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GENETIC DIFFERENTIATION AMONG KARYOTYPIC FORMS OF THE BLACK RAT, *RATTUS RATTUS*

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ABSTRACT

The black rat, *Rattus rattus*, consists of five karyotypic forms—2n = 42 (high C-banding); 2n = 42 (low C-banding); 2n = 40; 2n = 38; 2n = 42 Mauritius. Here, we use isozyme electrophoresis and microcomplement fixation to elucidate the genetic distance and phylogenetic relationship among each of the various karyotypic forms of *R. rattus* and *R. norvegicus*. The results show that (1) the 2n = 42 Mauritius black rat (2n = 42Mau) is genetically very similar to the 2n = 38 form, suggesting that this island population has undergone very rapid chromosomal evolution; (2) the 2n = 40 form from the highlands of Sri Lanka is genetically distinct from the 2n = 38 form from the lowlands; the genetic difference is probably insufficient, however, to prevent future introgression; (3) the level of genetic differentiation occurring between the 2n = 42 forms on the one hand and the 2n = 38, 2n = 40 and 2n = 42 Mau forms on the other support the hybrid incompatability data in suggesting that the two groups are either full species or incipient species; (4) in contrast to data from amino acid composition of transferrin and from restriction endonuclease digests of mtDNA, the present data suggest that the various karyotypic forms of *R. rattus* are phylogenetically more closely related to each other than any is to *R. norvegicus*, and that they are related by a series 2n = 42 → 2n = 40 → 2n = 38; (5) the *R. rattus/R. norvegicus* divergence occurred 2–8 million years ago, whereas the various chromosomal forms of *R. rattus* diverged over the last 4 million years.

THE black rat, *Rattus rattus*, has been the subject of numerous cytogenetic studies (YOSIDA 1980a). These studies have revealed a great wealth of chromosomal diversity within the species, including pericentric inversions, centric fusions, centric fissions, C-band polymorphisms and supernumerary chromosomes.

Yosida recognized the following five chromosomal groups within this species:

1. Southeast Asian (2n = 42 with high C-banding; includes *R.r. mindanensis, R.r. diardii, R.r. flavipectus*). This form has a gross karyotype which we believe is probably ancestral for the genus Rattus (BAVERSTOCK *et al.* 1977). Every element in the complement possesses procentric blocks of C-positive heterochromatin.

2. Japanese (2n = 42, with low C-banding; *R.r. tanezumi*). The gross kary-

otype of this form is the same as the Southeast Asian form. However, seven of the 12 pairs of large telocentrics lack procentric C-blocks.

3. Ceylonese (2n = 40; *R.r. kandianus*). This form is found in the highlands of Sri Lanka. It differs karyotypically from the Southeast Asian form by a single centric fusion involving chromosomes *11* and *12*.

4. Oceanian (2n = 38; *R.r. rattus*). The karyotype of this form differs from the Southeast form by two centric fusions, one of which is also found in the 2n = 40 form. Oceanian black rats are found from India westward into the Middle East and have been transported, presumably by man, to Europe, Africa, the Americas and Australia. They are also found in the lowlands of Sri Lanka.

5. Mauritius (2n = 42Mau; *R.r. rattus*). This form is found exclusively on the island of Mauritius. It differs karyotypically from the Southeast Asian form by the two centric fusions observed in the Oceanian black rat and two additional centric fissions.

The variety of karyotypic forms found within the black rat suggests the possibility of the existence of cryptic species (BAVERSTOCK 1981). Moreover, data from the analysis of amino acid composition of transferrin and from restriction endonuclease digests of mitochondrial DNA suggest the possibility that the 2n = 42 forms of *R. rattus* may be more closely related to *R. norvegicus* than to the fusion forms of *R. rattus* (MORIWAKI, SATO and ISUCHIYA 1971; HAYASHI *et al.* 1979).

The mtDNA data have been disputed by BROWN and SIMPSON (1981), who found that the mtDNA of all forms of *R. rattus* they examined was more like each other than any was to the mtDNA of *R. norvegicus*. However, their data raised a second problem—the mtDNA of the 2n = 38 form of *R. rattus* is more like the mtDNA of the 2n = 42 form than it is like the 2n = 40 form.

We, therefore, undertook a detailed molecular study of the genetic relationships between the various karyotypic forms of *R. rattus* and of *R. norvegicus*, using the techniques of isozyme electrophoresis and microcomplement fixation. Of special interest were the following problems:

1. The Mauritius black rat (2n = 42Mau) differs from the 2n = 38 form by two fixed centric fissions. YOSIDA (1980a) has suggested that this rat reached the island about 400 years ago as a 2n = 38 form which subsequently underwent two centric fissions that have become fixed. If this were true, it would be a case of remarkably rapid chromosomal evolution. The possibility remains, however, that the black rat has been on the island for a much longer period of time or that the 42Mau karyotype arose elsewhere long before it arrived on the island. How different genetically are the 2n = 38 and 2n = 42Mau forms?

2. On the island of Sri Lanka, the 2n = 40 form occurs on the highlands and the 2n = 38 form on the lowlands, with limited hybridization where they meet. Are these forms distinct genetic types?

3. Are there "cryptic species" within *R. rattus* as currently recognized?

4. What are the evolutionary relationships between the various forms of *R. rattus* and *R. norvegicus*, and how old are the divergences?

The present study was undertaken to provide answers to these questions.

