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Genetic Differentiation in Indonesian and French Rats of the Subgenus *Rattus*

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Key Word Index – *Rattus*; Muridae; rodent systematics; polymorphism; protein electrophoresis.

Abstract – The allozyme polymorphism at 28 loci was examined in four Indonesian species of *Rattus* (*R. diardii, R. tiomanicus, R. argentiventer* and *R. exulans*) and in two French species (*R. rattus* and *R. norvegicus*). *R. diardii* was the most polymorphic species ($H = 0.089$) and *R. tiomanicus* and *R. exulans* the least polymorphic ($H = 0.026$). The six species were found to differ from one another by 2–7 diagnostic loci. The use of allozyme characters for systematic identification is discussed. Genetic relationships between the six species were established using two different methods. In both cases, the results were different from those published earlier. These results are discussed in view of the representativeness of the loci investigated.

Introduction

Small mammals, in particular rodents of the genus *Rattus*, are responsible in Indonesia for much agricultural damage [1–3]. A comprehensive rodent control can only be developed when the biology of these pests is well understood and this requires that each population is correctly assigned to a species by some characters.

In South Asia rats have undergone considerable morphological differentiation so that the species and subspecies status of many "forms" is not clearly defined. Morphological and cytological studies are not sufficient [4] and recently several authors [5–8] attempted to investigate the use of biochemical characters. Although only a few loci were analysed, the results indicated clearly that electrophoretic studies of allozymes represented an excellent tool for species identification.

In the present report, we describe the allozyme polymorphism at 28 loci for four Indonesian species (*Rattus diardii, R. exulans, R. argentiventer* and *R. tiomanicus*) and compare it to that of European *R. rattus* and *R. norvegicus*. In addition, we also examined a sample of *R. exulans* from Saigon, Vietnam.

Results

Allozyme Polymorphism

R. diardii was represented by 13 individuals captured at Cipaku, a suburb of Bogor. This species was found polymorphic for eight loci, *Amy, α-Gpd, Hpd, Id-2, Mdh-1, Me-1, Np* and *Sdh* (Table 1). All polymorphic loci appeared to be in Hardy–Weinberg equilibrium and the mean heterozygosity at the 28 loci was estimated at 8.9%.

R. tiomanicus was represented by 15 individuals collected at Tajur, a suburb of Bogor. One of these rats demonstrated an abnormal phenotype at the *Np* locus and was excluded from the sample shown in Table 1. This rat was homozygous for the Np^{150} allele whereas the remaining rats were homozygous for Np^{100}. *R. tiomanicus* was polymorphic for four loci (*Amy, Glo, Id-1* and *Me-1*), and the population appeared in Hardy–Weinberg equilibrium. The mean heterozygosity at 28 loci was estimated at 2.6%.

R. argentiventer was represented by 16 individuals captured in a rice field near Cirebon, a town in West Java. Among this sample, two rats had abnormal genotypes at two loci: they were homozygous for the $Id\text{-}1^{100}$ and the Hbb^{S} alleles whereas the remaining rats of this sample were homozygous for the $Id\text{-}1^{120}$ and Hbb^{SL} alleles, respectively; in addition, they demonstrated a Np^{100} allele which was absent in the other rats of the sample. When these two rats are excluded from the sample, *R. argentiventer* appears polymorphic at four loci (*Amy, Hpd, Ipo* and *Np*) that are in Hardy–Weinberg equilibrium. The mean heterzygosity at 28 loci was estimated at 5.0% in this species.

R. exulans was represented by 15 individuals captured in Ciloto, a small mountain town 25 km east of Bogor; three individuals captured at Karawang near Djakarta; and two individuals

