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Differentiation of 70 Populations in the *Rana nigromaculata* Group by the Method of Electrophoretic Analyses

By

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ABSTRACT

Seventeen kinds of enzymes and two kinds of blood proteins extracted from 1777 frogs of 47 populations of *Rana nigromaculata*, 220 frogs of 12 populations of *R. brevipoda brevipoda* and 340 frogs of 11 populations of *R. brevipoda porosa* were analyzed by the method of horizontal starch-gel electrophoresis. There were 7.6 phenotypes produced by 4.1 alleles on the average at each of 28 loci. The gene frequencies were described in 25 of the 28 loci other than three which had each a single allele. The fixation index (Fst), average heterozygosity, proportion of polymorphic loci and mean number of alleles per locus were calculated in all the populations of *R. nigromaculata* and *R. brevipoda*.

Genetic distances were estimated by the method of NEI (1975) on the basis of the gene frequencies at 28 loci controlling 17 enzymes and two blood proteins. The genetic distances between the 47 populations of *R. nigromaculata* and the 23 populations of *R. brevipoda* were 0.133~0.768, 0.546 on the average. The genetic distances among the 45 populations of *R. nigromaculata* other than the Suwon and Beijing were 0.000 ~0.206, while those among the 12 populations of *R. brevipoda brevipoda* and those among the 11 populations of *R. brevipoda porosa* were 0.009~0.134 and 0.000~0.066, respectively.

Of 10 districts where *R. nigromaculata* and *R. brevipoda* were sympatric, Shibata, Niigata Prefecture, was most peculiar in that the two species were 0.133 in genetic distance. In the Matsumoto and Maibara districts, the genetic distances were 0.417 and 0.375, respectively, while in the other seven districts, they were 0.509~0.737.

A dendrogram was drawn for the 47 populations of *R. nigromaculata* and the 23 populations of *R. brevipoda* on the basis of the genetic distances among them by the UPGMA method. *R. brevipoda* seems to have invaded Japan earlier than *R. nigromaculata*, and to have produced *R. brevipoda porosa* by frequent hybridization with *R. nigromaculata* mainly at the Shibata district and introgression of *R. nigromaculata* genes for a long period.

INTRODUCTION

The specific name *Rana nigromaculata* was given by HALLOWELL (1860). He reported at the same time on *Rana marmorata* collected from Shimoda, Shizuoka Prefecture. COPE (1868) named pond frogs collected from Kanagawa as *Tomopterna porosa*. STEJNEGER (1907) placed *R. marmorata* and *T. porosa* as synonyms of *R.*

nigromaculata. According to him, the Japanese pond frogs distributed in Honshu, Shikoku and Kyushu belonged to only one species, *R. nigromaculata*. SCHMIDT (1927), TERENTJEV (1927) and OKADA (1931), moreover, considered that all the pond frogs distributed in Japan belonged to only one subspecies, *R. nigromaculata nigromaculata*.

In 1941, ITO found that there were two types of pond frogs in Nagoya district. One type was *Rana nigromaculata nigromaculata*, while the other was named *Rana nigromaculata brevipoda* by himself. MORIYA (1951) discovered the sympatric distribution of these two subspecies in Okayama district and found that the *R. nigromaculata brevipoda* was more uniform in color and pattern than that distributed in Nagoya district. He observed in detail the differences in morphological, ecological and embryological characters between the two subspecies found in Okayama district. The frogs which were assumed to be natural hybrids between the two subspecies had been seldom found in Okayama district in spite of the easiness of artificial production of hybrids between them. The two subspecies seemed to be isolated from each other mainly by ecological factors. MORIYA (1960b) confirmed that the males of reciprocal hybrids between the two subspecies were almost completely sterile. On the basis of MORIYA's observations and experiments, MOORE (1955, 1960, 1962) repeatedly expressed the view that each of *Rana nigromaculata nigromaculata* and *Rana nigromaculata brevipoda* was a valid species.

On the other hand, MORIYA (1954) collected numerous specimens of the pond frogs from various districts of Japan, and examined their morphological characters. On the basis of their morphological and distributional differences, he divided Japanese pond frogs into the following five races: 1) *nigromaculata* common, 2) Niigata intermediate, 3) Tokyo intermediate, 4) Nagoya *brevipoda*, and 5) Okayama *brevipoda*. Of these races, the Niigata intermediate was elucidated by KAWAMURA and NISHIOKA (1973, 1977, 1979) to be a mixture of the Tokyo intermediate race, natural hybrids between the latter and the *nigromaculata* common race, and the offspring of natural hybrids (KAWAMURA, 1962). They also clarified that natural hybridization was frequently occurring between the *nigromaculata* common race and the Tokyo intermediate race in Niigata district. According to MORIYA (1960a, b), the *nigromaculata* common race differed from the other four races in many respects of reproduction, and the males of reciprocal hybrids between the former race and the latter four races were very poor in reproductive ability, while the females seemed to be quite fertile. Mainly on the basis of MORIYA's observations, KAWAMURA (1962) considered it reasonable to divide Japanese pond frogs into the following two species, *Rana nigromaculata* HALLOWELL and *Rana brevipoda* ITO, and one subspecies, *Rana brevipoda porosa* (COPE). KAWAMURA and NISHIOKA (1978) produced reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* and their offspring which were repeatedly backcrossed by the paternal and maternal species until B_4 offspring were produced. Recently, MATSUI and HIKIDA (1985) proposed *Rana porosa porosa* in place of *Rana brevipoda porosa* on the basis of description on only three specimens by COPE (1868). In contrast to KAWAMURA's consideration that *R. brevipoda porosa* was derived from

hybridization between *R. nigromaculata* and *R. brevipoda*, MATSUI and HIKIDA stated that the hybrid status of *porosa* has not been demonstrated, in disregard of the actual existence of natural hybrids between the two species and their offspring. These natural hybrids and their offspring are easily found, especially in recent years in most sympatric areas of both species.

