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Systematic Evolution of 40 Populations of *Rana rugosa* Distributed in Japan Elucidated by Electrophoresis

By

Midori NISHIOKA[1], Yumiko KODAMA[1], Masayuki SUMIDA[1] and Masashi RYUZAKI[2]

[1]*Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Higashihiroshima 724, Japan*

and

[2]*Department of Biology, Kitasato University, School of Medicine, Sagamihara, Kanagawa 228, Japan*

ABSTRACT

Fourteen enzymes extracted from skeletal muscles and livers and two kinds of blood proteins were obtained from 614 *Rana rugosa* SCHLEGEL of 40 populations and analyzed by the method of starch-gel electrophoresis in order to elucidate the genetic differentiation of these 40 populations. It was found that there were 25 loci, where 5.8 phenotypes controlled by 3.4 alleles on the average were observed. The gene frequencies were described in 24 of the 25 loci other than the Hb–I which showed a single phenotype controlled by only one gene. The fixation index (Fst), the mean proportions of heterozygous loci, the mean proportions of polymorphic loci and the mean number of alleles per locus were calculated.

Genetic distances were estimated by the method of NEI (1975) on the basis of the gene frequencies at 25 loci controlling 14 enzymes and two blood proteins. The genetic distances among the 40 populations were 0.003~0.492. The largest of them was found between the Shiojiri population of Nagano Prefecture in the Chubu region and the Nagayo population of Nagasaki Prefecture in the Kyushu region. Among the 27 populations of the eastern group consisted of the Hokkaido, Tohoku, Kanto, Chubu, Hokuriku and Kinki regions, the genetic distances were 0.003~0.292, while among the 13 populations of the western group consisted of the Chugoku, Shikoku and Kyushu regions, they were 0.010~0.128.

A dendrogram was drawn on the basis of genetic distances among the 40 populations by the UPGMA method. The ancestor of *Rana rugosa* seemed to be first divided into two groups; the eastern group containing 26 populations and the western group containing 14 populations. The eastern group was then divided into three subgroups; northern subgroup containing seven populations, intermediate subgroup containing 10 populations and southern subgroup containing nine populations. The western group is distributed in the Kyushu, Shikoku, Chugoku regions and a part of the Kinki region. Of the eastern group, the southern subgroup is distributed in a part of the Kinki region and in most parts of the Chubu region. The intermediate subgroup is distributed in the Kanto region and a part of the Chubu region. The northern subgroup is distributed in the Hokkaido and Tohoku regions and a part of the Chubu region.

It was interesting that the four main branches of the dendrogram of *Rana rugosa* drawn on the basis of the genetic distances almost corresponded to the distribution of

the four kinds of sex-determining mechanisms found in this species.

INTRODUCTION

The existence of two different types of sex-determining mechanisms has been recognized in a small frog species, *Rana rugosa* SCHLEGEL, widely distributed in Japan. At first, KAWAMURA and NISHIOKA (1977) reported that *Rana rugosa* distributed around Hiroshima City was of the male heterogametic. Subsequently, TOBISHIMA and SAITOH (1989) reported that this species collected from Aomori and Iwate Prefectures, situated in the northern part of Japan, was of the female heterogametic in contrast to that from Hiroshima. As it was very interesting that there are two different types of sex-determining mechanisms in one and the same species, NISHIOKA, MIURA and SAITOH (1993) performed an experiment to re-confirm this phenomenon by using the specimens collected from Kumano, Hiroshima Prefecture, and those collected from Hirosaki, Aomori Prefecture. It was quite clear that the specimens from Hiroshima were of the XX–XY type and those from Hirosaki were of the ZW–ZZ type in sex-determining mechanism. On the other hand, NISHIOKA, MIURA and HANADA (1990) preliminarily examined the local differences of the sex chromosomes in 17 populations of *Rana rugosa*. It was found that six populations including the Sapporo population situated in the Hokkaido region, the Akita population and the most part of the Inawashiro population situated in the Tohoku region, the Niigata and Kanazawa populations situated in the Hokuriku region and the Katata population situated in the Kinki region were of the ZW–ZZ type which was nearly the same as that of the Hirosaki population. In contrast, three populations including the Gotsu and Kumano populations situated in the Chugoku region and the Nagayo population situated in the Kyushu region were of the XX–XY type which was the same as that of the Hiroshima population.

The remaining eight of the 17 populations whose sex-determining mechanisms were analyzed, consisted of four populations, the Daigo, Maebashi, Machida and Isehara populations, of the Kanto region and four other populations, the Hamakita, Miyakoda and Yonezu populations of Shizuoka Prefecture situated in the Chubu region and the Toba population of Mie Prefecture situated in the Kinki region. The former four populations were of the XX–XY type in which the X chromosome could not be distinguished from the Y chromosome in shape and banding pattern, although both X and Y chromosomes of these populations differed from those of the Kumano population in banding pattern. The latter four populations of the Chubu and Kinki regions were of the XX–XY type in which the X chromosome differed from the Y chromosome in shape and banding pattern.

In this study, the relationship of the four types of sex-determining mechanisms and the intraspecific differentiation clarified by electrophoretic analyses of enzymes and blood proteins will be examined in order to elucidate the evolution of the sex-determining mechanisms.

TABLE 1

Specimens of *Rana rugosa* used in the present study

Region	Prefecture	Station	No. of frogs			Population (No.)	
			Total	Female	Male		
Hokkaido	Hokkaido	Sapporo-city	16	15	1	Sapporo	(1)
Tohoku	Aomori	Hirosaki-city	18	2	16	Hirosaki	(2)
	Akita	Akita-city, Toyoiwaishidazaka	17	7	10	Akita	(3)
	Fukushima	Yama-gun, Inawashiro-cho	20	4	16	Inawashiro	(4)
Kanto	Ibaraki	Kuji-gun, Daigo-cho	22	14	8	Daigo	(5)
	〃	Hitachiota-city	14	8	6	Hitachiota	(6)
	Tochigi	Hoga-gun, Mashiko-cho	13	6	7	Mashiko	(7)
	〃	Ashikaga-city	11	5	6	Ashikaga	(8)
	Gunma	Maebashi-city, Kamikoide-cho	17	10	7	Maebashi	(9)
	Tokyo	Akigawa-city	17	8	9	Akigawa	(10)
	〃	Machida-city, Koyamadai	11	9	2	Machida	(11)
	Chiba	Kamogawa-city	23	8	15	Kamogawa	(12)
	Kanagawa	Isehara-city, Isehara	20	18	2	Isehara	(13)
Chubu	Shizuoka	Takata-gun, Kannami-cho	9	3	6	Kannami	(14)
	〃	Shita-gun, Oigawa-cho	20	14	6	Oigawa	(15)
	〃	Hamakita-city, Kifune	20	10	10	Hamakita	(16)
	〃	Hamamatsu-city, Wada-cho	20	10	10	Hamamatsu	(17)
	〃	Hamamatsu-city, Miyakoda-cho	20	8	12	Miyakoda	(18)
	〃	Hamamatsu-city, Yonezu-cho	20	13	7	Yonezu	(19)
	〃	Iwata-gun, Toyoda-cho	20	14	6	Toyoda	(20)
	Nagano	Shiojiri-city	3	3	0	Shiojiri	(21)
	〃	Azuma-gun, Miasa-mura	2	1	1	Miasa	(22)
Hokuriku	Niigata	Murakami-city, Hayakawa	20	10	10	Murakami	(23)
	Ishikawa	Kanazawa-city	23	1	22	Kanazawa	(24)
Kinki	Shiga	Otsu-city, Katata-cho	23	14	9	Katata	(25)
	Mie	Toba-city	3	1	2	Toba	(26)
	Wakayama	Wakayama-city, Kamizaki	1	0	1	Wakayama	(27)
Chugoku	Tottori	Tottori-city, Fushino	20	15	5	Tottori	(28)
	Shimane	Gotsu-city, Arifuku-cho	16	5	11	Gotsu	(29)
	Okayama	Okayama-city, Mitsu-cho	20	11	9	Okayama	(30)
	Hiroshima	Hutami-gun, Kisa-cho	20	11	9	Kisa	(31)
	〃	Higashihiroshima-city, Shiwa-cho	9	7	2	Shiwa	(32)
	〃	Aki-gun, Kumano-cho	21	17	4	Kumano	(33)
	〃	Hiroshima-city, Ushitaasahi	19	5	14	Hiroshima	(34)
	〃	Otake-city, Mikuradake	1	0	1	Otake	(35)
	Yamaguchi	Yamaguchi-city, Ogori-cho	1	1	0	Yamaguchi	(36)
Shikoku	Tokushima	Katsuura-gun, Katsuura-cho	7	5	2	Katsuura	(37)
	Ehime	Matsuyama-city, Suehiro-cho	16	13	3	Matsuyama	(38)
Kyushu	Nagasaki	Nishihigaki-gun, Nagayo-cho	21	12	9	Nagayo	(39)
	Miyazaki	Hyuga-city, Hiraiwa	20	15	5	Hyuga	(40)
Total			614	333	281	40 populations	

MATERIALS AND METHODS

Rana rugosa SCHLEGEL used in the present study consisted of 333 females and 281 males, 614 in total, collected from 40 places in Hokkaido, Honshu, Shikoku and Kyushu. The collecting stations, numbers of frogs and sexual ratios are shown in Table 1. Twelve enzymes extracted from skeletal muscles, two enzymes from livers and two kinds of blood proteins were analyzed by the method of horizontal starch-gel electrophoresis. The kinds of enzymes and blood proteins analyzed, their abbreviations, E. C. Nos., samples and buffer systems are shown in Table 2. The method of electrophoresis has been reported by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was performed by the methods of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. The detection of blood proteins was made by the amino-black staining method.

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 a definite locus, fixation index (Fst) by WRIGHT (1978) was utilized to indicate the degree of genetic differentiation among different populations. The genetic variations of different populations were quantitatively shown by the mean proportions of polymorphic loci per population and mean proportions of heterozygous loci per individual (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974).

The genetic relationships among populations were evaluated by calculating the genetic distances (D) by the method of NEI (1972). A dendrogram was drawn on the basis of the genetic distances by the unweighted pair-group arithmetic average

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
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	T-C pH 7.0
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	〃	〃
Mannose phosphate isomerase	MPI	5.3.1.8	〃	T-C pH 7.0
Peptidase	Pep	3.4.3.1	〃	T-B-E pH 8.0
Phosphoglucomutase	PGM	2.7.5.1	〃	〃
Superoxide dismutase	SOD	1.15.1.1	〃	〃
Sorbitol dehydrogenase	SORDH	1.1.1.14	Liver	T-C pH 7.0
Serum albumin	Ab	—	Blood serum	T-B-E pH 8.0
Hemoglobin	Hb	—	Erythrocyte	T-B-E pH 8.6

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

(UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975).

OBSERVATION

I. Electrophoretic patterns and alleles

There were numerous phenotypes controlled by genes at 25 loci in the 14 kinds of enzymes and two kinds of blood proteins (Table 3). The electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, --- in the order of mobility from fast to slow, and the multiple alleles were shown by *a, b, c,* --- (Fig. 1).

At the Hb-I locus of the 25 loci, there was a single phenotype. At six loci, the AK, CK, α-GDH, GPI, IDH-A and SOD-B loci, two or three phenotypes produced by two alleles, *a* and *b,* were observed. At nine loci, the AAT-B, Fum, LDH-A, MDH-A, MDH-B, Pep-A, Pep-C, PGM and SOD-A loci, three to six phenotypes controlled by three alleles, *a, b* and *c,* were observed. At five loci, the IDH-B, LDH-B, Pep-D, Ab and Hb-II loci, four to eight phenotypes controlled by four alleles, *a, b, c* and *d,* were observed. At two loci, the AAT-A and Pep-B loci, 10 or 11 phenotypes controlled by five alleles, *a, b, c, d* and *e,* were observed. At the remaining two loci, the MPI and SORDH loci, 16 or 22 phenotypes controlled by seven alleles, *a, b, c, d, e, f* and *g,* were observed. At all the 25 loci, 5.8 phenotypes controlled by 3.4 alleles on the average were observed (Table 3).

TABLE 3

Number of phenotypes and alleles at 25 loci in 40 populations of *Rana rugosa*

Locus	No. of phenotypes	No. of alleles	Locus	No. of phenotypes	No. of alleles
AAT-A	10	5	MPI	22	7
AAT-B	3	3	Pep-A	4	3
AK	3	2	Pep-B	11	5
CK	3	2	Pep-C	6	3
Fum	4	3	Pep-D	6	4
α-GDH	2	2	PGM	4	3
GPI	2	2	SOD-A	4	3
IDH-A	2	2	SOD-B	3	2
IDH-B	8	4	SORDH	16	7
LDH-A	3	3	Ab	4	4
LDH-B	8	4	Hb-I	1	1
MDH-A	3	3	Hb-II	7	4
MDH-B	6	3	Average	5.8	3.4

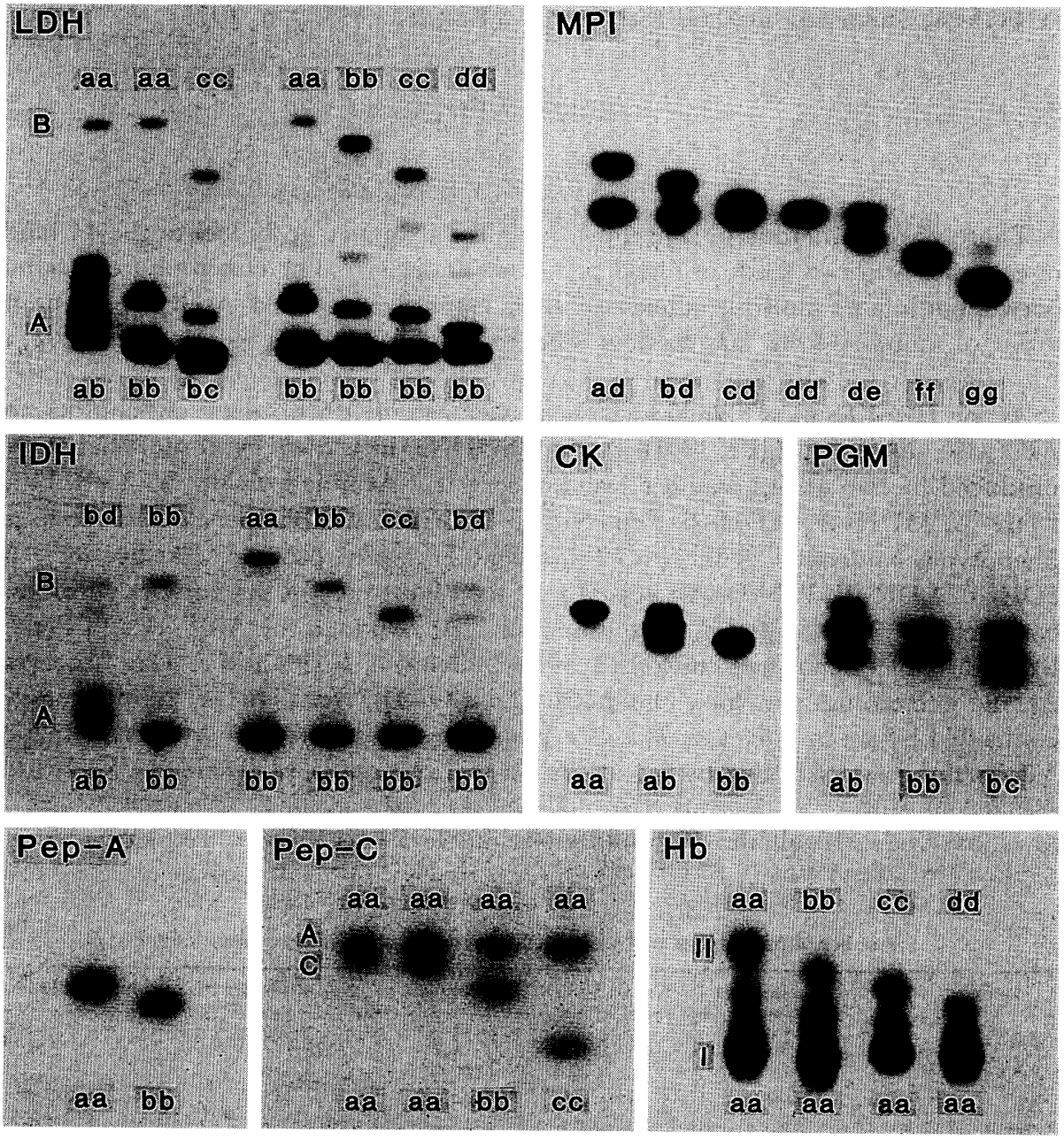

Fig. 1. Electrophoretic patterns of seven enzymes, LDH, MPI, IDH, CK, PGM, Pep-A and Pep-C, and one blood protein, Hb, in 40 populations of *Rana rugosa*.

II. Gene frequency

Of the 25 loci, the Hb-I locus showed a phenotype, AA, controlled by a gene. The gene frequencies at the other 24 loci were as follows (Table 4~6).

1. AAT-A locus

When the electrophoretic patterns at the AAT-A locus were analyzed in the 40 populations, 10 phenotypes, AA, CC, DD, EE, AB, AD, BD, BE, CD and DE, produced by five alleles, *a~e*, were observed. When the gene frequencies of the 40

populations were examined, 29 populations including the Sapporo population, three of the Tohoku region, eight of the Kanto region except the Maebashi population, nine of the Chubu region, two of the Hokuriku region, three of the Kinki region and three of the Chugoku region, the Tottori, Shiwa and Yamaguchi populations, had only allele *d*. In three populations, the Maebashi, Okayama and Matsuyama populations, allele *d* was very high in frequency, being 0.912 ~0.975, while in addition to allele *d*, the Maebashi and Okayama populations had allele *a* in frequencies of 0.088 and 0.025, respectively, and the Matsuyama population had allele *c* in a frequency of 0.033. In six populations, the Gotsu, Kisa, Kumano, Hiroshima and Otake populations of the Chugoku region and the Katsuura population of the Shikoku region, allele *d* was high in frequency, being 0.469~0.786, and allele *a* was 0.031~0.500. In addition to alleles *d* and *a*, alleles *b* and *e* were 0.375 and 0.125, respectively, in frequency, in the Gotsu population, allele *e* was 0.050 in the Kisa population, and allele *c* was 0.024 and 0.118 in the Kumano and Hiroshima populations, respectively. In the Nagayo and Hyuga populations of the Kyushu region, allele *e* was high in frequency, being 0.714 and 0.450, respectively, while in addition to allele *e*, alleles *b* and *d* were 0.238 and 0.048, respectively, in the Nagayo population and alleles *d*, *a* and *b* were 0.375, 0.125 and 0.050, respectively, in the Hyuga population (Table 4; Fig. 2).

2. AAT-B locus

When the electrophoretic patterns at the AAT-B locus were analyzed in the 40 populations, three phenotypes, BB, AB and BC, produced by three alleles, *a*~*c*, were observed. In all the 40 populations, allele *b* was high in frequency, being 0.857~1.000. In six of these populations, the Sapporo, Inawashiro, Mashiko, Ashikaga, Hamamatsu and Katsuura populations, allele *a* was found in frequencies of 0.025~0.136, and in seven populations, the Daigo, Hitachiota, Maebashi, Hamakita, Yonezu, Toyoda and Hiroshima populations, allele *c* was found in frequencies of 0.029~0.143, in addition to allele *b*. All the remaining 27 populations had only allele *b* (Table 4; Fig. 3).