MATERIALS AND METHODS

Specimens used were from laboratory colonies maintained at the National Institute of Genetics in Misima. These colonies were established from animals collected as follows: *R.r. tanezumi* (2n = 42, Japanese type)—collected from Anami Island in 1978—*42 R.r. (Jap)*; *R.r. flavipectis* (2n = 42, Southeast Asian type)—collected in Hong Kong in 1973—*42 R.r. (HK)*; *R.r. kandianus* (2n = 40)—collected in Sri Lanka in 1973—*40 R.r. (SL)*; *R.r. rufescens* (2n = 38)—collected from Trincomelle in Sri Lanka in 1978—*38 R.r. (SL)*; *R.r. rattus* (2n = 38) Oceanian—collected from Adelaide, Australia in 1980—*38 R.r. (Aus)*; *R.r. rufescens* (2n = 38)—collected from the Seychelles Islands in 1978—*38 R.r. (SI)*; *R. rattus* Mauritius (2n = 42Mau)—collected from Mauritius in 1978—*42 R.r. (Mau)*. *R. norvegicus* (*R. nor.*) was the Porton laboratory strain, I.M.V.S. Adelaide. *R. losea* (*R. los.*) (2n = 42; from Thailand in 1976), *R. exulans* (*R. ex.*) (2n = 42; from Thailand in 1976) and *R. villosissimus* (*R. vill.*) (2n = 50; from Australia 1980) were included as outgroups (see FARRIS 1972). Three specimens of each form were studied electrophoretically except for the Mauritius black rat where only two individuals were available. Animals were killed and liver, kidney and plasma frozen immediately. A total of 45 loci was screened for electrophoretic variation using cellulose acetate (Cellogel) as previously described (BAVERSTOCK *et al.* 1980). The loci scored were: Aconitase (*Acon-1* and *Acon-2*); Acid phosphatase (*Acp-1* and *Acp-2*); Adenosine deaminase (*Ada*); Alcohol dehydrogenase (*Adh*); Adenylate kinase (*Ak-1* and *Ak-2*); Albumin (*Alb*); Alkaline phosphatase (*Ap-1* and *Ap-2*); Carbonic anhydrase (*Ca*); Diaphorase (*Dia*); Enolase (*Enol*); β-Galactosidase (*βGal*); Glyoxalase (*Glo*); Glutamate oxaloacetate transaminase (*Got-1* and *Got-2*); Glycollate oxidase (*Gox*); α-Glycerophosphate dehydrogenase (*αGpd*); Glucose-phosphate isomerase (*Gpi*); Glucuronidase (*Gus*); Hydroxybutyrate dehydrogenase (*Hbdh*); Isocitrate dehydrogenase (*Idh-1* and *Idh-2*); Lactate dehydrogenase (*Ldh-1* and *Ldh-2*); Malate dehydrogenase (*Mdh-1* and *Mdh-2*); Malic enzyme (*Me*); Nucleoside phosphorylase (*Np*); Peptidases, val-leu (*Pep-A*), leu-gly-gly (*Pep-B*); lys-leu (*Pep-Cl* and *Pep-C2*); phe-pro (*Pep-D*); 6-Phosphogluconate dehydrogenase (*6-Pgd*); Phosphoglycerate kinase (*Pgk*); Phosphoglucomutase (*Pgm*); Pyruvate kinase (*Pk-1* and *Pk-2*); Superoxide dismutase (*Sod-1* and *Sod-2*); Sorbitol dehydrogenase (*Sordh*); Transferrin (*Trf*). Alleles were designated according to electrophoretic mobility with *a* being most anodal.

Genetic distances were calculated as NEI *D*'s (corrected for small samples, NEI 1978) or as "fixed" differences (BAVERSTOCK, WATTS and COLE 1977).

The present study is based on only a small number of representatives of each form. Nevertheless, it has been amply demonstrated that estimates of genetic distance and of fixed differences are not greatly influenced by small sample size (SARICH 1977; BAVERSTOCK, WATTS and COLE 1977; NEI 1978; GORMAN and RENZI 1979). Moreover, our estimates of genetic distance have been corrected for small sample size (NEI 1978). For these reasons we believe that the conclusions of the present study are unlikely to be altered greatly by using larger sample sizes.

Microcomplement fixation (MC'F) of albumin followed the protocol of CHAMPION *et al.* (1974). Antisera were prepared in rabbits (three per antigen) to purified albumin of Oceanian *R. rattus* (2n = 38) collected in Adelaide, of *R. norvegicus* (laboratory strain) and of *R. villosissimus* collected from southwest Queensland, Australia.

RESULTS

Electrophoretic data: Table 1 shows allele frequencies at 45 loci for seven populations of *R. rattus*, along with *R. norvegicus*, *R. losea* and *R. exulans*. A matrix of percent fixed differences and of corrected NEI *D*'s is shown in Table 2. Phenetic clusterings by UPGMA (SNEATH and SOKAL 1973) and phylogenetic analyses by the distance Wagner method (FARRIS 1972) for both sets of data are shown in Figures 1–4. All show essentially the same features, namely,

1. All three 2n = 38 populations of *R. rattus* form a group with a maximum of one fixed difference between them. Corrected NEI *D*'s range from 0.022 to 0.033 (Table 2).