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TABLE 1. ALLELIC FREQUENCIES IN SIX *RATTUS* SPECIES

		Species (number of animals used in italics)							
Isoenzyme*		*norvegicus*	*rattus*	*diardii*	*argentiventer*	*tiomanicus*	Ciloto	*exulans* Saigon	Djakarta
Alb	*103*	0	0	0	0	0	1.00	1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	0	0	0
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Amy	*100*	1.00	1.00	0.89	0.64	0.89	0.23	0	0.16
	85	0	0	0.11	0.36	0.11	0.70	1.00	0.84
	70	0	0	0	0	0	0.07	0	0
		3	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Glo	*100*	1.00	1.00	1.00	1.00	0.93	0.87	1.00	1.00
	75	0	0	0	0	0.07	0.10	0	0
	60	0	0	0	0	0	0.03	0	0
		10	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Got-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67
	70	0	0	0	0	0	0	0	0.33
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Got-2	*100*	0.38	1.00	1.00	1.00	1.00	0	0	0
	65	0.62	0	0	0	0	1.00	1.00	1.00
		12	*8*	*13*	*14*	*14*	*15*	*2*	*3*
α-Gpd	*100*	1.00	0	0	0	0	0	0	0
	75	0	1.00	0.83	1.00	1.00	1.00	1.00	1.00
	60	0	0	0.17	0	0	0	0	0
		11	*9*	*12*	*14*	*14*	*15*	*2*	*3*
Hbb	S^L	0	0	0	1.00	0	0	0	0
	S^R	0	0	0	0	0	1.00	1.00	1.00
	P	1.00	1.00	1.00	0	1.00	0	0	0
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Hpd	*120*	0	0.07	0.15	0	0	0	0	0
	100	0.82	0.86	0.85	0.86	1.00	0.96	0.50	0.83
	80	0.18	0.07	0	0.14	0	0.04	0.50	0.17
		11	*7*	*13*	*14*	*14*	*13*	*2*	*3*
Id-1	*120*	0	0	0	1.00	0.04	0	0	0
	100	1.00	1.00	1.00	0	0.96	1.00	1.00	1.00
		9	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Id-2	*100*	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00
	30	0	0	0.04	0	0	0	0	0
		11	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Ipo	*100*	1.00	1.00	1.00	0.82	1.00	1.00	1.00	1.00
	90	0	0	0	0.07	0	0	0	0
	10	0	0	0	0.11	0	0	0	0
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Ldh-B	*100*	1.00	0.37	1.00	1.00	1.00	1.00	1.00	1.00
	85	0	0.63	0	0	0	0	0	0
		12	*8*	*13*	*14*	*14*	*15*	*2*	*3*
Mdh-1	*110*	0	0	0.35	0	0	0	0	0
	100	1.00	1.00	0.65	1.00	1.00	1.00	1.00	1.00
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Mdh-2	*100*	0.87	0.31	1.00	1.00	1.00	1.00	1.00	1.00
	50	0.13	0.69	0	0	0	0	0	0
		12	*8*	*13*	*14*	*14*	*15*	*2*	*3*
Me-1	*150*	0	0	0.12	0	0.79	0	0	0
	125	0	1.00	0.84	0	0.21	1.00	1.00	1.00
	100	1.00	0	0.04	1.00	0	0	0	0
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Mpi	*R*	0.95	1.00	0	1.00	1.00	1.00	1.00	1.00
	L	0.05	0	1.00	0	0	0	0	0
		11	*8*	*12*	*14*	*14*	*15*	*2*	*3*
Np	*200*	0	0	0	0.75	0	0	0	0
	150	0	1.00	0	0.25	0	1.00	1.00	1.00
	130	0	0	0.42	0	0	0	0	0
	100	1.00	0	0.54	0	1.00	0	0	0
	80	0	0	0.04	0	0	0	0	0
		11	*9*	*13*	*14*	*14*	*15*	*2*	*3*
6-Pgd	*100*	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	L	0.08	0	0	0	0	0	0	0
		12	*9*	*13*	*14*	*14*	*15*	*2*	*3*
Pgi	*125*	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	100	1.00	0	0	0	0	0	0	0
		11	*9*	*13*	*14*	*14*	*15*	*2*	*3*

TABLE 1. (*continued*)

Isoenzyme*		Species (number of animals used in italics)							
		norvegicus	*rattus*	*diardii*	*argentiventer*	*tiomanicus*	Ciloto	*exulans* Saigon	Djakarta
Pk	*105*	0	0	0	0	0	1.00	1.00	1.00
	100	1.00	0	0	1.00	1.00	0	0	0
	90	0	1.00	1.00	0	0	0	0	0
		11	*9*	*12*	*14*	*14*	*15*	*2*	*3*
Sdh	*100*	1.00	1.00	0.15	1.00	1.00	1.00	1.00	1.00
	80	0	0	0.12	0	0	0	0	0
	50	0	0	0.73	0	0	0	0	0
		11	*9*	*13*	*14*	*14*	*15*	*2*	*3*