The present study was designed to clarify the present status and the origin of the Japanese pond frogs by electrophoretic analyses of enzymes and blood proteins.

Electrophoretic analyses of enzymes and blood proteins have been performed to elucidate the intraspecific or interspecific differentiation in *Rana tagoi* by NISHIOKA, OHTA and SUMIDA (1987), *Buergeria, Rhacophorus* and *Polypedates* by NISHIOKA, SUMIDA, OHTA and SUZUKI (1987), *Rana narina* by NISHIOKA, UEDA and SUMIDA (1987), *Bufo japonicus* by KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI (1990), 13 *Bufo* species and subspecies by NISHIOKA, SUMIDA, UEDA and WU (1990), *Rana limnocharis* by NISHIOKA and SUMIDA (1990), *Hyla* by NISHIOKA, SUMIDA and BORKIN (1990), six pond frog species by NISHIOKA and SUMIDA (1992), and 12 brown frog species by NISHIOKA, SUMIDA, BORKIN and WU (1992).

MATERIALS AND METHODS

The materials used in the present study consisted of 2337 frogs of 70 populations collected from various districts of Japan, Korea and China during the 10 years between 1977 and 1986. They included 1777 frogs of 47 populations belonging to *Rana nigromaculata*, 340 frogs of 11 populations belonging to *R. brevipoda porosa* and 220 frogs of 12 populations belonging to the Nagoya and Typical races of *R. brevipoda brevipoda* (MORIYA, 1954). These frogs were frozen and preserved in REVCO's stocker at −80°C. The collecting stations, dates, numbers of frogs and population names are shown in Tables 1 and 2.

Of the 47 populations of *R. nigromaculata*, the Sakata was collected by Mr. T. IGARASHI, three of the Matsumoto, Chino and Nagoya were by Mr. R. SHIMOYAMA, the Ina was by Dr. K. TATEISHI, the Yamaguchi was by Dr. H. SAMBUICHI, the Nangoku was by Dr. K. UTSUMI, the Matsuyama was by Mr. Y. MYOREI, the Sasebo was by Mr. M. ODA, two of the Munakata and Suwon were by Dr. M. KURAMOTO, the Kagoshima was by Dr. S. ISHIKUBO, the Oita was by Mr. S. SATO, and the Beijing was by Dr. Chih-Ye CHANG.

Of the 23 populations of *R. brevipoda*, the Maki of *R. b. porosa* was collected by Mr. K. KINEBUCHI, four of the Nagano, Iiyama, Shinano and Matsumoto of the same subspecies and the Tsu of the Nagoya race of *R. b. brevipoda* by Mr. R. SHIMOYAMA and the Ina of the Nagoya race of the same subspecies by Dr. K. TATEISHI.

Fourteen kinds of enzymes of skeletal muscles, three kinds of enzymes of livers and two kinds of blood proteins extracted from frogs were analyzed by the method of horizontal starch-gel electrophoresis. The kinds of enzymes and blood proteins analyzed, their abbreviations, E. C. Nos., samples used for electrophoresis and buffer systems are shown in Table 3. The method of electrophoresis has been

TABLE 1

Collecting station, prefecture, area, date, number of *Rana nigromaculata* and the name of population