TABLE 1

Allele frequencies (percent) at 45 loci in ten populations of Rattus

Locus	Al-lele	42 R.r. (Jap)	42 R.r. (HK)	40 R.r. (SL)	38 R.r. (SL)	38 R.r. (Aus)	38 R.r. (SI)	42 R.r. (Mau)	*R. nor.*	*R. los.*	*R. ex.*
Acon-2	*a*							25			
	b				84	100	67	25			
	c	100	100	100	16		33	50			
	d								100		
	e										100
	f									100	
Acp-2	*a*	100	100	100	100	100	100	100			
	b								100	100	100
Ada	*a*								100		16
	b										84
	c	100	100	100	100	100	100	100		100	
Adh	*a*								100		100
	b	100	100	100	100	100	100	100		100	
Ak-2	*a*									75	
	b	50	33	100	100	100	100	100		25	100
	c	50	67						100		
Alb	*a*										100
	b	100	100	100	100	100	100	100	100	100	
Ap-1	*a*		100								
	b			50	100	100	84	100			50
	c	84									
	d	16		50			16		100	100	
	e										50
Ca	*a*	33	100	100	100	100	100	100			16
	b	67							100	100	84
βGal	*a*								100		
	b	100	100			16				?	
	c										100
	d			100	100	84	100	100			
Got-1	*a*										100
	b	100	100	100	100	100	100	100	100	100	
Gox	*a*			100	100	100	100	100	100	100	100
	b	100	100								
αGpd	*a*								100		
	b	100	100	100	100	100	100	100		100	100
Gpi	*a*	100	100	100	100	100	100	100		100	100
	b								100		

TABLE 1—Continued

Locus	Allele	42 R.r. (Jap)	42 R.r. (HK)	40 R.r. (SL)	38 R.r. (SL)	38 R.r. (Aus)	38 R.r. (SI)	42 R.r. (Mau)	*R. nor.*	*R. los.*	*R. ex.*
Gus	*a*			100							
	b		100								
	c				100	100	50	100	?	?	?
	d	100									
	e						50				
Hbdh	*a*								100		100
	b			100	84	100	67	50		100	
	c	100	100		16		33	50			
Ldh-2	*a*	100	84	100	100	100	100	100	100	100	100
	b		16								
Mdh-1	*a*	100	100	100	100	100	100	100		100	100
	b								100		
Mdh-2	*a*	100	16								
	b		84	100	100	100	100	100	100	100	100
Me	*a*	100	100	100	100	100	100	100			
	b								100		100
	c									100	
Np	*a*										100
	b		16	100	100	100	100	100			
	c	100	84						100	100	
Pep-A	*a*										16
	b	100	100	100	100	100	100	100	100	100	84
Pep-C1	*a*										100
	b		33	100	50						
	c								50		
	d	100	67		50	100	100	100	50	25	
	e									25	
	f									50	
Pep-C2	*a*										100
	b	100	100	100	100	100	100	100	100	25	
	c									75	
Pep-D	*a*	16				100					
	b			100	100		100	100	100	100	100
	c	84	100								
6Pgd	*a*	100		100	50	100	100	50		100	100
	b		100								
	c				50			50	100		
Pk-1	*a*								100	100	100
	b	100	100	100	100	100	100	100			

TABLE 1—Continued

Locus	Allele	42 R.r. (Jap)	42 R.r. (HK)	40 R.r. (SL)	38 R.r. (SL)	38 R.r. (Aus)	38 R.r. (SI)	42 R.r. (Mau)	*R. nor.*	*R. los.*	*R. ex.*
Pk-2	*a*										100
	b								100		
	c	100	100	100	100	100	100	100		100	
Sord-h	*a*	100									
	b		100	100	100	100	100	100	100	100	100
Trf	*a*	100	50								
	b								100		
	c										100
	d							25			
	e		50								
	f				67	?		75			
	g			100							
	h									100	
	i				33		100				

The following 15 loci were invariant across all populations—*Acon-2, Acp-1, Ak-1, Ap-2, Dia, Enol, Glo, Got-2, Idh-1, Idh-2, Ldh-1, Pep-B, Pgk, Pgm, Sod-1, Sod-2.*

TABLE 2

Genetic distances between Rattus populations

		A	B	C	D	E	F	G	H	I	J
42 R. r. (Jap)	A	—	0.127	0.286	0.289	0.259	0.252	0.265	0.558	0.370	0.612
42 R. r. (HK)	B	9	—	0.229	0.223	0.231	0.213	0.211	0.507	0.375	0.599
40 R. r. (SL)	C	22	18	—	0.072	0.101	0.076	0.096	0.430	0.267	0.454
38 R. r. (SL)	D	20	16	4	—	0.033	0.022	0.020	0.458	0.278	0.442
38 R. r. (Aus)	E	18	16	9	2	—	0.031	0.055	0.525	0.289	0.462
38 R. r. (SI)	F	18	16	7	0	2	—	0.039	0.491	0.271	0.439
42 R. r. (Mau)	G	20	16	7	0	2	2	—	0.443	0.298	0.418
R. nor.	H	39	39	36	36	40	36	34	—	0.366	0.411
R. los.	I	26	28	21	21	21	19	21	28	—	0.391
R. ex.	J	45	43	34	34	35	34	32	32	33	—

Upper matrix, corrected NEI *D*'s; Lower matrix, percent fixed differences.

2. *R. rattus* from Mauritius (2n = 42) is a fourth member of this group, with a maximum of one fixed difference between it and the 2n = 38 forms, and NEI *D*'s ranging from 0.020 and 0.055 (Table 2).

3. *R.r. kandianus*, the 2n = 40 form from Sri Lanka, is closer to this group than to any other, but is, nevertheless, genetically distinct from it, with NEI *D*'s ranging from 0.072 to 0.101. Significantly, at two of the 45 loci (*Gus* and *Trf*) it shares no alleles with the 2n = 38 form collected from the lowlands of Sri Lanka.