*Iso enzyme abbreviations are given in the Experimental. The loci *Acp, Car, Es-14, Gda-1, G-6-Pdh, Ldh-A* and *PGM* are monomorphic for the same allele in all populations.

captured near Saigon, Vietnam. The Ciloto population was polymorphic at three loci (*Amy, Glo* and *Hpd*) that appeared in Hardy–Weinberg equilibrium. The mean heterozygosity at 28 loci was estimated at 2.7%. Djakarta and Saigon populations were too small to allow any firm conclusions. They both closely resemble the Ciloto population but appear to have a slightly different polymorphism (Table 1).

R. norvegicus was represented by nine individuals captured in La Clastre, a village 10 km north of Montpellier, France. This population was polymorphic at five loci (*Got-1, Hpd, Mdh-2, Mpi* and *6-Pgd*) that appeared in Hardy–Weinberg equilibrium. The mean heterozygosity at 28 loci was estimated at 4.4%.

R. rattus was represented by nine individuals captured in the Island of Port Cros, south of Toulon, France. This population was polymorphic at three loci (*Hpd, Ldh-B* and *Mdh-2*) that appeared in Hardy–Weinberg equilibrium. The mean heterozygosity at 28 loci was estimated at 4.1%.

Comparison of Allozyme Polymorphism of Indonesian, Malayan and French Rats

Chan [5] investigated the allozyme polymorphism of Malayan *R. norvegicus, R. diardii, R. tiomanicus, R. exulans* and *R. argentiventer*. His drawings of the zymograms allow us to compare his data directly with ours at seven loci, i.e. *Acp, Ldh-B, 6-Pgd, Pgi, Mdh-1, Ipo* and *Np*. The Javan and Malayan populations are very similar (including an identical polymorphism for *Mdh-1* in *R. diardii*). The only differences were: (a) in *R. argentiventer*, a polymorphism at the *Np* and *Ipo* loci in Java and a monomorphism in Malaya; and (b) in *R. diardii*, a polymorphism for three alleles in Java and for two alleles in Malaya at the *Np* locus.

French and Malayan populations of *R. norvegicus* seem similar at the *Acp, Ldh-A, Mdh-1* and *Pgi* loci. The *6-Pgd* locus is polymorphic in both populations but allelic frequencies are different: three alleles are present in rats from Malaya and only two in those from France, and the *6-Pgd*^{100} allele (equivalent to the *6-Pgd*^{1} of Chan), has a higher frequency in France than in Malaya. Both populations are monomorphic at the *Np* locus but for different alleles (the French allele has the same electrophoretic mobility as that of *R. tiomanicus* whereas the Malayan allele is faster).

Species Identification

All species considered in the present study are different from one another by at least two diagnostic loci (Table 2). Some of the diagnostic loci have unique alleles that are found in none of the other species and can be considered as "identification loci". When the six species studied here are considered together, no identification locus exists for *R. tiomanicus, R. rattus* and *R. diardii*, whereas one is found in *R. argentiventer* (*Hbb*^{SL}), two in *R. norvegicus* (*α-Gpd*^{100} and *Pgi*^{100}), and three in *R. exulans* (*Alb*^{103}, *Hbb*^{SR} and *Pk*^{106}). In Indonesia, where only four of the species considered are present, *Pk*^{90} and *Mpi*^{L} become identification loci of *R. diardii,* and *Got-2*^{85} for *R. exulans*; therefore, the analysis of only two loci (*Hbb* and *Pk* or *Hbb* and *Mpi*) would be sufficient to identify any given individual.