Area	Prefecture	Station	Date	No. of frogs	Population	(No.)
Tohoku	Aomori	Namioka-city	Sep. 1986	19	Namioka	(1)
	Akita	Akita-city	Aug. 1978	6	Akita	(2)
	Yamagata	Sakata-city	〃	48	Sakata	(3)
Hokuriku	Niigata	Shibata-city	〃	16	Shibata	(4)
		Niigata-city	〃	20	Niigata	(5)
		Kashiwazaki-city	〃	26	Kashiwazaki	(6)
		Joetsu-city	〃	36	Joetsu	(7)
	Toyama	Toyama-city	〃	37	Toyama	(8)
	Ishikawa	Kanazawa-city	〃	55	Kanazawa	(9)
	Fukui	Sakai-gun, Mikuni	〃	25	Mikuni	(10)
Chubu	Nagano	Matsumoto-city	Oct. 1983	30	Matsumoto	(11)
		Okaya-city	Aug. 1978	64	Okaya	(12)
		Chino-city, Miyagawa	Sep. 1983	30	Chino	(13)
		Ina-city	June 1979	2	Ina	(14)
		Iida-city	Aug. 1978	40	Iida	(15)
	Yamanashi	Kitakoma-gun, Sutama	〃	35	Sutama	(16)
	Shizuoka	Mishima-city	June 1979	67	Mishima	(17)
	Aichi	Nagoya-city	Oct. 1981	30	Nagoya	(18)
		Inazawa-city, Osato	Oct. 1979	5	Inazawa	(19)
Kinki	Shiga	Sakata-gun, Maibara	1978～1986	21	Maibara	(20)
	Mie	Ueno-city	Oct. 1979	48	Igaueno	(21)
	Wakayama	Shingu-city	〃	6	Shingu	(22)
	Osaka	Higashiosaka-city	〃	38	Higashiosaka	(23)
	Hyogo	Himeji-city	〃	6	Himeji	(24)
Chugoku	Tottori	Tottori-city	Sep. 1980	64	Tottori	(25)
	Shimane	Matsue-city	〃	75	Matsue	(26)
		Gotsu-city	〃	23	Gotsu	(27)
	Yamaguchi	Hagi-city	Sep. 1978	66	Hagi	(28)
		Yamaguchi-city, Yahara	〃	52	Yamaguchi	(29)
	Hiroshima	Hiroshima-city	1976～1980	60	Hiroshima	(30)
		Aki-gun, Kumano	1976～1986	56	Kumano	(31)
		Kure-city, Hiro	May 1981	19	Hiro	(32)
		Miyoshi-city	Sep. 1986	19	Miyoshi	(33)
		Shobara-city	May 1981	19	Shobara	(34)
	Okayama	Asakuchi-gun, Konko	1976～1978	83	Konko	(35)
Shikoku	Kagawa	Takamatsu-city	Sep. 1978	42	Takamatsu	(36)
	Kochi	Nangoku-city	June 1979	32	Nangoku	(37)
	Ehime	Uwajima-city	〃	39	Uwajima	(38)
		Matsuyama-city	〃	23	Matsuyama	(39)
Kyushu	Fukuoka	Munakata-city	Sep. 1978	58	Munakata	(40)
	Nagasaki	Sasebo-city	〃	58	Sasebo	(41)
	Kumamoto	Kumamoto-city	Oct. 1978	49	Kumamoto	(42)
	Kagoshima	Kagoshima-city	〃	60	Kagoshima	(43)
	Miyazaki	Miyazaki-city	Sep. 1980	56	Miyazaki	(44)
	Oita	Oita-city	May 1979	55	Oita	(45)
Korea		Suwon	June 1977	38	Suwon	(46)
China		Beijing	Sep. 1979	21	Beijing	(47)
Total				1777	47 populations	

TABLE 2

Collecting station, prefecture, date, number of *Rana brevipoda* and the name of population

	Prefecture	Station	Date	No. of frogs	Population (No.)
R. b. porosa	Iwate	Morioka-city	Aug. 1978	26	Morioka (48)
		Nishiiwai-gun, Hiraizumi	〃	9	Hiraizumi (49)
		Ichinoseki-city	〃	39	Ichinoseki (50)
	Fukushima	Sukagawa-city	June 1979	43	Sukagawa (51)
	Tochigi	Utsunomiya-city	Aug. 1978	56	Utsunomiya (52)
	Niigata	Shibata-city	〃	38	Shibata (53)
		Nishikanbara-gun, Maki	Aug. 1977	29	Maki (54)
	Nagano	Nagano-city	Sep. 1977	19	Nagano (55)
		Iiyama-city	Oct. 1981	30	Iiyama (56)
		Kamiminochi-gun, Shinano	〃	8	Shinano (57)
		Matsumoto-city	Aug. 1982	43	Matsumoto (58)
R. b. brevipoda Nagoya race	Nagano	Ina-city	June 1979	69	Ina (59)
	Aichi	Inazawa-city, Osato	Oct. 1979	1	Inazawa (60)
	Gifu	Hashima-city	May 1977	1	Gifuhashima (61)
	Shiga	Sakata-gun, Maibara	1978～1986	46	Maibara (62)
	Mie	Tsu-city	Oct. 1981	30	Tsu (63)
		Ueno-city	Oct. 1979	4	Igaueno (64)
	Osaka	Higashiosaka-city	〃	20	Higashiosaka (65)
	Hyogo	Amagasaki-city	May 1977	1	Amagasaki (66)
R. b. brevipoda Typical race	Okayama	Kurashiki-city, Tamashima	May 1981	4	Tamashima (67)
		Asakuchi-gun, Konko	June 1978	38	Konko (68)
	Hiroshima	Shobara-city	May 1981	4	Shobara (69)
		Aki-gun, Kumano	May 1977	2	Kumano (70)
Total				560	23 populations

TABLE 3

Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	E.C.No.	Sample	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	3.5.4.4	〃	〃
Adenylate kinase	AK	2.7.4.3	〃	〃
Alcohol dehydrogenase	ADH	1.1.1.1	Liver	T-B-E pH 8.0
Creatine kinase	CK	2.7.3.2	Skeletal muscle	〃
Fumarase	Fum	4.2.1.2	Liver	〃
α-Glycerophosphate dehydrogenase	α-GDH	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	〃	T-B-E pH 8.0
Isocitrate dehydrogenase	IDH	1.1.1.42	〃	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	〃	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	〃	〃
Malic enzyme	ME	1.1.1.40	〃	T-C pH 7.0
Mannose phosphate isomerase	MPI	5.3.1.8	〃	〃
Peptidase	Pep	3.4.3.1	Liver	T-B-E pH 8.0
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	Skeletal muscle	T-C pH 7.0
Phosphoglucomutase	PGM	2.7.5.1	〃	T-B-E pH 8.0
Superoxide dismutase	SOD	1.15.1.1	〃	〃
Serum albumin	Ab	—	Blood serum	〃
Hemoglobin	Hb	—	Erythrocyte	T-B-E pH 8.6

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

reported previously by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was performed by the agar overlay method of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. The detection of blood proteins was made by the amido-black staining method.