4. The two 2n = 42 forms, *R.r. tanezumi* and *R.r. flavipectus*, are genetically distinct, with five fixed differences between them and a NEI *D* of 0.127. They, nevertheless, form a grouping (Figures 1–4) that is distinct from the remaining

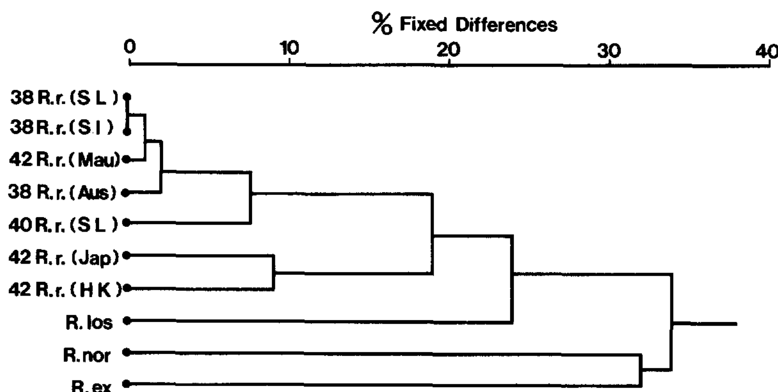

FIGURE 1.—Phenetic clustering based on fixed allelic differences (%) by UPGMA of seven populations of *R. rattus*, *R. norvegicus*, *R. losea* and *R. exulans*.

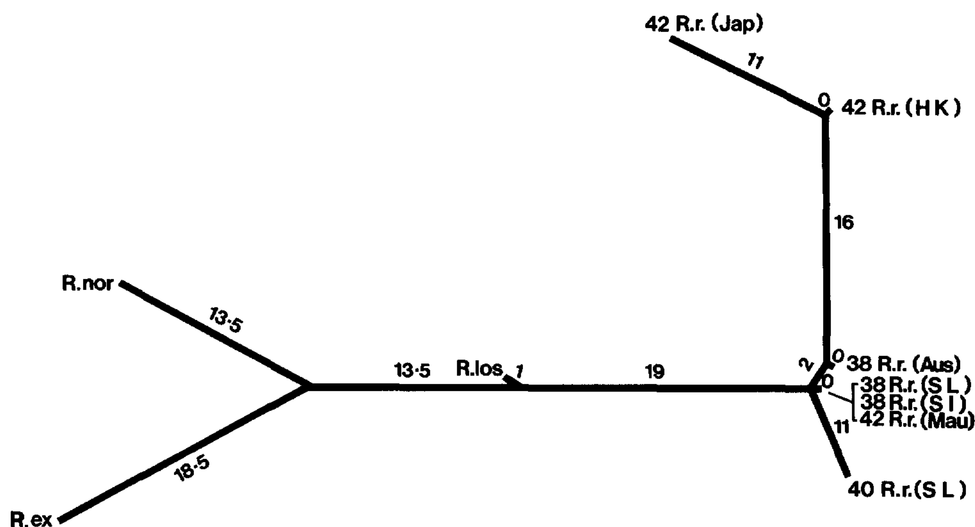

FIGURE 2.—Wagner tree based on fixed allelic differences (%).

forms from which they differ by fixed differences at an average of nine loci and an average NEI *D* of 0.25.

5. All forms of *R. rattus* cluster with each other before any clusters with *R. norvegicus*, *R. losea* or *R. exulans*.

Albumin immunological data: Table 3 shows the albumin immunological distance (AID) of six forms of *R. rattus* and of *R. norvegicus*, *R. villosissimus*, *R. losea* and *R. exulans* to antibodies against albumin of *R. villosissimus*, *R. norvegicus* and Oceanian *R. rattus* from Australia. All forms of *R. rattus* have albumins that are much more similar to the albumin of Oceanian *R. rattus* than to that of *R. norvegicus*. *R. rattus* and *R. norvegicus* are, however, approximately equidistant from *R. villosissimus*.

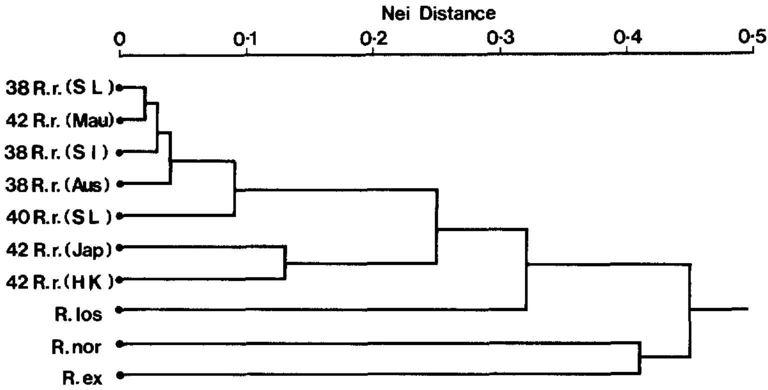

FIGURE 3.—Phenetic clustering based on NEI D'.