We have mentioned the existence of two aberrant rats in the "*argentiventer*" sample and of one in the "*tiomanicus*" sample. We have attempted to determine their species status using identification loci. The three rats were homozygous for *Hbb*^{S} and *Mpi*^{R} alleles so they cannot be *R. argentiventer* (*Hbb*^{SL}), *R. diardii* (*Mpi*^{L}) or *R. exulans* (*Hbb*^{SR}). However, it is not possible to conclude that they belong to *R. tiomanicus* on the basis of these negative results. At this stage, it becomes necessary to compare the

TABLE 2. DIAGNOSTIC LOCI AND NEI'S GENETIC DISTANCES (*D*) OF SIX *RATTUS* SPECIES

	norvegicus	*rattus*	*diardii*	*argentiventer*	*tiomanicus*	*exulans*
norvegicus	—	0.259	0.260	0.226	0.130	0.338
rattus	5 { *α-Gpd, Me-1, Np, Pgi, Pk*	–	0.140	0.234	0.140	0.224
diardii	3 { *α-Gpd, Pgi, Pk*	2 { *Mpi, Np*	–	0.274	0.139	0.306
argentiventer	5 { *α-Gpd, Hbb, Id-1, Np, Pgi*	4 { *Hbb, Id-1, Me-1, Pk*	5 { *Id-1, Hbb, Mpi, Np, Pk*	–	0.147	0.289
tiomanicus	3 { *α-Gpd, Me-1, Pgi*	2 { *Np, Pk*	2 { *Mpi, Pk*	3 { *Np, Hbb, Me-1*	–	0.250
exulans	7 { *Alb, α-Gpd, Hbb, Me-1, Np, Pgi, Pk*	4 { *Alb, Got-2, Hbb, Pk*	6 { *Alb, Got-2, Hbb, Mpi, Np, Pk*	6 { *Alb, Got-2, Hbb, Id-1, Me-1, Pk*	5 { *Alb, Got-2, Hbb, Np, Pk*	–

phenotypes of these rats with those of *R. tiomanicus* for all available loci. The two rats of the "*argentiventer*" sample were homozygous for the *Mpi*^{L} allele which is absent in *R. tiomanicus*. The rat of the "*tiomanicus*" sample was homozygous for the *Np*^{150} allele which is absent in all the *R. tiomanicus* species examined. This demonstrates that the three aberrant rats belong to none of the four Indonesian species studied here.

Genetic Relationships of the Rat Species Studied

The genetic differentiation of the six *Rattus* species analysed in the present study can be estimated by different means, such as the number of diagnostic loci between pairs of species (Table 2) or by Nei's [9] genetic distances (Table 2). Both methods allow us to conclude that *R. exulans* is the species showing the highest genetic differentiation from the others, and that *R. tiomanicus*/*R. norvegicus* and *R. diardii*/*R. rattus* constitute two closely related groups. Dendrograms constructed according to the method of Sokal and Sneath [10] clearly show these relationships (Fig. 1A).

An interesting way to group different species according to genetic affinities is to consider the *R. exulans* identification loci (*Alb, Hbb* and *Pk*). *R. exulans* is the only species with a unique *Alb* allele (*Alb*^{103}), and two groups based on *Alb* genotypes

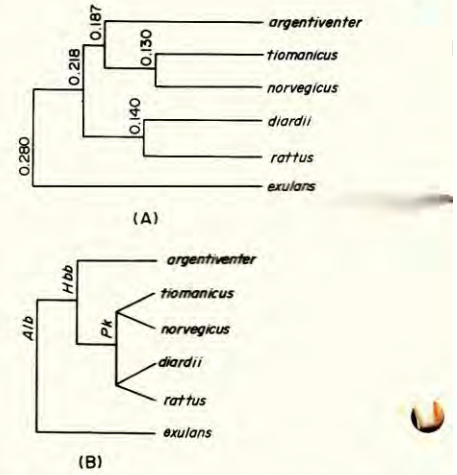

FIG. 1. GENETIC RELATIONSHIPS BETWEEN SIX SPECIES OF *RATTUS*. (A) Based on Nei's genetic distances; (B) based on the identification loci.

can be formed: group 1 with *exulans* and group 2 with the five other species. In group 2, *R. argentiventer* is the only species to have a unique allele at the *Hbb* locus and can, on this basis, be separated from the other species in group 2a. Group 2b can then be divided into two new groups according to the *Pk* genotypes: the *rattus/diardii* group is characterized by Pk^{80}, and the *tiomanicus/norvegicus* group by Pk^{100}. The only difference between the dendrogram constructed from this information (Fig. 1B) and that constructed from Nei's genetic distances is that the latter indicates that *R. argentiventer* is a little closer to the *tiomanicus/norvegicus* group than to the *rattus/diardii* group.