3. AK locus

When the electrophoretic patterns at the AK locus were analyzed in the 40 populations, three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*, were observed. Of the 40 populations, 30 populations including the Sapporo population, the three populations of the Tohoku region, the two populations of the Hokuriku region, the three populations of the Kinki region, the nine populations of the Chugoku region, the two populations of the Shikoku region, the two populations of the Kyushu region, the seven populations of the Chubu region other than the Shiojiri and Hamamatsu populations and the Kamogawa population of the Kanto region had only allele *b*. In the Hamamatsu population of the Chubu region and five populations of the Kanto region, the Hitachiota, Ashikaga, Akigawa, Machida and Isehara populations, allele *b* was very high in frequency, being 0.714~0.975, and allele *a* was 0.025~0.286. In the remaining three popula-

TABLE 4

Gene frequencies at 10 loci, AAT-A, AAT-B, AK, CK, Fum, α-GDH,

Population	Sample size	AAT-A					AAT-B			AK		CK	
		a	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*a*	*b*	*a*	*b*
Sapporo	16				1.000		0.031	0.969			1.000	1.000	
Hirosaki	18				1.000			1.000			1.000	1.000	
Akita	17				1.000			1.000			1.000	1.000	
Inawashiro	20				1.000		0.025	0.975			1.000	1.000	
Daigo	22				1.000			0.909	0.091	0.455	0.545	1.000	
Hitachiota	14				1.000			0.857	0.143	0.286	0.714	1.000	
Mashiko	13				1.000		0.077	0.923		0.576	0.423	1.000	
Ashikaga	11				1.000		0.136	0.864		0.182	0.818	1.000	
Maebashi	17	0.088			0.912			0.882	0.118	0.324	0.676	1.000	
Akigawa	17				1.000			1.000		0.088	0.912	1.000	
Machida	11				1.000			1.000		0.273	0.727	1.000	
Kamogawa	23				1.000			1.000			1.000	1.000	
Isehara	20				1.000			1.000		0.150	0.850	1.000	
Kannami	9				1.000			1.000			1.000	1.000	
Oigawa	20				1.000			1.000			1.000	1.000	
Hamakita	20				1.000			0.950	0.050		1.000	1.000	
Hamamatsu	20				1.000		0.025	0.975		0.025	0.975	1.000	
Miyakoda	20				1.000			1.000			1.000	1.000	
Yonezu	20				1.000			0.900	0.100		1.000	1.000	
Toyoda	20				1.000			0.925	0.075		1.000	1.000	
Shiojiri	3				1.000			1.000		1.000		1.000	
Miasa	2				1.000			1.000			1.000	1.000	
Murakami	20				1.000			1.000			1.000	1.000	
Kanazawa	23				1.000			1.000			1.000	0.761	0.239
Katata	23				1.000			1.000			1.000	0.783	0.217
Toba	3				1.000			1.000			1.000	1.000	
Wakayama	1				1.000			1.000			1.000	0.500	0.500
Tottori	20				1.000			1.000			1.000	0.250	0.750
Gotsu	16	0.031	0.375		0.469	0.125		1.000			1.000	0.438	0.563
Okayama	20	0.025			0.975			1.000			1.000	0.175	0.825
Kisa	20	0.400			0.550	0.050		1.000			1.000	0.050	0.950
Shiwa	9				1.000			1.000			1.000		1.000
Kumano	21	0.190		0.024	0.786			1.000			1.000	0.024	0.976
Hiroshima	19	0.265		0.118	0.618			0.971	0.029		1.000	0.088	0.912
Otake	1	0.500			0.500			1.000			1.000	1.000	
Yamaguchi	1				1.000			1.000			1.000		1.000
Katsuura	7	0.286			0.714		0.071	0.929			1.000	0.714	0.286
Matsuyama	16			0.033	0.967			1.000			1.000	0.133	0.867
Nagayo	21		0.238		0.048	0.714		1.000			1.000		1.000
Hyuga	20	0.125	0.050		0.375	0.450		1.000			1.000	1.000	

GPI, IDH-A, IDH-B and LDH-A, in 40 populations of *Rana rugosa*

Fum			α-GDH		GPI		IDH-A		IDH-B				LDH-A		
a	*b*	*c*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*c*	*d*	*a*	*b*	*c*
	1.000			1.000	1.000			1.000	0.281	0.719				1.000	
	1.000			1.000	1.000			1.000	1.000					1.000	
	1.000			1.000	1.000			1.000	0.912	0.088				1.000	
	1.000			1.000	1.000			1.000	0.675	0.325				1.000	
0.024	0.929	0.048		1.000	1.000			1.000		0.773	0.227			1.000	
0.357	0.643			1.000	0.857	0.143	0.036	0.964		0.821	0.179			1.000	
0.192	0.808			1.000	0.923	0.077		1.000		0.654	0.346			1.000	
	1.000			1.000	0.864	0.136		1.000		0.227	0.636	0.136		1.000	
	1.000			1.000	0.971	0.029		1.000		0.118	0.882			1.000	
0.029	0.971			1.000	1.000			1.000		0.324	0.676			1.000	
0.091	0.909			1.000	1.000			1.000		0.364	0.636			1.000	
	1.000			1.000	1.000			1.000		0.217	0.761	0.022		1.000	
0.125	0.875		0.025	0.975	1.000			1.000		0.500	0.500			0.950	0.050
	1.000			1.000	1.000			1.000		0.944	0.056			1.000	
0.025	0.950	0.025		1.000	1.000			1.000	0.050	0.500	0.450			1.000	
	1.000			1.000	1.000			1.000		1.000				1.000	
0.075	0.925			1.000	1.000			1.000	0.075	0.450	0.475			1.000	
	1.000			1.000	1.000			1.000	0.050	0.375	0.575			1.000	
0.250	0.750			1.000	1.000			1.000	0.075	0.475	0.450			1.000	
	1.000			1.000	1.000			1.000		0.300	0.700			1.000	
	1.000			1.000	1.000			1.000			1.000			1.000	
	1.000			1.000	1.000			1.000	0.500	0.500				1.000	
	1.000			1.000	1.000			1.000	0.450	0.550				1.000	
0.043	0.957			1.000	1.000		0.022	0.978		0.609	0.348	0.043		1.000	
	0.913	0.087		1.000	0.957	0.043		1.000		0.630	0.370			1.000	
0.500	0.500			1.000	1.000			1.000		0.667	0.333			1.000	
	1.000			1.000	1.000			1.000		1.000				1.000	
	1.000		0.075	0.925	1.000			1.000		1.000				1.000	
0.063	0.938			1.000	1.000			1.000		1.000				1.000	
	1.000			1.000	0.975	0.025		1.000		1.000				1.000	
0.025	0.975			1.000	0.975	0.025		1.000		1.000				1.000	
0.111	0.833	0.056		1.000	1.000			1.000		1.000				0.944	0.056
0.024	0.976			1.000	0.976	0.024		1.000	0.048	0.952				1.000	
	1.000			1.000	1.000			1.000		1.000				1.000	
	1.000			1.000	1.000			1.000		1.000			0.500	0.500	
	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			1.000	0.933	0.067		1.000		1.000				1.000	
	0.857	0.143		1.000	1.000			1.000		1.000				1.000	
	1.000			1.000	1.000			1.000		1.000				1.000	

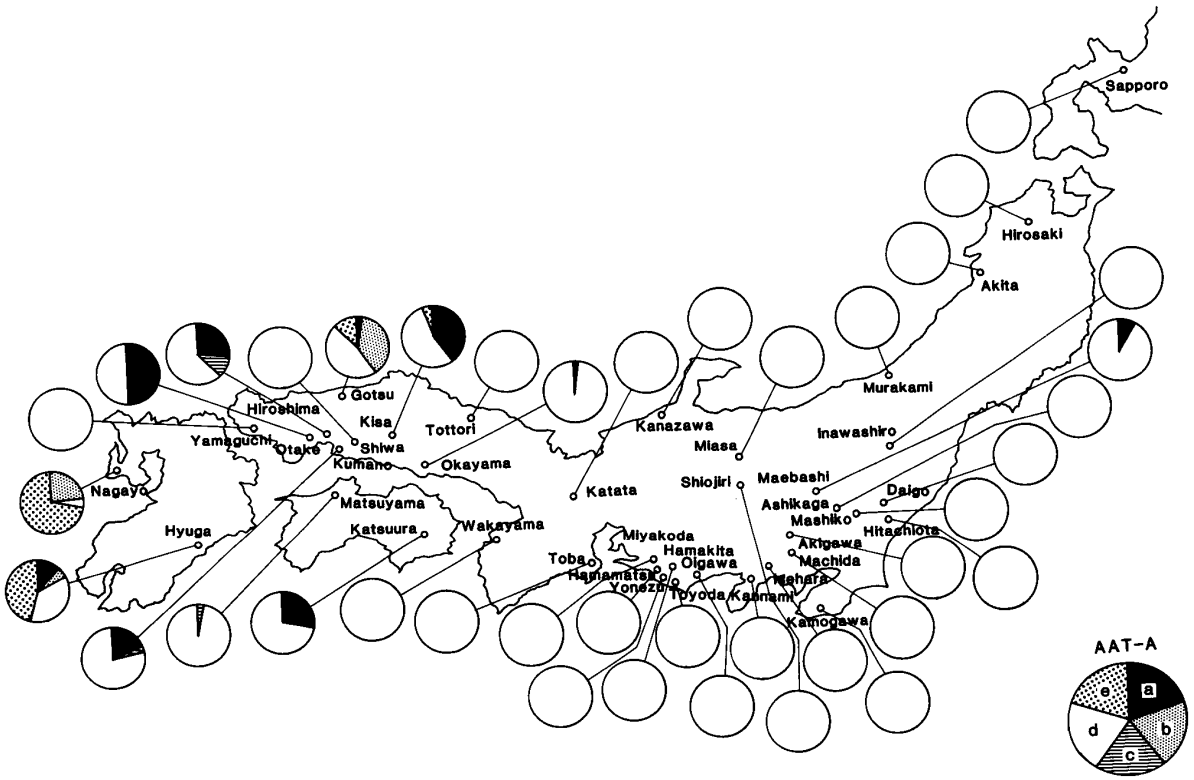

Fig. 2. Geographic distribution of AAT-A alleles among 40 populations of *Rana rugosa*.

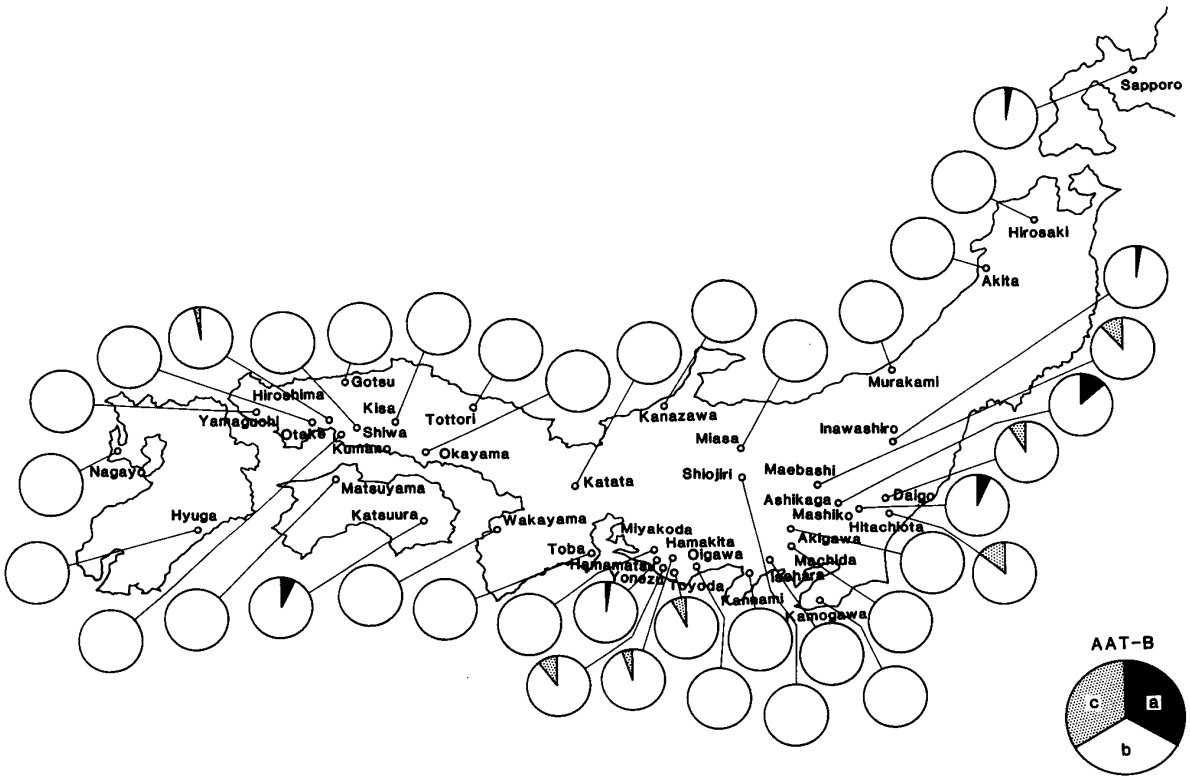

Fig. 3. Geographic distribution of AAT-B alleles among 40 populations of *Rana rugosa*.

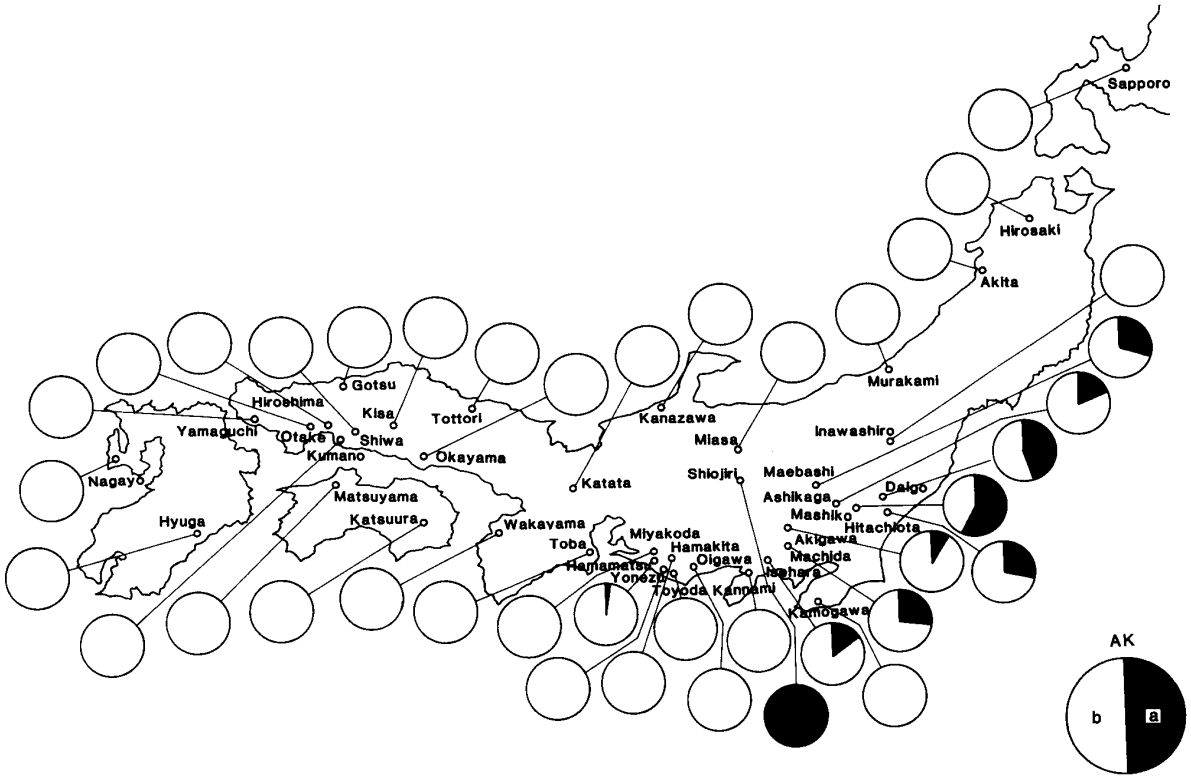

Fig. 4. Geographic distribution of AK alleles among 40 populations of *Rana rugosa*.

tions of the Kanto region, the Daigo, Mashiko and Maebashi populations, allele *b* was 0.545, 0.423 and 0.676, respectively, in frequency, and allele *a* was 0.455, 0.576 and 0.324, respectively. In the Shiojiri population of the Chubu region, there was only allele *a* (Table 4; Fig. 4).

4. CK locus

When the electrophoretic patterns at the CK locus were analyzed in the 40 populations, three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*, were observed. Of the 40 populations, 26 populations including the Sapporo population, the three populations of the Tohoku region, the nine populations of the Kanto region, the nine populations of the Chubu region, the Murakami population of the Hokuriku region, the Toba population of the Kinki region, the Otake population of the Chugoku region, and the Hyuga population of the Kyushu region, had only allele *a*. In three populations including the Kanazawa population of the Hokuriku region, the Katata population of the Kinki region and the Katsuura population of the Shikoku region, allele *a* was 0.714~0.783 and allele *b* was 0.217~0.286 in frequency. The only one frog of the Wakayama population of the Kinki region had alleles *a* and *b* both in a frequency of 0.500. In the Gotsu population of the Chugoku region, alleles *b* and *a* were 0.563 and 0.438, respectively, in frequency. The Shiwa and Yamaguchi populations of the Chugoku region and the Nagayo population of the Kyushu region had only allele *b*, while six populations including the Tottori, Okayama, Kisa, Kumano and Hiroshima

populations of the Chugoku region and the Matsuyama population of the Shikoku region had allele *b* in frequencies of 0.750~0.976 and allele *a* in frequencies of 0.024~0.250 (Table 4; Fig. 5).

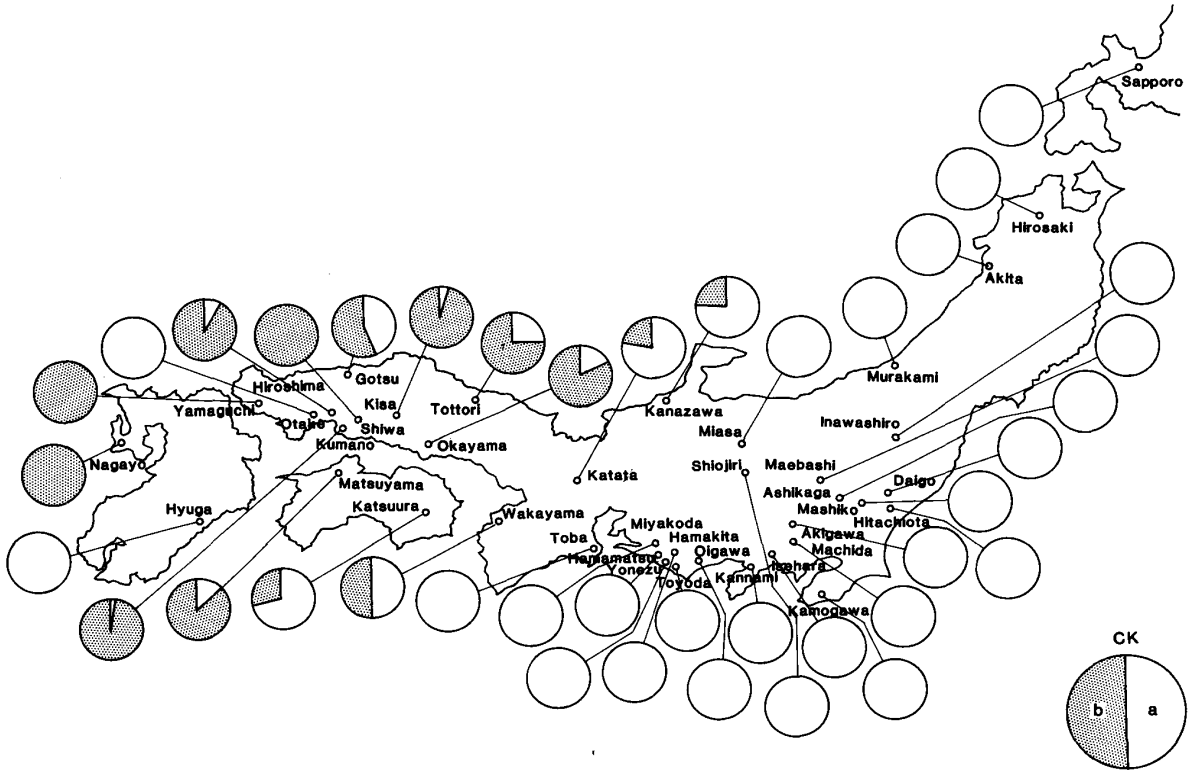

Fig. 5. Geographic distribution of CK alleles among 40 populations of *Rana rugosa*.

5. Fum locus

When the electrophoretic patterns at the Fum locus were analyzed in the 40 populations, four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*~*c*, were observed. In the Hitachiota, Yonezu and Toba populations, allele *b* was 0.500~0.750, and allele *a* was 0.250~0.500 in frequency. In the Daigo, Oigawa and Shiwa populations, allele *b* was 0.833~0.950, allele *a* was 0.024~0.111 and allele *c* was 0.025~0.056 in frequency. In the Katata and Nagayo populations, allele *b* was 0.913 and 0.857, respectively, and allele *c* was 0.087 and 0.143, respectively, in frequency. In the Mashiko, Akigawa, Machida, Isehara, Hamamatsu, Kanazawa, Gotsu, Kisa and Kumano populations, allele *b* was 0.808 ~0.976 and allele *a* was 0.024~0.192 in frequency. In the remaining 23 populations, there was only allele *b* (Table 4; Fig. 6).

6. α-GDH locus

When the electrophoretic patterns at the α-GDH locus were analyzed in the 40 populations, two phenotypes, BB and AB, produced by two alleles, *a* and *b*, were observed. Of the 40 populations, 38 except the Isehara and Tottori populations had only allele *b*. In the Isehara and Tottori populations, allele *b* was 0.975 and

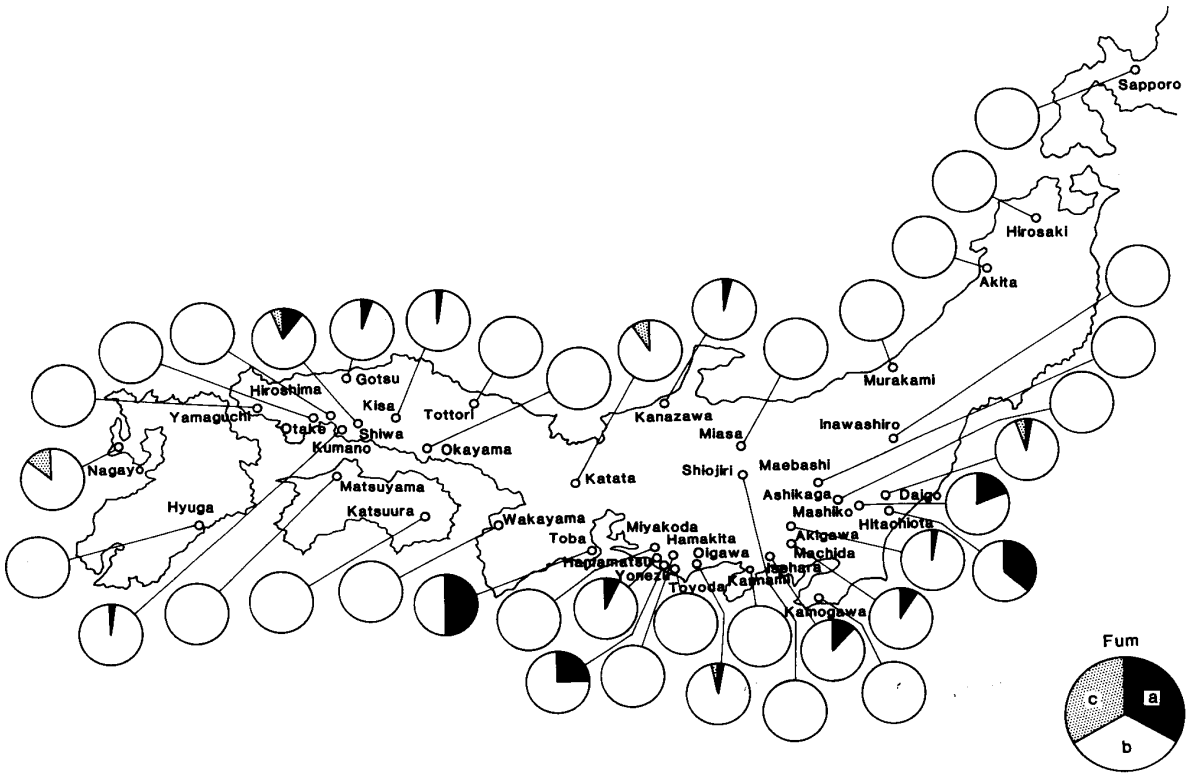

Fig. 6. Geographic distribution of Fum alleles among 40 populations of *Rana rugosa*.