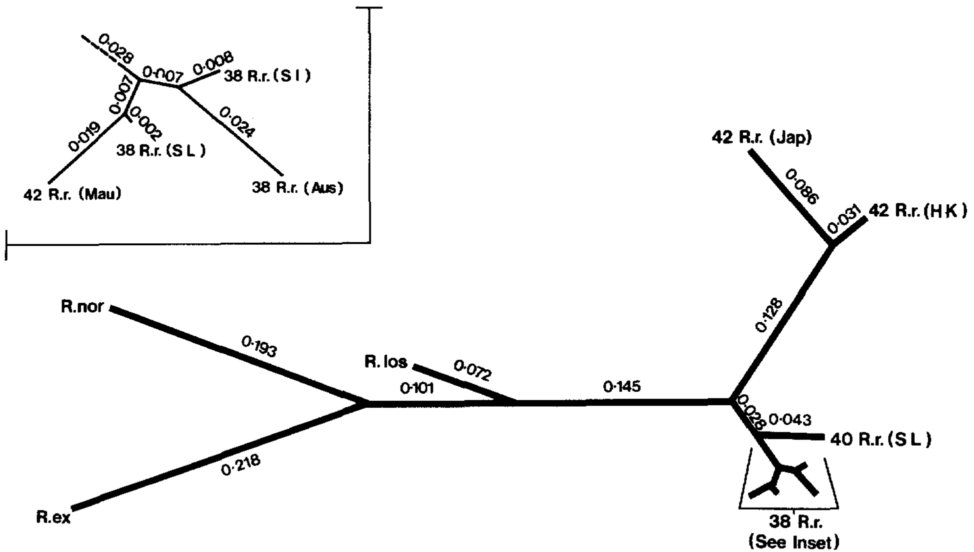

FIGURE 4.—Wagner tree based on NEI D'.

DISCUSSION

The 2n = 42 Mauritius black rat: The black rat on Mauritius possesses a diploid number of 42, with two fusions (the 11/12 and 4/7 common to the 2n = 38 form) and two additional fissions (involving the small metacentrics). YOSIDA (1980a) suggested that the 2n = 38 form of the black rat arrived in Mauritius with the Portuguese during the 16th century and subsequently underwent two chromosomal fissions, which have since become fixed. An additional fission occurs as a polymorphism. If this interpretation is correct, then the rate of chromosomal evolution in the Mauritius black rat has been far more rapid than the average rate for rodents of about 0.2 changes per million years (WILSON *et al.* 1975). An alternative explanation is that the black rat has

TABLE 3

Albumin cross reactivity among Rattus forms

Antigen		Antiserum		
		38 R. r. (Aus)	*R. nor.*	*R. vill.*
42 R.r. (Jap)	group III and IV	2	7	13
42 R.r. (HK)	group III and IV	2	7	12
40 R.r. (SL)	group II	1	6	10
38 R.r. (Aus)	group I	0	8	9
38 R.r. (SI)	group I	1	6	9
42 R.r. (Mau)	group I	1	7	10
R. nor.		8	0	10
R. los.		1	4	7
R. ex.		10	4	15
R. vill.		13	9	0

Values are means of at least two determinations.

been on Mauritius for a much longer period of time (BAVERSTOCK 1981). The present data show that the Mauritius black rat is genetically very similar to the 2n = 38 forms (NEI *D* from 0.02 to 0.06) and are, therefore, consistent with YOSIDA'S interpretation. This is not to deny the possibility that the black rat has been on Mauritius for considerably longer than 400 years, rather that larger genetic difference beween the Mauritius black rat and the 2n = 38 forms of the black rat would be contrary to YOSIDA'S hypothesis, and such were not found.

The black rat in Sri Lanka: Two chromosomal forms of black rat occur in Sri Lanka, a 2n = 40 form (with an 11/12 fusion) on the central highlands and a 2n = 38 form (with an 11/12 and a 4/7 fusion) on the coastal lowlands. Occasional specimens are found that have 39 chromosomes due to heterozygosity for the 4/7 fusion.

The present data show that the 2n = 38 lowland and 2n = 40 highland forms are genetically distinct, but that the 2n = 38 form is not very different from 2n = 38 forms from other parts of the world. It seems most likely, therefore, that the 2n = 40 form is endemic and the 2n = 38 form is a recent immigrant, presumably transported there by human agency. The presence of 2n = 39 individuals on the island indicates that the two forms are hybridizing. Moreover, laboratory studies indicated that hybrids are partially fertile, and it is, therefore, possible that the two forms are in the process of undergoing genetic introgression.

Cryptic species: Among the seven populations of *R. rattus* sampled, four groups emerge: group I, the 2n = 38 populations (including the 2n = 42Mau); group II, the 2n = 40 form from Sri Lanka; group III, the 2n = 42 form from Hong Kong; and group IV, the 2n = 42 form from Japan. Groups I and II differ genetically at 4–9% of loci, whereas groups III and IV differ at 9% loci. The corresponding NEI *D*'s are 0.07–0.10 and 0.13, respectively. It is not possible to state categorically that group I and group II are or are not different species, since, in some groups of rodents, different species share the same electrophoretic alleles at all loci studied, with NEI *D*'s as low as 0.002, *e.g.*, Spalax (NEVO

and SHAW 1972) and Thomomys (NEVO *et al.* 1974). Such cases are, however, relatively unusual, and NEI *D*'s of less than 0.2 are more typical of those between subspecies of rodents (AYALA 1975). We, therefore, suggest that the members of groups I and II are the same biological species. The same applies to members of groups III and IV. Groups I/II and III/IV, on the other hand, have fixed differences at 19% of the loci. The lower 95% limit of this estimate is 14%. As we have shown elsewhere, rodent taxa differing at such a large proportion of electrophoretic loci are usually (although not always) different species (BAVERSTOCK, WATTS and COLE 1977). An alternative way of looking at this problem is to consider NEI *D*'s. The groups under consideration have a NEI *D* of about 0.25. Again, this is typical of the level of differentiation characterizing different species of rodents (AYALA 1975). On the genetic data alone, therefore, we would consider groups I and II to be members of a single species and groups III and IV to be members of single species. Groups I/II and III/IV, however, are probably separate biological species.

