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Biochemical Systematics of Three Species of the Japanese Long-Tailed Field Mice; *Apodemus speciosus*, *A. giliacus* and *A. argenteus*

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ABSTRACT—The genetic relationships among two chromosomal races (2n=48 and 2n=46) of *Apodemus speciosus speciosus*, two insular subspecies *A. s. ainu* (2n=48) and *A. s. navigator* (2n=46), and two related species *A. giliacus* (2n=48+B's) and *A. argenteus* (2n=46) were examined by electrophoretic analyses of 17 different enzymes. The biochemical dendrogram constructed from the Nei's genetic distances showed the following: (1) Allozymic differentiation between the two chromosomal races was exceedingly low, being equivalent to that observed between local populations of the same species, although they distribute parapatrically with a narrow zone of hybridization. (2) The insular subspecies *A. s. ainu* and *A. s. navigator* have slightly differentiated from *A. s. speciosus* at an allozymic level, and their differentiation has occurred in almost the same divergence time. (3) In a phylogenetic aspect, *A. speciosus* was more closely related to *A. giliacus* than to *A. argenteus*. These findings are well consistent with the karyological relationships among these three *Apodemus* species so far reported. The avarage value of genetic distances between the two chromosomal races was 0.033, so far as 26 genetic loci were concerned. Such a low genetic distance suggests that the raciation of *Apodemus s. speciosus* has taken place lately in an evolutionary time scale: only some a hundred thousand years ago. The present electrophoretic results strongly support our previous notion that the raciation event of *A. s. speciosus* might have advanced in a stasipatric mode somewhere in a southern part of Japan.

INTRODUCTION

The large Japanese field mouse, *Apodemus speciosus speciosus*, consists of two chromosomal races which have been produced by a Robertsonian chromosome rearrangement [1–5]. They distribute parapatrically, forming a narrow zone of hybridization known as "Toyama-Hamamatsu line" which partitions the field mouse into the northern and southern populations at the middle region of the mainland of Japan, Honshu. This hybrid zone has been reported to be at most 20 km in width [6]. According to Corbet [7], *A. speciosus* is endemic to Japan and classified into eight subspecies containing the nominate subspecies *A. s. speciosus* and seven insular subspecies. Karyologically, they can be divided into two subgroups: Group I is the 2n=48-type which is distributed only in the northward of the hybrid zone including Sado Island, and Group II is the 2n=46-type distributing in the southward of the hybrid zone including the neighbouring small islands such as Oki Islands and Tsushima Islands [5]. Karyosystematically, the Honshu population of the northward of the hybrid zone is regarded as a northern race of *A. s. speciosus* or race A, and that of the southward a southern race or race B.

Three possible differentiation processes of the chromosomal races of *A. speciosus* could be proposed as follows: (1) During the glacial period the 2n=48-type mice first came into Japan from China through the Korean Peninsula, and thereafter the 2n=46-type mice came through the same route [4]. (2) The 2n=48-type mice came into Japan

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through Sakhalin and the 2n=46-type ones through the Korean Peninsula, and they contacted at the middle region of Honshu. (3) The 2n=48-type mice came into Japan from China through the Korean Peninsula and the 2n=46-type mice were newly derived from the former type somewhere in the southern part of Japan, and the latter mice gradually extended their habitat, finally forming the existing pattern of distribution divided by the Toyama-Hamamatsu line [8]. It is self-evident, in the first and second views, that the two types of karyotype had already been differentiated somewhere in the east area of the Eurasian Continent before their migration into Japan. In either case, therefore, the genetic differentiation between the two karyological forms could be expected to be respectable. On the other hand, the third view can be regarded as one of the representative cases of the stasipatric mode of differentiation. If this is the case, the degree of genetic differentiation between the two karyological forms including the insular subspecies should be rather small. For clarifying which of these views is authentic, the biochemical estimation of their genetic distances would be one of the most informative and trustworthy, as described in similar cases of various taxonomic groups of animals [9, 10]. In addition, it would also provide valuable quantitative information on the genetic or evolutionary relationships among three *Apodemus* species distributing in Japan; *A. speciosus, A. giliacus* and *A. argenteus*.

With the background mentioned above, we have attempted an electrophoretic study to estimate the degree of the genetic differentiation among two chromosomal races of *A. s. speciosus,* two insular subspecies *A. s. ainu* and *A. s. navigator,* and two related species *A. giliacus* and *A. argenteus*.

MATERIALS AND METHODS

Animals

One hundred seventeen specimens of *Apodemus speciosus,* 3 specimens of *A. giliacus* and 5 specimens of *A. argenteus* were collected at various localities of Honshu, Hokkaido and the neighbouring islands from May, 1984 to March, 1986. Figure 1 shows the collecting lacalities and population numbers, of which the populations #1 to #7 correspond to the local populations of *A. s. speciosus* (#1, race A; #2, hybrid zone and #3~7, race B), the populations #8 and #9 to the insular subspecies *A. s. ainu* and *A. s. navigator,* and the populations #10 and #11 to the related species *A. giliacus* and *A. argenteus,* respectively. The collecting localities (the number of specimens examined) of each population were as follows: #1, Zatoh-ishi, Hirosaki (8), Hyakuzawa, Mt. Iwaki (4), Takinosawa, Lake Towada (7) and Mt. Ohdake, Hakkoda (4), Aomori Pref.; #2, Ina (52), Nagano Pref,; #3, Agematsu (11), Nagano Pref.; #4, Nachikatsu-ura (9), Wakayama Pref.; #5, Miyoshi (1), Hiroshima Pref.; #6, Mt. Daisen (3) Tottori Pref.; #7, Shiramine, Ishikawagun (2), Ishikawa Pref,; #8, Rishiri Island (1), Akkeshi (9) and Naganuma, Yuhbarigun (14), Hokkaido; #9, Tohgo, Oki Islands (2), Shimane Pref.; #10, Naganuma, Yuhbarigun (3), Hokkaido; #11, Zatoh-ishi, Hirosaki (2), Aomori Pref.; Shiramine, Ishikawagun (1), Ishikawa Pref. and Nachikatsu-ura (2), Wakayama Pref.

Specimens of the *A. speciosus* complex were identified in principle according to the collecting localities, but those from the hybrid zone by their karyotypes, since three karyological forms (race A, race B and their hybrid) can be found in this area. Chromosome preparation was made according to the protocol described in the previous paper [8]. Table 1 shows the number of specimens of three karyological forms in the populations #2 and #3. Ina (#2) can be regarded as the northern limit of the hybrid zone, and Agematsu (#3) as just the outside of the southern limit based on the composition of three karyological forms in the specimens captured.