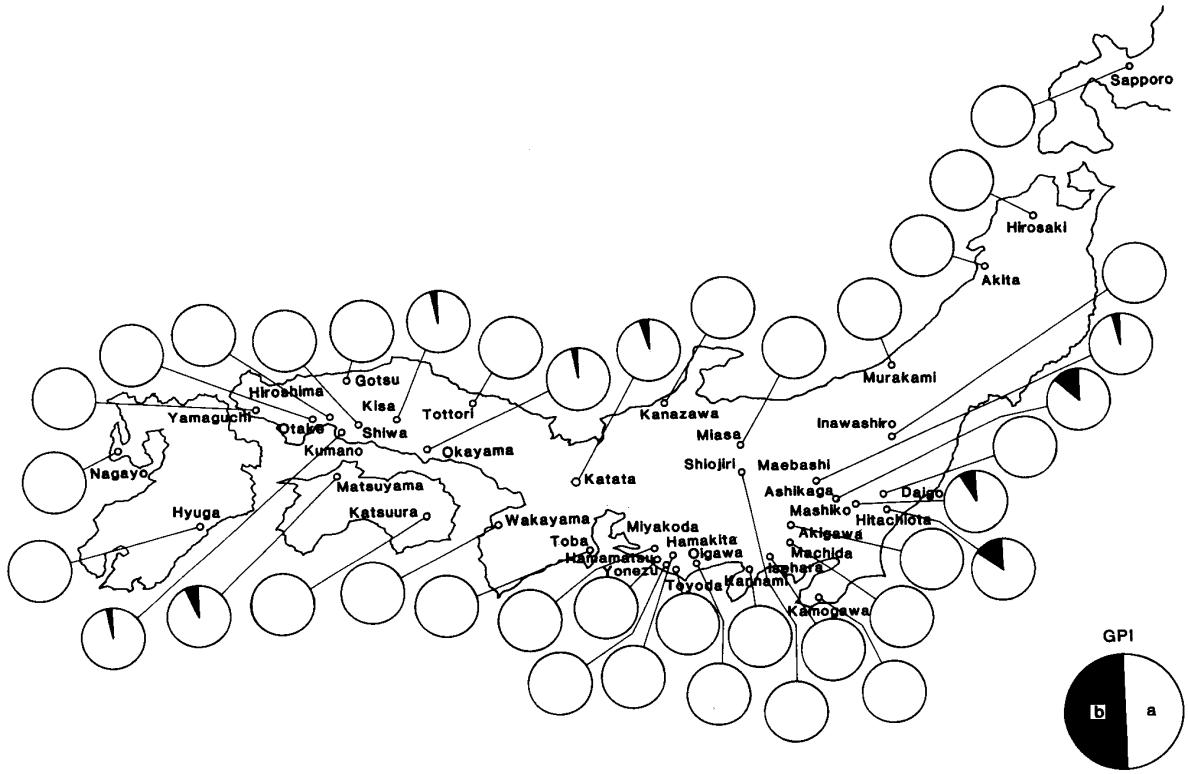

Fig. 7. Geographic distribution of GPI alleles among 40 populations of *Rana rugosa*.

0.925, respectively, while allele *a* was 0.025 and 0.075, respectively, in frequency (Table 4).

7. GPI locus

When the electrophoretic patterns at the GPI locus were analyzed in the 40 populations, two phenotypes, AA and AB, produced by two alleles, *a* and *b*, were observed. Of the 40 populations, nine populations including the Hitachiota, Mashiko, Ashikaga and Maebashi populations of the Kanto region, the Katata population of the Kinki region, the Okayama, Kisa and Kumano populations of the Chugoku region, and the Matsuyama population of the Shikoku region, had allele *a* in frequencies of 0.857~0.976 and allele *b* in frequencies of 0.024~0.143. The remaining 31 populations had only allele *a* (Table 4; Fig. 7).

8. IDH-A locus

When the electrophoretic patterns at the IDH-A locus were analyzed in the 40 populations, two phenotypes, BB and AB, produced by two alleles, *a* and *b*, were observed. In the Hitachiota and Kanazawa populations, allele *b* was 0.964 and 0.978, respectively, in frequency, and allele *a* was 0.036 and 0.022, respectively. The remaining 38 populations had only allele *b* (Table 4).

9. IDH-B locus

When the electrophoretic patterns at the IDH-B locus were analyzed in the 40 populations, eight phenotypes, AA, BB, CC, AB, AC, BC, BD and CD, produced by four alleles, *a*~*d*, were observed. Of the 40 populations, the Wakayama population and the 13 populations situated in the west of Wakayama had only allele *b*, except that the Kumano population had allele *a* in a frequency of 0.048 in addition to allele *b*. Of the 26 populations situated in the east of Wakayama, the Hamakita population had only allele *b* and the Sapporo, Daigo, Hitachiota and Kannami populations had allele *b* in frequencies of 0.719~0.944. In addition to allele *b*, the Sapporo population had allele *a* in a frequency of 0.281, while the the Daigo, Hitachiota and Kannami populations had allele *c* in frequencies of 0.056 ~0.227. While allele *b* was found in frequencies of 0.550~0.667 in five populations, the Mashiko, Murakami, Kanazawa, Katata, and Toba populations, allele *a* was found in a frequency of 0.450 in the Murakami population, and alleles *c* and *d* were found in frequencies of 0.348 and 0.043, respectively, in the Kanazawa population, in addition to allele *b*. In the Katata, Mashiko and Toba populations, allele *c* was found in frequencies of 0.370, 0.346 and 0.333, respectively, in addition to allele *b*. The Isehara population had alleles *b* and *c* both in a frequency of 0.500, the Miasa population had alleles *a* and *b* both in a frequency of 0.500, and the Oigawa population had allele *b* in a frequency of 0.500 and alleles *c* and *a* in frequencies of 0.450 and 0.050, respectively. In the Yonezu population, alleles *b*, *c* and *a* were found in frequencies of 0.475, 0.450 and 0.075, respectively.

In contrast to these, the Miyakoda and Hamamatsu populations had allele *c* in frequencies of 0.575 and 0.475, respectively, allele *b* in frequencies of 0.375 and

0.450, respectively, and allele *a* in frequencies of 0.050 and 0.075, respectively. Five populations of the Kanto region, the Ashikaga, Maebashi, Akigawa, Machida and Kamogawa populations, and two populations of the Chubu region, the Toyoda and Shiojiri populations, had allele *c* in frequencies of 0.636~1.000. Of these populations, the Shiojiri population had only allele *c*. The Ashikaga and Kamogawa populations had allele *b* in frequencies of 0.227 and 0.217, respectively, and allele *d* in frequencies of 0.136 and 0.022, respectively, in addition to allele *c*. The remaining four populations, the Maebashi, Akigawa, Machida and Toyoda populations, had allele *b* in frequencies of 0.118~0.364 in addition to allele *c*. The Hirosaki population of the Tohoku region had only allele *a*, and the Akita and Inawashiro populations had allele *a* in frequencies of 0.912 and 0.675, respectively, and allele *b* in frequencies of 0.088 and 0.325, respectively (Table 4; Fig. 8).

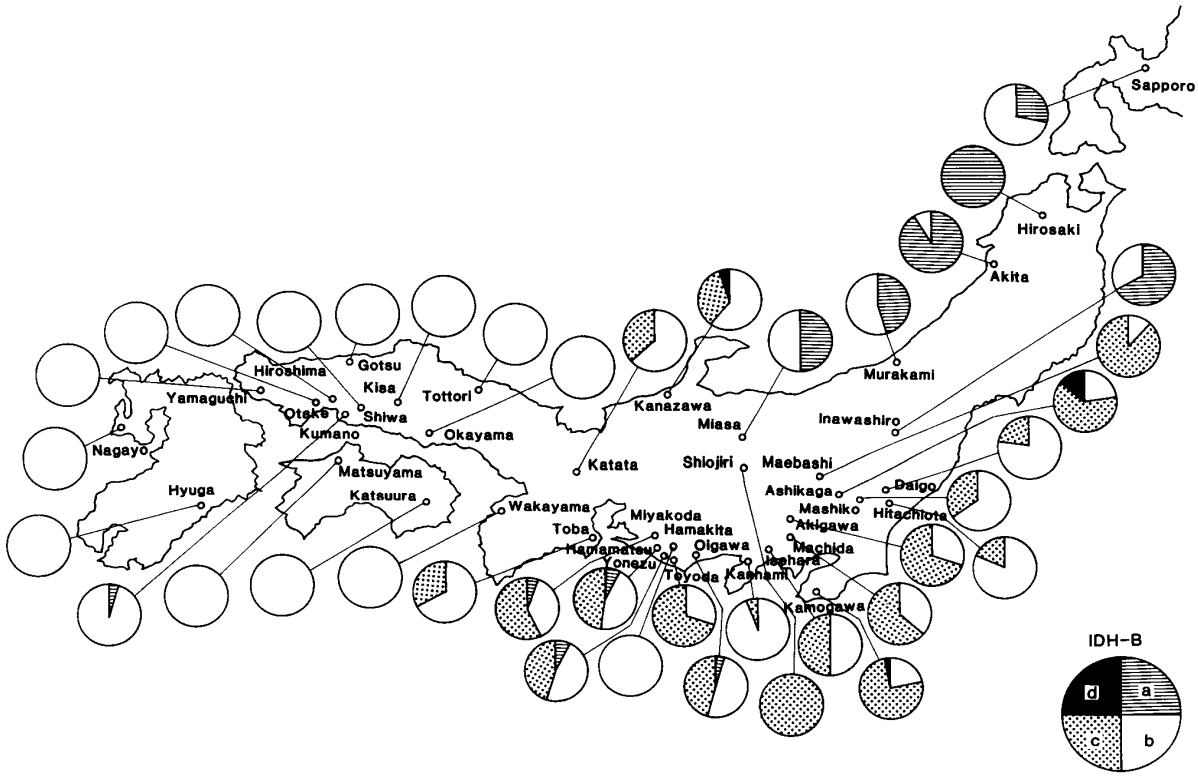

Fig. 8. Geographic distribution of IDH-B alleles among 40 populations of *Rana rugosa*.

10. LDH-A locus

When the electrophoretic patterns at the LDH-A locus were analyzed in the 40 populations, three phenotypes, BB, AB and BC, produced by three alleles, *a*~*c*, were observed. In the 40 populations, allele *b* was high in frequency, being 0.500~1.000. In addition to allele *b*, the Isehara and Shiwa populations had allele *c* in frequencies of 0.050 and 0.056, respectively. One frog of the Otake population had allele *a* in a frequency of 0.500 in addition to allele *b*. All the remaining 37 populations had only allele *b* (Table 4).

TABLE 5

Gene frequencies at seven loci, LDH-B, MDH-A, MDH-B,

Population	Sample size	LDH-B				MDH-A			MDH-B				
		a	*b*	*c*	*d*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*
Sapporo	16			1.000			1.000				1.000		
Hirosaki	18			1.000			1.000				1.000		
Akita	17			1.000			1.000				1.000		
Inawashiro	20			1.000			1.000				1.000		
Daigo	22			1.000			1.000				1.000	0.023	0.795
Hitachiota	14			1.000			0.964	0.036			1.000		0.107
Mashiko	13			1.000			0.962	0.038			1.000		0.154
Ashikaga	11			1.000			1.000				1.000	0.091	0.318
Maebashi	17			0.824	0.176		1.000				1.000		0.882
Akigawa	17			1.000			1.000				1.000		0.765
Machida	11			1.000			1.000				1.000		0.727
Kamogawa	23			1.000			1.000				1.000	0.043	0.370
Isehara	20			1.000			1.000				1.000		0.625
Kannami	9	1.000					1.000				1.000		
Oigawa	20	1.000					1.000				1.000		
Hamakita	20	0.875	0.125				1.000				1.000		
Hamamatsu	20	0.900	0.100				1.000				1.000		
Miyakoda	20	0.975	0.025				1.000				1.000		
Yonezu	20	0.900	0.100				1.000				1.000		
Toyoda	20	0.850	0.150				1.000				1.000		
Shiojiri	3		0.167	0.833			1.000				1.000		1.000
Miasa	2			1.000			1.000				1.000		
Murakami	20			1.000			1.000				1.000		
Kanazawa	23	0.065	0.804	0.109	0.022		1.000				1.000		
Katata	23	0.957	0.043				1.000				1.000	0.022	0.283
Toba	3	1.000					1.000				1.000		
Wakayama	1	1.000					1.000				1.000		0.500
Tottori	20	1.000				0.150	0.850		0.400	0.600			0.025
Gotsu	16	1.000					1.000		0.938	0.063			
Okayama	20	0.875	0.125			0.025	0.975		0.650	0.300	0.050	0.075	0.100
Kisa	20	1.000					1.000		1.000				0.100
Shiwa	9	1.000					1.000		0.611	0.389			
Kumano	21	1.000					1.000		0.738	0.262			
Hiroshima	19	1.000					1.000		0.971	0.029			
Otake	1	1.000					1.000		0.500	0.500			
Yamaguchi	1	1.000					1.000		1.000				
Katsuura	7	1.000					1.000		0.071		0.929		0.143
Matsuyama	16	1.000					1.000		0.367	0.100	0.533		
Nagayo	21	1.000					0.976	0.024	0.738	0.262			0.071
Hyuga	20	1.000					1.000		1.000				

MPI, Pep-A, Pep-B and Pep-C, in 40 populations of *Rana rugosa*

MPI					Pep-A			Pep-B					Pep-C		
c	*d*	*e*	*f*	*g*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*
0.094			0.063	0.844	1.000			0.071	0.929						1.000
			0.194	0.806	1.000			0.028	0.972					0.806	0.194
	0.206		0.412	0.382	1.000				1.000					0.471	0.529
	0.300		0.300	0.400	1.000				0.650		0.350			0.100	0.900
	0.182				1.000			0.523	0.227		0.250		0.455	0.523	0.023
0.107	0.571	0.071	0.143		1.000				0.250		0.679	0.071	0.036	0.964	
0.077	0.769				1.000				0.154		0.692	0.154	0.308	0.692	
	0.455		0.136		1.000			0.091	0.318		0.500	0.091	0.364	0.636	
	0.118				1.000				0.382		0.441	0.176	0.294	0.706	
	0.235				1.000			0.029	0.353		0.529	0.088	0.088	0.912	
	0.227		0.045		1.000				0.455		0.500	0.045	0.045	0.955	
	0.348	0.087	0.152		1.000				0.413		0.587		0.326	0.674	
	0.225		0.150		1.000			0.075	0.625		0.275	0.025	0.075	0.925	
	0.667		0.333		1.000			0.056	0.444		0.389	0.111		1.000	
0.325	0.525		0.150		1.000				0.050		0.950		0.250	0.750	
0.375	0.450		0.150	0.025	1.000				0.100		0.900		0.050	0.950	
0.300	0.625		0.075		1.000				0.200		0.800		0.250	0.700	0.050
0.150	0.725		0.125		1.000				0.075		0.925		0.125	0.875	
	1.000				1.000				0.050		0.950		0.175	0.825	
0.350	0.425		0.225		1.000						1.000		0.125	0.875	
					1.000				0.667		0.333		1.000		
			0.500	0.500	1.000				1.000					0.500	0.500
	0.075	0.100	0.200	0.625	1.000			0.025	0.975				0.025	0.125	0.850
0.065	0.674		0.261		1.000			0.087	0.761		0.152		0.196	0.630	0.174
0.109	0.326	0.043	0.217		0.891	0.109		0.043	0.500		0.457		0.457	0.543	
	0.500		0.500		1.000				0.333		0.667		0.167	0.833	
			0.500			1.000					1.000		0.500	0.500	
	0.900		0.050	0.025		1.000			0.050		0.425	0.525	0.225	0.775	
	0.938		0.063			1.000					1.000		0.344	0.406	0.250
	0.625		0.200		0.050	0.900	0.050		0.025		0.900	0.075	0.400	0.600	
	0.850		0.050			1.000					0.950	0.050	0.450	0.550	
	1.000					1.000			0.056		0.944		0.056	0.944	
	1.000					1.000			0.024		0.976		0.048	0.952	
	1.000					1.000			0.059		0.941		0.176	0.824	
	1.000					1.000					1.000			1.000	
	0.500		0.500			1.000					1.000			1.000	
	0.857					1.000			0.214		0.786		0.286	0.643	0.071
	0.967	0.033				1.000					1.000		0.030	0.969	
0.024	0.214	0.024	0.667			1.000				0.310	0.690		0.214	0.786	
0.050	0.700		0.250			1.000					1.000		0.050	0.950	

11. LDH-B locus

When the electrophoretic patterns at the LDH-B locus were analyzed in the 40 populations, eight phenotypes, AA, BB, CC, DD, AB, BC, CD and BD, produced by four alleles, *a*~*d*, were observed. Of the 40 populations, 16 including the Sapporo population, the three of the Tohoku region, the nine of the Kanto region, the Shiojiri and Miasa populations of the Chubu region, the Murakami population of the Hokuriku region had allele *c* in very high frequencies, being 0.824~1.000. In addition to allele *c*, the Maebashi population had allele *d* in a frequency of 0.176, and the Shiojiri population had allele *b* in a frequency of 0.167. All the remaining 14 populations had only allele *c*.

In contrast to these populations, 23 populations including seven of the Chubu region and 16 of the Kinki, Chugoku, Shikoku and Kyushu regions had allele *a* in very high frequencies, being 0.850~1.000. Of these populations, the Hamakita, Hamamatsu, Miyakoda, Yonezu and Toyoda populations of the Chubu region, the Katata population of the Kinki region, and the Okayama population of the Chugoku region, had allele *b* in frequencies of 0.025~0.150 in addition to allele *a*. The remaining 16 populations had only allele *a*. The Kanazawa population of the Hokuriku region had allele *b* in a high frequency of 0.804, and alleles *c*, *a* and *d* in frequencies of 0.109, 0.065 and 0.022, respectively (Table 5; Fig. 9).

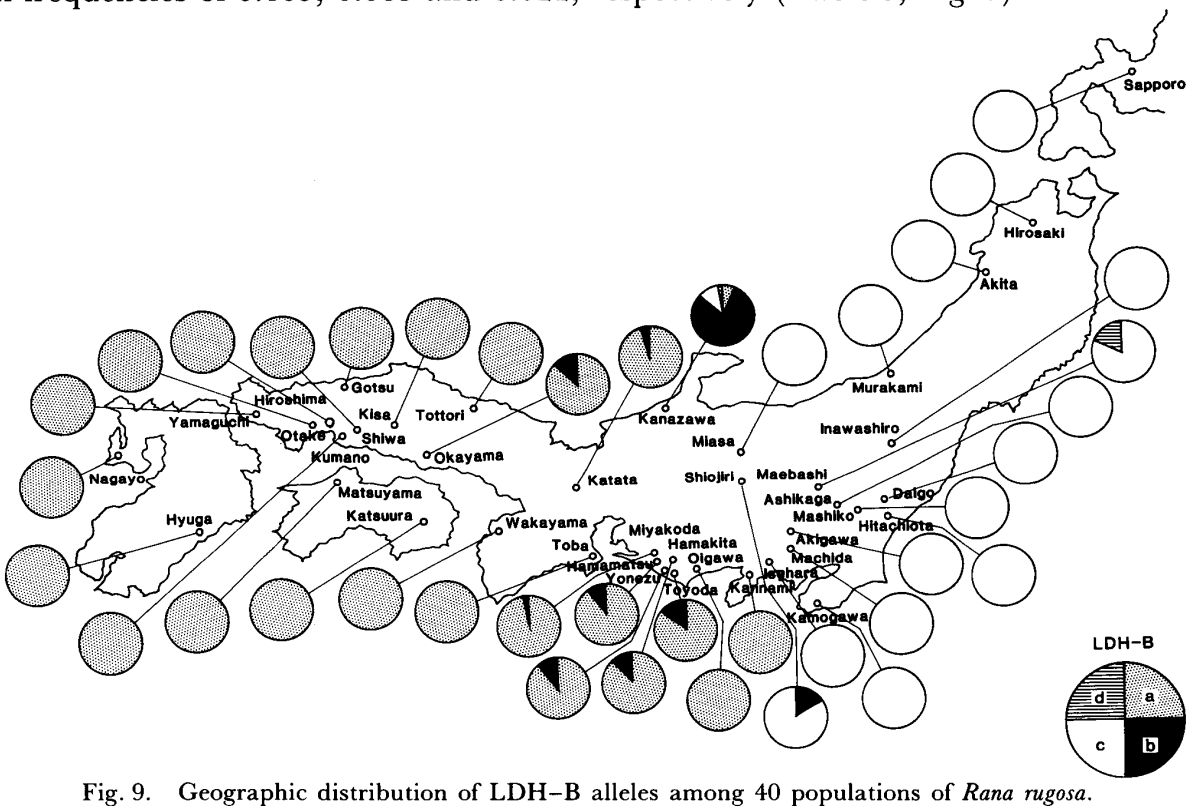

Fig. 9. Geographic distribution of LDH-B alleles among 40 populations of *Rana rugosa*.

12. MDH-A locus

When the electrophoretic patterns at the MDH-A locus were analyzed in the 40 populations, three phenotypes, BB, AB and BC, produced by three alleles, *a*~*c*,

were observed. All the 40 populations had allele *b* in high frequencies, being 0.850~1.000. In addition to allele *b*, the Hitachiota, Mashiko and Nagayo populations had allele *c* in frequencies of 0.024~0.038, and the Tottori and Okayama populations had allele *a* in frequencies of 0.150 and 0.025, respectively. All the remaining 35 populations had only allele *b* (Table 5).

13. MDH-B locus

When the electrophoretic patterns at the MDH-B locus were analyzed in the 40 populations, six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*~*c*, were observed. Of the 40 populations, the 27 populations situated in the east of Wakayama, including the Wakayama population, had only allele *c*, while in the remaining 13 western populations, numerous variations in the kind of alleles were found. In the seven populations of the Chugoku region other than the Tottori and Otake populations and the two populations of the Kyushu region, allele *a* was high in frequency, being 0.611~1.000. While three populations, the Kisa, Yamaguchi and Hyuga populations, had only allele *a*, the other six populations had allele *b* in frequencies of 0.029~0.389 in addition to allele *a*. The Okayama population had allele *c* in a frequency of 0.050 in addition to alleles *a* and *b*. One frog of the Otake population had alleles *a* and *b* both in a frequency of 0.500, and the Tottori population had alleles *b* and *a* in frequencies of 0.600 and 0.400, respectively. While the Katsuura population of the Shikoku region had allele *c* in a high frequency, being 0.929, and allele *a* in a frequency of 0.071, the Matsuyama population had alleles *c*, *a* and *b* in frequencies of 0.533, 0.367 and 0.100, respectively (Table 5; Fig. 10).