Data on hybridization in the laboratory support this point of view. Thus, F_1 hybrids between the 2n = 42 and 2n = 38 forms are largely sterile, whereas hybrids between the 2n = 38 and 2n = 40 forms are fertile (although with reduced litter size), and hybrids between the 2n = 38 form and the 2n = 42Mau form have a still higher fertility (YOSIDA 1980a,b).

The field data are somewhat equivocal on this point. In some areas the 2n = 38 and 2n = 42 forms are found together without interbreeding *e.g.*, in Sagar and Chikmagalus in India (YOSIDA 1980a; LAKHOTIA, RAO and JHANWAR 1973) and also in Cairo (BADR and BADR 1970), although this latter claim should be treated with some skepticism, since it is probable that the 2n = 42 form found in this study was in fact *R. norvegicus* (WHITE 1978).

On the other hand, two cases of hybridization and apparent introgression have emerged from the karyological studies (YOSIDA 1980a). On Eniwetok Island in the South Pacific, both chromosomal and transferrin data suggest hybridization and at least backcrossing between the 2n = 38 and 2n = 42 forms. This island occurs in an area where the 2n = 38 form is expected to occur. The island was mandated to Japan between the first and second World Wars, and presumably the Japanese black rat (2n = 42) was transported to the island during that time.

Similarly Chichijima Island off the south coast of Japan has a population of black rats which karyotypic and transferrin data show to be the result of hybridization and at least backcrossing of the 2n = 42 form and 2n = 38 form. In view of the large number of electrophoretically distinguishable differences between "pure" 2n = 42 and 2n = 38, it would be worthwhile studying both these island populations electrophoretically to determine the full extent of introgression between the forms.

If the chromosomal, electrophoretic and laboratory hybridization data are considered together, it seems that the 2n = 38 and 2n = 42 forms are best considered as incipient species. Where they meet, they may introgress, become sympatric without interbreeding or one may replace the other depending upon the prevailing biological conditions.

Evolutionary relationships: Based upon karyotypic studies, YOSIDA (1980a) pos-

tulated that the karyotypic forms of *R. rattus* were related by the transformation series 42 → 40 → 38 → 42Mau. This view is fully supported by the electrophoretic data presented here, namely, that the 2n = 38 and 2n = 42Mau forms are more closely related to each other than either is to the 2n = 40 form, and these three are more closely related to each other than any is to any 2n = 42 form. However, because the 2n = 42 karyotype is ancestral, it is not possible on karyotypic data alone to relate the different populations of 2n = 42 nor to relate independently derived karyotypes. In the present case, we have been able to show that the two 2n = 42 forms from Hong Kong and Japan are more closely related to each other than either is to any of the fusion forms.

R. norvegicus has a karyotype modified from the presumed ancestral Rattus karyotype by two pericentric inversions. It is, therefore, feasible on karyotypic data alone to derive *R. norvegicus* from the 2n = 42 forms of *R. rattus* after the divergence of the 2n = 40 form. This latter possibility is supported by studies of amino acid composition of transferrins (MORIWAKI, SATO and TSUCHIYA 1971) and of mitochondrial DNA restriction endonuclease digests (HAYASHI *et al.* 1979). Our data on both isoenzyme electrophoresis and MC'F of albumins present quite a different picture, namely, that all forms of *R. rattus* are more closely related to each other than any is to *R. norvegicus*. How are these data to be reconciled?

MORIWAKI, SATO and TSUCHIYA (1971) compared the amino acid composition of purified transferrin from a range of 2n = 42 subspecies of *R. rattus*, 2n = 38 *R. rattus* from New Guinea and *R. norvegicus*. According to them, the transferrin of the 2n = 38 *R. rattus* differed on average by 57 amino acids from the 2n = 42 forms of *R. rattus* and by 60 amino acids from *R. norvegicus*. The 2n = 42 form differed by only 25 amino acids from *R. norvegicus*. It is not at all clear, however, how they arrived at these conclusions. Moreover, the amino acid differences they propose seem far too large. For example, within *R. r. tanezumi* there are two electrophoretically distinguishable forms of transferrin—TfR and TfN. MORIWAKI, SATO and TSUCHIYA included both forms in their study and concluded that they differed by 18 amino acids. Such an allelic difference seems remarkably high in view of what is known about the usual pattern of allelic differences in proteins, where allelic forms typically differ by only one or two amino acids. Moreover, the number of amino acid substitutions they claim to have found between 2n = 42 forms of *R. rattus* average 28 differences. Again, this seems far too high for allelic forms. An alternative explanation is that the transferrins are not in fact homologous, but paralogous. The mitochondrial DNA data of HAYASHI *et al.* (1979) also seem problematical. A more extensive mtDNA analysis by BROWN and SIMPSON (1981) showed that *R. norvegicus* mtDNA differed from that of three karyotypic forms of *R. rattus* (2n = 42, n = 40 and 2n = 38) by about 16%, whereas karyotypic forms of *R. rattus* differed by a maximum of 9%, a result more in keeping with our molecular data.

