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Genetic Differentiation of the Japanese Brown Frog, *Rana japonica*, Elucidated by Electrophoretic Analyses of Enzymes and Blood Proteins

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ABSTRACT

Intraspecific differentiation of *Rana japonica* was examined by starch-gel electrophoretic analyses of 15 enzymes and three blood proteins in 505 frogs from 25 populations in Japan. These enzymes and blood proteins were controlled by genes at 25 loci, where 4.6 phenotypes were produced by 3.0 alleles on the average. The fixation indexes (Fst) coined by WRIGHT (1978) were calculated. Of the 25 loci analyzed, the AK and MDH-A loci were zero in Fst, while the Pep-A and Hb-II loci were very high in Fst, being 0.722 and 0.781, respectively. The other 21 loci were 0.020~0.490 in Fst. The mean proportions of polymorphic loci in each of the 25 populations ranged from 16.0% to 60.0% with an average of 41.8%, while the mean proportions of heterozygous loci per individual ranged from 4.0% to 18.1% with an average of 11.3%. The mean numbers of alleles per locus ranged from 1.08 to 1.76 with an average of 1.48.

Genetic distances among the 25 populations were estimated on the basis of gene frequencies at the 25 loci according to NEI (1975) and a dendrogram was drawn by using the UPGMA clustering method. It was found that *Rana japonica* first evolved into eastern and western groups. The eastern group was then divided into the Akita population and a subgroup containing eight populations, while the western group was differentiated into the Joetsu population which was followed by the Takatomi and Oki populations and finally branched out into several subgroups containing 13 populations. The genetic distances among the eight populations of the eastern group except the Akita population ranged from 0.005 to 0.100 with a mean of 0.036, while those among 15 populations of the western group except the Joetsu population ranged from 0.014 to 0.159 with a mean of 0.062. The genetic distances between nine populations of the eastern group and 16 populations of the western group ranged from 0.099 to 0.239 with a mean of 0.157.

INTRODUCTION

Rana japonica GÜNTHER is a slender brownish frog which usually inhabits plains and hillsides. This species breeds in the still water of rice fields, marshes and small pools. Occasionally it can be found co-habiting with the mountain brown frog, *Rana ornativentris*, which lives in mountainous districts.

Rana japonica is widely distributed in Kyushu, Shikoku and Honshu of Japan,

and is also found on the Oki, Yaku and Tanegashima Islands. Outside of Japan, this species is known to be distributed in China except for the northeastern region (OKADA, 1931). SUMIDA (1981) has reported that in Honshu, the Ichinoseki population is slightly differentiated morphologically, karyologically and biochemically from the Hiroshima population. The Ichinoseki population is, moreover, reproductively isolated by incomplete hybrid sterility from the Hiroshima population.

The genetic differentiations of anurans distributed in Japan and adjacent countries have been previously reported by NISHIOKA and her collaborators in several species. NISHIOKA, SUMIDA, OHTA and SUZUKI (1987) studied the genetic differences in *Buergeria japonica*. NISHIOKA, SUMIDA and BORKIN (1990) studied those in *Hyla japonica*, while NISHIOKA and SUMIDA (1990) studied those in *Rana limnocharis*. KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI (1990) reported the genetic differences in *Bufo japonicus*. NISHIOKA, SUMIDA and OHTANI (1992) re-

TABLE 1

Specimens of *Rana japonica* used in the present study

Prefecture	Station	No. of frogs			Population
		Total	Female	Male	
Iwate	Shiwa-gun, Shiwa-cho	3	1	2	Shiwa
〃	Ichinoseki-city, Sannoseki	37	19	18	Ichinoseki
Miyagi	Toda-gun, Wakuya-cho	24	13	11	Wakuya
Fukushima	Fukushima-city, Yamaguchi-cho	28	15	13	Fukushima
〃	Sukagawa-city	2	1	1	Sukagawa
Tochigi	Utsunomiya-city, Takanezawa-cho	29	14	15	Utsunomiya
Chiba	Sahara-city	19	10	9	Sahara
Kanagawa	Isehara-city, Sannomiya	18	10	8	Isehara
Akita	Akita-city, Toyoiwaishidazaka	43	28	15	Akita
Niigata	Joetsu-city	5	5	0	Joetsu
Toyama	Toyama-city	15	6	9	Toyama
Fukui	Sakai-gun, Mikuni-cho	21	17	4	Mikuni
Shizuoka	Shizuoka-city, Minaminumagami	28	17	11	Shizuoka
Gifu	Yamagata-gun, Takatomi-cho	21	13	8	Takatomi
Shiga	Otsu-city, Katata-cho	5	4	1	Katata
Hiroshima	Higashihiroshima-city, Saijo-cho	11	3	8	Saijo
〃	Hiroshima-city, Fuchu-cho	36	16	20	Hiroshima
〃	Yamagata-gun, Geihoku-cho	6	6	0	Geihoku
〃	Saiki-gun, Saiki-cho, Iinoyama	21	5	16	Saiki
Shimane	Oki-gun, Saigo-cho	5	3	2	Oki
〃	Ochi-gun, Ochi-cho	46	16	30	Ochi
〃	Nima-gun, Nima-cho	24	10	14	Nima
Yamaguchi	Mine-gun, Mitou-cho	1	1	0	Mitou
〃	Yamaguchi-city, Sayama	31	10	21	Yamaguchi
Fukuoka	Munakata-city, Akama	26	8	18	Munakata
Total		505	251	254	

ported the genetic differences in *Rana nigromaculata* and *R. brevipoda*, while NISHIOKA, KODAMA, SUMIDA and RYUZAKI (1993) reported the genetic differences in *Rana rugosa*.

The present study was performed in order to clarify biochemically the whole aspect of speciation in *Rana japonica* by starch-gel electrophoretic analyses. This study has been reported in part by NISHIOKA, SUMIDA, BORKIN and WU (1992).

MATERIALS AND METHODS

A total of 505 adult frogs, 251 females and 254 males, were collected from 25 stations in Honshu, Kyushu and the Oki Island of Japan. Of these frogs, those obtained from 13 populations were reported by NISHIOKA, SUMIDA, BORKIN and WU (1992). The frogs collected from 12 other stations were added to these in the present study. The collecting stations, population names, numbers of frogs and their sexes are shown in Table 1. Fifteen enzymes and three blood proteins were analyzed by the method of horizontal starch-gel electrophoresis. The kinds of enzymes and blood proteins analyzed, their abbreviations, Enzyme Commission numbers (Nomenclature Committee of Biochemistry, 1992), tissue samples used, and associated buffer systems are shown in Table 2. The details of electrophoretic method have been reported previously by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was carried out by the agar-overlay method outlined by HARRIS and HOPKINSON (1976). The detection of blood

TABLE 2
Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	E.C. No.	Sample	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	3.5.4.4	〃	〃
Adenylate kinase	AK	2.7.4.3	〃	〃
Creatine kinase	CK	2.7.3.2	〃	T-B-E pH 8.0
Fumarase	Fum	4.2.1.2	Liver	〃
α-Glycerophosphate dehydrogenase	α-GDH	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	〃	T-B-E pH 8.0
Isocitrate dehydrogenase	IDH	1.1.1.42	〃	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	〃	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	〃	〃
Malic enzyme	ME	1.1.1.40	〃	T-C pH 7.0
Mannose phosphate isomerase	MPI	5.3.1.8	〃	〃
Peptidase	Pep	3.4.3.1	Liver	T-B-E pH 8.0
Phosphoglucomutase	PGM	2.7.5.1	Skeletal muscle	〃
Superoxide dismutase	SOD	1.15.1.1	〃	〃
Serum albumin	Ab	—	Blood serum	〃
Serum protein C	Prot-C	—	〃	〃
Hemoglobin	Hb	—	Erythrocyte	T-B-E pH 8.6

T-C, Tris-citrate buffer T-B-E, Tris-borate-EDTA buffer

proteins was made by the amido-black staining method.

As a standard to indicate the degree of genetic differentiation found at a definite locus among local populations, the fixation index (Fst) coined by WRIGHT (1978) was utilized. When each of the multiple alleles existed in a frequency of more than 1% at a locus, this locus was regarded to be polymorphic. In order to show quantitatively the genetic variations of local populations, the mean proportions of heterozygous loci per individual specimen and the mean proportions of polymorphic loci per population were used (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974).

The genetic relationships among local populations were evaluated by calculating the genetic distances (D) (NEI, 1975, 1987). A dendrogram was drawn on the

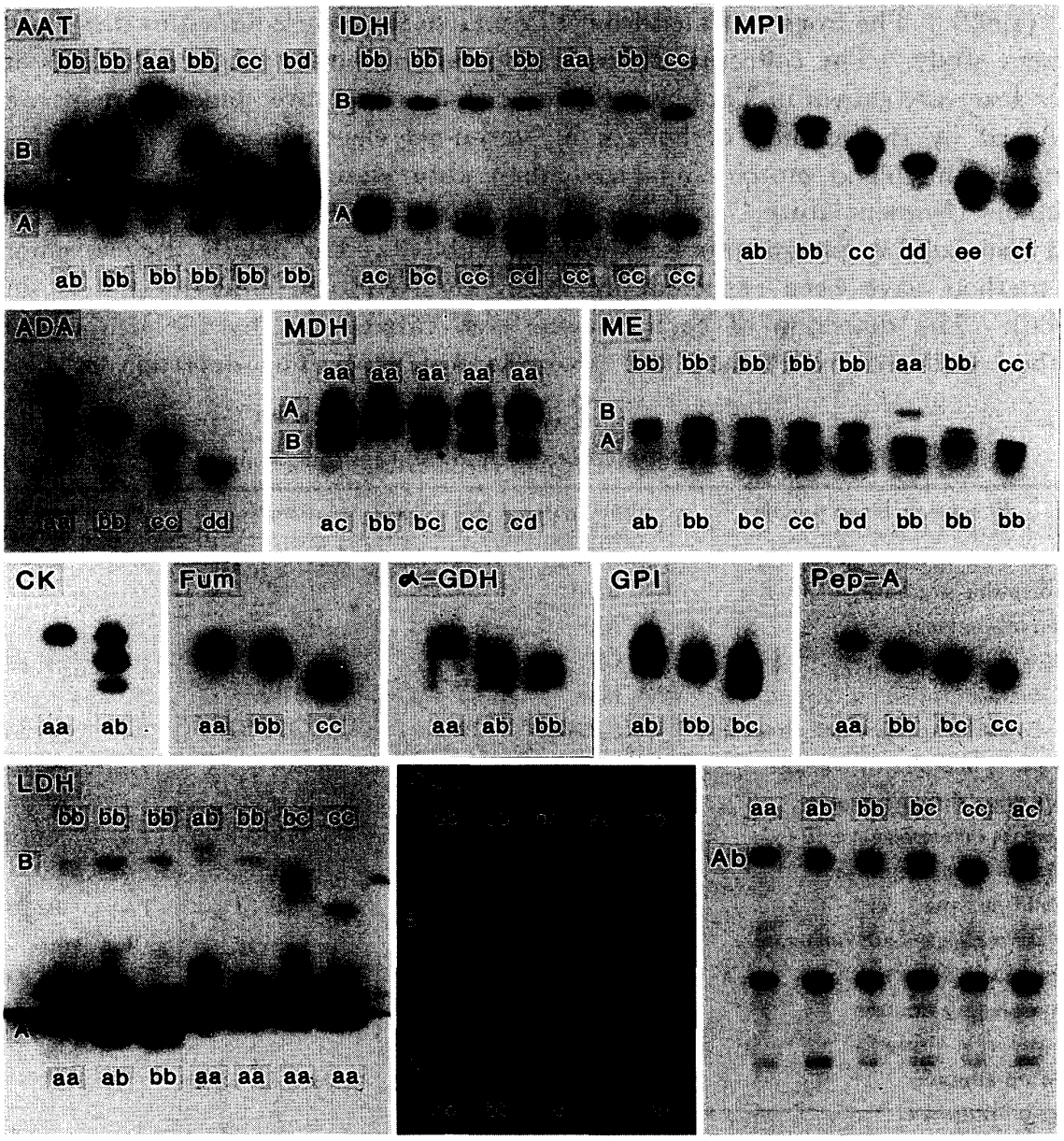

Fig. 1. Electrophoretic patterns of 13 enzymes, AAT, IDH, MPI, ADA, MDH, ME, CK, Fum, α-GDH, GPI, Pep-A, LDH and SOD, and one blood protein, Ab, in 25 populations of *Rana japonica*.

basis of the genetic distances by using the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975, 1987).

OBSERVATION

I. Electrophoretic patterns and allelomorphs

The electrophoretic patterns of 15 enzymes extracted from skeletal muscles and livers and three blood proteins were analyzed in 505 frogs of 25 populations. The results showed that these enzymes and blood proteins were controlled by the genes at 25 loci. The electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, *etc.* in the order of mobility from fast to slow, and the multiple alleles were shown by *a*, *b*, *c*, *etc.* (Fig. 1).

TABLE 3

Number and kind of alleles and phenotypes at 25 loci in 25 populations of *Rana japonica*

Locus	Alleles		Phenotypes	
	No.	Kind	No.	Kind
AAT-A	2	*a, b*	2	BB, AB
AAT-B	4	*a~d*	7	AA, BB, CC, AB, AC, BC, BD
ADA	4	*a~d*	9	AA, BB, CC, DD, AB, AC, BC, BD, CD
AK	1	*a*	1	AA
CK	2	*a, b*	2	AA, AB
Fum	3	*a~c*	6	AA, BB, CC, AB, AC, BC
α-GDH	2	*a, b*	3	AA, BB, AB
GPI	3	*a~c*	3	BB, AB, BC
IDH-A	4	*a~d*	4	CC, AC, BC, CD
IDH-B	3	*a~c*	6	AA, BB, CC, AB, AC, BC
LDH-A	2	*a, b*	3	AA, BB, AB
LDH-B	3	*a~c*	4	BB, CC, AB, BC
MDH-A	1	*a*	1	AA
MDH-B	4	*a~d*	5	BB, CC, AC, BC, CD
ME-A	4	*a~d*	5	BB, CC, AB, BC, BD
ME-B	3	*a~c*	5	AA, BB, CC, AB, BC
MPI	6	*a~f*	13	BB, CC, DD, EE, AB, AC, BC, BD, BE, CD, CE, CF, DE
Pep-A	3	*a~c*	5	AA, BB, CC, AB, BC
PGM	3	*a~c*	4	AA, BB, AB, BC
SOD-A	3	*a~c*	4	AA, BB, AB, BC
SOD-B	2	*a, b*	3	AA, BB, AB
Ab	3	*a~c*	6	AA, BB, CC, AB, AC, BC
Prot-C	4	*a~d*	8	AA, BB, CC, AB, AC, BC, BD, CD
Hb-I	2	*a, b*	2	AA, AB
Hb-II	3	*a~c*	4	AA, BB, CC, AB
Average	3.0		4.6	

Two of the 25 loci, the AK and MDH-A loci, showed a single phenotype produced by a single allele, *a*. At the AAT-A, CK, α-GDH, LDH-A, SOD-B and Hb-I loci, there were two to three phenotypes produced by two alleles, *a* and *b*. At the Fum, GPI, IDH-B, LDH-B, ME-B, Pep-A, PGM, SOD-A, Ab and Hb-II loci, three to six phenotypes were produced by three alleles, *a*, *b* and *c*. At the AAT-B, ADA, IDH-A, MDH-B, ME-A and Prot-C loci, there were four to nine phenotypes produced by four alleles, *a*, *b*, *c* and *d*. The MPI locus was the most polymorphic. Thirteen phenotypes were produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*. At these 25 loci, 4.6 phenotypes were produced by 3.0 alleles on the average (Table 3).