When each of the multiple alleles exists in a frequency of more than 1% at a locus, this locus is regarded to be polymorphic. As a measure for indicating the degree of genetic differentiation in a definite locus between different populations, fixation index (Fst) by WRIGHT (1978) was used. In order to show genetic variations quantitatively among different populations, the mean proportions of polymorphic loci per population and the mean proportions of heterozygous loci per individual (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974) were used.

The genetic relationships among populations, subspecies and species were evaluated by calculating the genetic distances (D) by the method of NEI (1975). A dendrogram was drawn on the basis of these genetic distances by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975).

OBSERVATION

I. Electrophoretic patterns and alleles

The electrophoretic patterns of 17 enzymes extracted from skeletal muscles and livers, and two blood proteins were analyzed in 2337 frogs of 70 populations including 47 populations of *Rana nigromaculata*, 12 populations of *R. b. brevipoda* and 11 populations of *R. b. porosa*. These enzymes and blood proteins were found to be controlled by genes at 28 loci. 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 three of the 28 loci, the AAT-A, CK and IDH-A loci, there was a single phenotype, AA, produced by allele *a*. At the MDH-A locus, two phenotypes, AA and AB, produced by alleles *a* and *b* were observed. At 10 loci, the ADH-A, AK, PGM, GPI, Pep-A, SOD-B, AAT-B, Fum, Pep-B and 6-PGD loci, there were four to six phenotypes produced by three alleles, *a*~*c*. At six loci, the LDH-A, ADH-B, ME-A, LDH-B, Pep-C and Hb loci, five to eight phenotypes produced by four alleles, *a*~*d*, were observed. At the ADA and Pep-D loci, 11 phenotypes produced by five alleles, *a*~*e*, were observed. At the α-GDH and MDH-B loci, seven and 12 phenotypes produced by six alleles, *a*~*f*, were found. At the MPI and Ab loci, 14 phenotypes produced by seven alleles, *a*~*g*, were observed. At the IDH-B locus, there were 18 phenotypes produced by eight alleles, *a*~*h*. The ME-B locus was the most polymorphic and 31 phenotypes produced by 11 alleles, *a*~*k*, were observed.

On the average, there were 7.6 phenotypes produced by 4.1 alleles at each of the 28 loci (Table 4).