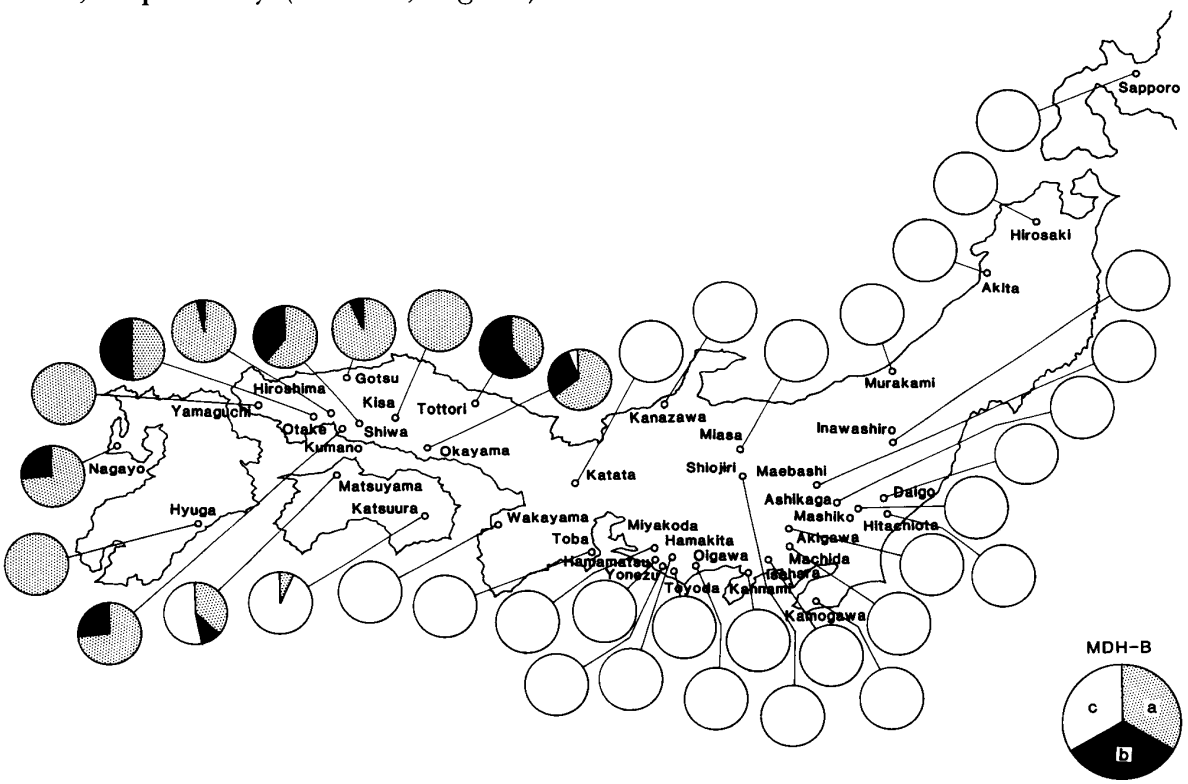

Fig. 10. Geographic distribution of MDH-B alleles among 40 populations of *Rana rugosa*.

14. MPI locus

When the electrophoretic patterns at the MPI locus were analyzed in the 40 populations, 22 phenotypes, BB, CC, DD, FF, GG, AB, AD, AF, BC, BD, BE, BF, CD, CE, CF, CG, DE, DF, DG, EF, EG and FG, produced by seven alleles, *a*~*g*, were observed. Of the 40 populations, 13 including the Mashiko population of the Kanto region, the Miyakoda and Yonezu populations of the Chubu region, the seven populations of the Chugoku region other than the Yamaguchi and Okayama populations, the two populations of the Shikoku region and the Hyuga population of the Kyushu region had allele *d* in very high frequencies, being 0.700~1.000. In 11 populations including the Hitachiota and Ashikaga populations of the Kanto region, the Kannami, Oigawa, Hamakita, Hamamatsu and Toyoda populations of the Chubu region, the Kanazawa population of the Hokuriku region, the Toba population of the Kinki region and the Okayama and Yamaguchi populations of the Chugoku region, allele *d* was fairly high in frequency, being 0.425~0.674, while in 10 populations including two populations of the Tohoku region, six populations of the Kanto region, one population of the Kinki region, and one population of the Kyushu region, allele *d* was low in frequency, being 0.118~0.348. The Murakami population of the Hokuriku region had allele *d* in a frequency of 0.075. The remaining five populations had no allele *d*.

Allele *b* was high in frequency, being 0.625~1.000, in six populations including the Daigo, Maebashi, Akigawa, Machida and Isehara populations of the Kanto region and the Shiojiri population of the Chubu region, while in 11 populations including the remaining four populations of the Kanto region, two populations, the Katata and Wakayama populations, of the Kinki region, three populations, the Tottori, Okayama and Kisa populations, of the Chugoku region, the Katsuura population of the Shikoku region, and the Nagayo population of the Kyushu region, allele *b* was low in frequency, being 0.025~0.500. The other 23 of the 40 populations had no allele *b*.

Allele *g* was high in frequency in the Sapporo and Hirosaki populations, being 0.844 and 0.806, respectively. The Murakami and Miasa populations had allele *g* in frequencies of 0.625 and 0.500, respectively. The Inawashiro and Akita populations had allele *g* in frequencies of 0.400 and 0.382, respectively. The Hamakita and Tottori populations each had allele *g* in a frequency of 0.025. The remaining 32 of the 40 populations had no allele *g*.

In the Nagayo population of the Kyushu region, allele *f* was high in frequency, being 0.667. In each of the Miasa, Toba, Wakayama and Yamaguchi populations, allele *f* was 0.500 in frequency. In the Akita, Kannami, Inawashiro, Kanazawa, Hyuga, Toyoda, Katata, Murakami, Okayama and Hirosaki populations, allele *f* was found in gradually decreasing frequencies from 0.412 to 0.194. In the Kamogawa, Isehara, Oigawa, Hamakita, Hitachiota, Ashikaga and Miyakoda populations, allele *f* also gradually decreased from 0.152 to 0.125 in frequency, and in the Hamamatsu, Sapporo, Gotsu, Tottori, Kisa and Machida populations, allele *f* gradually decreased from 0.075 to 0.045 in frequency. The

remaining 12 of the 40 populations had no allele *f*.

In five populations, the Oigawa, Hamakita, Hamamatsu, Miyakoda and Toyoda populations, of the Chubu region, allele *c* was found in frequencies of 0.150~0.375. In the Hitachiota and Mashiko populations of the Kanto region, allele *c* was 0.107 and 0.077, respectively, in frequency, and 0.109 in the Katata population of the Kinki region. In four populations, the Sapporo, Kanazawa, Nagayo and Hyuga populations, allele *c* was 0.024~0.094 in frequency. The remaining 28 of the 40 populations had no allele *c*. Allele *a* was found in frequencies of 0.022~0.091 in five populations, the Daigo, Ashikaga and Kamogawa populations, of the Kanto region and the Katata and Okayama populations. In the remaining 35 of the 40 populations, there was no allele *a*. Allele *e* was found only in six populations, the Hitachiota, Kamogawa, Murakami, Katata, Matsuyama and Nagayo populations, in frequencies of 0.024~0.100 (Table 5; Fig. 11).

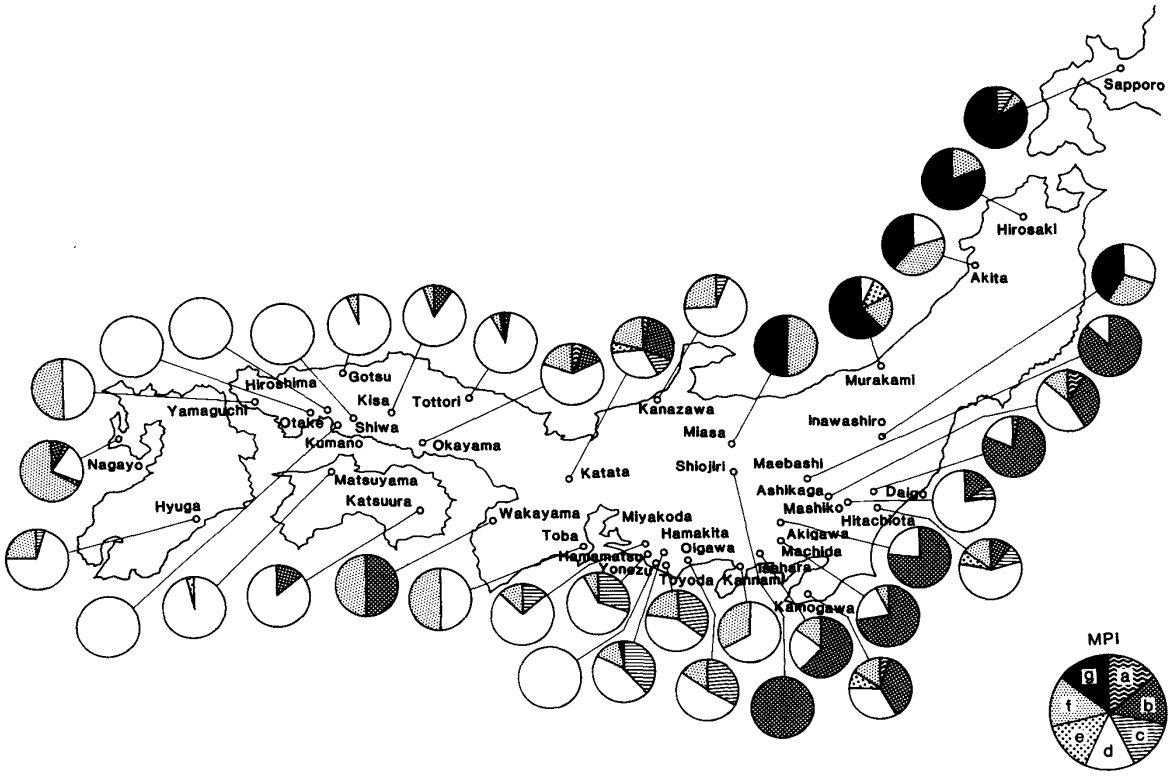

Fig. 11. Geographic distribution of MPI alleles among 40 populations of *Rana rugosa*.

15. Pep-A locus

When the electrophoretic patterns at the Pep-A locus were analyzed in the 40 populations, four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*~*c*, were observed. In 14 populations situated in the west of Wakayama including the Wakayama population, there was almost only allele *b*, except that the Okayama population had alleles *a* and *c* both in a frequency of 0.050, in addition to allele *b*. In 26 populations situated in the east of Wakayama, there was almost

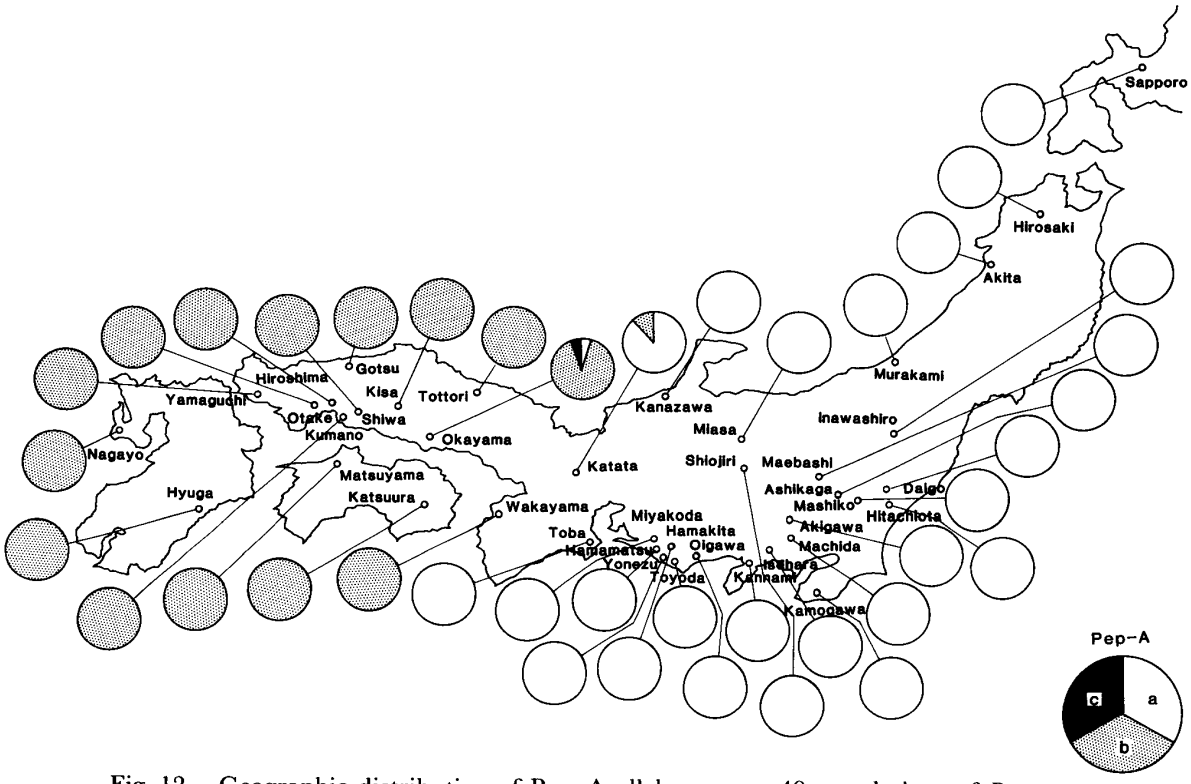

Fig. 12. Geographic distribution of Pep-A alleles among 40 populations of *Rana rugosa*.

only allele *a*, except that the Katata population had allele *b* in a frequency of 0.109 in addition to allele *a* (Table 5; Fig. 12).

16. Pep-B locus

When the electrophoretic patterns at the Pep-B locus were analyzed in the 40 populations, 11 phenotypes, AA, BB, CC, DD, EE, AB, AD, BD, BE, CD and DE, produced by five alleles, *a*~*e*, were observed. In 12 populations situated in the west of Wakayama including the Wakayama population except for the Tottori and Nagayo populations, and in six populations of the Chubu region other than the Kannami, Shiojiri and Miasa populations, allele *d* was very high in frequency, being 0.786~1.000. Of these 18 population, nine including the Shiwa, Kumano and Hiroshima populations of the Chugoku region, the Katsuura population of the Shikoku region, and the Oigawa, Hamakita, Hamamatsu, Miyakoda and Yonezu populations of the Chubu region, had allele *b* in frequencies of 0.024~0.214, in addition to allele *d*. The Kisa population had allele *e* in a frequency of 0.050, and the Okayama population had alleles *b* and *e* in frequencies of 0.025 and 0.075, respectively. The remaining seven populations had only allele *d*. In eight populations including six populations of the Kanto region other than the Daigo, Maebashi and Isehara populations, the Toba population of the Kinki region and the Nagayo population of the Kyushu region, allele *d* was fairly high in frequency, being 0.500~0.692. In six populations of the Kanto region and the Toba population, allele *b* was found in frequencies of 0.154~0.455, in addition to allele *d*.

In three populations, the Hitachiota, Mashiko and Machida populations, allele *e* was found in frequencies of 0.045~0.154. In the Ashikaga population, alleles *a* and *e* were found each in a frequency of 0.091 in addition to alleles *b* and *d*. In the Akigawa population, alleles *a* and *e* were 0.029 and 0.088, respectively, in frequency. In the Nagayo population, allele *c* was found in a frequency of 0.310 in addition to allele *d*. In the Maebashi population of the Kanto region, alleles *d*, *b* and *e* were found in frequencies of 0.441, 0.382 and 0.176, respectively.

Allele *b* was high in frequency, being 0.625~1.000, in nine populations including the Sapporo population, the three populations of the Tohoku region, the Isehara population of the Kanto region, the Shiojiri and Miasa populations of the Chubu region and the two populations of the Hokuriku region. In addition to allele *b*, the Sapporo, Hirosaki and Murakami populations had allele *a* in frequencies of 0.025~0.071, the Inawashiro and Shiojiri populations had allele *d* in frequencies of 0.350 and 0.333, respectively, the Kanazawa population had alleles *a* and *d* in frequencies of 0.087 and 0.152, respectively, and the Isehara population had alleles *d*, *a* and *e* in frequencies of 0.275, 0.075 and 0.025, respectively. The remaining two of the nine populations had only allele *b*. In the Katata population of the Kinki region, alleles *b*, *d* and *a* were found in frequencies of 0.500, 0.457 and 0.043, respectively. In the Kannami population, alleles *b*, *d*, *e* and *a* were found in frequencies of 0.444, 0.389, 0.111 and 0.056, respectively. In the Daigo population, allele *a* was 0.523 and alleles *b* and *d* were 0.227 and 0.250, respectively, in frequency. In the Tottori population, allele *e* was 0.525 and alleles *d* and *b* were 0.425 and 0.050, respectively, in frequency (Table 5; Fig. 13).

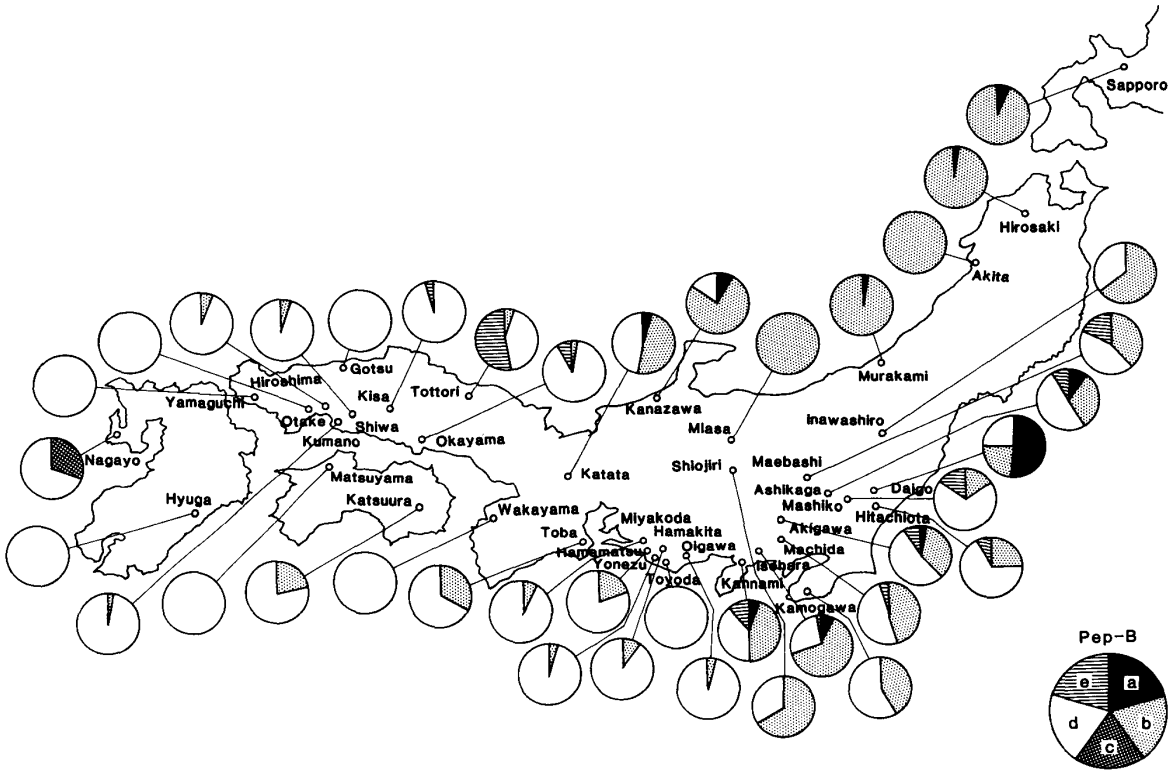

Fig. 13. Geographic distribution of Pep-B alleles among 40 populations of *Rana rugosa*.

17. Pep-C locus

When the electrophoretic patterns at the Pep-C locus were analyzed in the 40 populations, six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*~*c*, were observed. Of the 40 populations, 23 including the Hirosaki population of the Tohoku region, five populations of the Kanto region, seven populations of the Chubu region, the Toba population of the Kinki region, six populations of the Chugoku region other than the Gotsu, Okayama and Kisa populations, the Matsuyama population of the Shikoku region and the two populations of the Kyushu region, had allele *b* in high frequencies, being 0.700 ~1.000. Of these 23 populations, three populations of the Kannami, Otake and Yamaguchi populations had only allele *b*. In addition to allele *b*, the Hirosaki population had allele *c* in a frequency of 0.194, the Hamamatsu population had alleles *a* and *c* in frequencies of 0.250 and 0.050, respectively, and the remaining 18 populations had allele *a* in frequencies of 0.030~0.294. On the other hand, in 11 populations including four of Kanto region, the Daigo, Mashiko, Ashikaga and Kamogawa populations, the Miasa population of the Chubu region, the Kanazawa population of the Hokuriku region, the Katata and Wakayama populations of the Kinki region, the Okayama and Kisa populations of the Chugoku region, and the Katsuura population of the Shikoku region, allele *b* was 0.500~0.692 in frequency. Of these populations, the Miasa population had allele *c* in a frequency of 0.500, and three populations of the Daigo, Kanazawa and Katsuura populations had allele *a* in frequencies of 0.196~0.455 and allele *c* in frequencies of 0.023

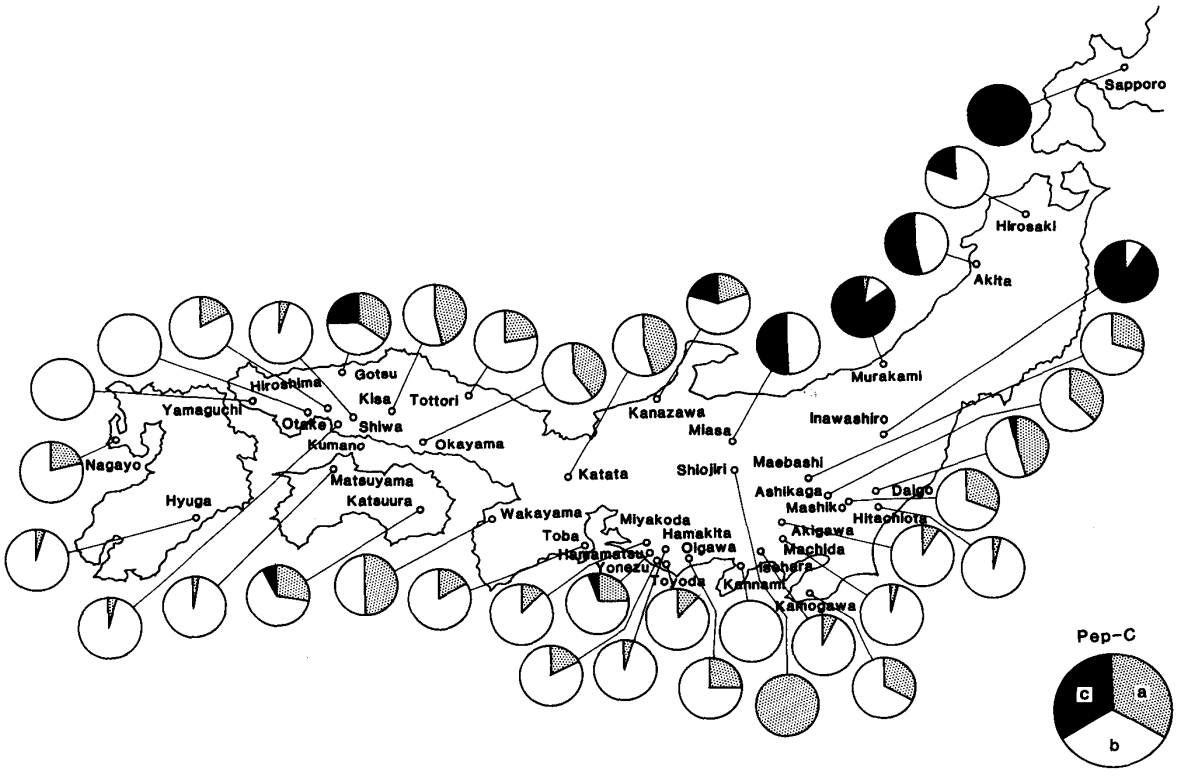

Fig. 14. Geographic distribution of Pep-C alleles among 40 populations of *Rana rugosa*.