II. Gene frequency

Of the 25 loci, the AK and MDH-A loci showed a single phenotype, AA, controlled by a single allele, *a*. The gene frequencies at the other 23 loci were as follows (Tables 4~6).

1. AAT-A locus

The electrophoretic patterns at the AAT-A locus obtained from 505 frogs of the

TABLE 4

Gene frequencies at 12 loci, AAT-A, AAT-B, ADA, AK, CK, Fum, α-GDH, GPI,

Population	Sample size	AAT-A		AAT-B				ADA				AK	CK		Fum		
		a	*b*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*a*	*b*	*a*	*b*	*c*
Shiwa	3		1.000		1.000			0.333		0.667		1.000	1.000			0.667	0.333
Ichinoseki	37		1.000		0.986	0.014		0.622	0.081	0.297		1.000	1.000		0.068	0.757	0.176
Wakuya	24		1.000		1.000			0.333	0.375	0.292		1.000	0.979	0.021	0.146	0.833	0.021
Fukushima	28		1.000		0.982	0.018			0.875	0.125		1.000	1.000		0.196	0.679	0.125
Sukagawa	2		1.000		1.000				1.000			1.000	1.000			0.750	0.250
Utsunomiya	29	0.052	0.948		1.000				0.621	0.379		1.000	1.000		0.017	0.948	0.034
Sahara	19		1.000	0.105	0.895				0.658	0.316	0.026	1.000	1.000			0.974	0.026
Isehara	18		1.000		1.000				0.722	0.278		1.000	1.000			1.000	
Akita	43		1.000		0.779	0.221		0.105		0.895		1.000	1.000			1.000	
Joetsu	5		1.000		0.800	0.200				1.000		1.000	1.000		0.100	0.900	
Toyama	15		1.000		0.767	0.233				0.967	0.033	1.000	1.000		0.033	0.967	
Mikuni	21		1.000		0.952	0.048				0.952	0.048	1.000	1.000			1.000	
Shizuoka	28	0.018	0.982		0.482	0.518		0.036	0.714	0.232	0.018	1.000	1.000		0.071	0.911	0.018
Takatomi	21		1.000		0.833	0.167			0.690	0.310		1.000	1.000			1.000	
Katata	5		1.000		0.200	0.800			0.600	0.400		1.000	1.000		0.200	0.500	0.300
Saijo	11		1.000		0.864	0.136			0.182	0.773	0.045	1.000	1.000			1.000	
Hiroshima	36		1.000	0.028	0.722	0.250			0.181	0.806	0.014	1.000	1.000		0.097	0.903	
Geihoku	6		1.000		0.750	0.250			0.250	0.417	0.333	1.000	1.000			1.000	
Saiki	21		1.000		0.548	0.452			0.524	0.190	0.286	1.000	1.000		0.381	0.619	
Oki	5		1.000		0.700	0.300		0.100	0.100	0.800		1.000	1.000		0.500	0.500	
Ochi	46		1.000		0.457	0.522	0.022		0.467	0.533		1.000	1.000		0.120	0.880	
Nima	24		1.000		0.750	0.229	0.021		0.729	0.271		1.000	1.000		0.083	0.917	
Mitou	1		1.000		1.000					1.000		1.000	1.000			1.000	
Yamaguchi	31		1.000		0.952	0.048			0.403	0.565	0.032	1.000	1.000		0.065	0.871	0.065
Munakata	26		1.000		0.827	0.173			0.308	0.538	0.154	1.000	1.000			0.827	0.173

25 populations showed two phenotypes, BB and AB, produced by two alleles, *a* and *b*. In gene frequency, allele *b* in the Utsunomiya and Shizuoka populations was 0.948 and 0.982, respectively, while allele *a* was 0.052 and 0.018, respectively. All the other 23 populations had only allele *b* (Table 4).

2. AAT-B locus

The electrophoretic patterns at the AAT-B locus obtained from 505 frogs of the 25 populations showed that there were seven phenotypes, AA, BB, CC, AB, AC, BC and BD, produced by four alleles, *a*, *b*, *c* and *d*. In the 21 populations other than the Shizuoka, Katata, Saiki and Ochi populations, allele *b* was very high in frequency, being 0.700~1.000. The Shiwa, Wakuya, Sukagawa, Utsunomiya, Isehara and Mitou populations had only allele *b*. The Sahara population had allele *a* in a frequency of 0.105 in addition to allele *b*, the Hiroshima population had alleles *a* and *c* in frequencies of 0.028 and 0.250, respectively, in addition to allele *b*, and the Nima population had alleles *c* and *d* in frequencies of 0.229 and 0.021, respectively, in addition to allele *b*. The other 12 populations, the Ichinoseki, Fukushima, Akita, Joetsu, Toyama, Mikuni, Takatomi, Saijo, Geihoku, Oki, Yamaguchi and Munakata populations, had allele *c* in frequencies of 0.014 ~0.300 in addition to allele *b*. In the Shizuoka and Saiki populations, allele *b* was

IDH-A, IDH-B, LDH-A and LDH-B, in 25 populations of *Rana japonica*

α-GDH		GPI			IDH-A				IDH-B			LDH-A		LDH-B		
a	*b*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*a*	*b*	*a*	*b*	*c*
	1.000		1.000				1.000			1.000		1.000			1.000	
	1.000		1.000				1.000			1.000		1.000			1.000	
	1.000	0.083	0.917				1.000			1.000		1.000			1.000	
	1.000		1.000		0.232		0.768			1.000		1.000			1.000	
0.250	0.750		1.000		0.250		0.750			1.000		1.000			1.000	
0.052	0.948	0.034	0.966		0.034		0.966			1.000		1.000			1.000	
	1.000		0.974	0.026	0.026		0.974			0.921	0.079	1.000			1.000	
	1.000	0.250	0.750				1.000		0.111	0.806	0.083	1.000			1.000	
	1.000	0.012	0.988				1.000			0.930	0.070	0.895	0.105	0.058	0.942	
0.400	0.600		1.000				1.000		0.100	0.900		1.000			1.000	
	1.000		1.000				1.000			1.000		1.000			1.000	
0.167	0.833	0.024	0.976				1.000			1.000		1.000			1.000	
	1.000	0.036	0.964				1.000		0.179	0.821		1.000			1.000	
	1.000		1.000				1.000			1.000		1.000			0.452	0.548
	1.000		1.000				1.000			1.000		1.000			1.000	
	1.000		1.000				0.909	0.091		1.000		1.000			1.000	
0.028	0.972	0.028	0.972				1.000		0.014	0.986		1.000			1.000	
0.083	0.917		1.000				1.000			1.000		1.000			1.000	
	1.000		1.000				1.000			0.929	0.071	1.000			1.000	
	1.000		1.000				1.000			1.000		1.000			1.000	
0.033	0.967		1.000			0.087	0.913			1.000		1.000			1.000	
0.167	0.833		1.000				1.000			0.938	0.063	1.000			1.000	
	1.000		1.000				1.000			1.000		1.000			1.000	
	1.000		1.000				1.000		0.016	0.484	0.500	1.000			1.000	
0.077	0.923		1.000				1.000			0.808	0.192	1.000			1.000	

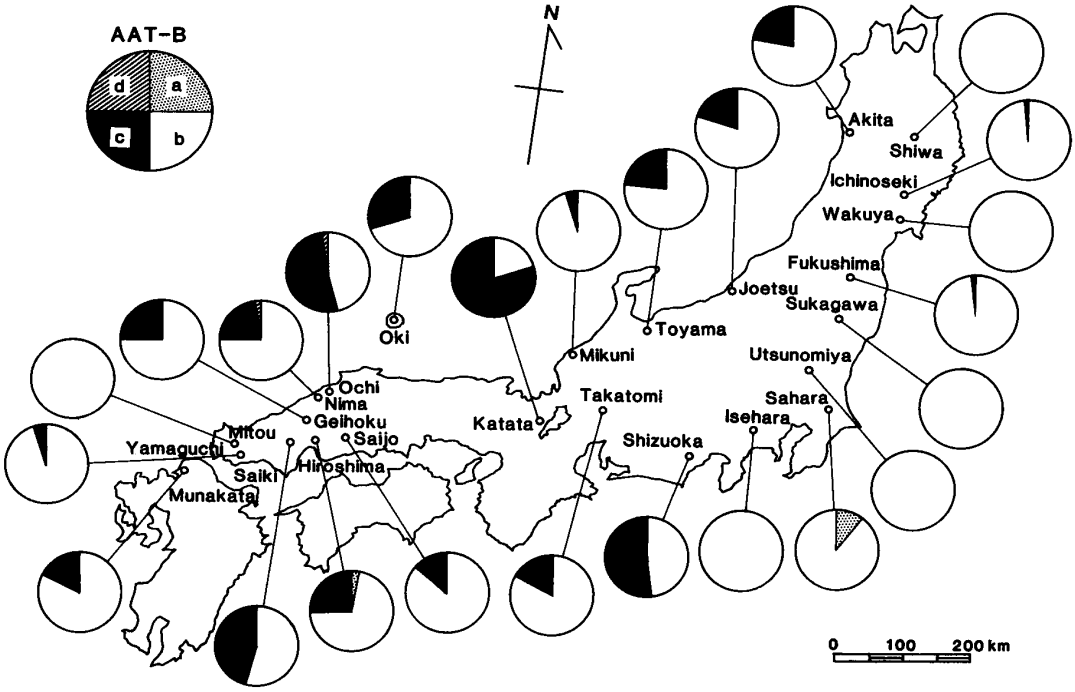

Fig. 2. Geographic distribution of AAT-B alleles among 25 populations of *Rana japonica*.

0.482 and 0.548, respectively, and allele *c* was 0.518 and 0.452, respectively, in frequency. In the Ochi population, alleles *b*, *c* and *d* were 0.457, 0.522 and 0.022, respectively, in frequency. In the Katata population, allele *c* was 0.800, and allele *b* was 0.200 in frequency (Table 4; Fig. 2).

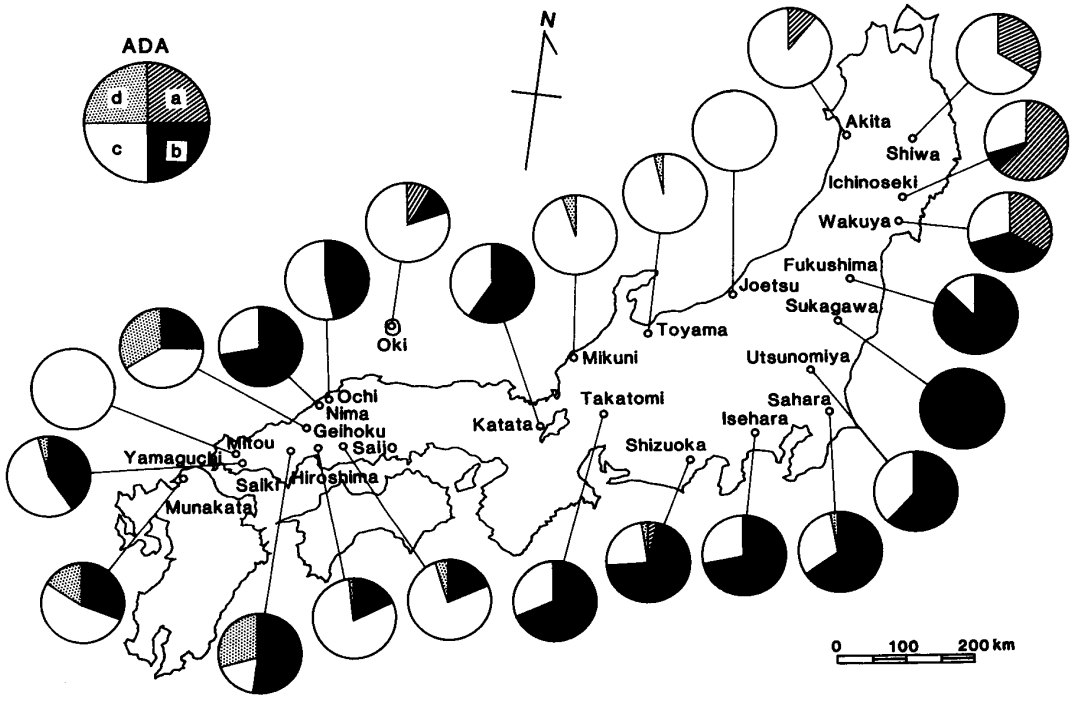

Fig. 3. Geographic distribution of ADA alleles among 25 populations of *Rana japonica*.

3. ADA locus

The electrophoretic patterns at the ADA locus obtained from 505 frogs of the 25 populations showed nine phenotypes, AA, BB, CC, DD, AB, AC, BC, BD and CD, produced by four alleles, *a*, *b*, *c* and *d*.

In 10 populations, the Fukushima, Sukagawa, Utsunomiya, Sahara, Isehara, Shizuoka, Takatomi, Katata, Saiki and Nima populations, allele *b* was high in frequency, being 0.524~1.000. The Sukagawa population had only allele *b*. In the Fukushima, Utsunomiya, Isehara, Takatomi, Katata and Nima populations, allele *c* was 0.125~0.400 in frequency, in addition to allele *b*. In the Sahara and Saiki populations, allele *c* was 0.316 and 0.190, respectively, and allele *d* was 0.026 and 0.286, respectively, in frequency, in addition to allele *b*. In the Shizuoka population, alleles *c*, *a* and *d* were 0.232, 0.036 and 0.018, respectively, in frequency, in addition to allele *b*. In nine populations, the Shiwa, Akita, Joetsu, Toyama, Mikuni, Saijo, Hiroshima, Oki and Mitou populations, allele *c* was high in frequency, being 0.667~1.000. The Joetsu and Mitou populations had only allele *c*. In the Shiwa and Akita populations, allele *a* was 0.333 and 0.105, respectively, in frequency, in addition to allele *c*. In the Toyama and Mikuni populations, allele *d* was 0.033 and 0.048, respectively, in frequency, in addition to allele *c*. In the Saijo and Hiroshima populations, allele *b* was 0.182 and 0.181, respectively, and allele *d* was 0.045 and 0.014, respectively, in frequency, in addition to allele *c*. In the Oki population, alleles *a* and *b* were each 0.100 in frequency, in addition to allele *c*. In the Ochi population, alleles *c* and *b* were 0.533 and 0.467, respectively, in frequency. In three populations, the Geihoku, Yamaguchi and Munakata populations, allele *c* was 0.417~0.565, allele *b* was 0.250~0.403, and allele *d* was 0.032~0.333 in frequency. In the Ichinoseki population, allele *a* was high in frequency, being 0.622, and alleles *c* and *b* were low in frequency, being 0.297 and 0.081, respectively. In the Wakuya population, alleles *a*, *b* and *c* were 0.333, 0.375 and 0.292, respectively, in frequency (Table 4; Fig. 3).