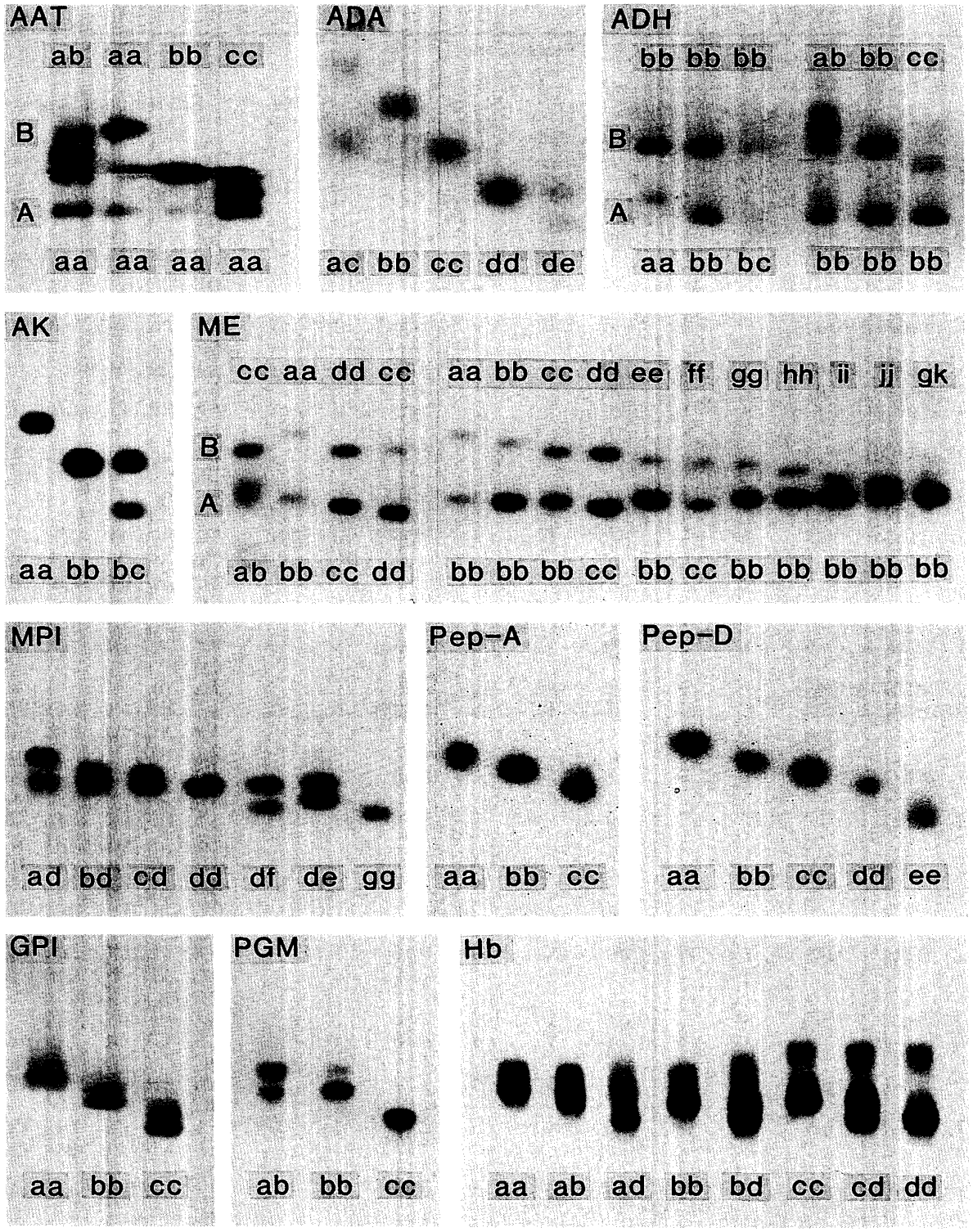

Fig. 1. Electrophoretic patterns of 10 enzymes, AAT, ADA, ADH, AK, ME, MPI, Pep-A, Pep-D, GPI and PGM, and one blood protein, Hb, in 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

TABLE 4

Number of phenotypes and alleles at 28 loci in *Rana nigromaculata* and *R. brevipoda*

Locus		*Rana nigromaculata*			*R. b. brevipoda* and *R. b. porosa*			Total			Kind of phenotypes
		No. of frogs	No. of alleles	No. of phenotypes	No. of frogs	No. of alleles	No. of phenotypes	No. of frogs	No. of alleles	No. of phenotypes	
1	AAT-A	1162	1	1	454	1	1	1616	1	1	AA
2	AAT-B	1162	3	6	454	3	3	1616	3	6	AA, BB, CC, AB, AC, BC
3	ADA	1162	5	9	454	3	4	1616	5	11	BB, CC, DD, EE, AB, AC, BC, BD, CD, CE, DE
4	ADH-A	1144	3	4	436	2	3	1580	3	4	AA, BB, AB, BC
5	ADH-B	1144	3	4	436	3	4	1580	4	6	BB, CC, DD, AB, BC, BD
6	AK	1162	2	2	454	2	3	1616	3	4	AA, BB, AB, BC
7	CK	1162	1	1	454	1	1	1616	1	1	AA
8	Fum	1144	3	6	436	2	2	1580	3	6	AA, BB, CC, AB, AC, BC
9	α-GDH	1733	6	7	500	2	3	2233	6	7	AA, CC, AC, BC, CD, CE, CF
10	GPI	1162	3	5	453	3	4	1616	3	5	AA, BB, CC, AB, BC
11	IDH-A	1733	1	1	500	1	1	2233	1	1	AA
12	IDH-B	1733	7	15	500	3	6	2233	8	18	BB, CC, DD, EE, FF, GG, AG, BC, BD, BE, BF, CE, CG, CH, DF, EF, EG, EH
13	LDH-A	1733	4	5	500	1	1	2233	4	5	CC, DD, AC, BC, CD
14	LDH-B	1733	4	5	500	3	6	2233	4	7	BB, CC, DD, AB, BC, BD, CD
15	MDH-A	1733	2	2	500	1	1	2233	2	2	AA, AB
16	MDH-B	1733	6	11	500	3	5	2233	6	12	AA, BB, CC, DD, EE, AB, AC, AE, BC, BD, BF, DF
17	ME-A	1162	4	6	454	2	2	1616	4	6	BB, CC, DD, AB, BC, BD
18	ME-B	1162	11	29	454	4	7	1616	11	31	AA, BB, CC, DD, EE, FF, GG, HH, II, JJ, AC, AG, AI, BC, BG, BI, CD, CE, CG, CH, CI, CJ, CK, DF, DG, EG, FG, GH, GI, GK, HI
19	MPI	1162	7	10	454	5	9	1616	7	14	AA, BB, CC, DD, EE, GG, AD, AE, BD, CD, CE, DE, DF, DG
20	Pep-A	1144	3	5	436	3	5	1580	3	5	AA, BB, CC, AB, BC
21	Pep-B	1144	3	6	436	3	4	1580	3	6	AA, BB, CC, AB, AC, BC
22	Pep-C	1144	4	5	434	3	6	1578	4	7	AA, BB, DD, AB, AD, BC, BD
23	Pep-D	1144	5	9	436	3	6	1580	5	11	AA, BB, CC, DD, EE, AB, AD, BC, BD, BE, DE
24	6-PGD	1162	3	6	454	3	5	1616	3	6	AA, BB, CC, AB, AC, BC
25	PGM	1162	3	4	454	2	2	1616	3	4	BB, CC, AB, BC
26	SOD-B	1733	2	3	500	3	5	2233	3	5	AA, BB, CC, AB, BC
27	Ab	1589	5	7	534	5	10	2123	7	14	AA, BB, CC, DD, EE, FF, AB, BC, BD, BE, BF, DE, EF, EG
28	Hb	1696	4	6	541	3	6	2237	4	8	AA, BB, CC, DD, AB, AD, BD, CD
Average		1355.0	3.9	6.4	468.5	2.6	4.1	1823.5	4.1	7.6	