~0.174, in addition to allele *b*. The other seven populations had allele *a* in frequencies of 0.308~0.500, in addition to allele *b*.

The Gotsu population of the Chugoku region had alleles *b*, *a* and *c* in frequencies of 0.406, 0.344 and 0.250, respectively, while the Shiojiri population had only allele *a*. The Sapporo population had only allele *c*, and the Akita and Inawashiro populations of the Tohoku region and the Murakami population of the Hokuriku region had allele *c* in frequencies of 0.529, 0.900 and 0.850, respectively. These three populations had allele *b* in frequencies of 0.100~0.471. The Murakami population of the Hokuriku region had allele *a* in a frequency of 0.025, in addition to alleles *c* and *d* (Table 5; Fig. 14).

18. Pep-D locus

When the electrophoretic patterns at the Pep-D locus were analyzed in the 40 populations, six phenotypes, AA, BB, CC, AB, BC and BD, produced by four alleles, *a*~*d*, were observed. Of the 40 populations, 39 other than the Sapporo population had allele *b* in high frequencies, being 0.643~1.000. In these populations, the Hitachiota population had allele *c* in a frequency of 0.357, and the Shiwa population had alleles *a* and *c* in frequencies of 0.278 and 0.056, respectively, in addition to allele *b*. Seven populations of the Mashiko, Akigawa, Machida, Miyakoda, Toyoda, Katata and Kisa populations had allele *a* in frequencies of 0.025~0.130, and allele *c* in frequencies of 0.025~0.136, in addition to allele *b*. The Tottori and Kumano populations had allele *a* in frequencies of 0.100 and 0.024, respectively, and the Murakami population had allele *d* in a frequency of 0.025, in addition to allele *b*. Thirteen populations including the Hirosaki population of the Tohoku region, the Isehara population of the Kanto region, the Kannami, Oigawa, Hamakita, Hamamatsu and Yonezu populations of the Chubu region, the Kanazawa population of the Hokuriku region, the Gotsu, Okayama and Hiroshima populations of the Chugoku region, the Matsuyama population of the Shikoku region and the Nagayo population of the Kyushu region had allele *c* in frequencies of 0.029~0.239 in addition to allele *b*. The remaining 14 populations had only allele *b*. The Sapporo population had alleles *b* and *c* in frequencies of 0.464 and 0.536, respectively (Table 6; Fig. 15).

19. PGM locus

When the electrophoretic patterns at the PGM locus were analyzed in the 40 populations, four phenotypes, BB, CC, AB and BC, produced by three alleles, *a*~*c*, were observed. The Kanazawa population had alleles *b* and *c* in frequencies of 0.652 and 0.348, respectively. The other 39 of the 40 populations had allele *b* in very high frequencies, being 0.925~1.000. In addition to allele *b*, three populations of the Akigawa, Toyoda and Gotsu populations had allele *a* in frequencies of 0.025~0.031 and three populations of the Miyakoda, Yonezu, and Tottori populations had allele *c* in frequencies of 0.025 or 0.075. The remaining 33 populations had only allele *b* (Table 6; Fig. 16).

TABLE 6

Gene frequencies at eight loci, Pep-D, PGM, SOD-A, SOD-B,

Population	Sample size	Pep-D				PGM			SOD-A			SOD-B	
		a	*b*	*c*	*d*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*
Sapporo	16		0.464	0.536			1.000			1.000		1.000	
Hirosaki	18		0.861	0.139			1.000			1.000		1.000	
Akita	17		1.000				1.000			1.000		1.000	
Inawashiro	20		1.000				1.000			1.000		1.000	
Daigo	22		1.000				1.000			1.000		1.000	
Hitachiota	14		0.643	0.357			1.000			1.000		1.000	
Mashiko	13	0.115	0.769	0.115			1.000			1.000		1.000	
Ashikaga	11		1.000				1.000			1.000		1.000	
Maebashi	17		1.000				1.000			1.000		1.000	
Akigawa	17	0.088	0.882	0.029		0.029	0.971			1.000		1.000	
Machida	11	0.045	0.818	0.136			1.000			1.000		1.000	
Kamogawa	23		1.000				1.000			1.000		0.935	0.065
Isehara	20		0.925	0.075			1.000			1.000		1.000	
Kannami	9		0.889	0.111			1.000			1.000		1.000	
Oigawa	20		0.900	0.100			1.000			1.000		1.000	
Hamakita	20		0.900	0.100			1.000			1.000		1.000	
Hamamatsu	20		0.925	0.075			1.000			1.000		1.000	
Miyakoda	20	0.075	0.900	0.025			0.925	0.075		1.000		1.000	
Yonezu	20		0.850	0.150			0.975	0.025		1.000		1.000	
Toyoda	20	0.075	0.825	0.100		0.025	0.975			1.000		1.000	
Shiojiri	3		1.000				1.000			1.000		1.000	
Miasa	2		1.000				1.000			0.750	0.250	1.000	
Murakami	20		0.975		0.025		1.000			1.000		1.000	
Kanazawa	23		0.761	0.239			0.652	0.348		0.761	0.239	1.000	
Katata	23	0.130	0.783	0.087			1.000			1.000		0.761	0.239
Toba	3		1.000				1.000			1.000		1.000	
Wakayama	1		1.000				1.000			1.000		1.000	
Tottori	20	0.100	0.900				0.975	0.025	0.050	0.950		1.000	
Gotsu	16		0.844	0.156		0.031	0.969			1.000		1.000	
Okayama	20		0.775	0.225			1.000			1.000		1.000	
Kisa	20	0.025	0.850	0.125			1.000			1.000		1.000	
Shiwa	9	0.278	0.667	0.056			1.000			1.000		1.000	
Kumano	21	0.024	0.976				1.000			0.976	0.024	1.000	
Hiroshima	19		0.971	0.029			1.000			0.971	0.029	1.000	
Otake	1		1.000				1.000			1.000		1.000	
Yamaguchi	1		1.000				1.000			1.000		1.000	
Katsuura	7		1.000				1.000			1.000		1.000	
Matsuyama	16		0.813	0.188			1.000			1.000		1.000	
Nagayo	21		0.953	0.048			1.000			1.000		1.000	
Hyuga	20		1.000				1.000			1.000		1.000	

SORDH, Ab, Hb–I and Hb–II, in 40 populations of *Rana rugosa*

SORDH							Ab				Hb–I	Hb–II			
a	*b*	*c*	*d*	*e*	*f*	*g*	*a*	*b*	*c*	*d*	*a*	*a*	*b*	*c*	*d*
	0.036			0.964				1.000			1.000		1.000		
				1.000				0.972		0.028	1.000		1.000		
				1.000				1.000			1.000		1.000		
				1.000				1.000			1.000		1.000		
	0.025			0.975				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			1.000	1.000			
	0.059			0.941			0.029	0.971			1.000	1.000			
				1.000			0.029	0.971			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	1.000			
	0.056			0.944				1.000			1.000			1.000	
				1.000			0.075	0.925			1.000			1.000	
				0.775	0.050	0.175		1.000			1.000		0.100	0.900	
				0.800	0.025	0.175		1.000			1.000		0.125	0.875	
				1.000				1.000			1.000		0.071	0.929	
				1.000				1.000			1.000		0.075	0.925	
				1.000			0.025	0.975			1.000		0.100	0.900	
				1.000				1.000			1.000	1.000			
				1.000				1.000			1.000		1.000		
				1.000				1.000			1.000		1.000		
	0.065	0.196		0.739				1.000			1.000		0.978		0.022
		0.065	0.022	0.913				1.000			1.000		0.395	0.053	0.553
				1.000				1.000			1.000		0.167		0.833
				1.000				1.000			1.000		1.000		
		0.325		0.675				1.000			1.000		0.275		0.725
				1.000			0.031	0.969			1.000		0.067		0.933
		0.525	0.050	0.425				0.950	0.050		1.000		0.125		0.875
0.025	0.025	0.650		0.300				1.000			1.000		0.125		0.875
	0.222	0.111	0.056	0.611				1.000			1.000		0.167		0.833
		0.238	0.071	0.690				0.929	0.071		1.000		0.452		0.548
0.088	0.176	0.088	0.265	0.382				1.000			1.000		0.579		0.421
		0.500		0.500				1.000			1.000				1.000
				1.000				1.000			1.000				1.000
		0.214	0.357	0.429				1.000			1.000				1.000
		0.406	0.313	0.281				1.000			1.000		0.063		0.938
0.100				0.900				1.000			1.000				1.000
0.025			0.200	0.775				1.000			1.000				1.000

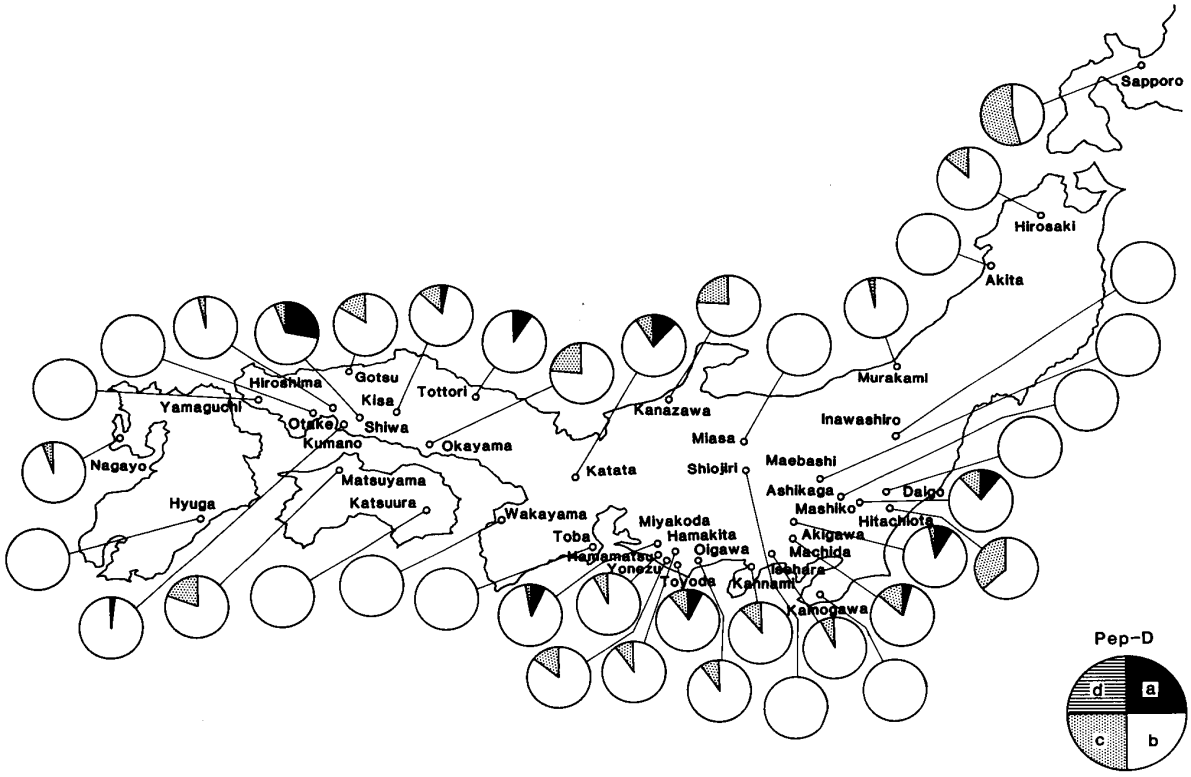

Fig. 15. Geographic distribution of Pep–D alleles among 40 populations of *Rana rugosa*.

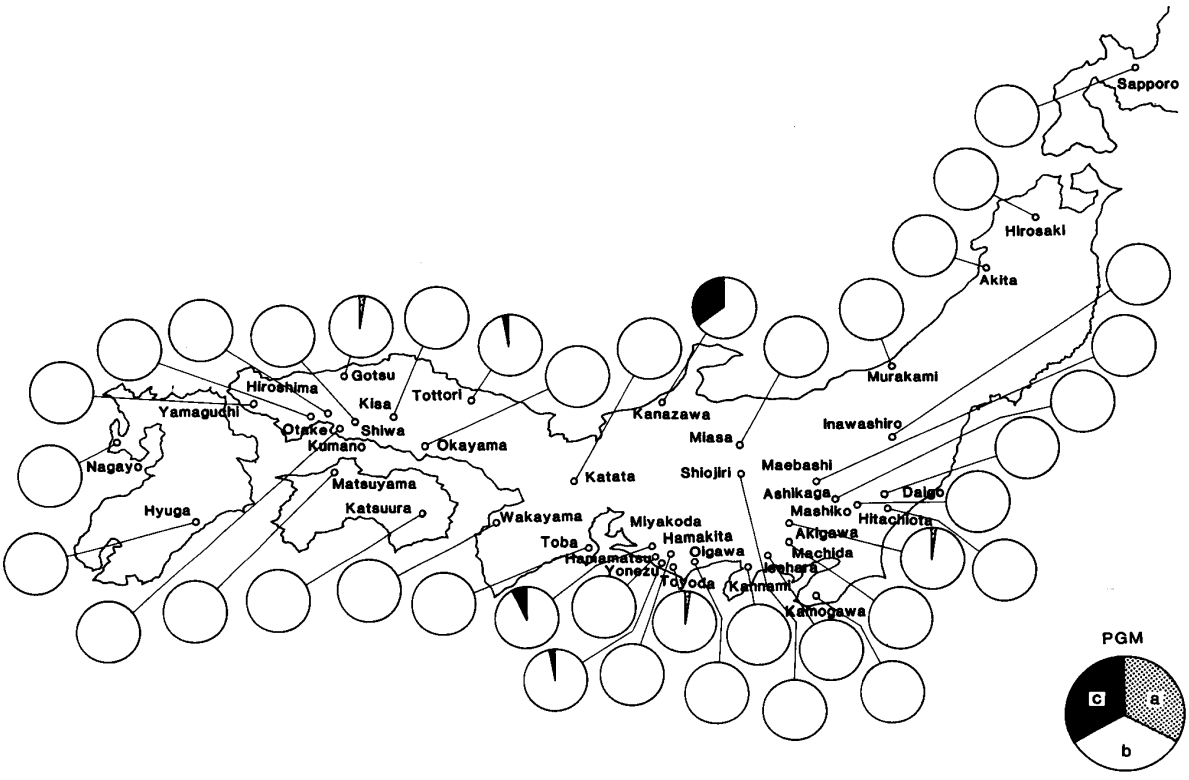

Fig. 16. Geographic distribution of PGM alleles among 40 populations of *Rana rugosa*.

20. SOD-A locus

When the electrophoretic patterns at the SOD-A locus were analyzed in the 40 populations, four phenotypes, BB, CC, AB and BC, produced by three alleles, *a*~*c*, were observed. All the 40 populations had allele *b* in very high frequencies, being 0.750~1.000. The Tottori population had allele *a* in a frequency of 0.050, and the Miasa, Kanazawa, Kumano and Hiroshima populations had allele *c* in frequencies of 0.024~0.250 in addition to allele *b*. The remaining 35 populations had only allele *b* (Table 6; Fig. 17).

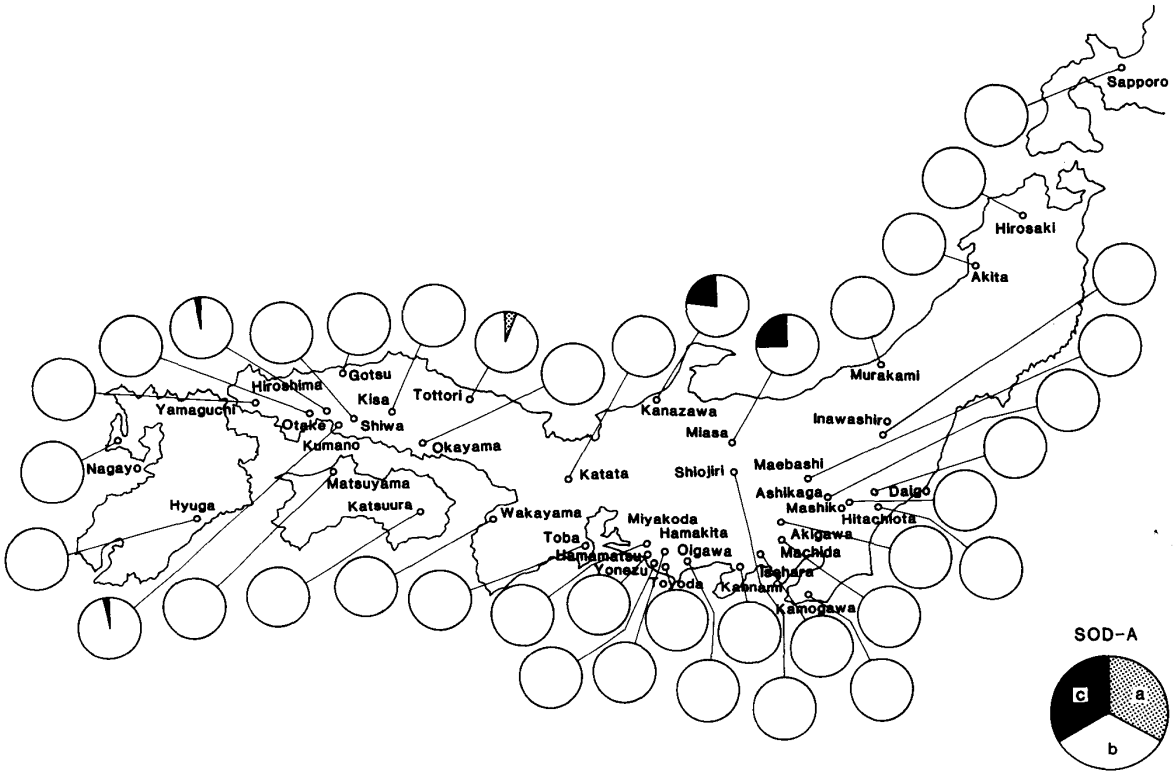

Fig. 17. Geographic distribution of SOD-A alleles among 40 populations of *Rana rugosa*.

21. SOD-B locus

When the electrophoretic patterns at the SOD-B locus were analyzed in the 40 populations, three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*, were observed. In the 40 populations, allele *a* was very high in frequency, being 0.761~1.000. While allele *a* was 0.935 and 0.761 and allele *b* was 0.065 and 0.239 in the Kamogawa and Katata populations, respectively, there was only allele *a* in the other 38 populations (Table 6; Fig. 18).

22. SORDH locus

When the electrophoretic patterns at the SORDH locus were analyzed in the 40 populations, 16 phenotypes, AA, BB, CC, DD, EE, GG, AC, AD, AE, BC, BE, CD, CE, DE, EF and EG, produced by seven alleles, *a*~*g*, were observed. Of the

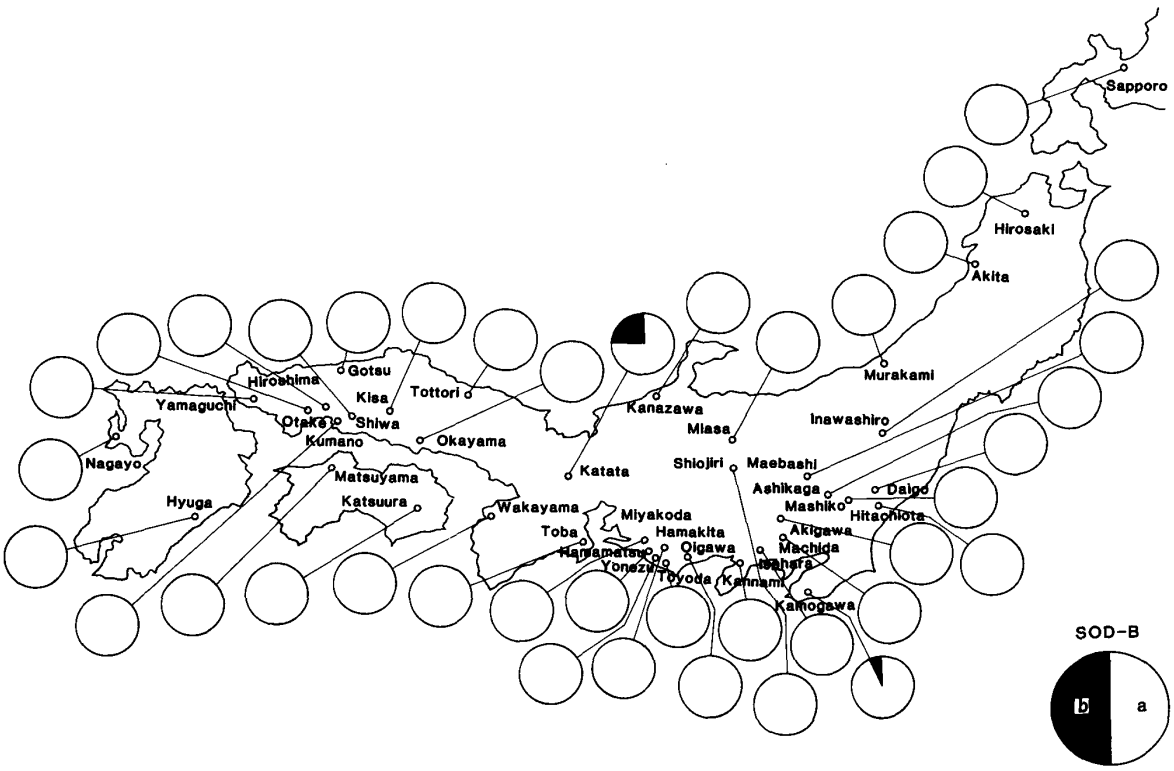

Fig. 18. Geographic distribution of SOD-B alleles among 40 populations of *Rana rugosa*.