4. CK locus

The electrophoretic patterns at the CK locus obtained from 505 frogs of the 25 populations showed two phenotypes, AA and AB, produced by two alleles, *a* and *b*. Every population other than the Wakuya population had only allele *a*. In the Wakuya population, alleles *a* and *b* were 0.979 and 0.021, respectively, in frequency (Table 4).

5. Fum locus

The electrophoretic patterns at the Fum locus obtained from 505 frogs of the 25 populations exhibited six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*, *b* and *c*.

The gene frequency of allele *b* was high, being 0.619~1.000 in 23 populations other than the Katata and Oki populations. The Isehara, Akita, Mikuni, Taka-

tomi, Saijo, Geihoku and Mitou populations had only allele *b*. In the Joetsu, Toyama, Hiroshima, Saiki, Ochi and Nima populations, allele *a* was 0.033~0.381 in frequency, in addition to allele *b*. In the Shiwa, Sukagawa, Sahara and Munakata populations, allele *c* was 0.026~0.333 in frequency, in addition to allele *b*. In the Ichinoseki, Wakuya, Fukushima, Utsunomiya, Shizuoka and Yamaguchi populations, allele *a* was 0.065~0.196 and allele *c* was 0.021~0.176 in frequency, in addition to allele *b*. In the Oki population, alleles *a* and *b* were each 0.500 in frequency. In the Katata population, alleles *b*, *c* and *a* were 0.500, 0.300 and 0.200, respectively, in frequency (Table 4; Fig. 4).

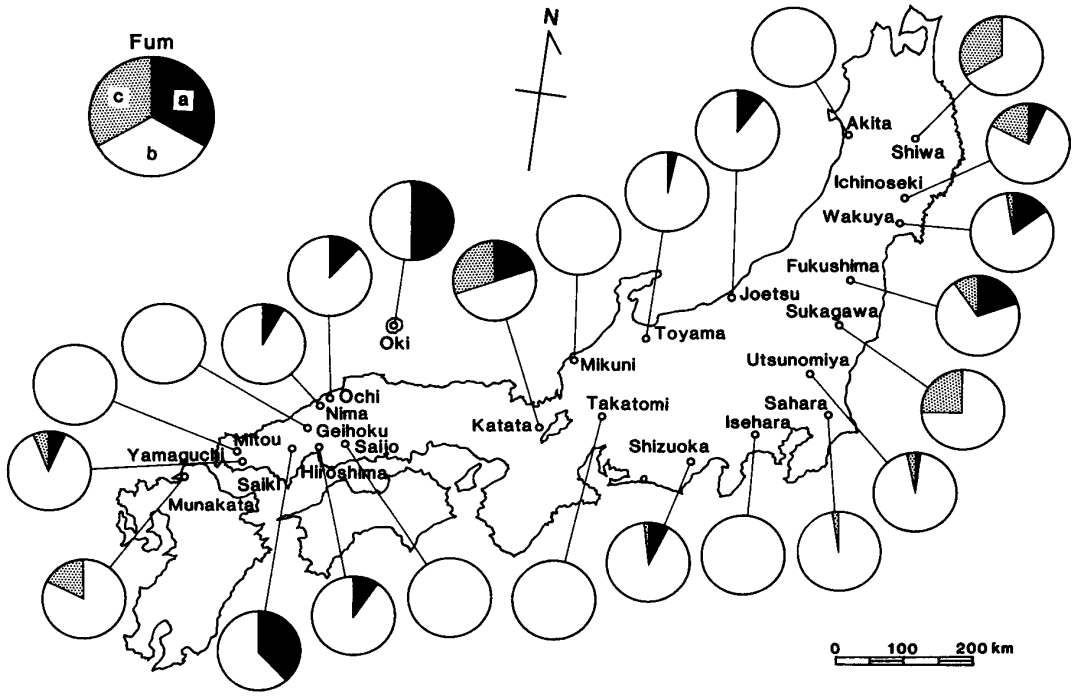

Fig. 4. Geographic distribution of Fum alleles among 25 populations of *Rana japonica*.

6. α–GDH

The electrophoretic patterns at the α–GDH locus obtained from 505 frogs of the 25 populations showed three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*. In gene frequency, allele *b* was very high in all the 25 populations, being 0.600~1.000. In the Sukagawa, Utsunomiya, Joetsu, Mikuni, Hiroshima, Geihoku, Ochi, Nima and Munakata populations, allele *a* was 0.028 ~0.400 in frequency, in addition to allele *b*. The other 16 populations had only allele *b* (Table 4; Fig. 5).

7. GPI locus

The electrophoretic patterns at the GPI locus obtained from 505 frogs of the 25 populations showed three phenotypes, BB, AB and BC, produced by three alleles, *a*, *b* and *c*. In gene frequency, allele *b* was very high in all the 25 populations,

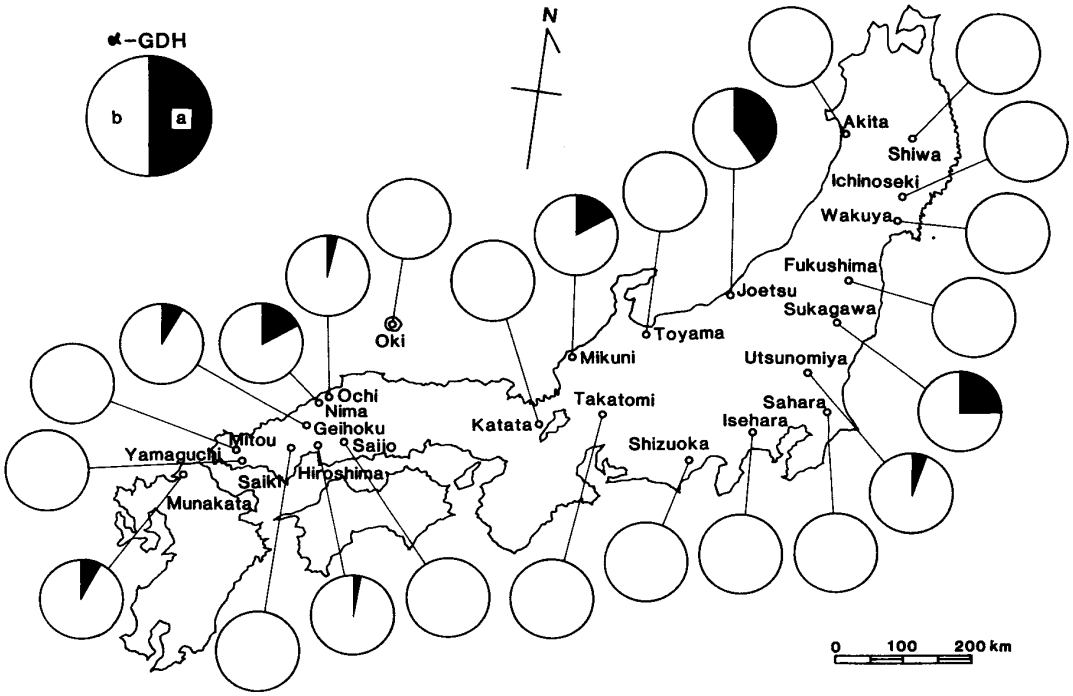

Fig. 5. Geographic distribution of α–GDH alleles among 25 populations of *Rana japonica*.

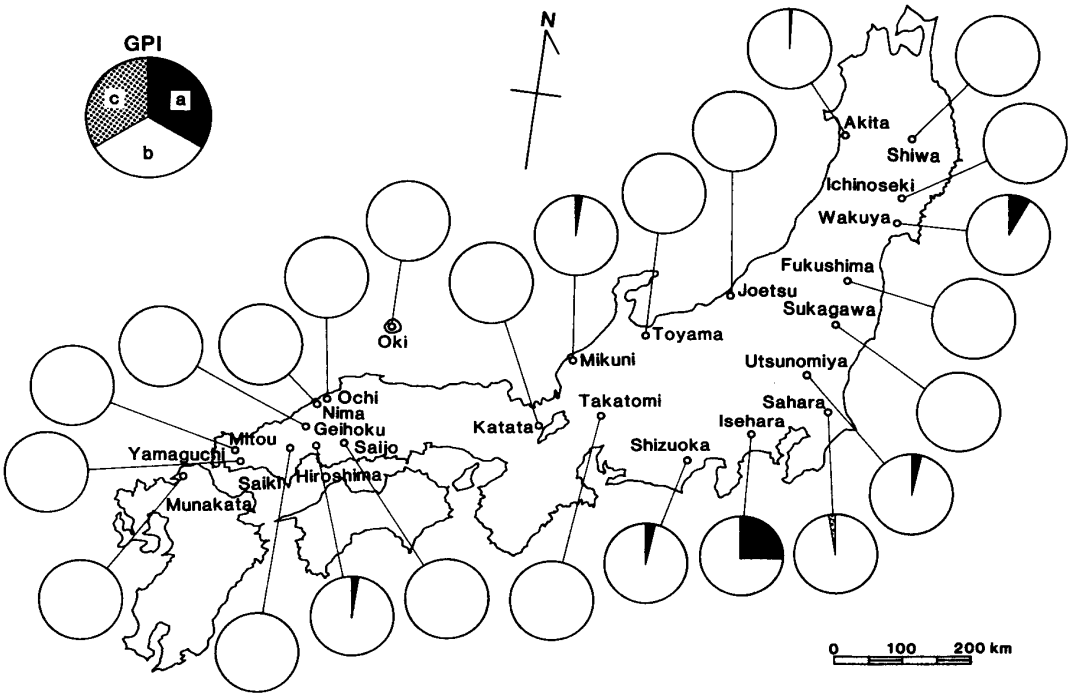

Fig. 6. Geographic distribution of GPI alleles among 25 populations of *Rana japonica*.

being 0.750~1.000. In the Wakuya, Utsunomiya, Isehara, Akita, Mikuni, Shizuoka and Hiroshima populations, allele *a* was 0.012~0.250 in frequency, in addition to allele *b*. In the Sahara population, allele *c* was 0.026 in frequency, in addition to allele *b*. The other 17 populations had only allele *b* (Table 4; Fig. 6).

8. IDH-A locus

The electrophoretic patterns at the IDH-A locus obtained from 505 frogs of the 25 populations showed four phenotypes, CC, AC, BC and CD, produced by four alleles, *a*, *b*, *c* and *d*. In gene frequency, allele *c* was very high in all the 25 populations, being 0.750~1.000. In the Fukushima, Sukagawa, Utsunomiya and Sahara populations, allele *a* was 0.026~0.250 in frequency, in addition to allele *c*. In the Saijo and Ochi populations, alleles *d* and *b* were 0.091 and 0.087, respectively, in frequency, in addition to allele *c*. The other 19 populations had only allele *c* (Table 4; Fig. 7).

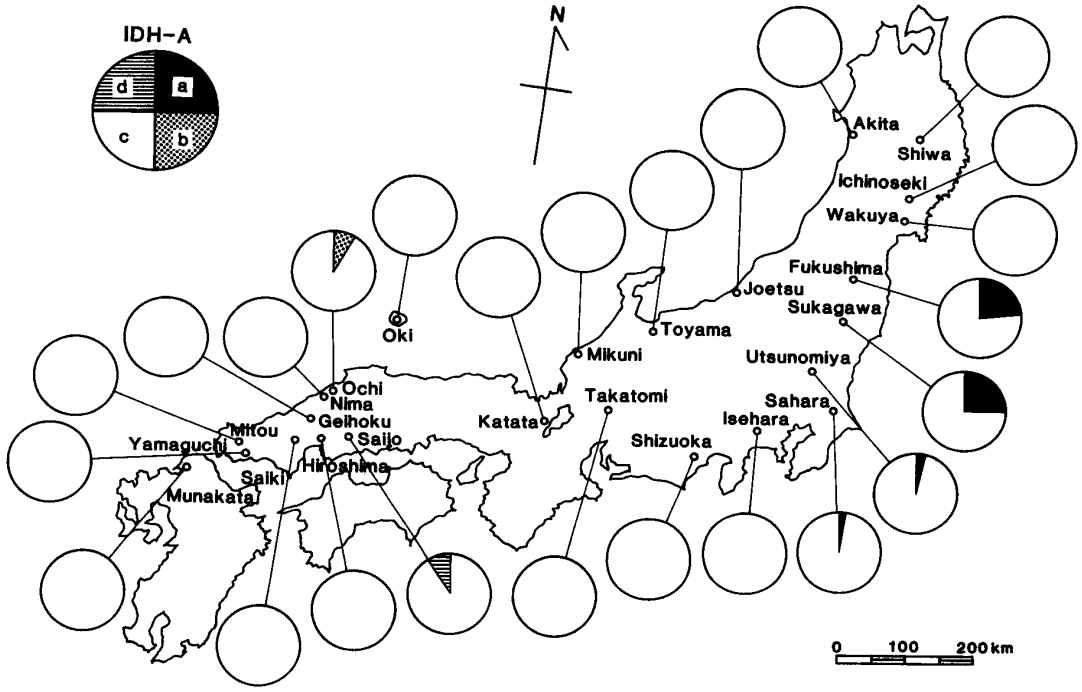

Fig. 7. Geographic distribution of IDH-A alleles among 25 populations of *Rana japonica*.

9. IDH-B

The electrophoretic patterns at the IDH-B locus obtained from 505 frogs of the 25 populations showed six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*, *b* and *c*. The Yamaguchi population had alleles *c*, *b* and *a* in frequencies of 0.500, 0.484 and 0.016, respectively. In 24 populations other than the Yamaguchi population, allele *b* was very high in frequency, being 0.806~1.000. In the Joetsu, Shizuoka and Hiroshima populations, allele *a* was 0.014~0.179 in frequency, in addition to allele *b*. In the Sahara, Akita, Saiki, Nima and Munakata populations, allele *c* was 0.063~0.192 in frequency, in addition to allele *b*. In the Isehara population, alleles *a* and *c* were 0.111 and 0.083, respectively, in frequency in addition to allele *b*. All the other 15 populations had only allele *b* (Table 4; Fig. 8).

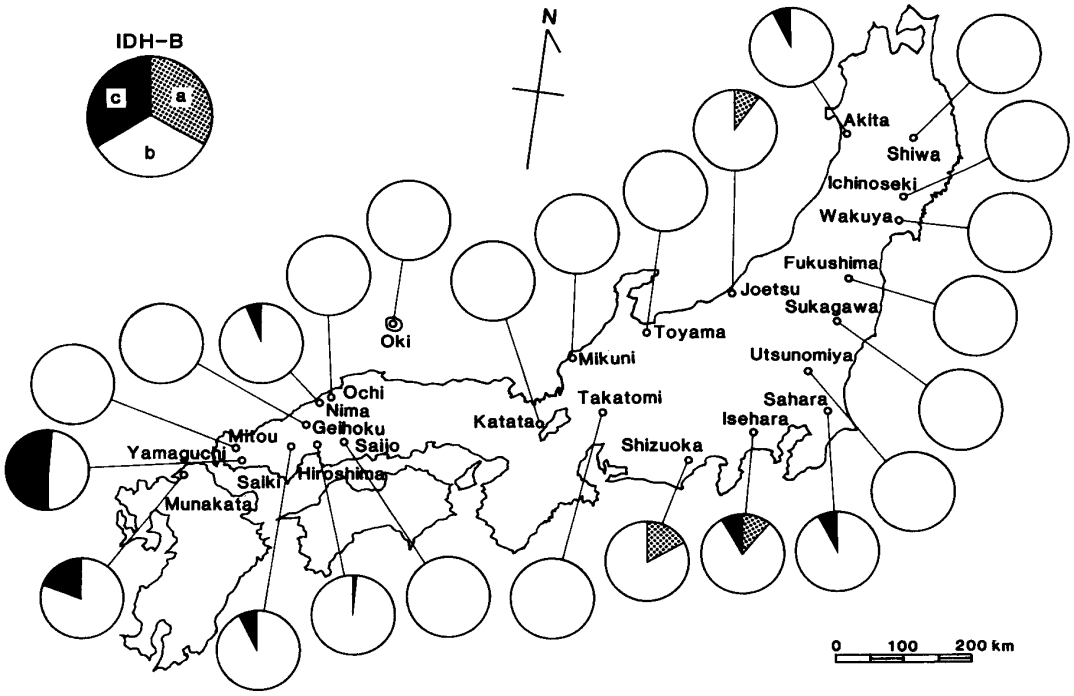

Fig. 8. Geographic distribution of IDH-B alleles among 25 populations of *Rana japonica*.