II. Gene frequency

As three of the 28 loci, the AAT-A, CK and IDH-A loci, had a single allele, the gene frequencies were described in the other 25 loci.

1. AAT-B locus

Electrophoretic patterns at the AAT-B locus were analyzed in 1616 frogs of 70 populations including 1162 frogs of 47 populations belonging to *Rana nigromaculata*

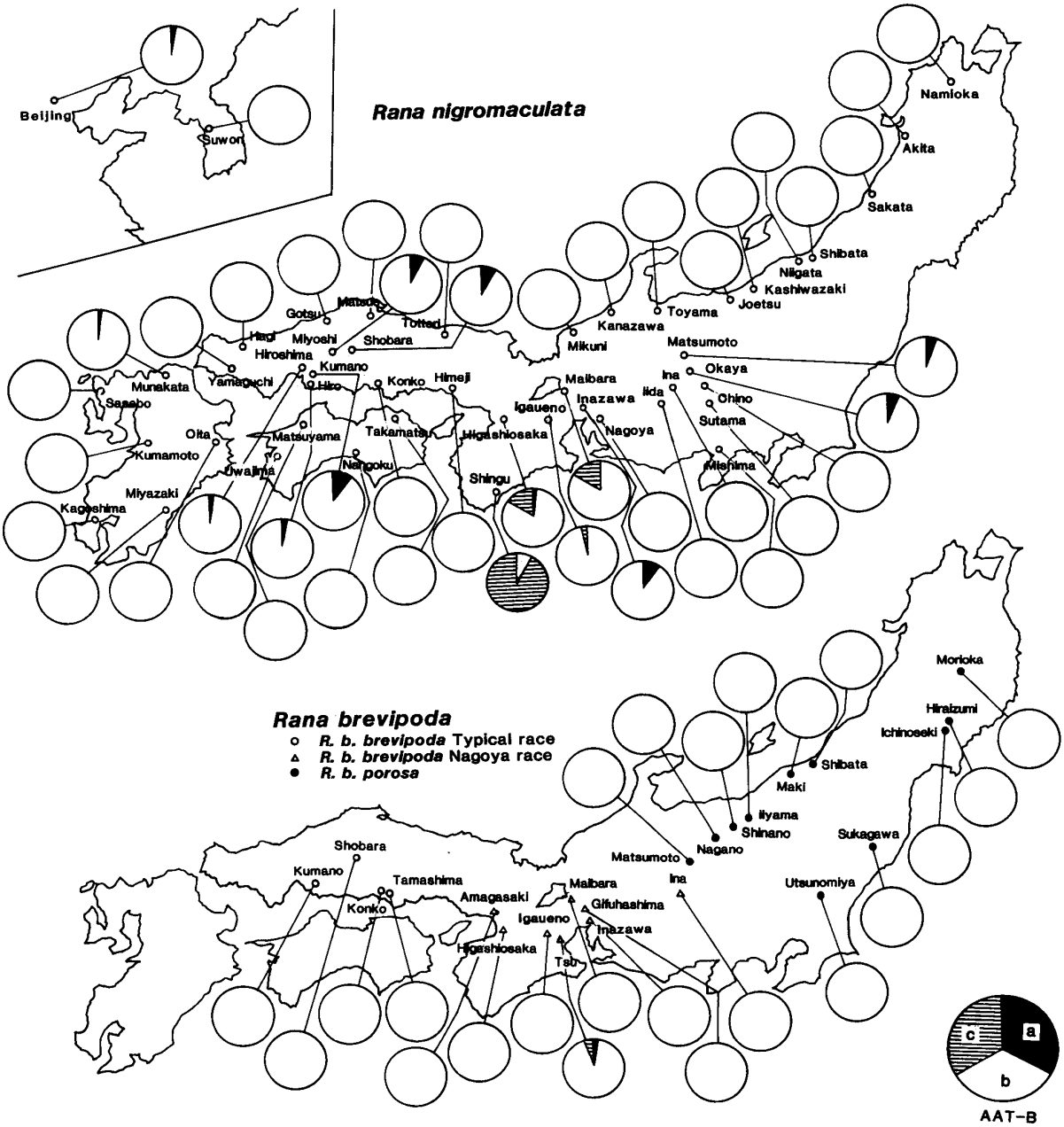

Fig. 2. Geographic distribution of AAT-B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