Electrophoresis

Liver, skeletal muscle and kidney were cut out from live specimens, and stored at −80°C until being analysed. The procedures for tissue preparation and polyacrylamide gel electrophoresis were almost the same as those described by Matsuoka [11, 12]. Each tissue was individually homogenized in 4 vols of cold 20 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 0.1 M KCl, using a Potter-Elvehjem glass homogenizer in an

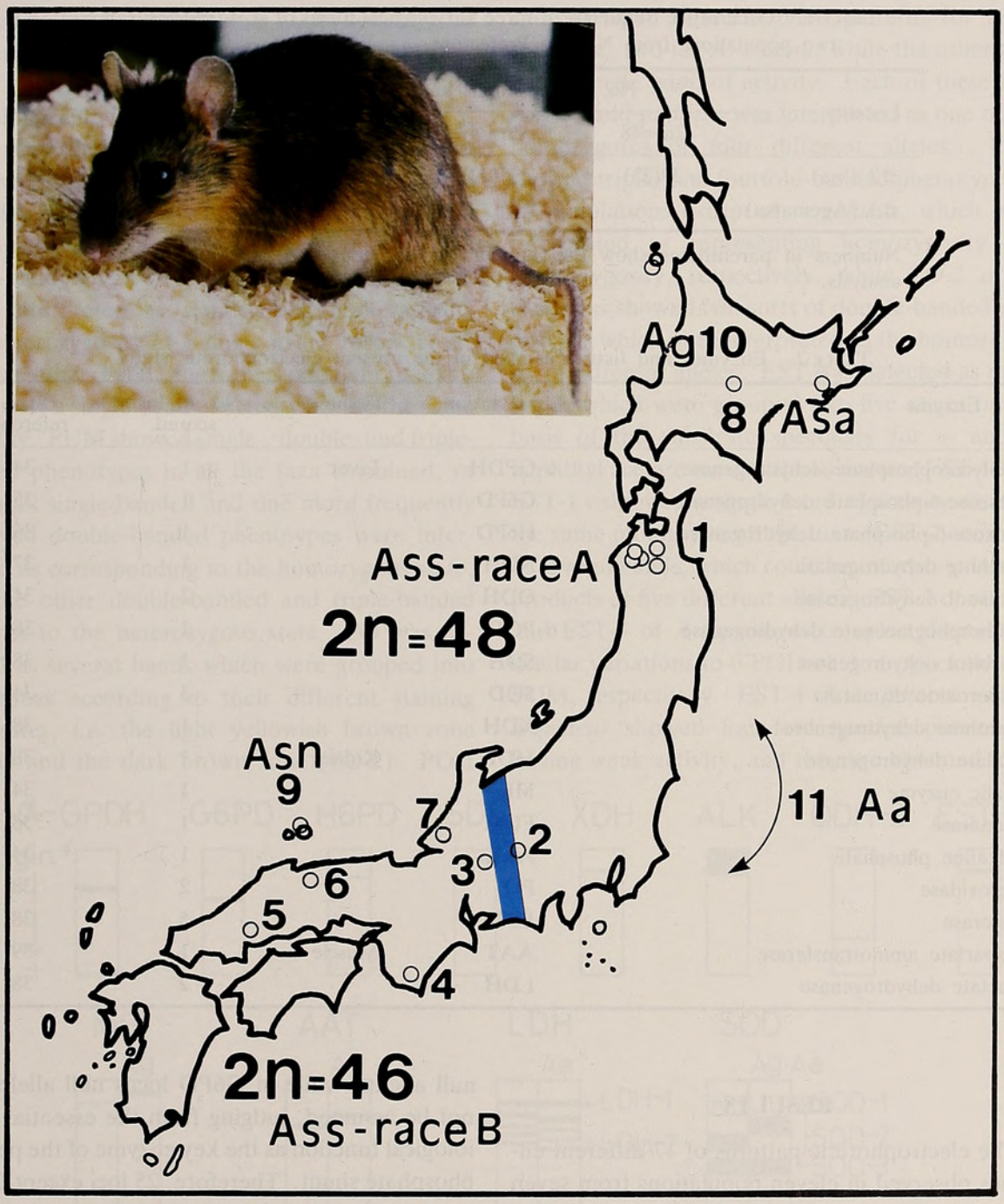

FIG. 1. Collecting localities (#1~#11) of eleven populations of three species of *Apodemus* examined. A thick line of middle Honshu represents a hybrid zone of the races A and B of *A. s. speciosus*. The inset of the upper left is a male race B (2n=46) mouse of *Apondemus s. speciosus* Ass: *A. speciosus speciosus,* Asa: *A. speciosus ainu,* Asn: *A. speciosus navigator,* Ag: *A. giliacus,* Aa: *A. argenteus.*

ice-water bath. After centrifugation at 6,100×g for 10 min at 4°C, the clear supernatant was used for electrophoresis. Electrophoresis was carried out on 7.5% polyacrylamide gel [13] using two different types of disc gel apparatuses: 3×11×75 mm plastic column and φ7×80 mm glass tube. After electrophoresis, seventeen enzymes could be detected on the gels in our electrophoretic system. The enzymes assayed in this study, their abbreviations, tissues used, number of loci scored in each enzyme and references for staining methods are listed in Table 2.

TABLE 1. Occurrence frequency of three karyological forms of *A. s. speciosus* in two populations from Nagano Prefecture

Locality	No. of specimens karyotyped			Total
	2n=48	2n=47	2n=46	
#2 (Ina)	29(23)	35(22)	7(7)	71(52)
#3 (Agematsu)			11(11)	11(11)

Numbers in parentheses show the number of specimens used for electrophoretic analysis.

TABLE 2. Enzymes and tissues assayed in the present electrophoretic study

Enzyme	Abbreviation	Tissue	No. of loci scored	Stain reference
α-Glycerophosphate dehydrogenase	α-GPDH	Liver	1	34
Glucose-6-phosphate dehydrogenase	G6PD		1	35
Hexose-6-phosphate dehydrogenase	H6PD		1	36
Nothing dehydrogenase	NDH		1	37
Octanol dehydrogenase	ODH		2	34
6-Phosphogluconate dehydrogenase	6-PGD		1	38
Sorbitol dehydrogenase	SDH		1	38
Superoxide dismutase	SOD		3	34
Xanthine dehydrogenase	XDH		1	38
Malate dehydrogenase	MDH	Kidney	1	38
Malic enzyme	ME		1	34
Fumarase	FUM		1	38
Alkaline phosphate	ALK		1	34
Peroxidase	PO		2	38
Esterase	EST		5	38
Aspartate aminotransferase	AAT	Muscle	1	39
Lactate dehydrogenase	LDH		2	38

RESULTS

The electrophoretic patterns of 17 different enzymes observed in eleven populations from seven taxa of *Apodemus* are diagrammatically shown in Figure 2. From these band patterns, 24~26 genetic loci were obtained in each population. Of them, 15 loci shown in the first and second row of the zymograms: α-GPDH, G6PD, H6PD, SDH, XDH, ALK, ODH-2, EST-1, ME, AAT, LDH-1, LDH-2 and SOD-1~3 were monomorphic in each population. In the course of this study, G6PD and SOD-1 could not be scored in *A. giliacus* and *A. argenteus*. The lack of SOD-1 locus in these species could be assumed as the homozygosity of null alleles, while in G6PD locus null alleles can not be assumed, judging from the essential physiological function as the key enzyme of the pentose phosphate shunt. Therefore, 25 loci except G6PD were used to compare the *A. speciosus* complex with *A. giliacus* and *A. argenteus*, and 24 loci except G6PD and SOD-1 were used for comparing *A. giliacus* with *A. argenteus*.