Discussion

The present investigation has confirmed that *Rattus* species have a low level of heterozygosity. As Chan found [5], *R. diardii* appears to be the most polymorphic species with a mean heterozygosity of 0.089, while *R. tiomanicus* and *R. exulans* are the least polymorphic ($H = 0.026$).

As far as species identification is concerned, we have shown that all the species studied are different from one another by at least two loci, including *R. tiomanicus* and *R. diardii* which Chan found identical using the loci he studied. *R. diardii* and *R. rattus* have been considered as two geographical subspecies by many authors (see review [4]). Cytological data obtained by one of us [11] confirmed that the Java *diardii* rats possessed 42 chromosomes whereas the French *R. rattus* is supposed to have only 38. It is known that polymorphisms in chromosome numbers exist in some *R. rattus* populations [12, 13], and this difference cannot be taken as an indication of genetic isolation (i.e. species formation). Our allozyme data, however, indicate that *diardii* and *rattus* are as different from one another as, for example, *diardii* and *tiomanicus*, which are two well-recognized species [4]. This observation, although it cannot be taken as definite proof, suggests that *diardii* and *rattus* have reached distinct species status.

Our attempt to determine the genetic relationships between rat species have given results quite different from those proposed by Chan [5], especially with respect to *R. norvegicus* and *R. exulans*. This is not surprising considering that all available methods of estimating genetic differentiation are based on the assumption that the loci studied are representative of the whole genome. The simple fact that we analysed 28 loci rather than nine, as did Chan, increases significantly the probability to have included differentiating loci among the sampled genome and is sufficient to explain that different conclusions were reached in both studies. An important point is to determine in which limits our sample of loci is more representative of the genome than that of Chan. Expressed differently, we can speculate how significantly our conclusions might be modified by the addition of further loci in future investigation. It is well known that the rate of evolution varies widely according to the loci considered and, therefore, genetic distances will also vary considerably according to the proportion of "fast" and "slow" evolving loci included in the sample. At the present time, very little is known about the rate of evolution of the loci coding allozymes, and it seems reasonable to consider the results on genetic differentiation obtained from this type of study as a working hypothesis for future investigation.

Experimental

All the rats studied were captured in the field by Tohari (for Indonesian specimens) and by Cheylan and Thaler (for French specimens). Rats were brought alive in the laboratory; their tissues were isolated and stored at $-70°$ for electrophoretic analysis.

Horizontal acrylamide electrophoresis was used to analyse amylases (Amy), and horizontal starch electrophoresis to study the other enzymic systems. Albumin (Alb) in plasma; and hemoglobin (Hbb), glyoxalase (Glo), nucleoside phosphate dehydrogenase (Np), and glutamate deaminase (Gda) in hemolysates were investigated using TEB 8.6 buffer systems [14]; T.C. 8.0 [14] buffer systems were used to analyse α-glycerophosphate-dehydrogenase (α-Gpd), 6-phosphogluconate-dehydrogenase (6-Pgd), phosphoglucomutase (Pgm), and phosphoglucoisomerase (Pgi) in liver homogenates; T.M.E. 6.9 [14] buffer systems to analyse glutamate-oxaloacetate-1 (Got-1), and indophenol oxidase (IPO) in liver homogenates and glucose-6-phosphate-dehydrogenase (G-6-Pd), esterase-14 (Es-14) and carbonic anhydrase (Car), in hemolysates; LiOH [14] buffer systems to analyse mannose-6-phosphate-isomerase (Mpi) in heart homogenates; Tris–PO_4 [14] buffer systems to investigate pyruvate kinase (Pk) in liver homogenates; T.C. 6.7 [14] buffer systems to study lactate-dehydrogenases A and B (Ldh-A and Ldh-B), isocitrate-dehydrogenase-1 (Id-1), malate-dehydrogenase-1 (Mdh-1) and malic-enzyme-1 (Me-1) in kidney or heart homogenates; T.C. 6.4 (= T.C. 6.7 adjusted to pH 6.4) buffer systems to analyse isocitrate-dehydrogenase-2 (Id-2), malate-dehydrogenase-2 (Mdh-2) and glutamate-oxalo-acetate-2 (Got-2) in heart homogenates and sorbitol-dehydrogenase (Sdh) in liver homogenates. Staining techniques were adapted from previously described studies [14, 15].

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