40 populations, 31 populations including the Wakayama population situated in the east of Wakayama, the Gotsu and Yamaguchi populations of the Chugoku region, and the two populations of the Kyushu region had allele *e* in very high frequencies, being 0.739~1.000. In addition to allele *e*, the Sapporo, Daigo, Maebashi and Kannami populations in eastern Japan had allele *b* in frequencies of 0.025~0.059, the Kanazawa population had alleles *b* and *c* in frequencies of 0.065 and 0.196, respectively, the Katata population had alleles *c* and *d* in frequencies of 0.065 and 0.022, respectively, and the Hamakita and Hamamatsu populations had allele *f* in frequencies of 0.050 and 0.025, respectively, and allele *g* in a frequency of 0.175 each. The Nagayo population of the Kyushu region had allele *a* in a frequency of 0.100 and the Hyuga population had alleles *a* and *d* in frequencies of 0.025 and 0.200, respectively, in addition to allele *e*. The remaining 21 populations had only allele *e*.

In four populations including the Tottori, Shiwa, Kumano and Otake populations of the Chugoku region, allele *e* was 0.500~0.690 in frequency. Of these populations, the Tottori and Otake populations had allele *c* in frequencies of 0.325 and 0.500, respectively, the Kumano population had alleles *c* and *d* in frequencies of 0.238 and 0.071, respectively, and the Shiwa population had alleles *b*, *c* and *d* in frequencies of 0.222, 0.111 and 0.056, respectively. The Hiroshima population had alleles *e*, *d*, *b*, *a* and *c* in frequencies of 0.382, 0.265, 0.176, 0.088 and 0.088, respectively. The Katsuura population of the Shikoku region had alleles *e*, *d* and *c* in frequencies of 0.429, 0.357 and 0.214, respectively. The Okayama, Kisa and

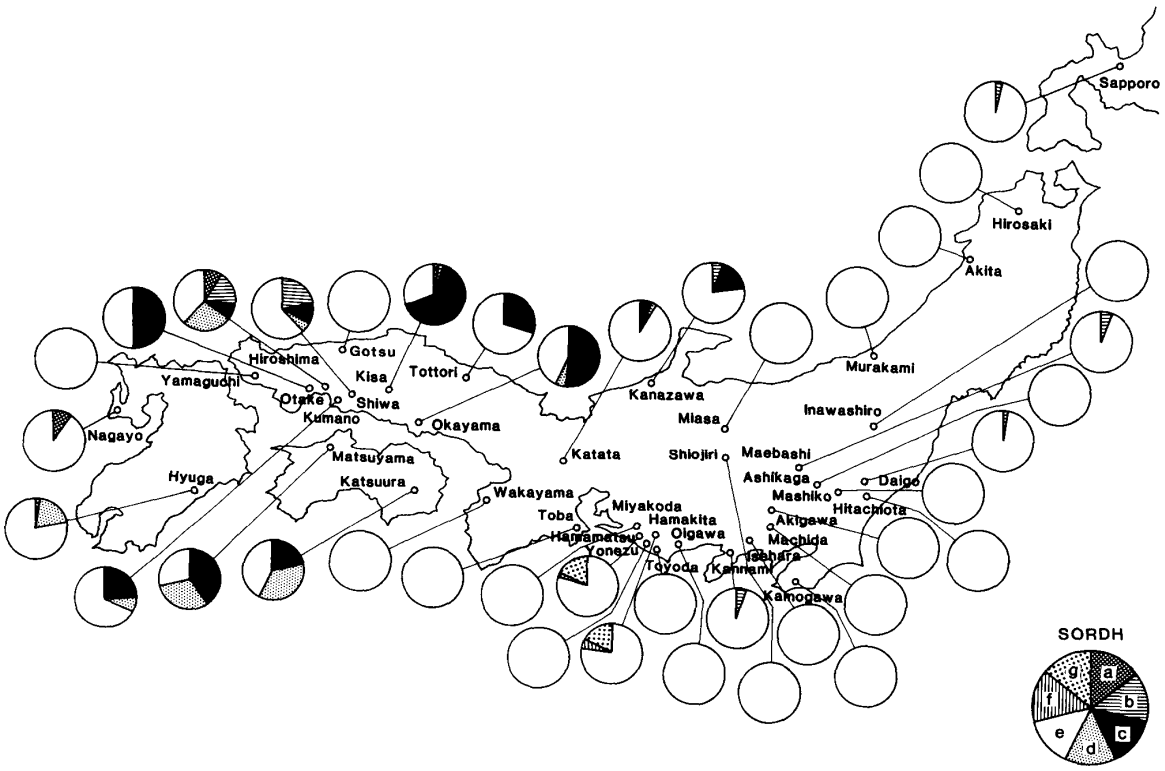

Fig. 19. Geographic distribution of SORDH alleles among 40 populations of *Rana rugosa*.

Matsuyama populations had allele *c* in frequencies of 0.406~0.650. In addition to allele *c*, the Okayama population had alleles *e* and *d* in frequencies of 0.425 and 0.050, respectively, the Kisa population had alleles *e*, *b* and *a* in frequencies of 0.300, 0.025 and 0.025, respectively, and the Matsuyama population had alleles *d* and *e* in frequencies of 0.313 and 0.281, respectively (Table 6; Fig. 19).

23. Ab locus

When the electrophoretic patterns at the Ab locus were analyzed in the 40 populations, four phenotypes, BB, AB, BC and BD, produced by four alleles, *a*~*d*, were observed. The 40 populations were very high in frequencies of allele *b*, being 0.925~1.000. In five populations of the Maebashi, Akigawa, Oigawa, Toyoda and Gotsu populations, allele *a* was 0.025~0.075 in frequency, in two populations, the Okayama and Kumano populations, allele *c* was 0.050 and 0.071, respectively, in frequency, and in the Hirosaki population, allele *d* was 0.028 in frequency, in addition to allele *b* (Table 6; Fig. 20).

24. Hb-II locus

When the electrophoretic patterns at the Hb-II locus were analyzed in the 40 populations, seven phenotypes, AA, BB, CC, DD, BC, BD and CD, produced by four alleles, *a*~*d*, were observed. Of the 40 populations, the nine populations of the Kanto region and the Shiojiri population of the Chubu region had only allele *a*. Eight populations, including the Sapporo population, the three populations of the

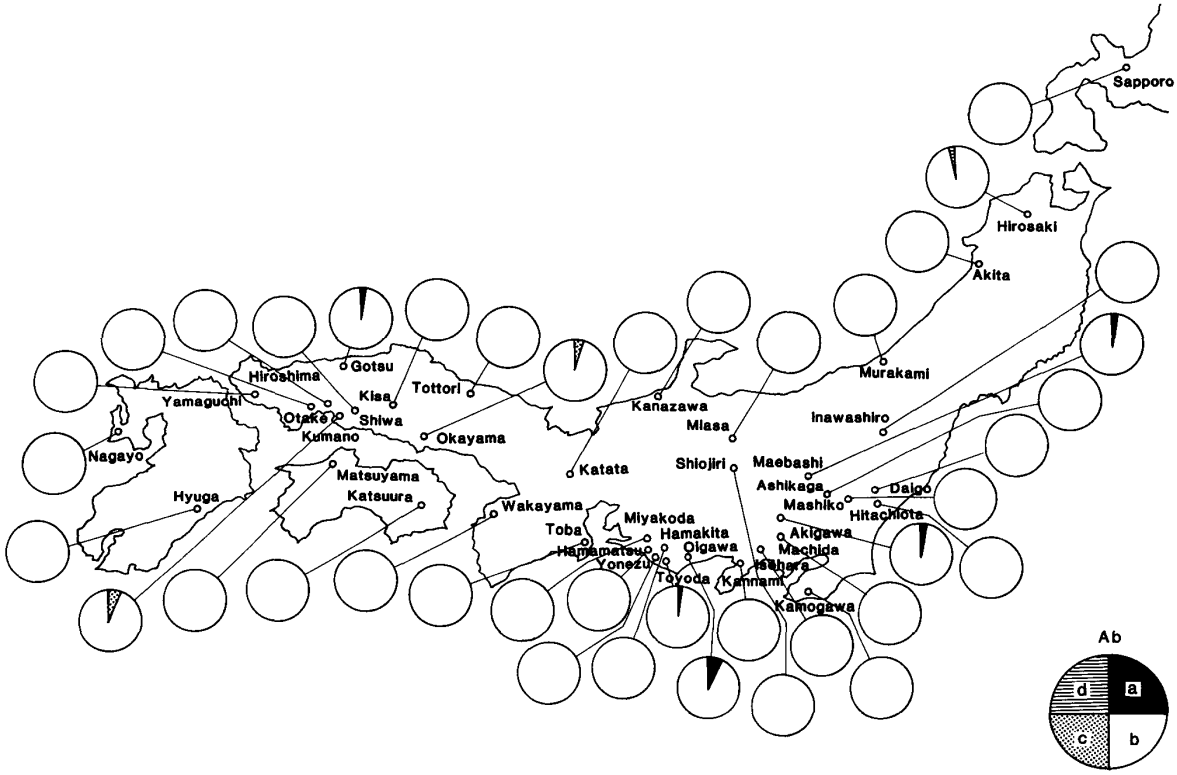

Fig. 20. Geographic distribution of Ab alleles among 40 populations of *Rana rugosa*.

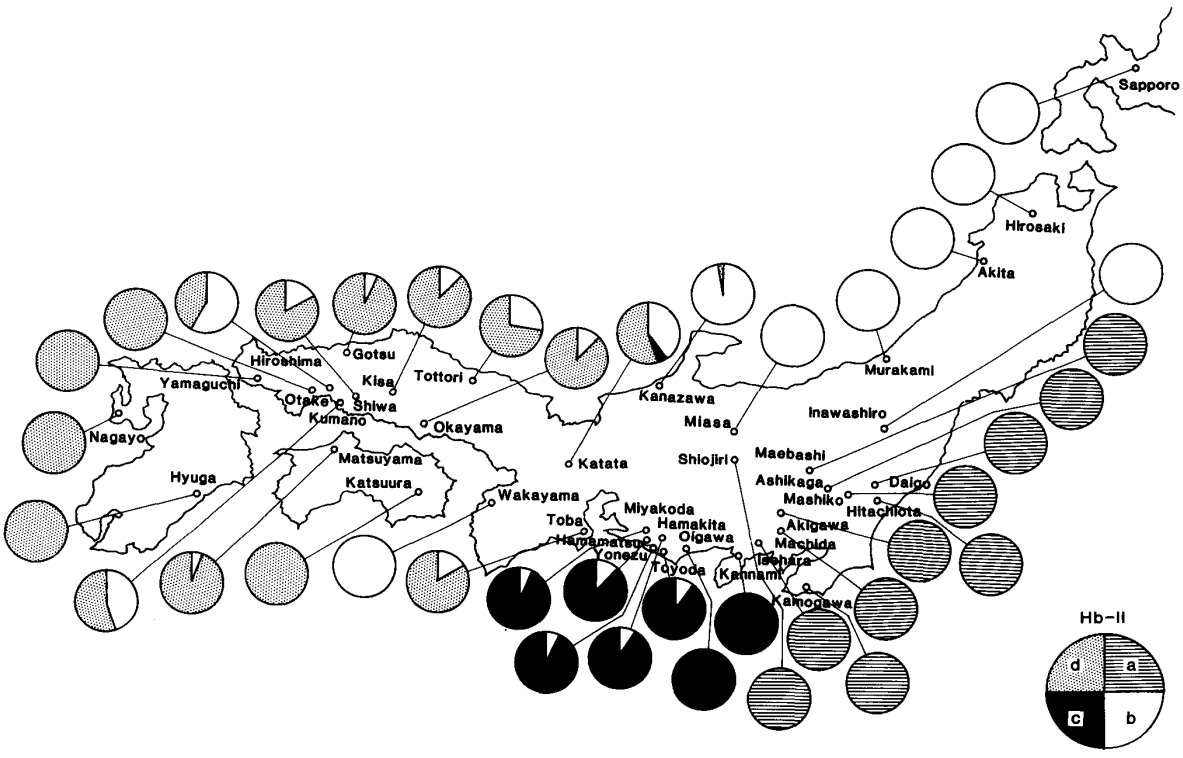

Fig. 21. Geographic distribution of Hb–II alleles among 40 populations of *Rana rugosa*.

Tohoku region, the Miasa population of the Chubu region, the two populations of the Hokuriku region and the Wakayama population of the Kinki region, were very high in frequencies of allele *b*, being 0.978~1.000, while only the Kanazawa population had allele *d* in a frequency of 0.022 in addition to allele *b*. The remaining seven populations had only allele *b*. The seven populations of the Chubu region other than the Shiojiri and Miasa populations had allele *c* in very high frequencies, being 0.875~1.000. Two populations, the Kannami and Oigawa populations, had only allele *c*, while the remaining five populations had allele *b* in frequencies of 0.071~0.125, in addition to allele *c*.

In 12 populations including the Toba population of the Kinki region, the seven populations of the Chugoku region other than the Kumano and Hiroshima populations, the two populations of the Shikoku region and the two populations of the Kyushu region, allele *d* was very high in frequency, being 0.725~1.000. Of these populations, seven including the Toba, Tottori, Gotsu, Okayama, Kisa, Shiwa and Matsuyama populations had allele *b* in frequencies of 0.063~0.275, in addition to allele *d*. In the Kumano population, alleles *d* and *b* were 0.548 and 0.452, respectively, in frequency, and in the Hiroshima population, alleles *b* and *d* were 0.579 and 0.421, respectively, in frequency. The Katata population had alleles *d*, *b* and *c* in frequencies of 0.553, 0.395 and 0.053, respectively (Table 6; Fig. 21).

III. Genetic variation

1. Fixation index (Fst)

The fixation index was calculated at 25 loci in 614 frogs belonging to the 40 populations of *Rana rugosa* by the method of WRIGHT (1978). When the gene frequencies at a definite locus are the same in all the 40 populations, the fixation index is zero, as no differentiation has occurred. When there is a characteristic allele at a definite locus in one or more populations, the fixation index is 1.000. The more advanced the differentiation in the locus, the higher becomes fixation index.

The results of examination of fixation indexes in the 40 populations of *R. rugosa* showed that the Pep-A locus was the most advanced in differentiation, being 0.979 in Fst. This seems due to the fact that the western populations have been differentiated from the eastern populations, as the 14 populations situated in the west of Wakayama including the Wakayama population had almost allele *b* in contrast to the 26 populations situated in the east of Wakayama which had almost allele *a*. Secondly, the LDH-B locus was 0.901 in Fst. This is attributable to the fact that 23 populations including 16 populations of the western Japan consisting of the Kinki, Chugoku, Shikoku and Kyushu regions and seven populations of Shizuoka Prefecture had almost allele *a*, while 16 populations including the Sapporo population, the three populations of the Tohoku region, the nine populations of the Kanto region, the two populations of Nagano Prefecture and the

TABLE 7
Fixation index at 25 loci in 40 populations of *Rana rugosa*

Locus	Fst	Locus	Fst
AAT-A	0.436	MPI	0.426
AAT-B	0.079	Pep-A	0.979
AK	0.514	Pep-B	0.476
CK	0.781	Pep-C	0.389
Fum	0.209	Pep-D	0.153
α-GDH	0.060	PGM	0.224
GPI	0.082	SOD-A	0.198
IDH-A	0.029	SOD-B	0.196
IDH-B	0.514	SORDH	0.332
LDH-A	0.414	Ab	0.045
LDH-B	0.901	Hb-I	0
MDH-A	0.092	Hb-II	0.863
MDH-B	0.795		

Murakami population of Niigata Prefecture, had almost allele *c*. The Hb-II locus was also large in Fst, being 0.863. This seems attributable to the fact that all the 10 populations including the nine populations of the Kanto region and the Shiojiri population of the Chubu region had only allele *a*, eight populations including the Sapporo population, the three populations of the Tohoku region, the two populations of the Hokuriku region, the Miasa population of the Chubu region and the Wakayama population of the Kinki region had almost allele *b*, seven populations of the Chubu region had almost allele *c*, and 12 populations including the Toba population of the Kinki region, the seven populations of the Chugoku region, the two populations of the Shikoku region and the two populations of the Kyushu region had almost allele *d*. The MDH-B locus was 0.795 in Fst. All the 27 populations situated in the east of Wakayama including the Wakayama population had only allele *c*. Of the other 13 populations, three populations, the Kisa, Yamaguchi and Hyuga populations, had only allele *a*, and the remaining 10 populations had allele *a* in frequencies of 0.071~0.971. Nine of the western 13 populations had allele *b* in frequencies of 0.029~0.600 and three populations had allele *c* in frequencies of 0.050~0.929. The CK locus was 0.781 in Fst. All the 24 populations of the eastern Japan including the Sapporo population, the 21 populations of the Tohoku, Kanto and Chubu regions, the Murakami population of the Hokuriku region and the Toba population of the Kinki region had only allele *a*, while among the 16 populations of the western Japan including the Kanazawa population of the Hokuriku region, the Katata and Wakayama populations of the Kinki region, and the 13 populations of the Chugoku, Shikoku and Kyushu regions, allele *b* was abundant in nine populations, allele *a* was abundant in five populations, and nearly an equal amount of alleles *a* and *b* was found in two populations.

Six loci, the AK, IDH-B, Pep-B, AAT-A, MPI and LDH-A loci, were 0.514~0.414 in decreasing order of Fst. Seven loci, the Pep-C, SORDH, PGM, Fum, SOD-A, SOD-B and Pep-D loci, were 0.389~0.153 in decreasing order of Fst. Six other loci, the MDH-A, GPI, AAT-B, α-GDH, Ab and IDH-A loci,

TABLE 8
Genetic variabilities at 25 loci in 40 populations of *Rana rugosa*

Population	Sample size	Mean proportion of heterozygous loci per individual (%)		Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
Sapporo	16	6.5	(5.7)	24.0	1.28
Hirosaki	18	4.2	(3.9)	20.0	1.20
Akita	17	4.5	(5.2)	12.0	1.16
Inawashiro	20	6.6	(7.1)	20.0	1.24
Daigo	22	11.5	(10.6)	32.0	1.48
Hitachiota	14	11.7	(13.7)	44.0	1.60
Mashiko	13	13.2	(13.1)	40.0	1.52
Ashikaga	11	12.6	(12.2)	28.0	1.48
Maebashi	17	10.9	(11.1)	44.0	1.52
Akigawa	17	8.7	(8.4)	36.0	1.48
Machida	11	10.2	(9.5)	28.0	1.36
Kamogawa	23	9.0	(8.5)	20.0	1.36
Isehara	20	10.8	(9.9)	36.0	1.48
Kannami	9	7.6	(6.0)	20.0	1.28
Oigawa	20	7.4	(8.1)	28.0	1.40
Hamakita	20	7.4	(7.8)	32.0	1.44
Hamamatsu	20	11.4	(11.8)	44.0	1.60
Miyakoda	20	7.9	(7.3)	32.0	1.44
Yonezu	20	9.0	(8.5)	36.0	1.40
Toyoda	20	9.6	(9.0)	36.0	1.44
Shiojiri	3	4.0	(2.9)	8.0	1.08
Miasa	2	14.0	(7.5)	16.0	1.16
Murakami	20	6.4	(5.6)	20.0	1.32
Kanazawa	23	16.9	(17.4)	52.0	1.80
Katata	23	16.9	(18.1)	52.0	1.84
Toba	3	16.0	(9.8)	24.0	1.24
Wakayama	1	12.0	(6.0)	12.0	1.12
Tottori	20	14.0	(13.9)	48.0	1.60
Gotsu	16	9.5	(10.5)	40.0	1.52
Okayama	20	15.6	(15.0)	56.0	1.80
Kisa	20	10.6	(10.2)	40.0	1.60
Shiwa	9	9.8	(9.6)	32.0	1.48
Kumano	21	9.0	(9.1)	52.0	1.60
Hiroshima	19	7.4	(10.2)	40.0	1.56
Otake	1	16.0	(8.0)	16.0	1.16
Yamaguchi	1	4.0	(2.0)	4.0	1.04
Katsuura	7	9.7	(11.2)	32.0	1.40
Matsuyama	16	8.1	(8.8)	36.0	1.44
Nagayo	21	10.1	(10.7)	36.0	1.56
Hyuga	20	6.0	(6.2)	16.0	1.32
Average	15.4	9.9	(9.3)	31.1	1.42

Parentheses show an expected value.

were 0.092~0.029 in decreasing order of Fst. The Hb-I locus was zero in Fst (Table 7).