The major features of variation in the remaining 11 polymorphic loci (ODH-1, 6-PGD, NDH, MDH, FUM, PO-1, PO-2 and EST-2~5) are summarized as follows: ODH-1 showed single- and triple-banded phenotypes only in *A. s. speciosus*. This variation was interpreted as a diallelic system at a single locus for a dimeric protein, with single-

banded patterns corresponding to the homozygous state and triple-banded patterns to the heterozygous state. In 6-PGD of *A. s. speciosus* and NADP-specific NDH of *A. s. speciosus* and *A. s. ainu,* single- and double-banded phenotypes were observed. This variation was interpreted as a diallelic system at a single locus coding for a monomeric protein. MDH showed double- and triple-banded phenotypes in *A. argenteus,* which were interpreted as representing homozygosity and heterozygosity at a single locus, respectively. On the other hand, the other populations exhibited only a single band of activity having the same mobility. FUM showed single-, double- and triple-banded phenotypes in all the taxa examined, of which all single-banded and one more frequently observed double-banded phenotypes were interpreted as corresponding to the homozygous state, and the other double-banded and triple-banded patterns to the heterozygous state. PO was detected as several bands which were grouped into two zones according to their different staining properties, *i.e.* the light yellowish brown zone (PO-1) and the dark brown zone (PO-2). PO-1 showed three different band patterns for *A. s. speciosus,* two for *A. s. ainu,* while the other taxa had a single band of activity. Each of these four single band patterns was interpreted as one of the homozygotes of four different alleles. PO-2 showed triple- and fourfold-banded phenotypes in 10 populations except *A. argenteus,* which were interpreted as representing homozygosity and heterozygosity, respectively, while PO-2 of *A. argenteus* showed two sorts of double-banded phenotypes which were interpreted as the homozygosity of different alleles. EST was detected as many bands which were grouped into five zones on the basis of the substrate specificity for α- and β-naphthyl acetate used as the substrate (EST-1～5). EST-1 exhibited a single band of activity showing the same mobility in all the taxa. EST-2 consisted of several bands, which could be interpreted as the products of five different alleles. EST-3 of all taxa and EST-4 of *A. speciosus* complex showed the similar variations to 6-PGD of *A. s. speciosus* and FUM, respectively. EST-4 of *A. giliacus* and *A. argenteus* showed four-fold banded phenotypes having weak activity, and they are assumed to be

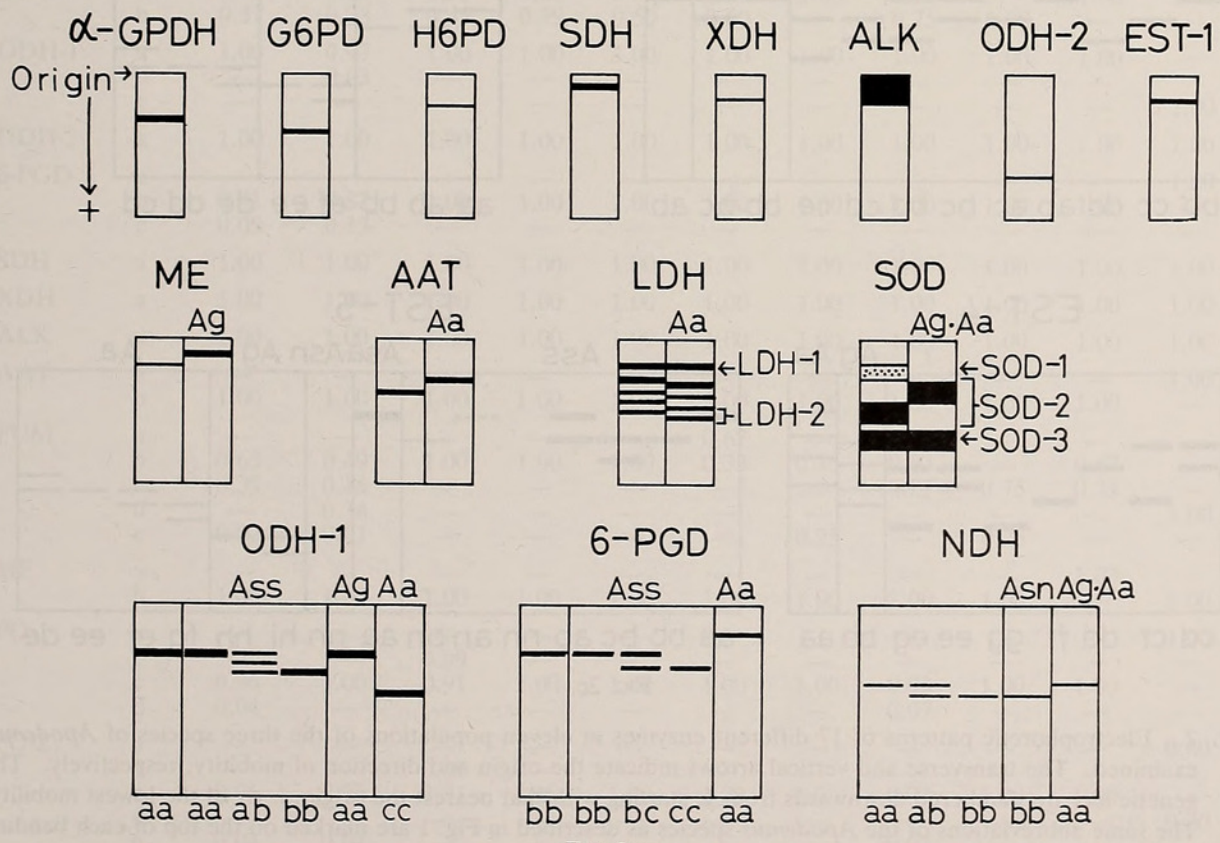

FIG. 2a.