10. LDH-A locus

The electrophoretic patterns at the LDH-A locus obtained from 505 frogs of the 25 populations showed three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*. In the Akita population, alleles *a* and *b* were 0.895 and 0.105, respectively, in frequency. All the other 24 populations had only allele *a* (Table 4).

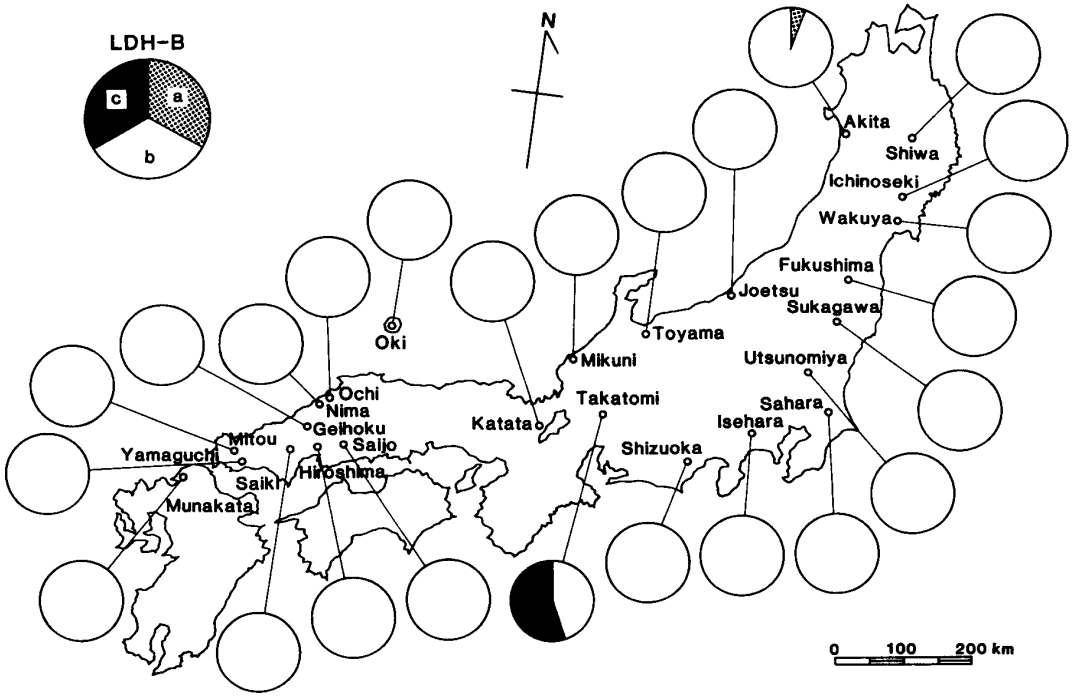

Fig. 9. Geographic distribution of LDH-B alleles among 25 populations of *Rana japonica*.

11. LDH-B locus

The electrophoretic patterns at the LDH-B locus obtained from 505 frogs of the 25 populations showed four phenotypes, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*. Every population other than the Akita and Takatomi populations had only allele *b*. In the Akita population, allele *a* was 0.058 and allele *b* was 0.942 in frequency. In the Takatomi population, alleles *b* and *c* were 0.452 and 0.548, respectively, in frequency (Table 4; Fig. 9).

12. MDH-B locus

The electrophoretic patterns of MDH-B obtained from 505 frogs of the 25 populations showed five phenotypes, BB, CC, AC, BC and CD, produced by four alleles, *a*, *b*, *c* and *d*. Every population other than the Oki, Ochi and Nima populations had allele *c* in very high frequencies of 0.667~1.000. The 12 populations, the Shiwa, Ichinoseki, Sukagawa, Utsunomiya, Sahara, Isehara, Joetsu,

TABLE 5

Gene frequencies at nine loci, MDH-A, MDH-B, ME-A, ME-B, MPI, Pep-A,

Population	Sample size	MDH-A	MDH-B				ME-A				ME-B		
		a	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*
Shiwa	3	1.000			1.000			1.000				1.000	
Ichinoseki	37	1.000			1.000			1.000			0.014	0.986	
Wakuya	24	1.000		0.042	0.958			1.000				1.000	
Fukushima	28	1.000		0.036	0.094			0.911		0.089	0.143	0.857	
Sukagawa	2	1.000			1.000			1.000				1.000	
Utsunomiya	29	1.000			1.000			0.983		0.017	0.069	0.931	
Sahara	19	1.000			1.000			1.000			0.105	0.895	
Isehara	18	1.000			1.000			1.000			0.056	0.944	
Akita	43	1.000			0.942	0.058		0.802	0.198		0.151	0.837	0.012
Joetsu	5	1.000			1.000			0.800	0.200			0.100	0.900
Toyama	15	1.000			1.000			1.000				1.000	
Mikuni	21	1.000			1.000		0.100	0.733		0.167	0.071	0.929	
Shizuoka	28	1.000		0.036	0.964			0.554	0.446			1.000	
Takatomi	21	1.000			1.000			1.000			0.667	0.333	
Katata	5	1.000			1.000			0.900	0.100			0.800	0.200
Saijo	11	1.000		0.136	0.864			1.000				1.000	
Hiroshima	36	1.000		0.181	0.819			1.000				0.833	0.167
Geihoku	6	1.000		0.250	0.750			1.000			0.083	0.917	
Saiki	21	1.000		0.333	0.667			0.762	0.238			0.905	0.095
Oki	5	1.000		0.700	0.300			1.000				1.000	
Ochi	46	1.000		0.446	0.554			1.000				1.000	
Nima	24	1.000		0.688	0.313			1.000			0.042	0.958	
Mitou	1	1.000			1.000			1.000				1.000	
Yamaguchi	31	1.000	0.065	0.113	0.823			0.887	0.113			0.871	0.129
Munakata	26	1.000		0.192	0.808			0.500	0.500			0.750	0.250

Toyama, Mikuni, Takatomi, Katata and Mitou populations, had only allele *c*. In the Wakuya, Fukushima, Shizuoka, Saijo, Hiroshima, Geihoku, Saiki and Munakata populations, allele *b* was 0.036~0.333 in frequency, in addition to allele *c*. The Akita population had allele *d* in a frequency of 0.058, and the Yamaguchi population had alleles *a* and *b* in frequencies of 0.065 and 0.113, respectively, in addition to allele *c*. In the Oki and Nima populations, allele *b* was high in frequency, being 0.700 and 0.688, respectively, and allele *c* was low in frequency, being 0.300 and 0.313, respectively. The Ochi population had alleles *b* and *c* in frequencies of 0.446 and 0.554, respectively (Table 5; Fig. 10).

13. ME-A locus

The electrophoretic patterns at the ME-A locus obtained from 505 frogs of the 25 populations showed five phenotypes, BB, CC, AB, BC and BD, produced by four alleles, *a*, *b*, *c* and *d*. In the Shizuoka and Munakata populations, allele *a* was 0.554 and 0.500, respectively, and allele *b* was 0.446 and 0.500, respectively, in

PGM, SOD-A and SOD-B, in 25 populations of *Rana japonica*

MPI						Pep-A			PGM			SOD-A			SOD-B	
a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*
		1.000				1.000				1.000			1.000			1.000
		0.905	0.054	0.041		0.946	0.054		0.027	0.973			1.000			1.000
		0.833	0.146	0.021		1.000			0.083	0.917		0.021	0.979			1.000
		0.232	0.625	0.143		0.946	0.054		0.143	0.857			1.000			1.000
			1.000			1.000				1.000			1.000			1.000
	0.052	0.379	0.431	0.138		0.966	0.034		0.086	0.914		0.017	0.983		0.138	0.862
	0.026	0.421	0.447	0.105		1.000			0.079	0.868	0.053		1.000		0.026	0.974
		0.472	0.444	0.083		1.000			0.111	0.889			1.000			1.000
		0.058	0.663	0.279		0.965	0.035		0.105	0.895			1.000		0.047	0.953
		1.000				0.900	0.100		0.100	0.900			1.000			1.000
	0.033	0.800	0.167			0.200	0.800		0.167	0.833			1.000		0.433	0.567
0.119	0.429	0.429	0.024				0.905	0.095	0.405	0.595			1.000		0.286	0.714
		0.982	0.018			0.071	0.893	0.036		1.000			0.929	0.071		1.000
		1.000					1.000			1.000			1.000			1.000
	0.100	0.900					0.400	0.600		1.000			1.000			1.000
	0.182	0.636	0.138		0.045	0.136	0.864			1.000			1.000			1.000
	0.028	0.958	0.014			0.153	0.847		0.014	0.986		0.153	0.847			1.000
	0.083	0.917					0.917	0.083		1.000		0.417	0.583			1.000
	0.167	0.714	0.119			0.071	0.881	0.048		1.000			1.000			1.000
0.100	0.700	0.100			0.100		0.800	0.200		1.000		0.100	0.900			1.000
	0.022	0.902	0.076			0.033	0.902	0.065	0.087	0.913		0.152	0.848			1.000
		0.958	0.042				0.854	0.146	0.188	0.813			1.000			1.000
		1.000					0.500	0.500		1.000			1.000			1.000
	0.371	0.613	0.016			0.016	0.952	0.032		1.000			1.000			1.000
		0.885	0.115				1.000		0.038	0.962		0.058	0.942			1.000

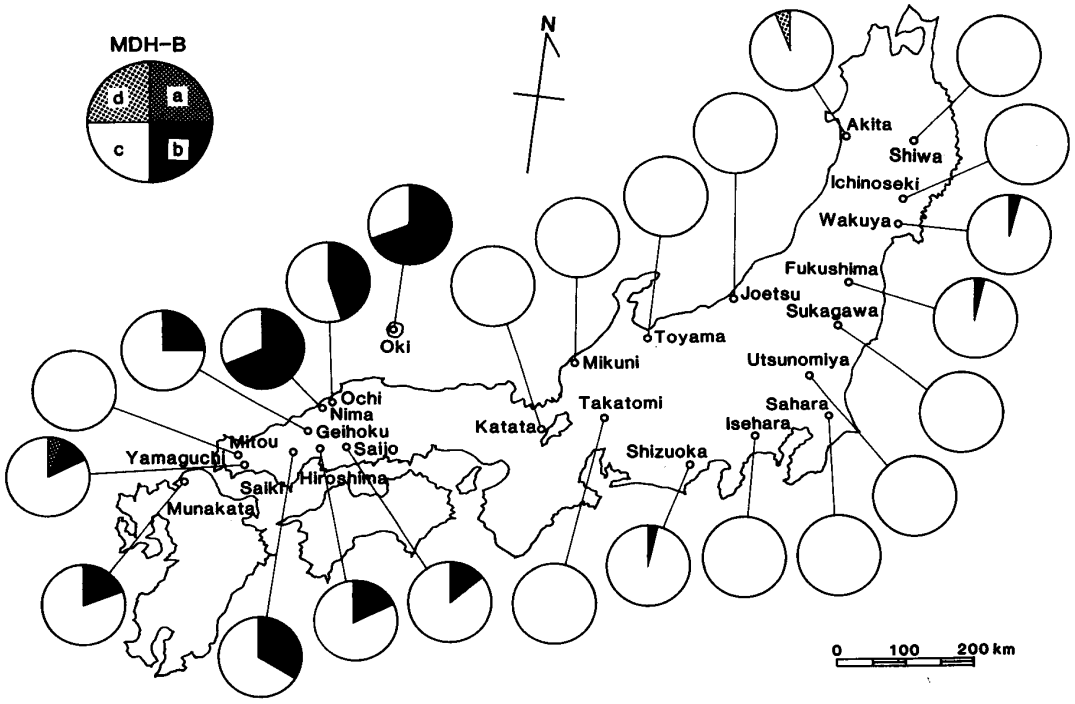

Fig. 10. Geographic distribution of MDH-B alleles among 25 populations of *Rana japonica*.

frequency. In 23 populations other than the Shizuoka and Munakata populations, allele *b* was very high in frequency, being 0.733~1.000. In the Akita, Joetsu, Katata, Saiki and Yamaguchi populations, allele *c* was 0.100~0.238 in frequency, in addition to allele *b*. In the Fukushima and Utsunomiya populations, allele *d* was 0.089 and 0.017, respectively, in frequency, in addition to allele *b*. In the Mikuni population, alleles *a* and *d* were 0.100 and 0.167, respectively, in

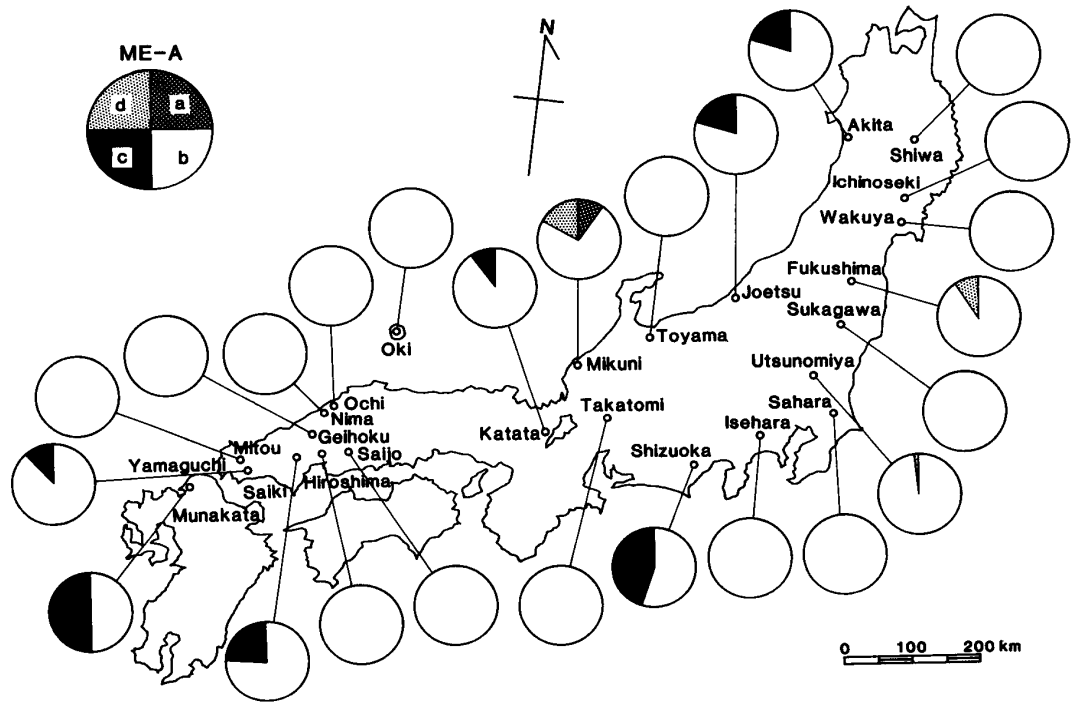

Fig. 11. Geographic distribution of ME-A alleles among 25 populations of *Rana japonica*.

frequency, in addition to allele *b*. The other 15 populations had only allele *b* (Table 5; Fig. 11).