2. Mean proportion of heterozygous loci

The mean proportion of heterozygous loci per individual at the 25 loci analyzed in each of the 40 populations of *Rana rugosa* was calculated. As shown in Table 8, it was found that the highest rate was 16.9% in each of the Kanazawa and Katata populations. The expected values in the two populations were 17.4% and 18.1%, 17.8% on the average. Five populations, the Toba, Otake, Okayama, Miasa and Tottori populations, were 16.0~14.0%, 15.1% on the average. The expected values were 15.0~7.5%, 10.8% on the average. The large differences between the observed and expected values seemed attributable to the fact that the sample sizes in the Toba, Otake and Miasa populations were small, having only one, two or three frogs. Six populations, the Mashiko, Ashikaga, Wakayama, Hitachiota, Daigo and Hamamatsu populations, were 13.2~11.4%, 12.1% on the average. The expected values were 13.7~6.0%, 11.2% on the average (12.3% on the average in five populations other than the Wakayama population having only one frog, that is, there was no difference between the observed and expected values). Five populations, the Maebashi, Isehara, Kisa, Machida and Nagayo populations, were 10.9~10.1%, 10.5% on the average. The expected values were 11.1~9.5%, 10.3% on the average. Seven populations, the Shiwa, Katsuura, Toyoda, Gotsu, Yonezu, Kumano and Kamogawa populations, were 9.8~9.0%, 9.4% on the average. The expected values were 11.2~8.5%, 9.5% on the average. Seven populations, the Akigawa, Matsuyama, Miyakoda, Kannami, Hiroshima, Oigawa and Hamakita populations, were 8.7~7.4%, 7.8% on the average. The expected values were 10.2~6.0%, 8.1% on the average. Four populations, the Inawashiro, Sapporo, Murakami and Hyuga populations, were 6.6~6.0%, 6.4% on the average. The expected values were 7.1~5.6%, 6.2% on the average. Four populations, the Akita, Hirosaki, Yamaguchi and Shiojiri populations, were 4.5~4.0%, 4.2% on the average. The expected values were 5.2~2.0%, 3.5% on the average. All the 40 populations were 16.9~4.0%, 9.9% on the average. The expected values were 18.1~2.0%, 9.3% on the average. In six populations, the Toba, Otake, Miasa, Wakayama, Yamaguchi and Shiojiri populations, which were very small, being one, two or three, in sample size, the differences between the observed and expected values were very large.

3. Mean proportion of polymorphic loci

When each of the alleles at one locus was contained at the rate of more than 1%, this locus was called polymorphic. When the mean proportions of polymorphic loci were estimated in each of the 40 populations of *Rana rugosa,* the highest value was 56.0% in the Okayama population. The following values were 52.0% in each of the Kanazawa, Katata and Kumano populations, 48.0% in the Tottori population, 44.0% in each of the Hitachiota, Maebashi and Hamamatsu populations, 40.0% in each of the Mashiko, Gotsu, Kisa and Hiroshima populations,

36.0% in each of the Akigawa, Isehara, Yonezu, Toyoda, Matsuyama and Nagayo populations, 32.0% in each of the Daigo, Hamakita, Miyakoda, Shiwa and Katsuura populations, 28.0% in each of the Ashikaga, Machida and Oigawa populations, 24.0% in each of the Sapporo and Toba populations, 20.0% in each of the Hirosaki, Inawashiro, Kamogawa, Kannami and Murakami populations, 16.0% in each of the Miasa, Otake and Hyuga populations, 12.0% in each of the Akita and Wakayama populations, 8.0% in the Shiojiri population, and 4.0% in the Yamaguchi population. The mean proportions of polymorphic loci in the 40 populations were 31.1% on the average (Table 8).

4. Mean number of alleles per locus

The largest mean number of alleles per locus among the 40 populations was 1.84 in the Katata population, followed by 1.80 in each of the Kanazawa and Okayama populations. The mean number of alleles per locus was 1.60 in each of the Hitachiota, Hamamatsu, Tottori, Kisa and Kumano populations, 1.56 in each of the Hiroshima and Nagayo populations, 1.52 in each of the Mashiko, Maebashi and Gotsu populations, 1.48 in each of the Daigo, Ashikaga, Akigawa, Isehara and Shiwa populations, 1.44 in each of the Hamakita, Miyakoda, Toyoda and Matsuyama populations, 1.40 in each of the Oigawa, Yonezu and Katsuura populations, 1.36 in each of the Machida and Kamogawa populations, 1.32 in each of the Murakami and Hyuga populations, 1.28 in each of the Kannami and Sapporo populations, 1.24 in each of the Inawashiro and Toba populations, 1.20 in the Hirosaki population, 1.16 in each of the Akita, Miasa and Otake populations, 1.12 in the Wakayama population, 1.08 in the Shiojiri population and 1.04 in the Yamaguchi population. The mean numbers of alleles per locus in the 40 populations were 1.42 on the average (Table 8).

IV. Genetic distances

Genetic distances were estimated by the method of Nei (1975) on the basis of the gene frequencies at 25 loci controlling 14 enzymes and two blood proteins obtained from 614 frogs of the 40 populations of *Rana rugosa* (Table 9). Of these populations, the eastern 27 populations consisted of the Sapporo population of the Hokkaido region, the three populations of the Tohoku region, the nine populations of the Kanto region, the nine populations of the Chubu region, the two populations of the Hokuriku region and the three populations of the Kinki region, while the remaining western 13 populations consisted of the nine populations of the Chugoku region, the two populations of the Shikoku region and the two populations of the Kyushu region.

Among these 40 populations, the smallest genetic distance was 0.003 between the Akigawa and Machida populations, while the largest was 0.492 between the Shiojiri and Nagayo populations. The genetic distances among the eastern 27 populations ranged from 0.003 to 0.292 with a mean of 0.117, while those among the western 13 populations ranged from 0.010 to 0.128 with a mean of 0.055.

1. The eastern 27 populations

The smallest of the genetic distances among the six populations (1~4, 22 and 23) including the Sapporo population of the Hokkaido region, the three populations of the Tohoku region, the Miasa population of the Chubu region and the Murakami population of the Hokuriku region, was 0.010 between the Inawashiro (4) and Murakami (23) populations, while the largest was 0.058 between the Sapporo (1) and Hirosaki (2) populations. The mean genetic distance among these six populations was 0.025 (Table 9).

The smallest of the genetic distances among the nine populations (5~13) of the Kanto region was 0.003 between the Akigawa (10) and Machida (11) populations, while the largest was 0.063 between the Hitachiota (6) and Maebashi (9) populations. The mean genetic distance among these nine populations was 0.025.

The smallest of the genetic distances among the seven populations (14~20) of

TABLE 9

Genetic identity(I) and genetic distance(D)

Population	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sapporo	1	—	.944	.953	.969	.854	.854	.840	.854	.831	.850	.854	.856	.868	.827	.805	.820	.826
Hirosaki	2	.058	—	.988	.963	.857	.862	.849	.881	.863	.880	.852	.885	.894	.842	.824	.829	.844
Akita	3	.048	.012	—	.986	.867	.866	.859	.892	.868	.883	.884	.894	.899	.852	.833	.835	.855
Inawashiro	4	.032	.037	.014	—	.876	.875	.874	.896	.867	.883	.881	.897	.893	.853	.848	.851	.868
Daigo	5	.158	.154	.143	.132	—	.953	.966	.970	.968	.970	.973	.961	.975	.864	.860	.865	.869
Hitachiota	6	.157	.149	.143	.133	.048	—	.985	.966	.936	.963	.970	.960	.970	.893	.887	.899	.894
Mashiko	7	.174	.164	.151	.134	.035	.015	—	.977	.953	.964	.971	.965	.965	.879	.886	.885	.896
Ashikaga	8	.158	.127	.115	.110	.030	.035	.023	—	.983	.987	.985	.995	.984	.879	.892	.877	.903
Maebashi	9	.185	.147	.141	.142	.032	.063	.048	.017	—	.990	.989	.983	.982	.853	.872	.853	.882
Akigawa	10	.163	.128	.125	.125	.030	.038	.037	.013	.010	—	.997	.991	.994	.876	.886	.877	.894
Machida	11	.158	.126	.124	.126	.028	.030	.030	.015	.011	.003	—	.987	.996	.877	.881	.875	.890
Kamogawa	12	.155	.122	.112	.109	.039	.041	.036	.005	.017	.009	.013	—	.986	.879	.896	.879	.905
Isehara	13	.141	.112	.106	.114	.025	.030	.036	.016	.019	.007	.004	.014	—	.888	.879	.879	.890
Kannami	14	.190	.173	.160	.158	.146	.113	.129	.129	.159	.132	.131	.129	.119	—	.975	.984	.976
Oigawa	15	.216	.193	.183	.165	.151	.120	.121	.114	.137	.121	.127	.110	.129	.025	—	.985	.996
Hamakita	16	.198	.188	.180	.161	.145	.107	.122	.131	.160	.131	.133	.129	.129	.016	.015	—	.984
Hamamatsu	17	.191	.169	.157	.142	.140	.112	.110	.102	.125	.112	.116	.100	.117	.024	.004	.016	—
Miyakoda	18	.221	.186	.176	.161	.154	.119	.116	.107	.131	.114	.120	.104	.124	.026	.004	.022	.005
Yonezu	19	.226	.199	.187	.168	.159	.109	.110	.115	.145	.125	.129	.115	.132	.029	.012	.027	.010
Toyoda	20	.218	.185	.179	.164	.155	.119	.121	.106	.123	.110	.116	.100	.123	.038	.006	.025	.009
Shiojiri	21	.238	.224	.207	.204	.065	.147	.093	.074	.048	.085	.076	.083	.088	.266	.227	.270	.208
Miasa	22	.035	.022	.011	.019	.128	.131	.145	.113	.139	.119	.117	.109	.096	.145	.178	.162	.154
Murakami	23	.016	.034	.017	.010	.133	.145	.152	.124	.151	.134	.133	.121	.113	.163	.189	.176	.163
Kanazawa	24	.111	.111	.093	.093	.152	.134	.141	.126	.152	.135	.134	.123	.120	.110	.132	.118	.104
Katata	25	.150	.142	.129	.123	.120	.121	.122	.103	.117	.107	.110	.097	.103	.056	.055	.060	.046
Toba	26	.199	.172	.157	.150	.149	.108	.123	.120	.146	.126	.125	.116	.117	.058	.059	.063	.054
Wakayama	27	.233	.232	.215	.188	.200	.209	.215	.205	.219	.203	.209	.199	.206	.145	.135	.123	.135
Tottori	28	.338	.330	.313	.294	.286	.258	.258	.271	.309	.277	.284	.274	.275	.170	.193	.176	.185
Gotsu	29	.347	.374	.344	.299	.300	.264	.262	.282	.326	.296	.302	.281	.300	.195	.188	.184	.189
Okayama	30	.343	.350	.333	.303	.283	.255	.260	.271	.307	.278	.283	.268	.280	.187	.188	.171	.184
Kisa	31	.420	.427	.406	.369	.348	.320	.317	.334	.370	.344	.351	.331	.349	.241	.239	.224	.233
Shiwa	32	.390	.381	.368	.341	.329	.268	.278	.306	.349	.305	.309	.303	.307	.198	.207	.189	.203
Kumano	33	.373	.350	.335	.309	.318	.267	.273	.292	.333	.295	.301	.290	.299	.191	.196	.179	.192
Hiroshima	34	.375	.364	.346	.317	.330	.287	.290	.308	.348	.315	.321	.306	.318	.205	.212	.191	.203
Otake	35	.398	.376	.361	.334	.312	.260	.267	.287	.324	.288	.294	.284	.291	.183	.190	.171	.187
Yamaguchi	36	.397	.371	.357	.336	.320	.275	.292	.298	.336	.298	.302	.293	.297	.199	.206	.190	.212
Katsuura	37	.289	.290	.269	.242	.221	.201	.200	.209	.240	.217	.222	.208	.215	.126	.134	.119	.125
Matsuyama	38	.367	.356	.345	.317	.298	.240	.251	.274	.315	.275	.279	.272	.279	.171	.178	.156	.172
Nagayo	39	.429	.412	.395	.377	.355	.333	.352	.348	.376	.348	.352	.340	.341	.245	.256	.240	.263
Hyuga	40	.367	.346	.331	.306	.287	.243	.254	.266	.300	.267	.272	.262	.269	.169	.173	.157	.175

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

the Chubu region other than the Shiojiri (21) and Miasa (22) populations was 0.004 between the Oigawa (15) and Hamamatsu (17) or Miyakoda (18) populations, while the largest was 0.038 between the Kannami (14) and Toyoda (20) populations. The mean genetic distance among these seven populations was 0.016. The smallest of the genetic distances between a group of the seven populations (14~20) and a group of the remaining two populations, the Shiojiri (21) and Miasa (22) populations, was 0.145 between the Kannami (14) and Miasa (22) populations, while the largest was 0.270 between the Hamakita (16) and Shiojiri (21) populations. The mean genetic distance between the group of these seven populations (14~20) and the group of the two populations (21 and 22) was 0.203. The genetic distance between the two populations (23 and 24) of the Hokuriku region was 0.093, while those among the three populations (25~27) of the Kinki region were 0.030~0.128 (Table 9).

among 40 populations of *Rana rugosa*

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
.802	.798	.804	.788	.965	.984	.895	.861	.819	.792	.713	.707	.710	.657	.677	.688	.687	.672	.672	.749	.693	.651	.693
.831	.820	.831	.800	.978	.967	.895	.868	.842	.793	.719	.688	.704	.652	.683	.705	.695	.687	.690	.749	.700	.662	.707
.839	.829	.836	.813	.989	.983	.911	.879	.855	.806	.732	.709	.717	.666	.692	.715	.708	.697	.700	.765	.708	.674	.718
.851	.845	.849	.815	.981	.990	.911	.884	.861	.828	.945	.742	.739	.691	.711	.734	.728	.716	.715	.785	.728	.686	.736
.857	.853	.856	.937	.880	.876	.859	.887	.862	.819	.751	.741	.753	.706	.720	.728	.719	.732	.726	.802	.742	.701	.751
.888	.897	.888	.863	.878	.865	.875	.886	.898	.811	.772	.768	.775	.726	.765	.765	.750	.771	.759	.818	.786	.717	.784
.890	.895	.886	.911	.865	.859	.868	.885	.884	.807	.773	.770	.771	.728	.757	.761	.748	.766	.747	.819	.778	.704	.776
.898	.891	.900	.929	.893	.884	.882	.902	.887	.815	.763	.754	.763	.716	.736	.747	.735	.751	.742	.812	.761	.706	.767
.877	.865	.884	.954	.870	.860	.859	.889	.864	.803	.734	.722	.736	.691	.705	.717	.706	.723	.715	.787	.730	.687	.741
.892	.882	.896	.918	.887	.875	.874	.898	.882	.816	.758	.744	.757	.709	.737	.745	.730	.750	.742	.805	.759	.706	.765
.887	.879	.891	.926	.889	.875	.874	.896	.882	.811	.752	.739	.753	.704	.735	.740	.725	.745	.740	.801	.757	.703	.762
.901	.892	.905	.921	.897	.886	.884	.907	.891	.820	.761	.755	.765	.718	.738	.749	.737	.753	.746	.812	.762	.712	.770
.883	.876	.884	.915	.908	.893	.887	.902	.890	.814	.760	.741	.756	.705	.735	.742	.728	.748	.743	.806	.757	.711	.764
.975	.971	.963	.767	.865	.849	.896	.945	.943	.865	.843	.823	.829	.786	.820	.826	.814	.833	.819	.882	.843	.783	.844
.996	.989	.994	.797	.837	.828	.876	.947	.942	.874	.825	.829	.829	.788	.813	.822	.809	.827	.814	.875	.837	.774	.841
.978	.974	.976	.764	.851	.838	.889	.942	.939	.884	.839	.832	.843	.799	.828	.836	.826	.843	.827	.888	.856	.786	.855
.995	.990	.991	.812	.857	.849	.902	.955	.947	.874	.831	.828	.832	.792	.816	.826	.816	.829	.809	.882	.842	.769	.839
—	.993	.995	.796	.839	.828	.887	.946	.943	.867	.829	.827	.826	.787	.818	.828	.814	.832	.814	.875	.841	.769	.842
.007	—	.983	.784	.828	.820	.886	.937	.945	.857	.829	.833	.826	.790	.824	.831	.817	.834	.808	.877	.845	.760	.839
.005	.017	—	.804	.839	.826	.885	.942	.938	.868	.812	.813	.818	.773	.803	.812	.798	.815	.807	.860	.827	.765	.832
.228	.244	.219	—	.812	.814	.801	.835	.788	.747	.654	.657	.669	.624	.617	.627	.622	.628	.629	.713	.638	.612	.651
.175	.189	.175	.208	—	.989	.923	.889	.865	.826	.741	.717	.728	.675	.700	.722	.716	.705	.714	.774	.717	.691	.730
.188	.198	.191	.206	.011	—	.911	.881	.849	.815	.736	.722	.724	.674	.693	.714	.710	.697	.698	.772	.709	.676	.718
.120	.120	.122	.222	.080	.093	—	.924	.894	.849	.802	.769	.798	.749	.773	.790	.786	.757	.757	.826	.793	.723	.764
.056	.065	.059	.181	.118	.126	.079	—	.970	.918	.872	.861	.877	.833	.852	.853	.842	.842	.851	.917	.873	.825	.859
.058	.056	.064	.238	.145	.164	.112	.030	—	.880	.853	.855	.855	.811	.843	.839	.821	.856	.851	.908	.863	.819	.873
.143	.154	.142	.292	.191	.204	.164	.086	.128	—	.890	.879	.898	.862	.875	.895	.890	.839	.886	.912	.889	.864	.863
.188	.187	.209	.424	.299	.307	.220	.137	.158	.116	—	.947	.974	.951	.974	.972	.955	.929	.951	.935	.961	.919	.921
.190	.183	.207	.421	.333	.326	.263	.150	.157	.129	.054	—	.963	.963	.959	.963	.959	.934	.962	.936	.945	.946	.966
.191	.191	.201	.403	.317	.324	.226	.132	.156	.107	.026	.038	—	.983	.978	.976	.966	.931	.969	.943	.978	.936	.933
.240	.236	.258	.472	.393	.395	.289	.182	.209	.148	.050	.037	.017	—	.965	.974	.976	.925	.956	.929	.965	.933	.930
.200	.194	.219	.482	.357	.367	.257	.160	.171	.134	.026	.042	.022	.036	—	.987	.973	.923	.973	.928	.978	.929	.925
.189	.185	.208	.467	.326	.337	.236	.159	.176	.110	.028	.038	.025	.027	.013	—	.990	.929	.973	.929	.974	.933	.933
.206	.202	.226	.474	.334	.342	.240	.172	.197	.177	.047	.042	.035	.025	.027	.010	—	.918	.959	.925	.963	.924	.934
.184	.182	.204	.466	.350	.362	.279	.172	.155	.176	.073	.068	.072	.078	.080	.074	.085	—	.906	.942	.931	.880	.959
.206	.213	.214	.464	.337	.359	.278	.161	.162	.121	.051	.038	.031	.045	.028	.027	.042	.099	—	.913	.957	.957	.942
.133	.131	.151	.338	.256	.259	.191	.087	.096	.092	.067	.066	.058	.074	.075	.074	.078	.060	.091	—	.966	.888	.939
.173	.169	.190	.449	.333	.344	.231	.136	.147	.117	.039	.057	.022	.036	.022	.026	.037	.072	.044	.035	—	.913	.926
.262	.274	.268	.492	.370	.392	.324	.192	.199	.147	.084	.055	.066	.069	.074	.070	.080	.128	.044	.119	.091	—	.932
.172	.175	.184	.430	.315	.331	.269	.152	.135	.147	.082	.035	.069	.073	.078	.069	.068	.041	.060	.063	.077	.071	—

distance(D) is given below.

2. The western 13 populations

The smallest of the genetic distances among the nine populations (28~36) of the Chugoku region in the western Japan was 0.010 between the Kumano (33) and Hiroshima (34) populations, while the largest was 0.099 between the Otake (35) and Yamaguchi (36) populations. The mean genetic distance was 0.043 among these nine populations. The smallest of the genetic distances among the four populations (37~40) of the Shikoku and Kyushu regions was 0.035 between the Katsuura (37) and Matsuyama (38) populations, while the largest was 0.119 between the Katsuura (37) and Nagayo (39) populations. The mean genetic distance among these four populations was 0.076 (Table 9).

3. The four populations (1~4) of the Hokkaido and Tohoku regions and other populations

The smallest of the genetic distances between a group of the four populations (1~4) including the Sapporo population of the Hokkaido region and the three populations of the Tohoku region and a group of the nine populations (5~13) of the Kanto region was 0.106 between the Akita (3) and Isehara (13) populations, while the largest was 0.185 between the Sapporo (1) and Maebashi (9) populations. The mean genetic distance between the group of the four populations (1~4) and the group of the nine populations (5~13) was 0.138 (Table 9).

The smallest of the genetic distances between the group of the former four populations (1~4) and a group of the eight populations (14~21) of the Chubu region other than the Miasa (22) population was 0.142 between the Inawashiro (4) and Hamamatsu (17) populations, while the largest was 0.238 between the Sapporo (1) and Shiojiri (21) populations. The mean genetic distance between the group of the four populations (1~4) and the group of the eight populations (14~21) was 0.186.

The smallest of the genetic distances between the group of the former four populations (1~4) and a group of the three populations (22~24) including the Miasa (22) population of the Chubu region and the two populations (23 and 24) of the Hokuriku region was 0.010 between the Inawashiro (4) and Murakami (23) populations, while the largest was 0.111 between the Kanazawa (24) and Sapporo (1) or Hirosaki (2) populations. The mean genetic distance between the group of the four populations (1~4) and the group of the three populations (22~24) was 0.048.

The smallest of the genetic distances between the group of the former four populations (1~4) and a group of the three populations (25~27) of the Kinki region was 0.123 between the Inawashiro (4) and Katata (25) populations, while the largest was 0.233 between the Sapporo (1) and Wakayama(27) populations. The mean genetic distance between the group of the four populations (1~4) and the group of the three populations (25~27) was 0.174.

The smallest of the genetic distances between the group of the former four populations (1~4) and a group of the 13 populations (28~40) in the western Japan

was 0.242 between the Inawashiro (4) and Katsuura (37) populations, while the largest was 0.429 between the Sapporo (1) and Nagayo (39) populations. The mean genetic distance between the group of the four populations (1~4) and the group of the 13 populations (28~40) was 0.350 (Table 9).

4. The nine populations (5~13) of the Kanto region and other populations

The smallest of the genetic distances between the group of the nine populations (5~13) of the Kanto region and a group of the 14 populations (14~27) of the Chubu, Hokuriku and Kinki regions was 0.048 between the Maebashi (9) and Shiojiri (21) populations, while the largest was 0.219 between the Maebashi (9) and Wakayama (27) populations. The mean genetic distance between the group of the nine populations (5~13) and the group of the 14 populations (14~27) was 0.128.