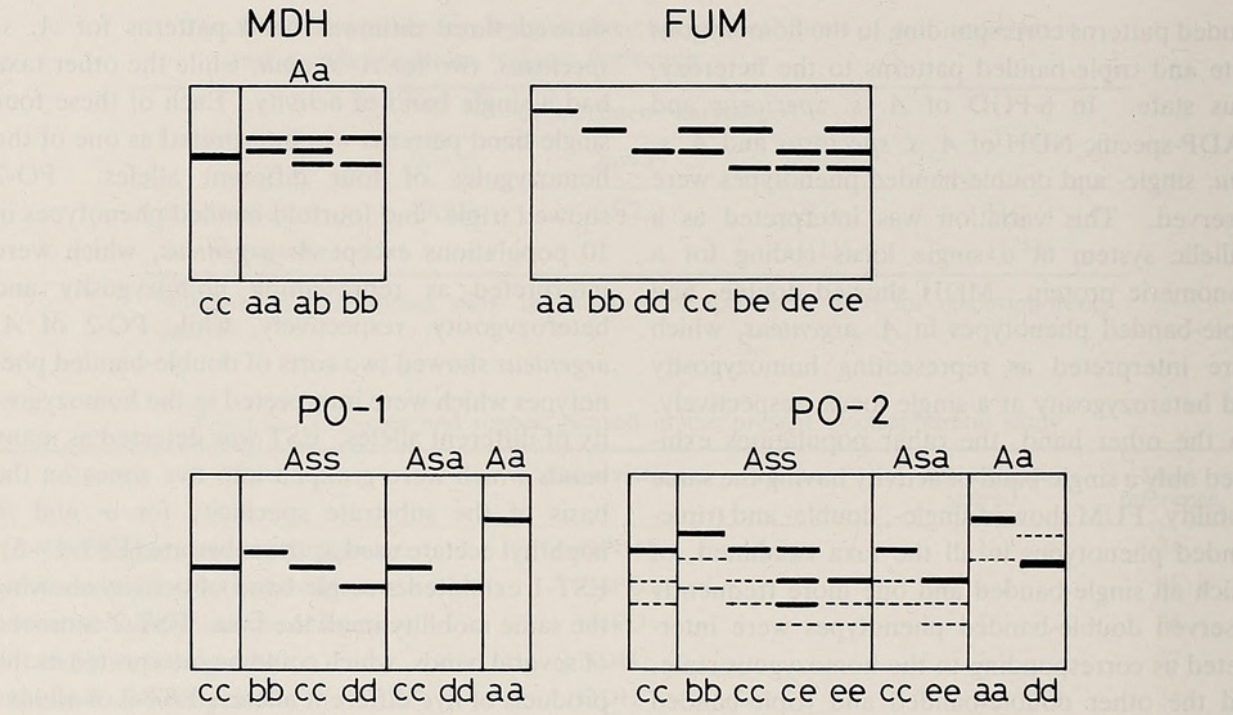

FIG. 2b.

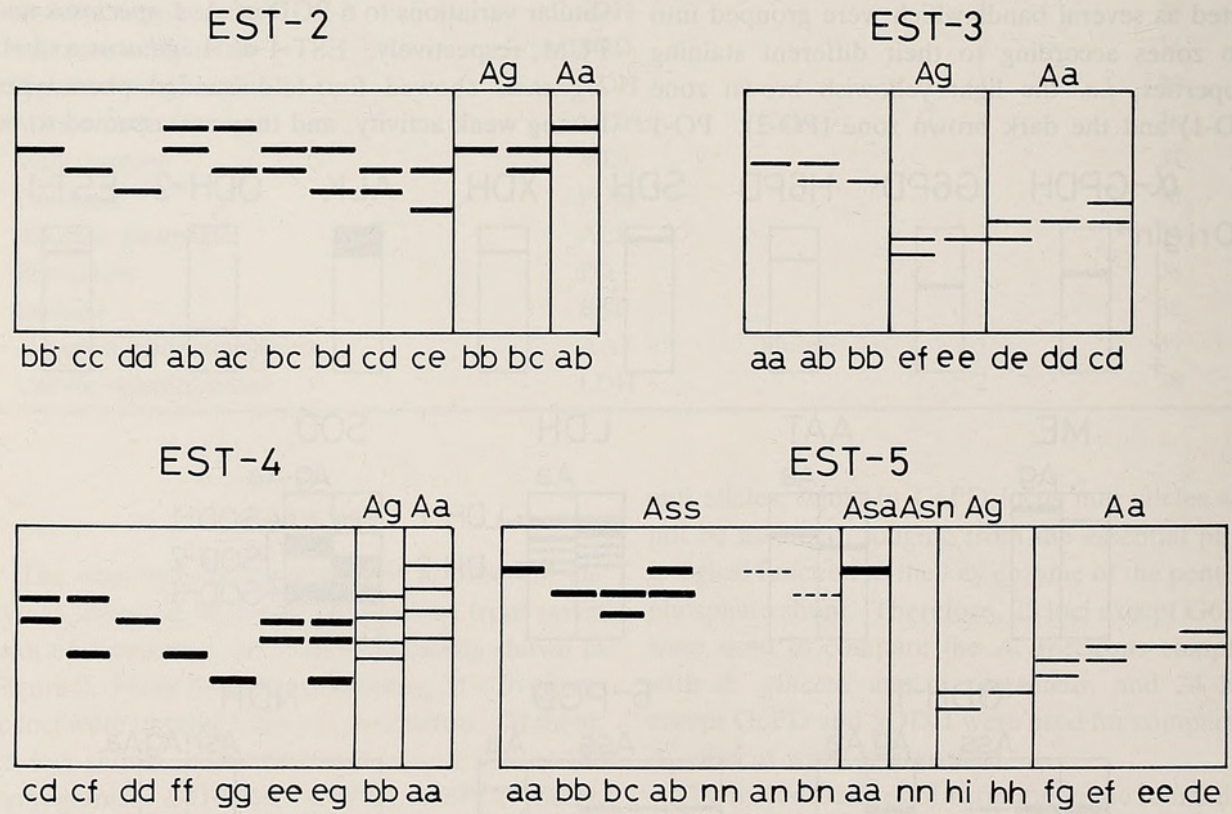

FIG. 2c.

FIG. 2. Electrophoretic patterns of 17 different enzymes in eleven populations of the three species of *Apodemus* examined. The transverse and vertical arrows indicate the origin and direction of mobitity, respectively. The genetic loci are numbered downwards from 1, starting with that nearest the origin, *i. e.*, of the lowest mobility. The same abbreviations of the *Apodemus* species as described in Fig. 1 are marked on the top of each banding pattern. No abbreviation represents the taxa other than those marked.

homozygotes of two different alleles. EST-5 was the most active zone of EST. However, the band of EST-5 was not detected in a few specimens. Therefore, several alleles including a null allele were assumed, so that the lack of band was interpreted as homozygosity of null allele and single weak band as heterozygosity with null allele.