TABLE 5

Gene frequencies at 25 loci in 70 populations of

Species	Population	Sample size	AAT-B			ADA					ADH-A			ADH-B				AK		
			a	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*a*	*b*	*c*
R. nigroma-	Namioka	19		1.000					1.000			1.000			1.000				1.000	
〃 *culata*	Akita	6		1.000					1.000			1.000			1.000				1.000	
〃	Sakata	48		1.000					1.000			1.000			0.983	0.017			1.000	
〃	Shibata	16		1.000					1.000			1.000			1.000				1.000	
〃	Niigata	20		1.000					1.000			1.000			1.000				1.000	
〃	Kashiwazaki	26		1.000					1.000			1.000			1.000				1.000	
〃	Joetsu	36		1.000					1.000			1.000			0.983	0.017			1.000	
〃	Toyama	37		1.000				0.167	0.833			1.000			0.967	0.033			1.000	
〃	Kanazawa	55		1.000				0.438	0.563			1.000			0.844	0.156			1.000	
〃	Mikuni	25		1.000				0.780	0.220			1.000			0.393	0.607			1.000	
〃	Matsumoto	30	0.050	0.950				0.817	0.183			1.000			1.000				1.000	
〃	Okaya	64	0.050	0.950				0.983	0.017			1.000			1.000				1.000	
〃	Chino	30		1.000				1.000				1.000			1.000				1.000	
〃	Ina	2		1.000				1.000				1.000			1.000				1.000	
〃	Iida	40		1.000				1.000				1.000			1.000				1.000	
〃	Sutama	35		1.000				1.000				1.000			1.000				1.000	
〃	Mishima	67		1.000				1.000				1.000		0.017	0.983				1.000	
〃	Nagoya	30		1.000				1.000				1.000			1.000				1.000	
〃	Inazawa	5	0.100	0.900				0.900	0.100			1.000			1.000				1.000	
〃	Maibara	21		0.833	0.167			0.667	0.333		0.119	0.881			1.000				1.000	
〃	Igaueno	48		0.979	0.021			0.885	0.115		0.010	0.990			1.000				1.000	
〃	Shingu	6		0.083	0.917			1.000				1.000			1.000				1.000	
〃	Higashiosaka	38	0.013	0.829	0.158			0.724	0.276		0.039	0.961			1.000				1.000	
〃	Himeji	6		1.000				0.833	0.167			1.000			0.750	0.250			1.000	
〃	Tottori	64		1.000				1.000				1.000				1.000			1.000	
〃	Matsue	75		1.000				1.000				1.000				1.000			1.000	
〃	Gotsu	23		1.000				1.000				1.000			0.300	0.700			1.000	
〃	Hagi	66		1.000				0.883	0.117			1.000			1.000				1.000	
〃	Yamaguchi	52		1.000				0.800	0.200			1.000			1.000				1.000	
〃	Hiroshima	60	0.017	0.983				0.850	0.150			1.000			0.750	0.250			1.000	
〃	Kumano	56	0.105	0.895				0.974	0.026			1.000			0.461	0.539			0.987	0.013
〃	Hiro	19	0.029	0.971				0.471	0.529			1.000			0.765	0.235			1.000	
〃	Miyoshi	19	0.079	0.921				0.763	0.237			1.000			0.368	0.632			1.000	
〃	Shobara	19	0.083	0.917				0.694	0.306			1.000			0.472	0.528			1.000	
〃	Konko	83		1.000				1.000				1.000			0.967	0.033			1.000	
〃	Takamatsu	42		1.000				0.917	0.083			1.000			1.000				1.000	
〃	Nangoku	32		1.000				0.050	0.950			1.000			1.000				1.000	
〃	Uwajima	39		1.000				1.000				1.000			1.000				1.000	
〃	Matsuyama	23		1.000				1.000				1.000			1.000				1.000	
〃	Munakata	58	0.017	0.983			0.067	0.667	0.267			1.000			0.950	0.050			1.000	
〃	Sasebo	58		1.000				0.767	0.233			1.000			0.950	0.050			1.000	
〃	Kumamoto	49		1.000			0.033	0.950	0.017			1.000			0.833	0.167			1.000	
〃	Kagoshima	60		1.000		0.250	0.367	0.383				1.000			0.933	0.067			1.000	
〃	Miyazaki	56		1.000		0.067	0.483	0.450				1.000			0.317	0.683			1.000	
〃	Oita	55		1.000			0.650	0.350				1.000			1.000				1.000	
〃	Suwon	38		1.000			0.306	0.639	0.056			0.972	0.028		1.000				1.000	
〃	Beijing	21	0.024	0.976			0.310	0.476	0.167	0.048		1.000			1.000				1.000	
R. b. porosa	Morioka	26		1.000					0.808	0.192		1.000			1.000				1.000	
〃	Hiraizumi	9		1.000					1.000			1.000			1.000				1.000	
〃	Ichinoseki	39		1.000					0.983	0.017		1.000			1.000				1.000	
〃	Sukagawa	43		1.000					1.000			1.000			1.000				1.000	
〃	Utsunomiya	56		1.000					0.750	0.250		1.000			0.844		0.156	0.156	0.844	
〃	Shibata	38		1.000					1.000			1.000			1.000				1.000	
〃	Maki	29		1.000					1.000			1.000			1.000				1.000	
〃	Nagano	19		1.000					1.000			1.000			1.000				1.000	
〃	Iiyama	30		1.000					1.000			1.000			1.000				1.000	
〃	Shinano	8		1.000					1.000			1.000			1.000				1.000	
〃	Matsumoto	43		1.000				0.105	0.895			1.000			1.000				1.000	
R. b. brevi-	Ina	69		1.000				0.091	0.909		0.939	0.061			1.000				1.000	
〃 *poda*	Inazawa	1		1.000					1.000		1.000				1.000				1.000	
〃	Gifuhashima	1		1.000					1.000		1.000				1.000				1.000	
〃	Maibara	46		1.000				0.033	0.967		0.903	0.097		0.032	0.968				1.000	
〃	Tsu	30	0.017	0.933	0.050				1.000		1.000				1.000				1.000	
〃	Igaueno	4		1.000					1.000		0.625	0.375			1.000				1.000	
〃	Higashiosaka	20		1.000				0.025	0.975		0.325	0.675			1.000				1.000	
〃	Amagasaki	1		1.000					1.000		1.000				1.000				1.000	
〃	Tamashima	4		1.000					1.000		1.000				1.000				1.000	
〃	Konko	38		1.000					1.000		1.000				1.000				1.000	
〃	Shobara	4		1.000					1.000		1.000				1.000				1.000	
〃	Kumano	2		1.000					1.000		1.000				1.000				1.000	

