wherever they inhabit. In the Asian-type rats *F* allele was observed only in those obtained from Kanpur in India, while it was fairly common in the Oceanian-type rats. An Asian-type transferrin allele, *N*, was detected in a considerably lower frequency in Wellington in New Zealand, Port Moresby in New Guinea and the Deccan plateau in India. A fairly small number of Oceanian-type rats having the other Asian-type transferrin allele, *R*, was found in Port Moresby and Cairns, Australia.

The six transferrin types demonstrated above, *R*₁, *R*₂, *C*₁, *C*₂, *D*₁ and *G*, were newly discovered in the present study, in addition to the remaining six *R*, *N*, *C*, *D*, *E* and *F*, reported previously (MORIWAKI, TSUCHIYA and YOSIDA 1969; MALECHA and TAMARIN 1969).

The geographic distribution of those transferrin alleles in Asia and Oceania described above is illustrated in Figure 3 for the region of the borderline of the two karyotypes. In general, the Asian-type rats (except those in northern India and Pakistan), have fast-moving transferrins such as *R*, *R*₁, *R*₂ and *N*, the Ceylon-type moderately moving ones such as *C*₁ and the Oceanian-type slowly moving ones such as *C*, *D*, *E* and *F*.

DISCUSSION

It has already been hypothesized based on the abundant cytogenetical data of various rats in Asia and Oceania that black rats having 42 chromosomes (Asian type) are presumably of an ancestral type which has evolved to Oceanian type (2N=38) through the intermediate Ceylon type (2N=40) (YOSIDA 1973). As the cause of those chromosome rearrangements, Yosida assumed the occurrence of two Robertsonian fusions. As shown in the present study, Asian-type rats mostly have *Tf-R*, *-R*₁, *-R*₂ and *-N* (*R*-series transferrins), and Oceanian type *Tf-C*, *-D* and *-E* (*C*-series transferrins). But exceptional cases were observed in northern India and Pakistan where all the rats having 42 chromosomes exhibited *Tf-C* and *Tf-E*. This is a crucial finding for revealing the differentiation process of the three cytotaxonomical types of *Rattus rattus*, because their divergence should have taken place in the India area long ago (YOSIDA *et al.* 1974). It is likely that in the relatively nearer past they have migrated all over the world. This is the case in the Oceanian *Rattus rattus* (YOSIDA, TSUCHIYA and MORIWAKI 1971b).

One possible explanation for these exceptional cases might be "Introgressive hybridization" (ANDERSON 1949) of *C*-series transferrin genes from the Oceanian-type population in the southern India and Pakistan area to the Asian-type one in the northern area. However, the evidence that the transferrin locus in those Asian-type rats had been completely replaced with *C*-series transferrin alleles, as far as the present data are concerned, seems to be hardly explained solely by the introgressive hybridization, although this may be the case in the other several populations which involved a lower frequency of transferrin alleles

probably introgressed from different cytotaxonomical types (see the data from Bangkok, Deccan pl., Cairns, Wellington and Port Moresby in Table 1).

The other likely possibility for those cases is that the separation of two rat groups having *C*-series transferrins and *R*-series ones had preceded the karyotypic differentiations from 42 to 38.

If we take into account this notion and the cytotaxonomical hypothesis mentioned above, a plausible differentiation process of *Rattus rattus* could be considered as follows: The presumptive ancestral rats have had 42 chromosomes and *Tf-X*, which might have originated in some part of the India-Indonesia area. They were separated into two groups probably by geographical isolation. We may imagine several geographical barriers in this area, for instance: Ganges River, Himalayan Mountains and Hindostan Plain in the inter-glacial periods. Such groups have propagated independently for a very large number of years, which seem to be long enough to develop *R*-series or *C*-series transferrins from *Tf-X* through numerous amino acid substitutions. Once *C*-series transferrins were established in the rat population with 42 chromosomes, the karyotypic differentiations had emerged. The length of time for them seems to be relatively smaller than that for transferrin evolution from *Tf-X* to either *Tf-R* or *Tf-C*, because in most rats in India, Pakistan, Ceylon and Oceania with chromosome numbers of either 42, 40 or 38, the transferrin alleles, *Tf^c*, *Tf^p* and *Tf^e*, are equally predominant. On the other hand, the rat populations with 42 chromosomes and *R*-series transferrins might have been expanded without karyotypic change to mainland China, Japan, the Indonesian islands and the Philippine islands accompanying further differentiation in transferrin alleles probably due to geographical isolation. As shown in Table 1, *Tf-R* is predominant in mainland China and Japan, *Tf-N* in Thailand, *Tf-R₁* in the Philippine islands and *Tf-R₂*

FIGURE 4—A hypothetical differentiation process of black rats having various transferrins and chromosome numbers. Arrows between *R*-series transferrins and *C*-series transferrins suggest the possible gene introgression.

in the Indonesian islands. This hypothetical process of differentiation is illustrated in Figure 4.

Even within the Asian-type rats, those from Japan exhibited the higher frequency of acrocentric chromosome No. 1, whereas those from India and Pakistan mostly had subtelocentric chromosome No. 1 (YOSIDA *et al.* 1974). The higher frequency of the subtelocentric chromosome No. 1 is rather common among the rats from northern India and Pakistan ($2N=42$), from Ceylon ($2N=40$) and from southern India ($2N=38$), regardless of the karyotypic difference. This finding may support the present argument that there were at least two groups of Asian-type rats, one with *R*-series transferrins and the other with *C*-series transferrins (See Figure 4).

According to the analytical data in a preliminary report (MORIWAKI, SATO and TSUCHIYA 1971), the difference between *Tf-R* and *Tf-C* in the amino acid composition is approximately 30 after correction for the blank experiment. Using these data, we attempted to get a rough idea on the length of time for divergence between *Tf-R* and *Tf-C*, though it must be considerably erroneous.

As almost a linear relationship between composition difference and sequence difference could be observed (MORIWAKI, SATO and TSUCHIYA 1971; HARRIS and TELLER 1973) in the range of less than 30 amino acid differences based on the sequence data on cytochrome-C and hemoglobins (DAYHOFF 1969), we may regard the difference between *Tf-R* and *Tf-C* as that in the sequence. For calculating the length of time since divergence, the amino acid sequence difference must be divided by a factor of 2, giving roughly 15 (KIMURA and OHTA 1971). As we do not know of any data on the rate of amino acid substitution in transferrin evolution, we used tentatively the value for fibrinopeptides, 9.0 per amino acid site per 10^9 years (DAYHOFF 1969). If the number of amino acid residues constituting this protein is assumed to be around 630 (HUDSON *et al.* 1973), the difference of 15 amino acids corresponds to the order of one million years.

As this seems to be a very rough estimation, the length of time since divergence of *Tf-R* and *Tf-C* can vary between the order of a million years and that of a hundred thousand years. But it may not be the order of ten thousand years.

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