The allele frequencies for all loci in 11 populations from three *Apodemus* species are given in Table 3. As evident from this table, the seven populations of *A. s. speciosus* (#1~#7) shared the same alleles in each locus and the diagnostic locus distinguishing the two chromosomal races could not be obtained. There was no diagnostic locus also in *A. s. speciosus, A. s. ainu* and A. s. navigator. Only one remarkable property common to the two insular subspecies was observed in EST-5. Although the locus was highly polymor-

TABLE 3. Allele frequencies at 26 genetic loci in eleven populations of the three species of *Apodemus*

Locus	Allele†	race A	Hybrid zone	race B					Asa	Asn	Ag	Aa
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
α-GPDH	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G6PD	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	NS	NS
H6PD	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LDH-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LDH-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
	b	—	—	—	—	—	—	—	—	—	—	1.00
MDH	a	—	—	—	—	—	—	—	—	—	—	0.70
	b	—	—	—	—	—	—	—	—	—	—	0.30
	c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
NDH	a	0.63	0.62	0.55	0.61	0.50	0.50	1.00	0.25	—	1.00	1.00
	b	0.37	0.38	0.45	0.39	0.50	0.50	—	0.75	1.00	—	—
ODH-1	a	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
	b	—	0.03	—	—	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—	—	—	—	1.00
ODH-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-PGD	a	—	—	—	—	—	—	—	—	—	—	1.00
	b	0.91	0.87	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
	c	0.09	0.13	—	—	—	—	—	—	—	—	—
SDH	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
XDH	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ALK	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAT	a	—	—	—	—	—	—	—	—	—	—	1.00
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
FUM	a	—	—	—	—	—	0.67	—	—	—	—	—
	b	0.63	0.49	1.00	1.00	1.00	0.33	0.75	0.93	—	0.67	—
	c	0.35	0.14	—	—	—	—	—	0.07	0.75	0.33	—
	d	—	0.36	—	—	—	—	—	—	—	—	1.00
	e	0.02	0.01	—	—	—	—	0.25	—	0.25	—	—
ME	a	—	—	—	—	—	—	—	—	—	1.00	—
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	1.00
PO-1	a	—	—	—	—	—	—	—	—	—	—	1.00
	b	—	—	0.09	—	—	—	—	—	—	—	—
	c	0.96	1.00	0.91	1.00	1.00	1.00	1.00	0.93	1.00	1.00	—
	d	0.04	—	—	—	—	—	—	0.07	—	—	—
PO-2	a	—	—	—	—	—	—	—	—	—	—	0.80
	b	—	—	0.09	—	—	—	—	—	—	—	—
	c	0.96	0.96	0.91	1.00	1.00	1.00	1.00	0.93	1.00	1.00	—
	d	—	—	—	—	—	—	—	—	—	—	0.20
	e	0.04	0.04	—	—	—	—	—	0.07	—	—	—

TABLE 3. Continued

Locus	Allele†	race A	Hybrid zone	race B					Asa	Asn	Ag	Aa
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
SOD-1	n	—	—	—	—	—	—	—	—	—	1.00	1.00
	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—
SOD-2	a	—	—	—	—	—	—	—	—	—	1.00	1.00
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—	—
SOD-3	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-2	a	0.02	0.03	—	—	—	—	—	0.04	—	—	0.50
	b	0.24	0.19	0.18	0.28	—	0.50	—	0.39	0.50	0.67	0.50
	c	0.67	0.61	0.73	0.72	1.00	0.50	1.00	0.21	0.25	0.33	—
	d	0.07	0.16	0.09	—	—	—	—	0.26	0.25	—	—
	e	—	0.01	—	—	—	—	—	—	—	—	—
EST-3	a	0.07	0.07	0.09	0.11	0.50	—	—	0.04	—	—	—
	b	0.93	0.93	0.91	0.89	0.50	1.00	1.00	0.96	1.00	—	—
	c	—	—	—	—	—	—	—	—	—	—	0.10
	d	—	—	—	—	—	—	—	—	—	—	0.80
	e	—	—	—	—	—	—	—	—	—	0.83	0.10
	f	—	—	—	—	—	—	—	—	—	0.17	—
EST-4	a	—	—	—	—	—	—	—	—	—	—	1.00
	b	—	—	—	—	—	—	—	—	—	1.00	—
	c	—	0.06	0.09	—	—	0.17	—	—	—	—	—
	d	—	0.09	0.18	0.11	—	—	—	0.07	—	—	—
	e	0.61	0.29	—	—	—	—	—	0.86	—	—	—
	f	0.35	0.50	0.73	0.89	1.00	0.83	0.50	0.07	0.75	—	—
	g	0.04	0.06	—	—	—	—	0.50	—	0.25	—	—
EST-5	n	0.48	0.37	0.18	0.11	1.00	0.67	—	—	1.00	—	—
	a	0.43	0.45	0.23	0.78	—	0.33	0.50	1.00	—	—	—
	b	0.09	0.16	0.59	0.11	—	—	0.50	—	—	—	—
	c	—	0.02	—	—	—	—	—	—	—	—	—
	d	—	—	—	—	—	—	—	—	—	—	0.10
	e	—	—	—	—	—	—	—	—	—	—	0.50
	f	—	—	—	—	—	—	—	—	—	—	0.30
	g	—	—	—	—	—	—	—	—	—	—	0.10
	h	—	—	—	—	—	—	—	—	—	0.67	—
	i	—	—	—	—	—	—	—	—	—	0.33	—

† Alleles are correspondingly lettered from "a", this being the allele of the lowest mobility.
NS: Not scored.
"n" represents null allele.

phic in *A. s. speciosus*, it was monomorphic in *A. s. ainu* and *A. s. navigator*, which showed only a single electromorph in all specimens. Interspecific comparison revealed that *A. speciosus* differed from *A. giliacus* in 5 loci (ME, SOD-2 and EST-3~5), while *A. speciosus* differed from *A. argenteus* in 11 loci (LDH-2, MDH, ODH-1, 6-PGD, AAT, PO-1, PO-2, SOD-2 and EST-3~5). Therefore, the four loci (SOD-2 and EST-3~5) were diagnostic, making distinction between *A. speciosus* and the other two *Apodemus* species possible.