14. ME-B

The electrophoretic patterns at the ME-B locus obtained from 505 frogs of the 25 populations showed five phenotypes, AA, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*. In the Joetsu population, allele *c* was very high in frequency, being 0.900, and allele *b* was low in frequency, being 0.100. The Takatomi population had alleles *a* and *b* in frequencies of 0.667 and 0.333, respectively. In 23 populations other than the Joetsu and Takatomi populations, allele *b* was very high in frequency, being 0.750~1.000. In the Ichinoseki, Fukushima, Utsunomiya, Sahara, Isehara, Mikuni, Geihoku and Nima populations, allele *a* was 0.014~0.143 in frequency, in addition to allele *b*. In the Katata, Hiroshima, Saiki, Yamaguchi and Munakata populations, allele *c* was 0.095~0.250 in frequency, in addition to allele *b*. In the Akita population, alleles *a* and *c* were 0.151 and 0.012, respectively, in frequency, in addition to allele *b*. The other nine populations had only allele *b* (Table 5; Fig. 12).

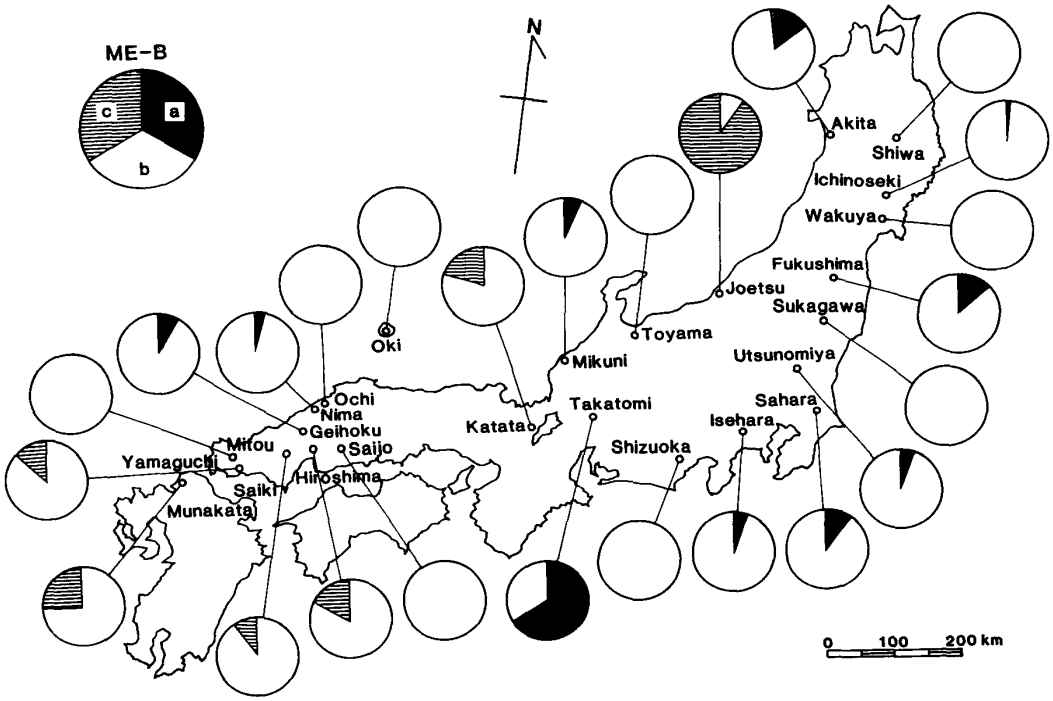

Fig. 12. Geographic distribution of ME-B alleles among 25 populations of *Rana japonica*.

15. MPI locus

The electrophoretic patterns at the MPI locus obtained from 505 frogs of the 25 populations showed 13 phenotypes, BB, CC, DD, EE, AB, AC, BC, BD, BE, CD, CE, CF and DE, produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*.

In 17 populations other than the Fukushima, Sukagawa, Utsunomiya, Sahara,

Isehara, Akita, Mikuni and Oki populations, allele *c* was very high in frequency, being 0.613~1.000. The Shiwa, Joetsu, Takatomi and Mitou populations had only allele *c*. In addition to allele *c*, allele *b* was 0.100 and 0.083 in frequency in the Katata and Geihoku populations, respectively, and allele *d* was 0.018~0.115 in frequency in the Shizuoka, Nima and Munakata populations. In the Toyama, Hiroshima, Saiki, Ochi and Yamaguchi populations, allele *b* was 0.022~0.371 and allele *d* was 0.014~0.167 in frequency, in addition to allele *c*. In the Ichinoseki and Wakuya populations, allele *d* was 0.054 and 0.146, respectively, and allele *e* was 0.041 and 0.021, respectively, in frequency, in addition to allele *c*. In the Saijo population, alleles *b*, *d* and *f* were 0.182, 0.136 and 0.045, respectively, in frequency, in addition to allele *c*. The Sukagawa population had only allele *d*. In the Fukushima and Akita populations, allele *d* was high in frequency, being 0.625 and 0.663, respectively, allele *c* was 0.232 and 0.058, respectively, and allele *e* was 0.143 and 0.279, respectively, in frequency. In the Oki population, allele *b* was high in frequency, being 0.700, and alleles *a*, *c* and *f* were low in frequency, being each 0.100. In the Utsunomiya, Sahara and Isehara populations, allele *c* was 0.379~0.472 in frequency, allele *d* was 0.431~0.447 in frequency and allele *e* was 0.083~0.138 in frequency. In the Utsunomiya and Sahara populations, allele *b* was 0.052 and 0.026, respectively, in frequency, in addition to alleles *c*, *d* and *e*. In the Mikuni population, alleles *b* and *c* were each 0.429, allele *a* was 0.119, and allele *d* was 0.024 in frequency (Table 5; Fig. 13).

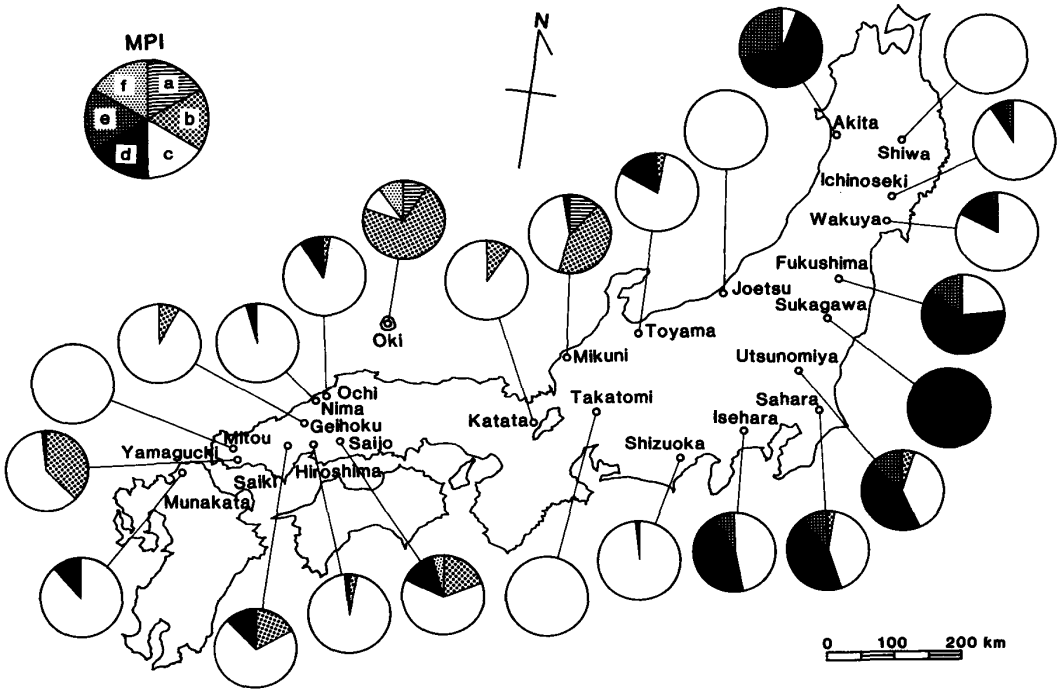

Fig. 13. Geographic distribution of MPI alleles among 25 populations of *Rana japonica*.

16. Pep–A locus

The electrophoretic patterns at the Pep–A locus obtained from 505 frogs of the

25 populations showed five phenotypes, AA, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*.

In 10 populations, the Shiwa, Ichinoseki, Wakuya, Fukushima, Sukagawa, Utsunomiya, Sahara, Isehara, Akita and Joetsu populations situated in the eastern region of Honshu, allele *a* was very high in frequency, being 0.900~1.000. In five populations, the Ichinoseki, Fukushima, Utsunomiya, Akita and Joetsu populations, allele *b* was 0.034~0.100 in frequency, in addition to allele *a*. The other five populations had only allele *a*. In 13 populations situated in the western Japan other than the Katata and Mitou populations, allele *b* was very high in frequency, being 0.800~1.000. Of these 13 populations, the Takatomi and Munakata populations had only allele *b*. In addition to allele *b*, allele *a* was 0.136~0.200 in frequency in the Toyama, Saijo and Hiroshima populations, allele *c* was 0.083 ~0.200 in frequency in the Mikuni, Geihoku, Oki and Nima populations, and alleles *a* and *c* were 0.033~0.071 and 0.032~0.065, respectively, in frequency, in the Shizuoka, Saiki, Ochi and Yamaguchi populations. In the Katata population, allele *c* was 0.600 and allele *b* was 0.400 in frequency. In the Mitou population, alleles *b* and *c* were each 0.500 in frequency (Table 5; Fig. 14).

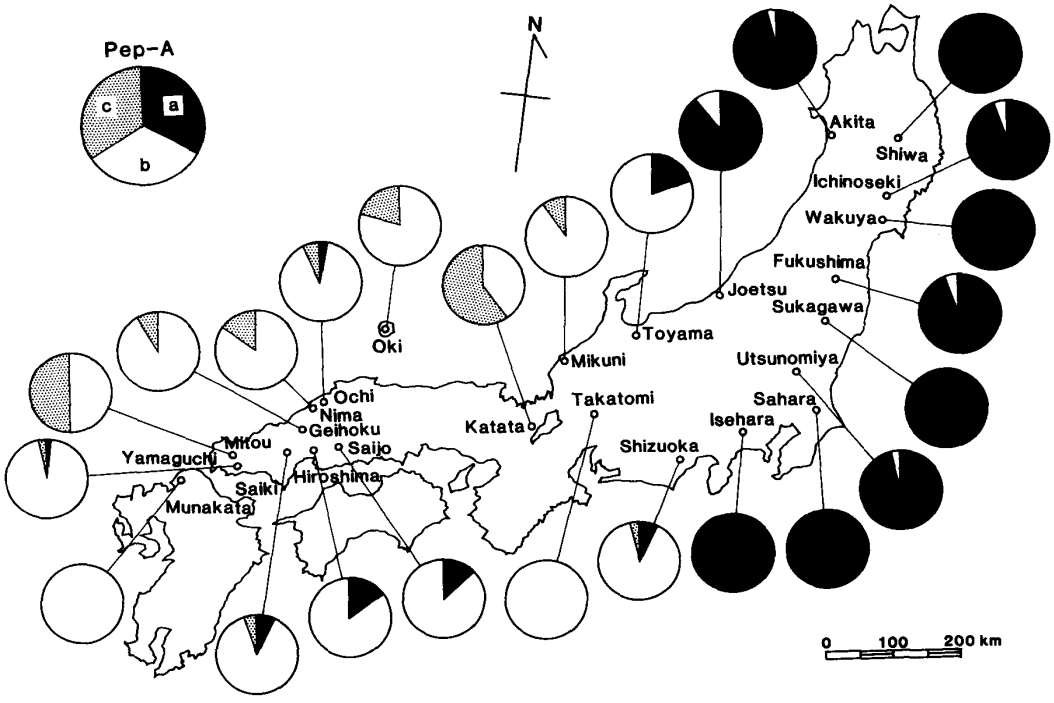

Fig. 14. Geographic distribution of Pep-A alleles among 25 populations of *Rana japonica*.

17. PGM locus

The electrophoretic patterns at the PGM locus obtained from 505 frogs of the 25 populations showed four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*, *b* and *c*. Allele *b* was high in frequency in all the 25 populations, being 0.595~1.000. In the Ichinoseki, Wakuya, Fukushima, Utsunomiya, Isehara, Akita, Joetsu, Toyama, Mikuni, Hiroshima, Ochi, Nima and Munakata popula-

tions, allele *a* was 0.014~0.405 in frequency, in addition to allele *b*. In the Sahara population, alleles *a* and *c* were 0.079 and 0.053, respectively, in frequency, in addition to allele *b*. The other 11 populations had only allele *b* (Table 5; Fig. 15).

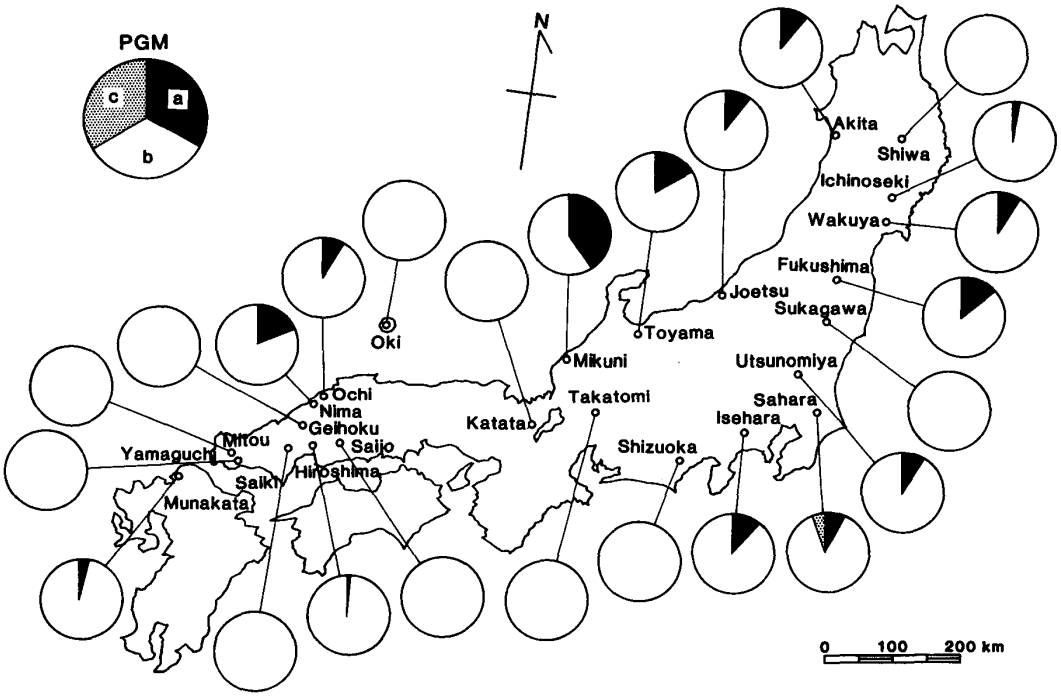

Fig. 15. Geographic distribution of PGM alleles among 25 populations of *Rana japonica*.