Interestingly, BROWN and SIMPSON (1981) found greater mtDNA sequence divergence between the 2n = 40 *R. rattus* from Sri Lanka and the 2n = 42 and 2n = 38 forms (5.3% – 9.6%) than between the 2n = 42 and 2n = 38 forms (3.4–4.5%). They concluded from strictly phenetic analysis of the data that the

2n = 40 form diverged before the separation of the 2n = 42 and 2n = 38 forms. Such a conclusion would require that the chromosome fusion common to both the 2n = 40 and 2n = 38 forms be due to convergence. Our electrophoretic data, however, are in accord with the chromosome data and support the closer relationship of the 2n = 40 and 2n = 38 forms. A phylogenetic analysis of the mtDNA data does not reconcile the data. Thus, a possible explanation for the mtDNA data is that the mtDNA of the 2n = 40 form has undergone a relatively rapid rate of sequence evolution. On this hypothesis, using *R. norvegicus* as an outgroup, the mtDNA of the 2n = 40 form would show more divergence from *R. norvegicus* mtDNA than would the mtDNA of other *R. rattus* forms. Such is not the case (see Table 2 of BROWN and SIMPSON). Nonetheless, there is some internal inconsistency in their data that suggests some sort of unequal rates of evolution. Thus, 2n = 42 and 2n = 38 mtDNAs show an average of 4.0% sequence divergence, 2n = 38 and 2n = 40 mtDNAs an average of 5.4% but 2n = 42 and 2n = 40 an average of 9.6%. To satisfy the tree triangle there must be zero evolution of mtDNA in the line leading to the 2n = 38 form.

One possible way in which the data may be reconciled is by convergence of restriction patterns in the 2n = 42 and 2n = 38 forms. This may be particularly common in restriction analyses, where the *loss* of restriction sites is the derived state. This is because, if (for example) a six-base cutter is used, there are six different ways of losing that site. Another way in which the data may be reconciled is by "reticulate evolution." An indicated, the 2n = 42 and 2n = 38 forms are largely reproductively isolated. However, in limited areas, hybridization occurs and presumably has occurred in the past. Thus, a scenario seems plausible in which the 2n = 42 form diverged first, followed by divergence of the 2n = 40 and 2n = 38 forms (as indicated by the chromosomal and electrophoretic data). A single 2n = 42 female then migrated into the geographic range of 2n = 38 and hybridized with it. The offspring would then be heterozygous for both of the two fusions and any nuclear genetic differences separating the forms, but they would be identical with the 2n = 42 mother for mtDNA. Repeated backcrossing would progressively dilute the chromosomal and nuclear genetic contribution of the 2n = 42 individual, but the mtDNA of the maternal line would be preserved. Thus, if all present day 2n = 38 individuals trace their maternal line back to that single immigrant, they will be genetically and chromosomally more like the ancestral 2n = 38 form, but their mtDNA will be more like the 2n = 42 form. Although it may seem *a priori* improbable that all 2n = 38 individuals could trace back to the single immigrant, it is well to remember that any species that becomes fixed for a new nuclear allele or a new mtDNA must trace its entire ancestry back to the single individual in which that mutant first occurred.

Dates of divergence: From the molecular data obtained here it is possible to determine the approximate dates of divergence of the various forms of *R. rattus* and of *R. rattus* from *R. norvegicus*. The relationship between NEI'S *D* and time depends to a large extent upon the proportions of "fast" and "slow" evolving loci studied (SARICH 1977). For many of the loci we used, we have no idea whether they should be classified as fast or slow, but there seems no reason to believe

TABLE 4

Heterozygosity estimates ($\hat{H}$) for the seven populations of R. rattus

Population	$\hat{H}$
42 R.r. (Jap)	0.04
42 R.r. (HK)	0.06
40 R.r. (SL)	0.01
38 R.r. (SL)	0.05
38 R.r. (Aus)	0.01
38 R.r. (SI)	0.04
42 R.r. (Mau)	0.06
	Mean 0.04

that the proportions are any different from those used in other studies, *i.e.*, about ¾ slow and ¼ fast. For such a mix, an electrophoretic *D* of 1.0 corresponds to a divergence time of about 20 million years (SARICH 1977; VAWTER, ROSENBLATT and GORMAN 1980). This gives a divergence time for *R. norvegicus* from *R. rattus* of about 8 MY, of the 2n = 42 forms of *R. rattus* from the 2n = 40, 38 and 42Mau forms of *R. rattus* of about 5 MY, of Japanese and Hong Kong 2n = 42 *R. rattus* of 2.5 MY and of *R.r. kandianus* (2n = 40) from the 2n = 38, 42 Mau forms of about 1.7 MY. The albumin immunological data suggest shorter times of divergence—about 4 MY for *R. rattus* and *R. norvegicus* and less than 1.5 MY for the various forms of *R. rattus.* However, it is worthwhile to recall that dating of such recent events is subject to a large variance for both albumin immunological data (MAXSON and MAXSON 1979) and electrophoretic data (SCHMITT 1978).

Levels of genetic variability: Because our sample sizes are very low, estimates of heterozygosity (corrected for small samples; NEI 1978) will be subject to a large variance. We indicate them here (Table 4) only because LEWONTIN (1973) and WHITE (1978) have suggested that *R. rattus* is unusual in possessing no genetic variability at 21 loci studied electrophoretically. Both refer to SEROV (1972). It is clear, however, that SEROV'S study related not to *R. rattus* but to *R. norvegicus.* As Table 4 shows, levels of electrophoretic variability in *R. rattus* are not unusually low for a mammal. PATTON, YANG and MYERS (1975) also showed that populations of the 2n = 38 *R. rattus* from the Galapagos Archipelago had a mean heterozygosity of about 0.03.