We could not find the different pattern of allozymes in either of the two chromosomal races of *A. s. speciosus* and also in the insular subspecies. Therefore, their genetic differences can be shown only by the slight differences of allele frequencies. Based on the allele frequencies data shown in Table 3, the genetic identity (I) and the genetic distance (D) between each population were calculated by the method of Nei [14]. Table 4 represents the matrices of I and D values between all pairs of 11 populations from the seven taxa of *Apodemus* examined. Figure 3 shows the biochemical dendrogram constructed from the genetic distance (D-value) matrix of Table 4, using the unweighted pair-group arithmetic average (UPGMA) clustering method of Sneath and Sokal [15].

TABLE 4. Genetic identities (above diagonal) and genetic distances (below diagonal) among eleven populations of the three species of *Apodemus*

	race A	Hybrid zone	race B					Asa	Asn	Ag	Aa
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
#1 *Ass* (Aomori)	—	.992	.973	.974	.957	.968	.965	.968	.943	.764	.479
#2 *Ass* (Ina, Nagano)	.008	—	.980	.981	.959	.975	.972	.961	.944	.760	.499
#3 *Ass* (Agematsu, Nagano)	.027	.020	—	.987	.966	.963	.976	.942	.921	.755	.468
#4 *Ass* (Wakayama)	.026	.019	.013	—	.961	.967	.974	.955	.914	.756	.465
#5 *Ass* (Hiroshima)	.044	.042	.035	.040	—	.956	.937	.897	.927	.738	.449
#6 *Ass* (Tottori)	.033	.025	.038	.034	.045	—	.942	.928	.956	.744	.471
#7 *Ass* (Fukui)	.036	.028	.024	.026	.065	.060	—	.922	.886	.758	.474
#8 *Asa*	.033	.040	.060	.046	.109	.075	.081	—	.901	.732	.454
#9 *Asn*	.059	.058	.082	.090	.076	.045	.121	.104	—	.708	.441
#10 *Ag*	.269	.274	.281	.280	.304	.296	.277	.312	.345	—	.517
#11 *Aa*	.736	.695	.759	.766	.801	.753	.747	.790	.819	.660	—

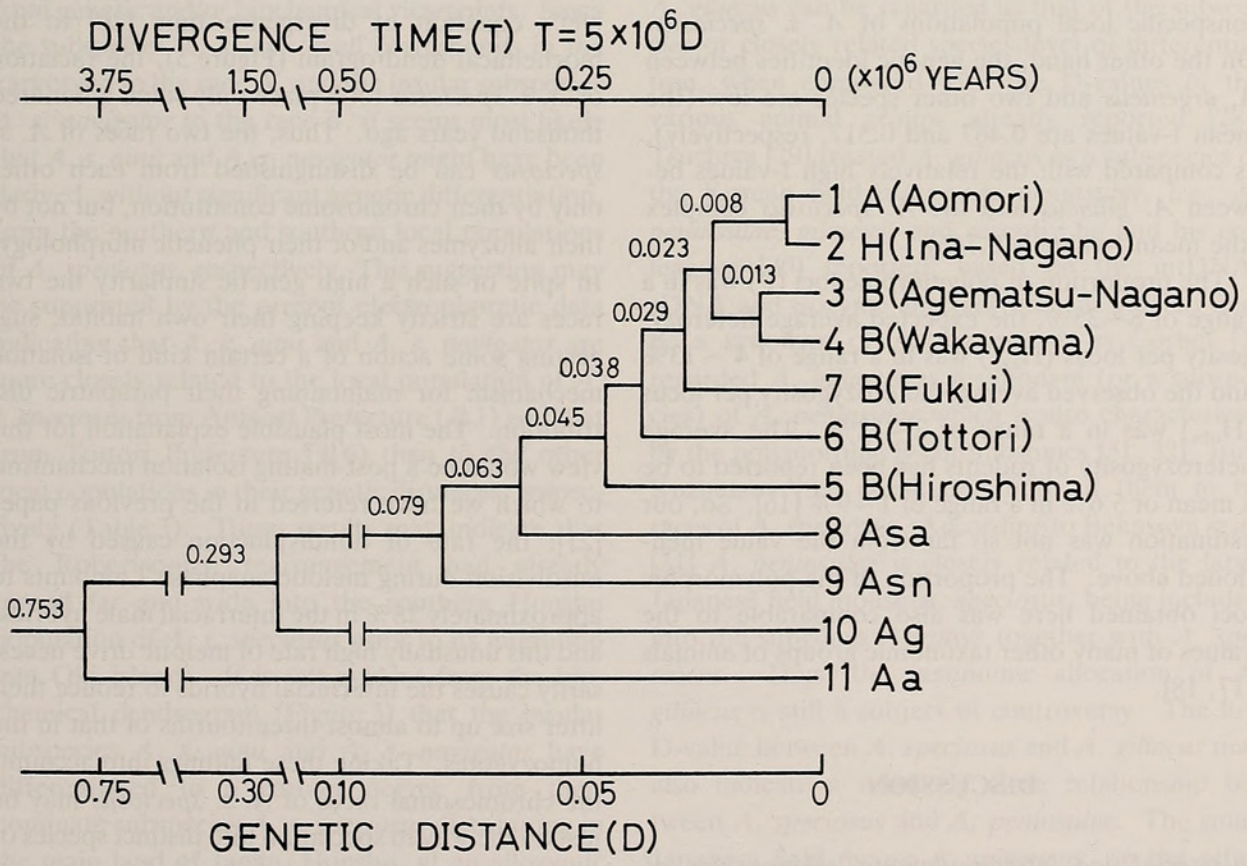

FIG. 3. A biochemical dendrogram showing the genetic relationships among eleven populations from seven taxa of three species of *Apodemus*, based on the Nei's genetic distances. A: pure race A population, B: pure race B population, H: mixed population of the race A, race B and their hybrids, others: the same as in Fig. 1.

As evident from Table 4, little differentiation is observed among seven local populations of *A. s. speciosus* (#1~#7) at a protein level. The genetic identity values (I) observed between them are a mean of 0.968 in a range of 0.937~0.992. Further, the difference of I-values between the three subspecies analyzed is also considerably small. The average I-value between *A. s. speciosus* and *A. s. ainu* is 0.939 in a range of 0.897~0.968, while that between *A. s. speciosus* and *A. s. navigator* 0.927 in a range of 0.886~0.956. The range of the intersubspecific I-values obtained overlapped with that of the interpopulational I-values of *A. s. speciosus* in the half of them. Moreover, *A. s. ainu* and *A. s. navigator* are more closely related to the local population of *A. s. speciosus* from Aomori Prefecture (#1) and that from Tottori Prefecture (#6) than to the other local populations, respectively. This finding is in good agreement with their geographic localities shown in Figure 1. Therefore, from a biochemical viewpoint the two insular subspecies analyzed may be considered to be the conspecific local populations of *A. s. speciosus*. On the other hand, the genetic identities between *A. argenteus* and two other species are low (the mean I-values are 0.467 and 0.517, respectively), as compared with the relatively high I-values between *A. giliacus* and the *A. speciosus* complex (the mean I-value is 0.746).