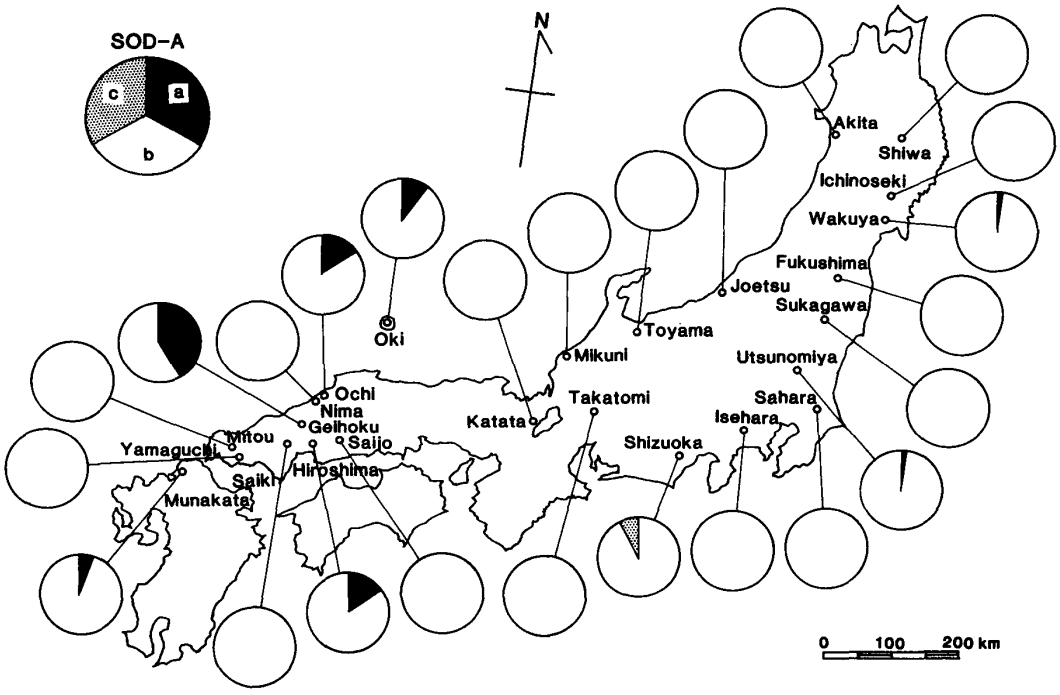

Fig. 16. Geographic distribution of SOD-A alleles among 25 populations of *Rana japonica*.

18. SOD-A locus

The electrophoretic patterns at the SOD-A locus obtained from 505 frogs of the 25 populations showed four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*, *b* and *c*. In all the 25 populations, allele *b* was high in frequency, being 0.583~1.000. In the Wakuya, Utsunomiya, Hiroshima, Geihoku, Oki, Ochi and Munakata populations, allele *a* was 0.017~0.417 in frequency, in addition to allele *b*. In the Shizuoka population, allele *c* was 0.071 in frequency, in addition to allele *b*. The other 17 populations had only allele *b* (Table 5; Fig. 16).

19. SOD-B locus

The electrophoretic patterns at the SOD-B locus obtained from 505 frogs of the 25 populations showed three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*. Allele *b* was high in frequency in all the 25 populations, being 0.567~1.000. In the Utsunomiya, Sahara, Akita, Toyama and Mikuni populations, allele *a* was 0.026~0.433 in frequency, in addition to allele *b*. The other 20 populations had only allele *b* (Table 5; Fig. 17).

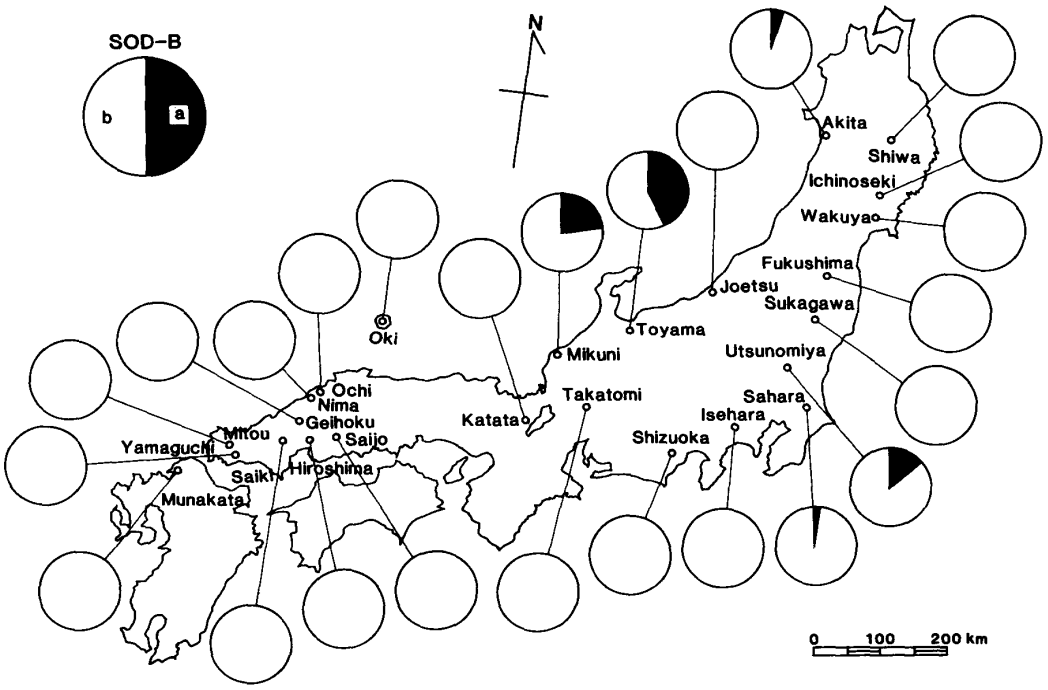

Fig. 17. Geographic distribution of SOD-B alleles among 25 populations of *Rana japonica*.

20. Ab locus

The electrophoretic patterns at the Ab locus obtained from 423 frogs of the 25 populations showed six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*, *b* and *c*.

Of the nine populations situated in the eastern region of Honshu, the seven populations other than the Ichinoseki and Sukagawa populations had allele *c* in

high frequencies, being 0.600~1.000. The Isehara and Akita populations had only allele *c*. The Shiwa, Wakuya, Fukushima, Utsunomiya and Sahara populations had allele *b* in frequencies of 0.119~0.400, in addition to allele *c*. The other two populations, the Ichinoseki and Sukagawa populations, had allele *c* in frequencies of 0.467 and 0.500, respectively, and allele *b* in frequencies of 0.533 and 0.500, respectively. Of the 15 populations including 13 populations situated in the western region of Honshu, the Munakata population in the Kyushu region and the Oki population of the Oki Island, the 12 populations other than the Takatomi, Saijo and Saiki populations had allele *b* in high frequencies, being 0.574~1.000. The Katata, Oki and Mitou populations had only allele *b*. In addition to allele *b*, the Shizuoka, Hiroshima, Geihoku, Ochi, Nima, Yamaguchi and Munakata populations had allele *a* in frequencies of 0.076~0.426, the Toyama population had allele *c* in a frequency of 0.067 and the Mikuni population had alleles *a* and *c* in frequencies of 0.154 and 0.231, respectively. The other three populations, the Takatomi, Saijo and Saiki populations, had alleles *a* and *b* in

TABLE 6

Gene frequencies at four loci, Ab, Prot-C, Hb-I and Hb-II, in 25 populations of *Rana japonica*

Population	Sample size	Ab			Prot-C				Hb-I		Hb-II		
		a	*b*	*c*	*a*	*b*	*c*	*d*	*a*	*b*	*a*	*b*	*c*
Shiwa	2		0.250	0.750		0.500	0.500		1.000				1.000
Ichinoseki	30		0.533	0.467	0.317	0.517	0.167		1.000				1.000
Wakuya	5		0.400	0.600	0.200	0.300	0.500		1.000				1.000
Fukushima	24		0.375	0.625	0.042	0.958			1.000				1.000
Sukagawa	1		0.500	0.500		1.000			1.000				1.000
Utsunomiya	21		0.119	0.881	0.048	0.929	0.024		1.000				1.000
Sahara	19		0.132	0.868		0.684	0.316		1.000				1.000
Isehara	14			1.000		0.964	0.036		1.000				1.000
Akita	42			1.000		1.000			1.000		1.000		
Joetsu	5	0.500	0.400	0.100		1.000			0.900	0.100	1.000		
Toyama	15		0.933	0.067		1.000			1.000		0.967	0.033	
Mikuni	13	0.154	0.615	0.231	0.154	0.808	0.038		1.000		0.769	0.231	
Shizuoka	17	0.235	0.765			0.971	0.029		1.000		0.118	0.882	
Takatomi	11	0.550	0.450			0.350	0.450	0.200	1.000		0.591	0.409	
Katata	5		1.000			1.000			1.000		0.200	0.800	
Saijo	11	0.636	0.364			0.955	0.045		1.000			1.000	
Hiroshima	34	0.426	0.574			0.632	0.368		1.000			1.000	
Geihoku	6	0.083	0.917			1.000			1.000			1.000	
Saiki	19	0.605	0.395			0.737	0.263		1.000		0.184	0.816	
Oki	1		1.000			0.500	0.500		1.000			1.000	
Ochi	46	0.076	0.924			0.978	0.022		1.000		0.304	0.696	
Nima	24	0.146	0.854			1.000			1.000		0.500	0.500	
Mitou	1		1.000			1.000			1.000		0.500	0.500	
Yamaguchi	31	0.371	0.629			0.984	0.016		1.000		0.129	0.871	
Munakata	26	0.160	0.840			0.900	0.100		1.000		0.865	0.135	

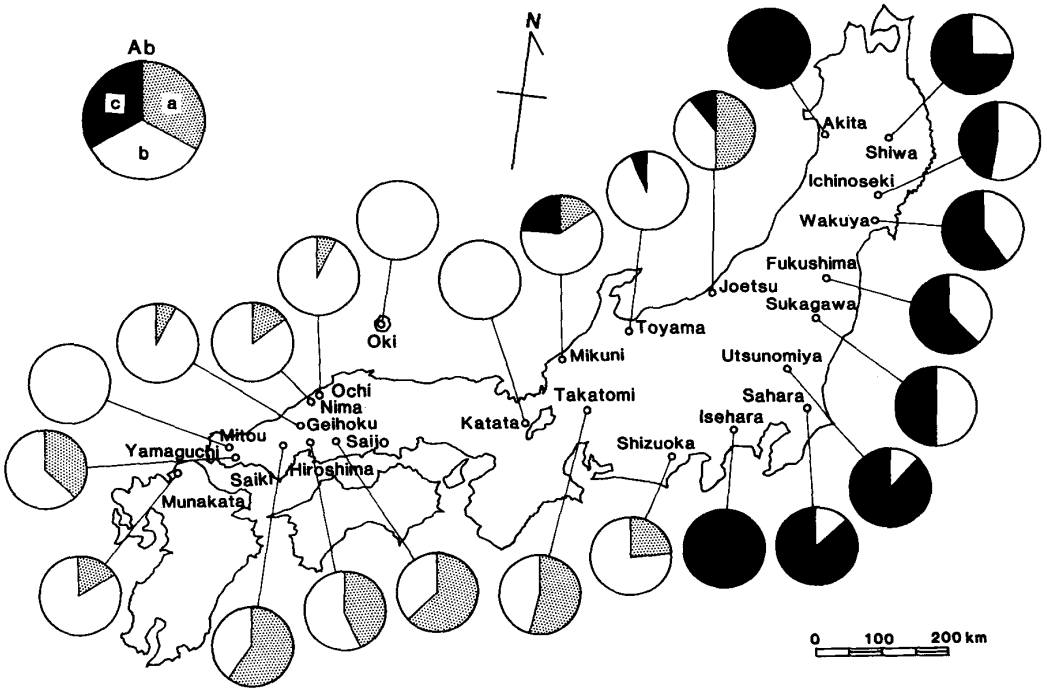

Fig. 18. Geographic distribution of Ab alleles among 25 populations of *Rana japonica*.

frequencies of 0.550~0.636 and 0.364~0.450, respectively. The Joetsu population had alleles *a*, *b* and *c* in frequencies of 0.500, 0.400 and 0.100, respectively (Table 6; Fig. 18).

21. Prot-C locus

The electrophoretic patterns at the Prot-C locus obtained from 423 frogs of the 25 populations showed eight phenotypes, AA, BB, CC, AB, AC, BC, BD and CD, produced by four alleles, *a*, *b*, *c* and *d*. In 20 populations other than the Shiwa, Ichinoseki, Wakuya, Takatomi and Oki populations, allele *b* was high in frequency, being 0.632~1.000. In addition to allele *b*, allele *c* was 0.016~0.368 in frequency in nine populations of the Sahara, Isehara, Shizuoka, Saijo, Hiroshima, Saiki, Ochi, Yamaguchi and Munakata populations, and allele *a* was 0.042 in frequency in the Fukushima population. In the Utsunomiya and Mikuni populations, allele *a* was 0.048 and 0.154, respectively, and allele *c* was 0.024 and 0.038, respectively, in frequency, in addition to allele *b*. The other eight populations had only allele *b*. In the Shiwa and Oki populations, alleles *b* and *c* were each 0.500 in frequency. In the Ichinoseki and Wakuya populations, allele *a* was 0.317 and 0.200, respectively, allele *b* was 0.517 and 0.300, respectively, and allele *c* was 0.167 and 0.500, respectively, in frequency. In the Takatomi population, alleles *c*, *b* and *d* were 0.450, 0.350 and 0.200, respectively, in frequency (Table 6; Fig. 19).