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LITERATURE CITED

AYALA, F. J., 1975 Genetic differentiation during the speciation process. Evol. Biol. **8:** 1–78.

BADR, F. M. and R. BADR, 1970 The somatic chromosomes of wild population of rats: numerical polymorphism. Chromosoma (Berl.) **57:** 397–403.

BAVERSTOCK, P. R., 1981 Book review—T. H. Yosida "Cytogenetics of the Black Rat." J. Mammal. **62:** 866–867.

BAVERSTOCK, P. R., C. H. S. WATTS, M. ADAMS and M. GELDER, 1980 Chromosomal and electrophoretic studies of Australian *Melomys* (Rodentia: Muridae). Aust. J. Zool. **28:** 553–574.

BAVERSTOCK, P. R., C. H. S. WATTS and S. R. COLE, 1977 Electrophoretic comparisons between allopatric populations of five Australian Pseudomyine rodents (Muridae). Aust. J. Biol. Sci. **30:** 471–485.

BAVERSTOCK, P. R., C. H. S. WATTS, J. T. HOGARTH, A. C. ROBINSON and J. F. ROBINSON, 1977 Chromosome evolution in Australian rodents. II. The *Rattus* group. Chromosoma (Berl.) **61:** 227–241.

BROWN, G. G. and M. V. SIMPSON, 1981 Intra- and interspecific variation of the mitochondrial genome in *Rattus norvegicus* and *Rattus rattus*: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. Genetics **97:** 125–143.

CHAMPION, A. B., E. M. PRAGER, D. WACHTER and A. C. WILSON, 1974 Microcomplement fixation. pp. 397–416. In: *Biochemical and Immunological Taxonomy of Animals,* Edited by C. A. WRIGHT. Academic Press, London.

FARRIS, J. S., 1972 Estimating phylogenetic trees from distance matrices. Am. Nat. **106:** 645–668.

GORMAN, G. C. and J. RENZI, JR., 1979 Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia, pp. 242–249.

HAYASHI, J. I., YONEKAWA, O. GOTOH, H. TAGASHIRA, K. MORIWAKI and T. H. YOSIDA, 1979 Evolutionary aspects of variant types of rat mitochondrial DNAs. Biochim. Biophys. Acta **564:** 202–211.

LAKHOTIA, S. C., S. R. V. RAO and S. C. JHANWAR, 1973 Studies on rodent chromosomes. VI. Coexistence of *Rattus rattus* with 38 and 42 chromosomes in South Western India. Cytologia (Tokyo) **38:** 403–410.

LEWONTIN, R. C., 1973 Population genetics. Annu. Rev. Genet. **7:** 1–17.

MAXSON, L. R. and R. D. MAXSON. 1979 Comparative albumin and biochemical evolution in plethodontid salamanders. Evolution **33:** 1057–1062.

MORIWAKI, K., T. SATO and K. TSUCHIYA, 1971 Difference in amino acid composition of serum transferrin among various species of *Rattus.* Annu. Rep. Natl. Inst. Genet. Jpn. **21:** 34–36.

NEI, M., 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:** 585–590.

NEVO, E., Y. J. KIM, C. R. SHAW and C. S. THAELER, JR., 1974 Genetic variation, selection and speciation in *Thomomys talpoides* pocket gophers. Evolution **28:** 1–23.

NEVO, E. and C. R. SHAW. 1972 Genetic variation in a subterranean mammal, *Spalax ehrenbergi.* Biochem. Genet. **7:** 235–241.

PATTON, J. L., S. Y. YANG and P. MYERS 1975 Genetic and morphological divergence among introduced rat populations (*Rattus rattus*) of the Galapagos Archipelago, Ecuador. Syst. Zool. **24,** 296–310.

SARICH, V. 1977 Rates, sample sizes and neutrality hypothesis for electrophoresis in evolutionary studies. Nature (Lond.) **265:** 24–28.

SCHMITT, L. H., 1978 Genetic variation in isolated populations of the Australian bush-rat, *Rattus fuscipes.* Evolution **32:** 1–14.

SEROV, O. L., 1972 Monomorphism of some structural genes in populations of wild and laboratory rats (*Rattus norvegicus*). Isozyme Bull. **5:** 38.

SNEATH, P. H. A. and R. R. SOKAL. 1973 *Numerical Taxonomy.* W. H. Freeman and Company, San Francisco.

VAWTER, A. T., R. R. ROSENBLATT and G. C. GORMAN, 1980 Genetic divergence among fishes of the eastern Pacific and the Caribbean: support for the molecular clock. Evolution **34:** 705–711.

WHITE, M. J. D., 1978 *Modes of Speciation.* W. H. Freeman and Company, San Francisco.

WILSON, A. C., G. L. BUSH, S. M. CASE and M. C. KING, 1975 Social structuring of mammalian populations and rate of chromosomal evolution. Proc. Natl. Acad. Sci. USA. **72:** 5061–5065.

YOSIDA, T. H., 1980a *Cytogenetics of the Black Rat. Karyotype Evolution and Species Differentiation.* University of Tokyo Press, Tokyo.

YOSIDA, T. H., 1980b Segregation of karyotypes in the F_2 generation of the hybrids between Mauritius and Oceanian type black rats with a note on their litter-size. Proc. Jpn. Acad. Ser. B **56:** 557–561.

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