The proportion of polymorphic loci (P) was in a range of 8~35%, the expected average heterozygosity per locus (H_{exp}) was in a range of 4 ~13% and the observed average heterozygosity per locus (H_{obs}) was in a range of 2~12%. The average heterozygosity of rodents has been reported to be a mean of 5.6% in a range of 1~9% [16]. So, our estimation was not so far from the value mentioned above. The proportion of the polymorphic loci obtained here was also comparable to the values of many other taxonomic groups of animals [17, 18].

DISCUSSION

Interracial and interpopulational relationships of A. s. speciosus

As clearly shown in Figure 3, the D-value between the race A (Aomori population: #1) and the mixed population of the race A, race B and hybrid form (Ina population: #2) is the smallest (D=0.008), although their interval is more than 1,000 km, and the Ina population is more closely related to the Aomori population than to the Agematsu population (#3) which is only 20 km away from Ina locating just on the northern limit of the hybrid zone. This fact may reflect that the mixed population of three karyological forms from the northern limit of the hybrid zone is under the genic influence of the race A to a certain degree. As a whole, *A. s. speciosus* showed a clinal pattern of genetic differentiation varying from north to south.

The interracial D-values were unexpectedly low, being 0.033 on the average in a range of 0.026~0.044. According to Selander *et al.* [19], the interracial D-values for rodents are in a range of 0.010~0.025. Accordingly, it may be reasonable to consider the two karyological forms of *A. s. speciosus* to be in the race level. Applying the Nei's equation of divergence time [20] to the biochemical dendrogram (Figure 3), the raciation of *A. s. speciosus* took place only some a hundred thousand years ago. Thus, the two races of *A. s. speciosus* can be distinguished from each other only by their chromosome constitution, but not by their allozymes and/or their phenetic morphology. In spite of such a high genetic similarity the two races are strictly keeping their own habitat, suggesting some action of a certain kind of isolation mechanism for maintaining their parapatric distribution. The most plausible explanation for this view would be a post-mating isolation mechanism, to which we have referred in the previous paper [21]: the rate of nondisjunction caused by the misdivision during meiotic anaphase I amounts to approximately 28% in the interracial male hybrids, and this unusually high rate of meiotic drive necessarily causes the interracial hybrids to reduce their litter size up to almost three-fourths of that in the homozygotes. Taking these findings into account, the chromosomal races of *A. s. speciosus* may be just on the way to speciation into distinct species or subspecies in spite of a low level of genetic differentiation. Similar mode of speciation has also been proposed to account for the differentiation of

the chromosomal races characterized by different chromosome numbers in two rodent groups: the mole rats of the *Spalax ehrenbergi* complex in Israel and the pocket gophers of the *Thomomys talpoides* complex in the southern Rockies of the United States [22, 23].

Intersubspecific relationships of A. speciousus

According to Britton and Thaler [24], the interpopulational and intersubspecific D-values of *Mus musculus* are 0.013 and 0.220, respectively. The average D-values between *A. s. speciosus* and *A. s. ainu* and that between *A. s. speciosus* and *A. s. navigator* were 0.063 and 0.076 respectively (Table 4). These values are rather equivalent to those between conspecific local populations of *Mus musculus* described above and of many other animal species previously reported [9, 10]. So these three subspecies have undergone little change at a structural gene level. Accordingly, both *A. s. ainu* and *A. s. navigator* can be considered to be still in the level of just local populations of *A. s. speciosus* from genetic and/or biochemical viewpoints. Since the subspecies *A. s. ainu* well corresponds in the karyotype to the race A, and the insular subspecies *A. s. navigator* to the race B, it seems most likely that *A. s. ainu* and *A. s. navigator* might have been derived, without significant genetic differentiation, from the northern and southern local populations of *A. speciosus,* respectively. This suggestion may be supported by the present electrophoretic data indicating that *A. s. ainu* and *A. s. navigator* are more closely related to the local population of *A. s. speciosus* from Aomori Prefecture (#1) and that from Tottori Prefecture (#6) than to the other local populations in their genetic distances, respectively (Table 3). These results may indicate that the Robertsonian rearrangement had already spread far and wide into the southern Honshu population of *A. s. speciosus* prior to its migration into Oki Islands. It is self-evident from the biochemical dendrogram (Figure 3) that the insular subspecies *A. s. ainu* and *A. s. navigator* have differentiated to a certain degree from their nominate subspecies *A. s. speciosus* distributing in the main land of Japan, Honshu, at an allozymic level, although their genetic distances are small.

Interspecific relationships of three Apodemus *species*

The field mouse *A. giliacus* was newly set up by Kobayashi and Hayata [25] as a distinct species distributing in Hokkaido. This species is characterized by varying number of B-chromosomes [26, 27]. However, the chromosomes of *A. giliacus* (2n =48+B's, NF=48) are highly homologous in their G-banding pattern to the race A of *A. s. speciosus* (2n=48, NF=56), excluding the B chromosomes and the sex chromosomes. Taking four pericentric inversions into consideration, their chromosomes mostly correspond to each other, whereas *A. argenteus* (2n=46, FN=52) chromosomes do not show such a high degree of G-band homology to those of *A. giliacus* as well as to those of *A. speciosus* (unpublished data). Therefore, their genetic relationships (Figure 2) revealed by the present electrophoretic study are well consistent with their karyological relationships. The average D-value 0.293 between *A. speciosus* and *A. giliacus* can be regarded as that of the subspecies or closely related species level of differentiation, when compared with the D-values of the various animal groups already reported [28]. Tsuchiya [29] treated *A. giliacus* as a subspecies of the Korean field mouse *A. peninsulae, i.e., A. peninsulae, giliacus,* and recently he and his colleagues [30] reported, based on the mtDNA, rDNA and isozyme analyses, that *A. giliacus* may be a synonym of *A. peninsulae,* as Corbet [7] regarded *A. giliacus* as a synonym (or a subspecies) of *A. peninsulae* which is also characterized by the polymorphic B-chromosomes [31, 32]. But, Kuznetzov [33] considered both of them to be races of *A. speciosus*. According to Bekasova *et al.* [31] *A. peninsulae* is closely related to the large Japanese field mouse *A. speciosus,* being included into the subgenus *Alsomys* together with *A. speciosus*. Thus, the taxonomic allocation of *A. giliacus* is still a subject of controversy. The low D-value between *A. speciosus* and *A. giliacus* may also indicate a relatively close relationship between *A. speciosus* and *A. peninsulae*. The small Japanese field mouse *A. argenteus,* on the other hand, differs considerably in morphology from *A. speciosus* and also from *A. giliacus,* although these

three species belong to the same subgenus *Alsomys* [31]. These biochemical and phenetic relationships are also well consistent with the karyological results already mentioned. The average D-value 0.753 between *A. argenteus* and the lineage of *speciosus-giliacus* is comparable to the values observed between different species or closely related genera of many other animals [28], and therefore the present results may suggest that *A. argenteus* may be remote to some extent in its affinity from the lineage of *speciosus-giliacus*.