22. Hb-I locus

The electrophoretic patterns at the Hb-I locus obtained from 423 frogs of the 25 populations showed two phenotypes, AA and AB, produced by two alleles, *a* and

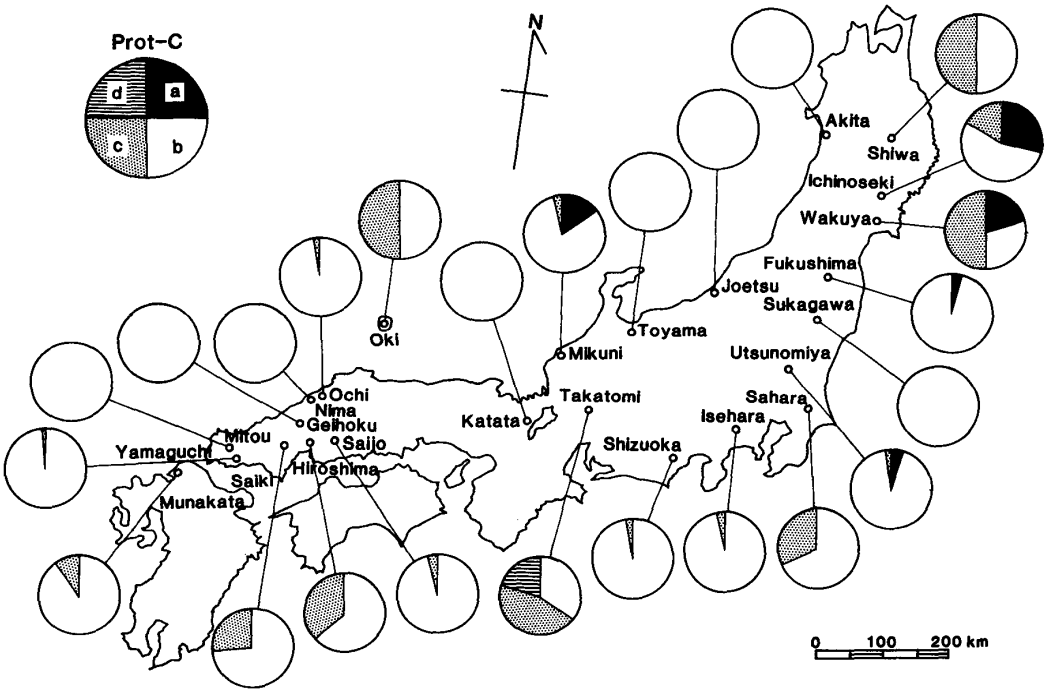

Fig. 19. Geographic distribution of Prot-C alleles among 25 populations of *Rana japonica*.

b. In the Joetsu population, allele *a* was 0.900 and allele *b* was 0.100 in frequency. All the other 24 populations had only allele *a* (Table 6).

23. Hb-II locus

The electrophoretic patterns at the Hb-II locus obtained from 423 frogs of the 25 populations showed four phenotypes, AA, BB, CC and AB, produced by three alleles, *a*, *b* and *c*. The eight populations situated in the eastern region of Honshu, the Shiwa, Ichinoseki, Wakuya, Fukushima, Sukagawa, Utsunomiya, Sahara and Isehara populations, had only allele *c*. In six populations including five populations situated in Honshu, the Akita, Joetsu, Toyama, Mikuni and Takatomi populations and the Munakata population in the Kyushu region, allele *a* was high in frequency, being 0.591~1.000. Of the six populations, the Akita and Joetsu populations had only allele *a*, and the other four populations had allele *b* in frequencies of 0.033~0.409 in addition to allele *a*. In nine populations including the eight populations situated in the western region of Honshu, the Shizuoka, Katata, Saijo, Hiroshima, Geihoku, Saiki, Ochi and Yamaguchi populations and the Oki population of the Oki Island, allele *b* was high in frequency, being 0.696~1.000. Of these nine populations, the Saijo, Hiroshima, Geihoku and Oki populations had only allele *b*, and the Shizuoka, Katata, Saiki, Ochi and Yamaguchi populations had allele *a* in frequencies of 0.118~0.304 in addition to allele *b*. In the Nima and Mitou populations, alleles *a* and *b* were each 0.500 in frequency (Table 6; Fig. 20).

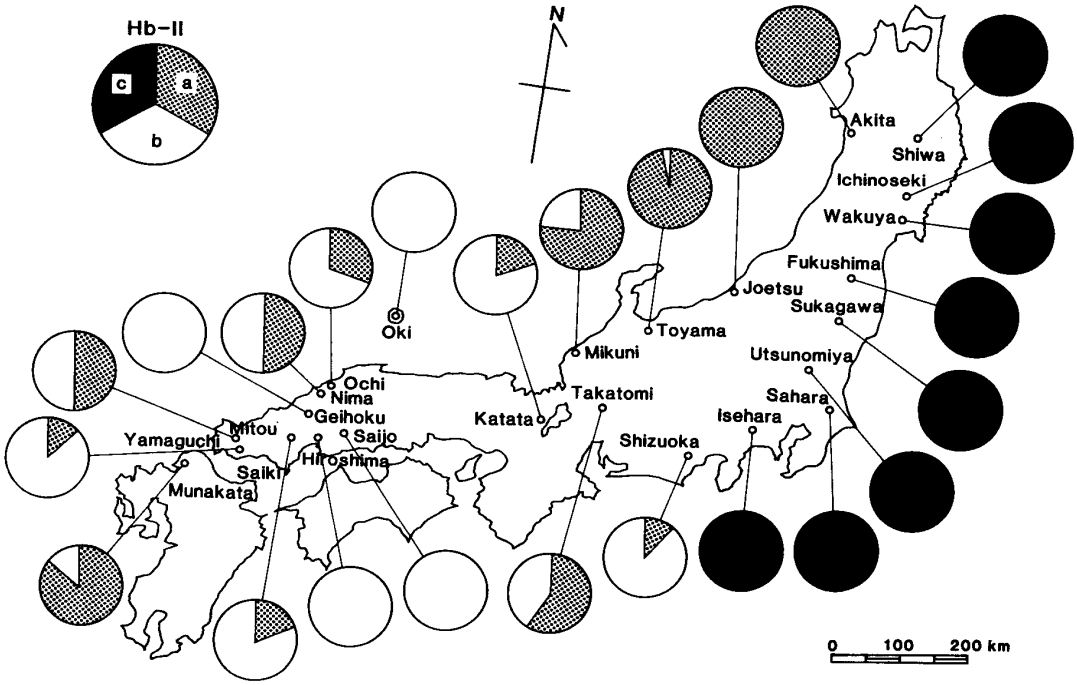

Fig. 20. Geographic distribution of Hb-II alleles among 25 populations of *Rana japonica*.

III. Genetic variation

1. Fixation index

The fixation index (Fst) was calculated according to WRIGHT (1978) at 25 loci in 505 frogs of the 25 populations (Table 7). When the gene frequencies at a definite locus are the same in all the 25 populations, the fixation index is zero. The fixation index is 1.000 when there is a characteristic allele at a definite locus in one

TABLE 7
Fixation index at 25 loci in 25 populations of *Rana japonica*

Locus	Fst	Locus	Fst
AAT-A	0.041	MDH-A	0
AAT-B	0.268	MDH-B	0.359
ADA	0.369	ME-A	0.254
AK	0	ME-B	0.417
CK	0.020	MPI	0.419
Fum	0.191	Pep-A	0.722
α-GDH	0.195	PGM	0.132
GPI	0.134	SOD-A	0.213
IDH-A	0.167	SOD-B	0.287
IDH-B	0.225	Ab	0.480
LDH-A	0.101	Prot-C	0.308
LDH-B	0.490	Hb-I	0.096
		Hb-II	0.781

or more populations. The higher the fixation index becomes, the more advanced is the genetic differentiation in the locus. The most advanced loci in differentiation were the Hb–II and Pep–A loci, being 0.781 and 0.722, respectively, in Fst. At these two loci, the genetic differentiation was distinct between the eastern and western populations. The LDH–B, Ab, MPI and ME–B loci were from 0.490 to 0.417 in Fst, the ADA, MDH–B and Prot–C loci were from 0.369 to 0.308 in Fst, the SOD–B, AAT–B, ME–A, IDH–B and SOD–A loci were from 0.287 to 0.213 in Fst, the α–GDH, Fum and IDH–A were from 0.195 to 0.167 in Fst, the GPI, PGM, LDH–A and Hb–I loci were from 0.134 to 0.096 in Fst, and the AAT–A and CK loci were 0.041 and 0.020, respectively, in Fst. These values at the 21 loci showed various degrees of genetic differentiation. The remaining two loci, the AK and MDH–A loci, were zero in Fst and were not differentiated (Table 7).

TABLE 8

Genetic variabilities at 25 loci in 25 populations of *Rana japonica*

Population	Sample size	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
Shiwa	3	7.1 (7.0)	16.0	1.16
Ichinoseki	37	9.6 (7.6)	36.0	1.52
Wakuya	24	11.3 (9.2)	40.0	1.56
Fukushima	28	12.2 (11.5)	48.0	1.56
Sukagawa	2	6.5 (8.7)	16.0	1.16
Utsunomiya	29	12.6 (10.1)	60.0	1.76
Sahara	19	10.8 (10.5)	48.0	1.64
Isehara	18	8.2 (8.5)	28.0	1.36
Akita	43	10.0 (9.8)	52.0	1.60
Joetsu	5	11.1 (9.6)	40.0	1.44
Toyama	15	8.4 (8.3)	36.0	1.36
Mikuni	21	15.9 (13.8)	52.0	1.72
Shizuoka	28	12.2 (9.8)	56.0	1.72
Takatomi	21	13.0 (12.2)	28.0	1.32
Katata	5	11.6 (10.4)	32.0	1.36
Saijo	11	9.3 (10.9)	32.0	1.44
Hiroshima	36	12.8 (12.2)	56.0	1.68
Geihoku	6	10.6 (14.0)	36.0	1.40
Saiki	21	18.1 (19.1)	48.0	1.60
Oki	5	12.6 (12.8)	32.0	1.44
Ochi	46	13.3 (13.7)	52.0	1.64
Nima	24	12.9 (13.2)	48.0	1.52
Mitou	1	4.0 (8.0)	48.0	1.08
Yamaguchi	31	13.6 (12.9)	48.0	1.32
Munakata	26	15.5 (15.4)	56.0	1.60
Average	20.2	11.3 (11.2)	41.8	1.48

Parentheses show an expected value.

2. Proportion of heterozygous loci

The mean proportion of heterozygous loci per individual was calculated on the 25 loci in 505 frogs of the 25 populations. The highest rate was 18.1% in the Saiki population. This was followed by 15.9% in the Mikuni population, 15.5% in the Munakata population, 13.6% in the Yamaguchi population, 13.3% in the Ochi population, 13.0% in the Takatomi population, 12.9% in the Nima population, 12.8% in the Hiroshima population, 12.6% in the Utsunomiya and Oki populations, and 12.2% in the Fukushima and Shizuoka populations. In the Katata, Wakuya and Joetsu populations, the rates progressively decreased from 11.6% to 11.1%. In the Sahara, Geihoku and Akita populations, the rates ranged from 10.8% to 10.0%. In the Ichinoseki, Saijo, Toyama, Isehara, Shiwa and Sukagawa populations, the rates became progressively lower ranging from 9.6% to 6.5%. The lowest rate was 4.0% in the Mitou population. The mean proportion of heterozygous loci per individual specimen was 11.3% on the average. There were no remarkable differences between the foregoing actual rates and the expected values, except in the Mitou population which had only one specimen (Table 8).

3. Proportion of polymorphic loci

The proportion of polymorphic loci which contain plural alleles at the rate of more than 1% was estimated in each of the 25 populations. The highest proportion of polymorphic loci was 60.0% in the Utsunomiya population. This was followed by 56.0% in the Shizuoka, Hiroshima and Munakata populations, 52.0% in the Akita, Mikuni and Ochi populations, 48.0% in the Fukushima, Sahara, Saiki, Nima, Mitou and Yamaguchi populations, 40.0% in the Wakuya and Joetsu populations, 36.0% in the Ichinoseki, Toyama and Geihoku populations, 32.0% in the Katata, Saijo and Oki populations, and 28.0% in the Isehara and Takatomi populations. The lowest was 16.0% in the Shiwa and Sukagawa populations. The proportions of polymorphic loci in the 25 populations were 41.8% on the average (Table 8).

4. Mean number of alleles per locus

The mean number of alleles at each of the 25 loci which control 15 enymes and three blood proteins in 505 frogs of the 25 populations was examined. The highest mean number of alleles per locus was 1.76 in the Utsunomiya population, followed by 1.72 in the Mikuni and Shizuoka populations. The mean numbers of alleles per locus in 10 populations, the Hiroshima, Sahara, Ochi, Akita, Saiki, Munakata, Wakuya, Fukushima, Ichinoseki and Nima populations, progressively decreased from 1.68 to 1.52. They ranged from 1.44 to 1.16 in 11 populations, the Joetsu, Saijo, Oki, Geihoku, Isehara, Toyama, Katata, Takatomi, Yamaguchi, Shiwa and Sukagawa populations. The smallest was 1.08 in the Mitou population. The mean numbers of alleles per locus in the 25 populations were 1.48 on the average (Table 8).

IV. Genetic distance

The genetic relationships among the 25 populations of *Rana japonica* distributed in Japan were assumed by estimating NEI's genetic distances on the basis of gene frequencies at 25 loci controlling 15 enzymes and three blood proteins obtained from 505 frogs (Table 9).

The genetic distances among the eight populations situated in eastern Japan, the Shiwa, Ichinoseki, Wakuya, Fukushima, Sukagawa, Utsunomiya, Sahara and Isehara populations, ranged from 0.005 between the Utsunomiya and Sahara populations to 0.100 between the Shiwa and Sukagawa populations, with a mean of 0.036 (Table 9).

The genetic distances among the 15 populations situated in western Japan, the Toyama, Mikuni, Shizuoka, Takatomi, Katata, Saijo, Hiroshima, Geihoku, Saiki, Oki, Ochi, Nima, Mitou, Yamaguchi and Munakata populations, ranged from

TABLE 9

Genetic identity (I) and genetic distance (D)

Population	No.	1	2	3	4	5	6	7	8	9	10	11
Shiwa	1	—	.986	.986	.935	.905	.959	.965	.951	.898	.873	.877
Ichinoseki	2	.014	—	.990	.946	.920	.959	.961	.949	.888	.870	.886
Wakuya	3	.014	.010	—	.956	.931	.969	.979	.964	.890	.859	.871
Fukushima	4	.067	.055	.045	—	.988	.987	.983	.980	.906	.838	.856
Sukagawa	5	.100	.083	.072	.012	—	.970	.965	.962	.887	.813	.832
Utsunomiya	6	.042	.042	.032	.013	.031	—	.995	.994	.930	.859	.873
Sahara	7	.035	.039	.022	.017	.035	.005	—	.992	.923	.853	.862
Isehara	8	.050	.052	.037	.020	.039	.006	.008	—	.922	.846	.851
Akita	9	.107	.119	.117	.098	.120	.073	.080	.081	—	.901	.906
Joetsu	10	.136	.139	.152	.176	.207	.153	.159	.167	.104	—	.910
Toyama	11	.131	.121	.138	.155	.184	.136	.149	.161	.099	.094	—
Mikuni	12	.141	.133	.145	.158	.191	.135	.148	.160	.102	.110	.024
Shizuoka	13	.161	.137	.146	.148	.174	.151	.154	.160	.185	.160	.087
Takatomi	14	.168	.157	.151	.180	.218	.177	.165	.189	.194	.146	.105
Katata	15	.163	.143	.159	.156	.182	.168	.171	.184	.191	.152	.092
Saijo	16	.129	.122	.132	.140	.166	.126	.133	.142	.141	.134	.074
Hiroshima	17	.117	.115	.118	.160	.194	.144	.142	.161	.166	.124	.073
Geihoku	18	.155	.134	.149	.164	.189	.158	.165	.175	.193	.165	.077
Saiki	19	.152	.137	.136	.142	.172	.149	.146	.161	.174	.154	.099
Oki	20	.189	.179	.180	.206	.234	.210	.208	.239	.219	.236	.127
Ochi	21	.153	.132	.143	.149	.176	.151	.156	.167	.166	.146	.055
Nima	22	.165	.140	.145	.144	.168	.150	.155	.162	.177	.148	.069
Mitou	23	.113	.104	.123	.152	.182	.135	.144	.155	.128	.102	.031
Yamaguchi	24	.150	.135	.147	.147	.172	.142	.146	.152	.163	.143	.077
Munakata	25	.149	.134	.148	.159	.185	.158	.161	.173	.133	.091	.039

Genetic identity (I) is given above the diagonal and genetic

0.014 between the Saijo and Hiroshima populations to 0.159 between the Takatomi and Oki populations, with a mean of 0.062. The genetic distances between a group of the eight eastern populations and a group of the 15 western populations ranged from 0.104 between the Ichinoseki and Mitou populations to 0.239 between the Isehara and Oki populations, with a mean of 0.156 (Table 9).