The smallest of the genetic distances between the group of the former nine populations (5~13) and the group of the 13 populations (28~40) in the western Japan was 0.200 between the Mashiko (7) and Katsuura (37) populations, while the largest was 0.376 between the Maebashi (9) and Nagayo (39) populations. The mean genetic distance between the group of the nine populations (5~13) and the group of the 13 populations (28~40) was 0.292 (Table 9).

5. The nine populations (14~22) of the Chubu region and other populations

a. Seven populations(14~20) of the Chubu region

The smallest of the genetic distances between a group of the seven populations (14~20) of the Chubu region other than the Shiojiri and Miasa populations (21 and 22) in Nagano Prefecture and a group of the two populations (25 and 26) including the Katata and Toba of the Kinki region was 0.046 between the Hamamatsu (17) and Katata (25) populations, while the largest was 0.065 between the Yonezu (19) and Katata (25) populations. The mean genetic distance between the group of the seven populations (14~20) and the group of the two populations (25 and 26) was 0.058.

The smallest of the genetic distances between the group of the former seven populations (14~20) and a group of the three populations (23, 24 and 27) including the two populations of the Hokuriku region and the Wakayama population of the Kinki region was 0.104 between the Hamamatsu (17) and Kanazawa (24) populations, while the largest was 0.198 between the Yonezu (19) and Murakami (23) populations. The mean genetic distance between the group of the seven populations (14~20) and the group of the three populations (23, 24 and 27) was 0.146.

The smallest of the genetic distances between the group of the former seven populations (14~20) and the group of the 13 populations (28~40) in the western Japan was 0.119 between the the Hamakita (16) and Katsuura (37) populations, while the largest was 0.274 between the Yonezu (19) and Nagayo (39) populations. The mean genetic distance between the group of the seven populations (14~20) and the group of the 13 populations (28~40) was 0.195 (Table 9).

b. Two populations (21 and 22) of the Chubu region

The smallest of the genetic distances between a group of the two populations (21 and 22) of the Chubu region and the group of the two populations (23 and 24) of the Hokuriku region was 0.011 between the Miasa (22) and Murakami (23) populations, while the largest was 0.222 between the Shiojiri (21) and Kanazawa (24) populations. The mean genetic distance between the group of the two populations (21 and 22) and the group of the two populations (23 and 24) was 0.130.

The smallest of the genetic distances between the group of the former two populations (21 and 22) and the group of the three populations (25~27) of the Kinki region was 0.118 between the Miasa (22) and Katata (25) populations, while the largest was 0.292 between the Shiojiri (21) and Wakayama (27) populations. The mean genetic distance between the group of the two populations (21 and 22) and the group of the three populations (25~27) was 0.194.

The smallest of the genetic distances between the group of the former two populations (21 and 22) and the group of the 13 populations (28~40) in the western Japan was 0.256 between the Miasa (22) and Katsuura (37) populations, while the largest was 0.492 between the Shiojiri (21) and Nagayo (39) populations. The mean genetic distance between the group of the two populations (21 and 22) and the group of the 13 populations (28~40) was 0.389 (Table 9).

6. The two populations (23 and 24) of the Hokuriku region and other populations

The smallest of the genetic distances between the group of the two populations (23 and 24) of the Hokuriku region and the group of the three populations (25~27) of the Kinki region was 0.079 between the Kanazawa (24) and Katata (25) populations, while the largest was 0.204 between the Murakami (23) and Wakayama (27) populations. The mean genetic distance between the group of the two populations (23 and 24) and the group of the three populations (25~27) was 0.142.

The smallest of the genetic distances between the group of the former two populations (23 and 24) and the group of the 13 populations (28~40) in the western Japan was 0.191 between the Kanazawa (24) and Katsuura (37) populations, while the largest was 0.395 between the Murakami (23) and Kisa (31) populations. The mean genetic distance between the group of the two populations (23 and 24) and the group of the 13 populations (28~40) was 0.298 (Table 9).

7. The three populations (25~27) of the Kinki region and other populations

The smallest of the genetic distances between the group of the three populations (25~27) of the Kinki region and the group of the 13 populations (28~40) in the western Japan was 0.087 between the Katata (25) and Katsuura (37) populations, while the largest was 0.209 between the Toba (26) and Kisa (31) populations. The mean genetic distance between the group of the three populations (25~27) and the group of the 13 populations (28~40) was 0.148 (Table 9).

V. Dendrogram

A dendrogram was drawn on the basis of the genetic distances among the 40 populations of *Rana rugosa* by the UPGMA method (SNEATH and SOKAL, 1973; NEI, 1975). It showed that *Rana rugosa* was first divided into the eastern and western groups. The former contained 26 populations (1~26) of the eastern Japan, while the latter contained 14 populations (27~40) of the western Japan. The eastern group was then divided into three subgroups, northern, intermediate and southern.

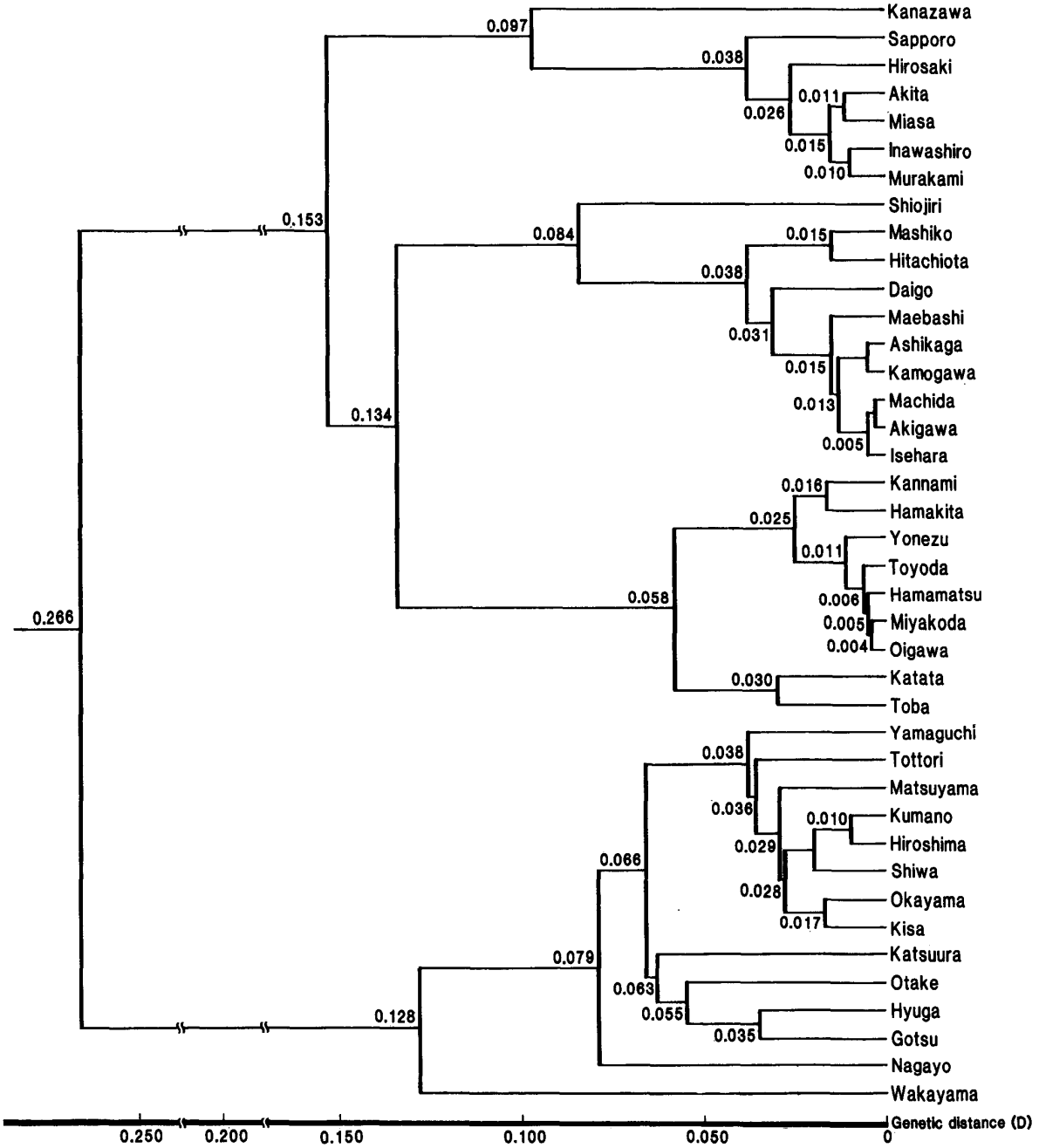

Fig. 22. Dendrogram for 40 populations of *Rana rugosa* based on genetic distances.

The first subgroup contained seven populations including the Sapporo population of the Hokkaido region, the Hirosaki, Akita and Inawashiro populations of the Tohoku region, the Miasa population of the Chubu region and the Murakami and Kanazawa populations of the Hokuriku region. The second subgroup contained 10 populations including the nine populations of the Kanto region, the Daigo, Hitachiota, Mashiko, Ashikaga, Maebashi, Akigawa, Machida, Kamogawa and Isehara populations, and the Shiojiri population distributed in the Chubu region. The third subgroup contained nine populations including the seven (14~20) distributed in Shizuoka Prefecture, the Kannami, Oigawa, Hamakita, Hamamatsu, Miyakoda, Yonezu and Toyoda populations, and two populations, the Katata and Toba populations, distributed in the Kinki region (Fig. 22).

DISCUSSION

The dendrogram of *Rana rugosa* drawn on the basis of the genetic distances among the 40 populations by the UPGMA method showed that this species was first divided into the eastern and western groups. The eastern group was then divided into three subgroups, the northern, intermediate and southern.

The northern subgroup consisted of seven populations, the Kanazawa (24), Sapporo (1), Hirosaki (2), Akita (3), Miasa (22), Inawashiro (4) and Murakami (23) populations. The genetic distances among these seven populations ranged from 0.010 between the Inawashiro and Murakami populations to 0.111 between the Kanazawa and Sapporo or Hirosaki populations with a mean of 0.046. The sex chromosomes in six of these seven populations other than the Miasa population were preliminarily examined by NISHIOKA, MIURA and HANADA (1990), and it was clarified that all of them were of the ZW–ZZ type. Thus, the northern subgroup of the eastern group of *R. rugosa* seemed to be the populations which were of the ZW–ZZ type in sex-determining mechanism and distributed in the Hokkaido, Tohoku and Hokuriku regions.

The intermediate subgroup of the eastern group shown in the dendrogram of *R. rugosa* consisted of 10 populations, the Shiojiri (21), Mashiko (7), Hitachiota (6), Daigo (5), Maebashi (9), Ashikaga (8), Kamogawa (12), Machida (11), Akigawa (10) and Isehara (13) populations. Nine of these 10 populations other than the Shiojiri population which is situated in the Chubu region are all situated in the Kanto region. The genetic distances among these 10 populations ranged from 0.003 between the Akigawa and Machida populations to 0.147 between the Hitachiota and Shiojiri populations with a mean of 0.037, while the genetic distances between these 10 populations and the former seven populations (1~4, 22~24) of the northern subgroup ranged from 0.096 to 0.238 with a mean of 0.143. The sex chromosomes were examined in six of these 10 populations, the Hitachiota, Daigo, Maebashi, Kamogawa, Machida and Isehara populations. It was found that they were not morphologically distinguishable from each other and the sex-determining mechanism was obscure (NISHIOKA, MIURA and HANADA, 1990; NISHIOKA, HANADA and MIURA, unpublished).

The southern subgroup of the eastern group shown in the dendrogram of *R. rugosa* consisted of nine populations, the Kannami (14), Hamakita (16), Yonezu (19), Toyoda (20), Hamamatsu (17), Miyakoda (18), Oigawa (15), Katata (25) and Toba (26) populations. Seven of these nine populations other than the Katata and Toba populations, which are situated in the Kinki region, are all situated in the Chubu region. The genetic distances among these nine populations ranged from 0.004 between the Oigawa population and the Hamamatsu or Miyakoda population to 0.065 between the Yonezu and Katata populations with a mean of 0.033. The genetic distances between these nine populations and the former seven populations (1~4, 22~24) of the northern subgroup ranged from 0.079 to 0.226 with a mean of 0.164, while the genetic distances between these nine populations and the former 10 populations (5~13, 21) of the intermediate subgroup ranged from 0.097 to 0.270 with a mean of 0.134. The sex chromosomes were examined in six of these nine populations, the Hamakita, Yonezu, Hamamatsu, Miyakoda, Toba and Katata populations (NISHIOKA, MIURA and HANADA, 1990). It was found that they were of the ZW–ZZ type only in the Katata population, while they were of the XX–XY type in the other five populations. However, it was noteworthy that the Y chromosome of the XY pair differed from the X chromosome in shape. It seems to be important that the sex-determining mechanism in the Katata population was of the ZW–ZZ type in contrast to those of the other populations of this subgroup.

The western group shown in the dendrogram of *R. rugosa* consisted of 14 populations, the Yamaguchi (36), Tottori (28), Matsuyama (38), Kumano (33), Hiroshima (34), Shiwa (32), Okayama (30), Kisa (31), Katsuura (37), Otake (35), Hyuga (40), Gotsu (29), Nagayo (39) and Wakayama (27) populations. Of these 14 populations, the Wakayama population is situated in the Kinki region, the Katsuura and Matsuyama populations in the Shikoku region, the Nagayo and Hyuga populations in the Kyushu region, and the remaining nine populations in the Chugoku region. The genetic distances among these 14 populations ranged from 0.010 between the Kumano and Hiroshima populations to 0.148 between the Wakayama and Kisa populations with a mean of 0.065. The genetic distances between these 14 populations (27~40) and the seven populations (1~4, 22~24) of the northern subgroup which are of the ZW–ZZ type in sex-determining mechanism ranged from 0.164 to 0.429 with a mean of 0.323. Those between the above 14 populations and the 10 populations (5~13, 21) of the intermediate subgroup which were of the obscure type ranged from 0.199 to 0.492 with a mean of 0.301, while those between the foregoing 14 populations and the nine populations (14~20, 25, 26) of the southern subgroup which are of the XX–XY type in sex-determining mechanism ranged from 0.086 to 0.274 with a mean of 0.183. KAWAMURA and NISHIOKA (1977), KASHIWAGI (1993) and NISHIOKA, MIURA and SAITOH (1993) have reported that *R. rugosa* distributed around Hiroshima is of the XX–XY type in sex-determining mechanisms on the basis of the results of breeding experiments using the sex-reversed males. Although the sex chromosomes were examined in five populations of the western group, the Okayama,

Hiroshima, Kumano, Gotsu and Nagayo populations, by NISHIOKA, MIURA and HANADA (1990), and NISHIOKA, HANADA and MIURA (unpublished), there was no heterozygous chromosome pair, but all the 13 chromosome pairs were completely homozygous in shape and C- or LR-banding patterns. Thus, this type of sex-determining mechanism may be called to be of the XX–XY type having no morphological differences between the X and Y chromosomes.

Of the four types of sex-determining mechanisms, the ZW–ZZ type, obscure type, XX–XY type having morphological differences between the X and Y chromosomes and XX–XY type having no morphological differences between the X and Y chromosomes, the last type seems to be the most primitive type which the ancestors of this species had when they invaded Japan. After these ancestors occupied the wide western areas of a mild climate, they gradually invaded the eastern areas. While the western group has kept the XX–XY type in sex-determining mechanism until the present age, the eastern group divided into three subgroups, each of which had developed a characteristic sex-determining mechanism. Such a differentiation must have required a very long time. It seems to be important to compare the genetic distances among populations of *R. rugosa* with those of various other species distributed in Japan in order to ascertain the oldness of the differentiation of the sex-determining mechanisms in this species.

The genetic distances between the Sapporo population (1) and the 14 western populations (27~40) of *R. rugosa* ranged from 0.233 between the Sapporo and Wakayama (27) populations to 0.429 between the Sapporo and Nagayo (39) populations with a mean of 0.362, while those between the Shiojiri (21) population and the 14 western populations ranged from 0.292 between the Shiojiri and Wakayama (27) populations to 0.492 between the Shiojiri and Nagayo (39) populations with a mean of 0.434.

In *Bufo* distributed widely in Japan, the genetic distances among the 39 populations of *Bufo japonicus* including *B. j. japonicus, B. j. montanus, B. j. torrenticola* and *B. j. yakushimensis* were very small, that is, they ranged from 0.003 between the Hirosaki and Namioka populations to 0.271 between the Namioka and Ono populations with a mean of 0.074 (KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI, 1990). In contrast, the genetic distances between *B. j. miyakonis* distributed on Miyako Island and the above 39 populations of *B. japonicus* ranged from 0.366 between the Miyako and Zama populations to 0.521 between the Miyako and Ono populations with a mean of 0.418. This seems to show that *miyakonis* is very different subspecies from the others. The genetic distances between the two populations of *B. japonicus torrenticola* and the 27 eastern populations of *B. japonicus* ranged from 0.085 to 0.205 with a mean of 0.126, while those between the former two populations and the 10 western populations ranged from 0.044 to 0.127 with a mean of 0.066. NISHIOKA, SUMIDA, UEDA and WU (1990) also reported in *Bufo* that the genetic distances between *Bufo japonicus gargarizans* from China and five other subspecies, *B. j. japonicus, B. j. montanus, B. j. yakushimensis, B. j. torrenticola* and *B. j. miyakonis,* ranged from 0.112 between *gargarizans* and *miyakonis* to 0.383 between *gargarizans* and *japonicus* from Kagoshima with a mean of 0.305. The

genetic distances between *B. j. gargarizans* from Taiwan and the above five subspecies ranged from 0.235 between *gargarizans* and *japonicus* from Zama to 0.366 between *gargarizans* and *miyakonis* with a mean of 0.294.

In *Hyla,* NISHIOKA, SUMIDA and BORKIN (1990) reported on the genetic distances between 11 populations, the Sakhalin, Kunashiri, Sapporo, Setana, Hirosaki, Ichinoseki, Odawara, Maibara, Hiroshima, Tsushima and Suwon (Korea) populations, of *Hyla japonica.* Among nine of these populations other than the Suwon and Tsushima populations, the genetic distances ranged from 0.012 between the Sakhalin and Sapporo populations to 0.102 between the Kunashiri and Hiroshima populations with a mean of 0.048. The genetic distances between the Suwon population and nine of the above populations other than the Tsushima population ranged from 0.137 between the Suwon and Hiroshima populations to 0.201 between the Suwon and Kunashiri populations with a mean of 0.167. The genetic distances between the Tsushima population and nine of the above populations other than the Suwon (Korea) populations ranged from 0.050 between the Tsushima and Hiroshima populations to 0.132 between the Tsushima and Kunashiri populations with a mean of 0.094. The Suwon and Tsushima populations were 0.059 in genetic distance. These seem to show that the genetic distances in *Rana rugosa* are very large in comparison with those in *Hyla.*

NISHIOKA, SUMIDA and OHTANI (1992) ascertained that the genetic distances among 45 populations of *Rana nigromaculata* distributed in Japan ranged from 0.0002 between the Okaya and Sutama populations to 0.206 between the Tottori or Matsue and Namioka populations with a mean of 0.078. Those between the Suwon population (Korea) and the above 45 populations ranged from 0.061 between the Suwon and Kumamoto populations to 0.146 between the Suwon and Namioka populations with a mean of 0.098. Those between the Beijing population (China) and the above 45 populations ranged from 0.109 between the Beijing and Matsumoto populations to 0.229 between the Beijing and Namioka populations with a mean of 0.147. Those between the Namioka population in the Tohoku region and nine populations of the Chubu region ranged from 0.156 to 0.204 with a mean of 0.180, while those between the Namioka population and the 11 populations of the Chugoku region ranged from 0.080 between the Namioka and Hiroshima populations to 0.206 between the Namioka population and the Tottori or Matsue population with a mean of 0.127. These also seem to show that the genetic distances in *R. rugosa* are very large as compared with those of *R. nigromaculata.*

In European green frogs, NISHIOKA and SUMIDA (1992) reported that the genetic distances among three populations of *Rana lessonae* from Luxembourg, Poland and Firenze ranged from 0.016 to 0.166 with a mean of 0.115. Those among three populations of *Rana esculenta* from Wien, Ukraine and Heidelberg ranged from 0.027 to 0.083 with a mean of 0.064, while those among three populations of *Rana ridibunda* from Belgorod, Roscoff and Adana ranged from 0.055 to 0.086 with a mean of 0.074. On the other hand, those between the three populations of *R. lessonae* and the three populations of *R. esculenta* ranged from 0.164 to 0.245 with a

mean of 0.197. Those between the three populations of *R. esculenta* and the three populations of *R. ridibunda* ranged from 0.165 to 0.289 with a mean of 0.231.

In brown frogs, NISHIOKA, SUMIDA, BORKIN and WU (1992) examined the genetic distances among 13 populations of *Rana japonica*. The genetic distances between the seven northern populations of *R. japonica* including the Morioka, Ichinoseki, Wakuya, Fukushima, Sukagawa, Utsunomiya and Isehara populations and the six southern populations of *R. japonica* including the Shizuoka, Toyama, Fukui, Hiroshima, Geihoku and Oki populations ranged from 0.055 to 0.182 with a mean of 0.102. It was found that the genetic distances in *R. rugosa* are very large as compared with those in *R. japonica*.

In *Rana limnocharis*, NISHIOKA and SUMIDA (1990) examined the genetic distances among six populations including the Okayama, Higashihiroshima, Hiroshima, Okinawa, Iriomote and Taiwan populations. The genetic distances between the Taiwan population and the other four populations other than the Iriomote population ranged from 0.137 to 0.169 with a mean of 0.153, and those between the Iriomote population and the other five populations ranged from 0.276 to 0.345 with a mean of 0.305.

NISHIOKA, OHTA and SUMIDA (1987) reported on genetic distances in *Rana tagoi*. The genetic distances among six populations of *R. t. tagoi*, the Nabara, Omogo, Kurama, Ono, Hirado and Oki populations, ranged from 0.031 between the Nabara and Omogo populations to 0.283 between the Nabara and Oki populations with a mean of 0.160. Those between *R. t. yakushimensis* and the above six populations of *R. t. tagoi* ranged from 0.182 to 0.335 with a mean of 0.274. Although the populations of *R. tagoi* are geographically and topographically isolated from each other, it seems to be strange that the populations of *R. rugosa* which are popular in distribution and ecology are remarkably larger in genetic distance than those of *R. tagoi*. This seems to be attributable to oldness of the origin of *R. rugosa*.

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