The outline of these consideration is simply depicted as a schematic diagram in Figure 4. The diagram shows the stasipatric mode of differentiation of *A. s. speciosus* which can be considered to have been derived from the ancestral form common to *A. agrarius*, since the latter is the only species in Eurasian Continent which shows the

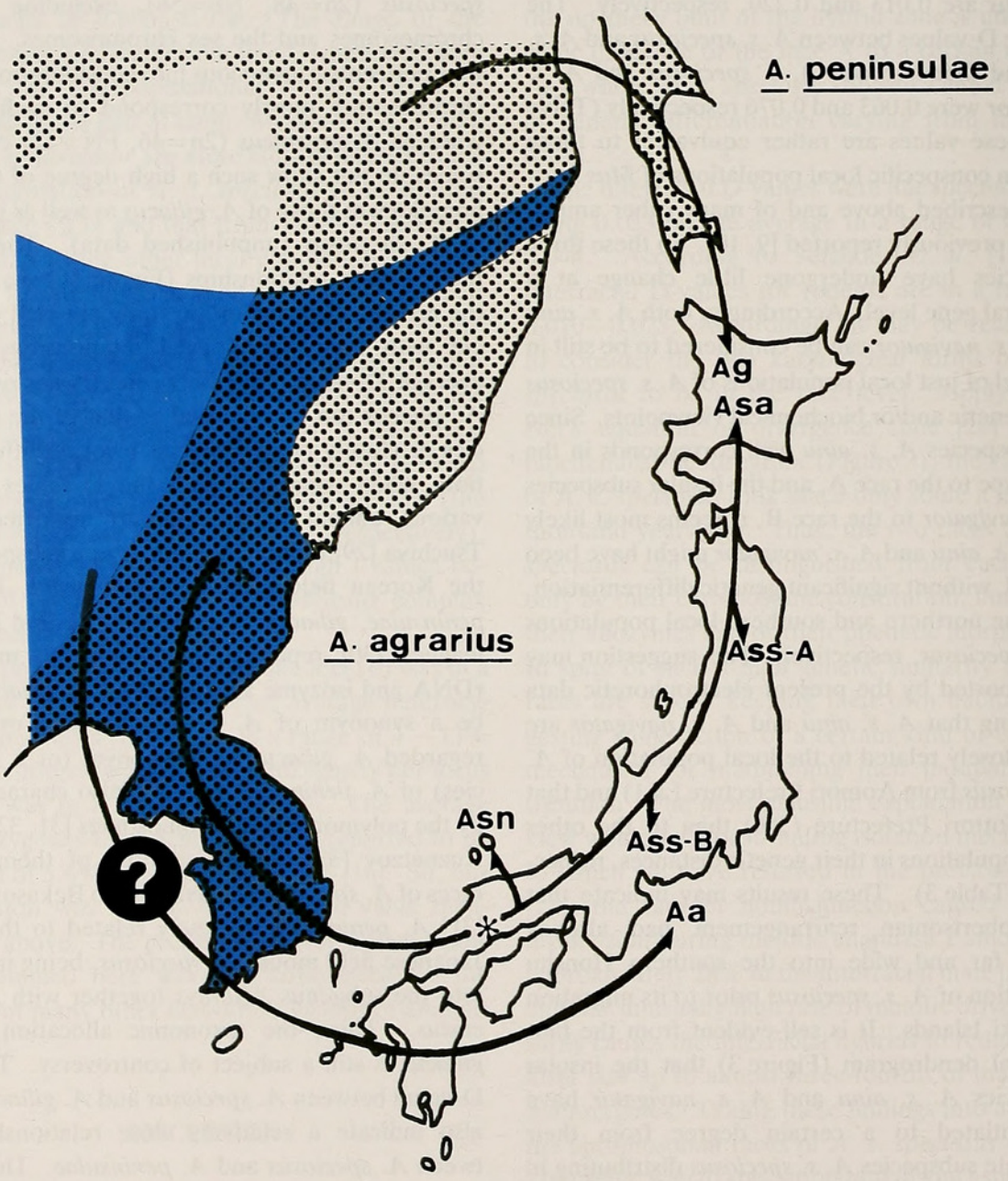

Fig. 4. Probable migration routes of three *Apodemus* species into Japan. Asterisk indicates the generation of a Robertsonian fusion rearrangement somewhere in the southern part of Japan. The shaded part in the continental side represents the distribution area of *A. agrarius* and dotted one that of *A. peninsulae*.

karyotype almost identical to that of the former (race A), and what is more it is distributed in Far East including Korean Peninsula [8]: the chromosomal variants (2n=46) of the ancestral form caused by the Robertsonian fusion rearrangement arised somewhere in the south part of Japan, and these variants have been established as a southern race after the acquirement of some advantages overwhelming the parental 2n=48-type mice. The insular subspecies *A. s. navigator* might have diverged from a local population of *A. speciosus* in which the Robertsonian rearrangement had already spread far and wide, and the other insular subspecies *A. s. ainu* might have maintained the 2n =48 karyotype of *A. speciosus* without any chromosome alteration. It is quite confirmative, based on their distribution area and B-chromosome characteristics, that *A. giliacus* has a very close affinity with the Korean field mouse *A. peninsulae,* and its ancestral population has migrated through Sakhalin into Hokkaido and diverged there as a new form, *A. giliacus* according to Kobayashi and Hayata [25] or *A. peninsulae giliacus* according to Corbet [7] and Tsuchiya [29].

The ancestral form of *A. argenteus* can not be inferred from the karyological characteristics, as the karyotype of *A. argenteus* is largely different from any of the *Apodemus* species distributing in the Eurasian Continent. The detailed chromosome banding analysis and the comparative biochemical surveys would be needed for elucidation of this subject.

The comparative electrophoretic study of the Japanese *Apodemus* and the continental *Apodemus* such as *A. agrarius, A. peninsulae, A. sylvaticus* and *A. chevrieri,* which are distributed in Far East including East Siberia, Korean Peninsula and North East China, would be required to elucidate the speciation process of the *Apodemus* group of Japan.

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