The genetic distances between the Akita population and the group of the eight eastern populations ranged from 0.073 between the Utsunomiya and Akita populations to 0.120 between the Sukagawa and Akita populations, with a mean of 0.099. Those between the Akita population and the group of the 15 western populations ranged from 0.099 between the Akita and Toyama populations to 0.219 between the Akita and Oki populations, with a mean of 0.162. The genetic distances between the Joetsu population and the group of the eight eastern populations ranged from 0.136 between the Shiwa and Joetsu populations to 0.207 between the Sukagawa and Joetsu populations, with a mean of 0.161. Those between the Joetsu population and the group of the 15 western populations ranged from 0.091

among 25 populations of *Rana japonica*

12	13	14	15	16	17	18	19	20	21	22	23	24	25
.869	.851	.864	.849	.879	.890	.856	.859	.828	.858	.848	.893	.861	.861
.875	.872	.855	.867	.885	.892	.875	.872	.836	.876	.870	.901	.874	.874
.865	.864	.860	.853	.877	.888	.861	.872	.835	.867	.865	.884	.864	.862
.854	.863	.835	.855	.869	.852	.849	.867	.814	.861	.866	.859	.864	.853
.826	.841	.804	.834	.847	.824	.828	.842	.791	.838	.845	.834	.842	.831
.874	.859	.838	.846	.882	.866	.854	.862	.811	.860	.860	.873	.868	.854
.862	.857	.848	.843	.875	.868	.848	.864	.812	.856	.856	.866	.864	.851
.852	.852	.828	.832	.868	.852	.839	.851	.788	.846	.850	.857	.859	.841
.903	.831	.823	.826	.868	.847	.825	.841	.803	.847	.838	.880	.850	.875
.896	.852	.864	.859	.875	.884	.848	.858	.790	.864	.863	.903	.867	.913
.976	.917	.900	.912	.929	.929	.925	.906	.880	.946	.933	.970	.926	.962
—	.910	.902	.892	.942	.932	.923	.911	.900	.931	.925	.958	.939	.952
.095	—	.920	.964	.957	.962	.967	.974	.899	.973	.956	.938	.962	.954
.103	.084	—	.890	.921	.934	.911	.929	.853	.916	.919	.907	.918	.926
.114	.036	.117	—	.926	.940	.946	.947	.906	.964	.937	.942	.930	.925
.059	.044	.083	.077	—	.986	.969	.968	.930	.962	.943	.956	.978	.933
.070	.039	.069	.062	.014	—	.977	.970	.940	.972	.948	.959	.969	.939
.081	.034	.093	.056	.032	.023	—	.958	.931	.981	.961	.957	.964	.940
.093	.027	.074	.054	.032	.030	.043	—	.935	.968	.959	.921	.964	.945
.106	.107	.159	.098	.072	.062	.071	.068	—	.940	.920	.909	.930	.886
.071	.027	.087	.037	.039	.029	.019	.032	.061	—	.985	.958	.959	.956
.078	.045	.085	.065	.058	.054	.040	.042	.084	.015	—	.945	.952	.957
.043	.064	.097	.060	.045	.042	.044	.082	.096	.043	.057	—	.952	.955
.063	.039	.086	.072	.022	.032	.036	.036	.073	.042	.050	.050	—	.952
.049	.047	.077	.078	.070	.063	.062	.056	.121	.045	.044	.046	.049	—

distance (D) is given below.

between the Joetsu and Munakata populations to 0.236 between the Joetsu and Oki populations, with a mean of 0.140. The genetic distance between the Akita and Joetsu populations was 0.104 (Table 9).

V. Dendrogram

A dendrogram was drawn for the 25 populations on the basis of the genetic distances among them by the UPGMA method (SNEATH and SOKAL, 1973; NEI, 1975, 1987). The dendrogram showed that *Rana japonica* distributed in Japan was first divided into the eastern group containing nine populations and the western group containing 16 populations. The eastern group was then differentiated into the Akita population and a subgroup containing eight populations, while the western group first differentiated the Joetsu population, which was followed by the Takatomi and Oki populations, and finally the group was divided into several subgroups containing 13 populations (Fig. 21).

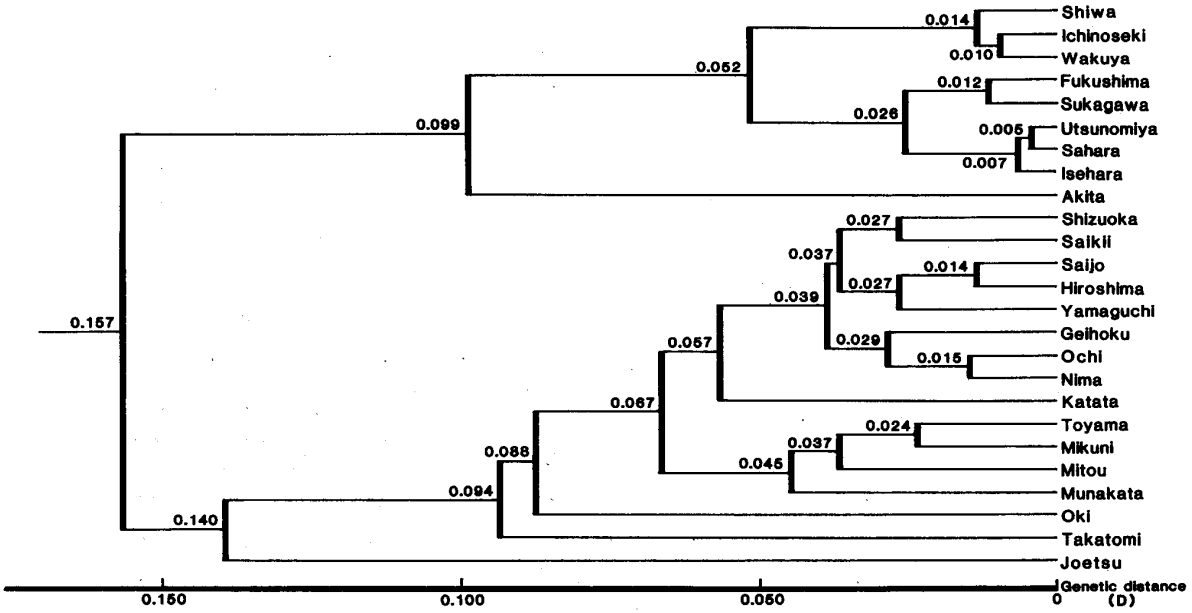

Fig. 21. Dendrogram for 25 populations of *Rana japonica* based on genetic distances.

DISCUSSION

The genetic distances have been studied in various amphibians at the level of populations, local races and subspecies by numerous authors. In *Aneides flavipunctatus*, the mean genetic distance among 13 populations was 0.093, while the mean genetic distance among three groups distributed in three disjunct regions was 0.164 (LARSON, 1980). In *Cynops pyrrhogaster*, the mean genetic distance among 23 populations was 0.060, while it was 0.156 between 20 northern and three southern

populations (HAYASHI and MATSUI, 1988). In *Cynops ensicauda*, the mean genetic distance among 15 populations was 0.035, while it was 0.078 between four populations in Amami and 11 populations in Okinawa (HAYASHI and MATSUI, 1988). In *Desmognathus fuscus*, the mean genetic distance among 13 populations was 0.205, while it was 0.322 between 10 northern and three southern populations (TILLEY and SCHWERDTFEGER, 1981). In *Taricha t. torosa*, the mean genetic distance among three populations was 0.093, while it was 0.131 between two northern populations and one southern population (HEDGECOCK, 1976).

In *Buergeria japonica*, the mean genetic distance among four populations was 0.116, while it was 0.208 between the Okinawa and three other populations (NISHIOKA, SUMIDA, OHTA and SUZUKI, 1987). In *Hyla regilla*, the mean genetic distance among 17 populations was 0.14, while the mean genetic distance among three groups consisting of 10 southern, three central and four Oregon populations was 0.20 (CASE, HANELINE and SMITH, 1975). In *Hyla japonica*, the mean genetic distance among 11 populations was 0.075, while it was 0.156 between the Korea population and 10 populations in Japan (NISHIOKA, SUMIDA and BORKIN, 1990). In *Bufo japonicus*, the mean genetic distance among 39 populations was 0.074, while it was 0.130 between 12 western and 27 eastern populations (KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI, 1990). In *Rana tagoi*, the mean genetic distance among seven populations was 0.192, while it was 0.232 between four land populations and three island populations (NISHIOKA, OHTA and SUMIDA, 1987). In *Rana limnocharis*, the mean genetic distance among six populations was 0.077, while it was 0.305 between the Iriomote population and five other populations (NISHIOKA and SUMIDA, 1990). In *Rana nigromaculata*, the mean genetic distance among 45 populations was 0.078, while it was 0.086 between 21 western and 24 eastern populations (NISHIOKA, SUMIDA and OHTANI, 1992). In *Rana b. brevipoda*, the mean genetic distance among 12 populations was 0.066, while it was 0.092 between four western populations (Typical race) and eight eastern populations (Nagoya race)(NISHIOKA, SUMIDA and OHTANI, 1992).

NISHIOKA, KODAMA, SUMIDA and RYUZAKI (1993) reported that the genetic distances among 40 populations of *R. rugosa* were 0.003~0.492, 0.181 on the average. The genetic differentiation between the eastern and western groups was distinctly recognizable at the Pep-A, LDH-B and MDH-B loci, being 0.979, 0.901 and 0.795, respectively, in Fst. The Hb-II locus was also large in Fst, being 0.863. A dendrogram drawn on the basis of genetic distances among the 40 populations by the UPGMA method (NEI, 1975) showed that *R. rugosa* has differentiated into the western group containing 14 populations and the eastern group which divided again into three subgroups; the northern subgroup of seven populations, the intermediate subgroup of 10 populations and the southern subgroup of nine populations. These four main branches of the dendrogram of *R. rugosa* almost corresponded to the distribution of the four kinds of sex-determining mechanisms found in this species, which has been reported by NISHIOKA, HANADA, MIURA and RYUZAKI (1994).

The present study revealed that the genetic distances among the 25 populations

of *Rana japonica* were 0.005~0.239, 0.110 on the average, while those between nine eastern populations including the Ichinoseki population and 16 western populations including the Hiroshima population were 0.099~0.239, 0.157 on the average. The genetic differentiation between the eastern and western groups was distinctly recognizable at the Hb–II and Pep–A loci, being 0.781 and 0.722, respectively, in Fst. At the Hb–II locus, eight populations of the eastern group excluding the Akita population had only allele *c*, while the 16 populations of the western group and the Akita population had alleles *a* and *b*. At the Pep–A locus, allele *a* was predominant in the nine populations of the eastern group and the Joetsu population, while allele *b* was predominant in 15 populations of the western group excluding the Joetsu population. At the Ab locus, the eastern group had a high frequency of allele *c*, while 13 populations of the western group excluding the Joetsu, Toyama and Mikuni populations had no allele *c*. This allele was also found in three populations, the Joetsu, Toyama and Mikuni, however, at a very low frequency. A dendrogram drawn from the genetic distances among these populations showed that the ancestor of *Rana japonica* was roughly divided into the eastern and western groups. It may be probable that after this species was divided into these two groups, they came into contact with each other in the norhwestern region of Honshu and introgressions occurred among the populations of this region.

SUMIDA (1981) and SUMIDA and NISHIOKA (1991) who analyzed the karyotypes in 16 of the 25 populations of *R. japonica* found that there were slight but distinct differences in the centromere position of chromosomes No. 6 and No. 9 between the eastern and western groups. When intraspecific hybrids were produced between these two groups of populations, overwhelmingly numerous males were obtained. In reciprocal hybrids, the ratios of males were 92.4% and 90.3% on the average. The testes of these males were more or less abnormal in inner structure. In contrast, when the matings were made between the males and females of the populations belonging to the same group, nearly the same number of males and females were produced, and the testes of the males obtained from these matings were normal in inner structure. SUMIDA (1994) who observed the chromosome behavior at spermatogenesis in males of the northern and southern populations of *R. japonica* and their reciprocal hybrids noticed that most of the meiotic chromosomes in the parental populations were ring-shaped bivalents, whereas those of reciprocal hybrids were characterized by a remarkable increase in univalents and rod-shaped bivalents. SUMIDA and NISHIOKA (1993) reported that the eastern population of *R. japonica* slightly differed from the western population in the reproductive isolating mechanism with *R. tsushimensis*. SUMIDA and NISHIOKA (1994) who also examined the linkage relationships between the sex-determining genes and 11 loci controlling eight enzymes and one blood protein in the crosses involving 30 males heterozygous at these loci from 10 local populations found that the locus linked with the sex-determining genes differs with the local populations. Thus, it is noteworthy to mention that the genetic differentiation of *Rana japonica* into the eastern and western groups was accompanied with incom-

plete hybrid male sterility and differences of the sex-linked genes.

The genetic variabilities in allopatric populations were reviewed in various amphibian species by NEVO, BEILES and BEN-SHLOMO (1984) and by NEVO and BEILES (1991). Acccording to them, the mean proportion of heterozygous loci per individual specimen and the mean proportion of polymorphic loci per population were 7.5±1.3% and 23.3±2.9%, respectively, in 22 species of genus *Rana*. In 47 populations of *Rana nigromaculata*, the two parameters showing genetic variabilities, the mean proportion of heterozygous loci per individual and the mean proportion of polymorphic loci per population were 6.1% and 27.8%, respectively. In 23 populations of *Rana brevipoda*, these two parameters were 7.5% and 24.1%, respectively (NISHIOKA, SUMIDA and OHTANI, 1992). In six populations of *Rana limnocharis*, they were 8.5% and 23.1%, respectively (NISHIOKA and SUMIDA, 1990), and in 40 populations of *Rana rugosa*, they were 9.9% and 31.1%, respectively (NISHIOKA, KODAMA, SUMIDA and RYUZAKI, 1993), while in seven populations of *Rana tagoi*, they were very high, being 16.1% and 55.2%, respectively.

The present study showed that the genetic variabilities in the 25 populations of *Rana japonica* were comparatively high, being 11.3% and 41.8% in the mean proportion of heterozygous loci per individual and the mean proprotion of polymorphic loci per population, respectively. DESSAUER, NEVO and CHUANG (1975) have described that the heterozygosity is high in organisms living in ecologically variable environments and suggested that the high degree of genetic variabilities operates as an adaptive strategy in the ecologically variable environments where they live. It is evident that this interpretation is principally applicable to *Rana japonica*.

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