Rana nigromaculata and *Rana brevipoda* (I)

Fun			α-GDH						GPI			IDH-B							LDH-A			
a	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*a*	*b*	*c*	*d*
1.000					1.000				0.026	0.974		1.000										1.000	
1.000					1.000					1.000		1.000										1.000	
1.000					0.990	0.010				1.000		0.948				0.052						1.000	
1.000			0.438		0.563					1.000		0.781					0.219					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				0.067	0.933		0.903				0.097						1.000	
1.000					1.000				0.150	0.850		0.892				0.108						1.000	
1.000					1.000					1.000		0.564				0.436						1.000	
1.000					1.000					1.000		0.660				0.340					0.100	0.900	
1.000			0.083		0.917					1.000		0.883				0.017	0.100					1.000	
1.000					1.000					1.000		0.953					0.047					1.000	
1.000					1.000				0.017	0.983		0.983				0.017						1.000	
1.000			0.250		0.750					1.000		1.000										1.000	
1.000					1.000					1.000		0.875				0.088	0.038					1.000	
1.000					1.000					1.000		1.000										1.000	
1.000					1.000					1.000		1.000										1.000	
0.983	0.017				1.000				0.083	0.900	0.017	0.683				0.317						1.000	
1.000					1.000					0.900	0.100	0.800				0.200						1.000	
1.000			0.095		0.905					1.000		0.786				0.048	0.167					1.000	
1.000			0.021		0.979					1.000		0.783				0.196	0.022					1.000	
1.000					1.000					1.000		1.000										1.000	
1.000			0.013		0.974	0.013				1.000		0.461				0.526	0.013					1.000	
1.000					1.000					1.000		0.417				0.583						1.000	
1.000					1.000					1.000		0.039	0.063			0.898						1.000	
0.983	0.017				1.000					1.000		0.007	0.027			0.967						0.993	0.007
0.900	0.100				1.000					1.000		0.130	0.739			0.130						1.000	
0.783	0.217				1.000					1.000		0.015	0.970			0.015						1.000	
0.033	0.533	0.433			1.000					1.000		0.240	0.644			0.115						1.000	
1.000					1.000				0.100	0.900		0.950				0.050						1.000	
1.000					1.000					1.000		0.750				0.250						1.000	
1.000					1.000				0.029	0.971		0.474				0.526						1.000	
1.000					1.000					1.000		0.842				0.158						1.000	
0.972	0.028				1.000				0.056	0.944		0.816				0.184						1.000	
1.000					1.000				0.033	0.967		0.880				0.102	0.018					1.000	
1.000					1.000					1.000		0.774				0.226						1.000	
1.000					1.000					1.000		0.953				0.047						1.000	
1.000					1.000					1.000		0.282				0.718						1.000	
1.000					1.000				0.065	0.935		0.022	0.391			0.587						0.761	0.239
0.900	0.100			0.034	0.966					1.000		0.043	0.957									0.948	0.052
1.000					1.000				0.017	0.983			0.181			0.819				0.121		0.879	
1.000					1.000					1.000		0.010	0.418			0.571						1.000	
1.000					1.000					1.000		0.033	0.158			0.808						1.000	
0.717	0.283				1.000					0.833	0.167		0.036			0.964						1.000	
0.983	0.017				1.000					1.000						1.000						1.000	
0.028	0.944	0.028			0.895		0.026	0.079		0.917	0.083		0.224			0.513		0.211	0.053			1.000	
0.024	0.952	0.024			1.000					0.929	0.071	0.071						0.929				1.000	
1.000			1.000							1.000				0.346		0.654						1.000	
1.000			1.000							1.000				0.500		0.500						1.000	
1.000			1.000							1.000				0.410		0.590						1.000	
1.000			1.000						0.500	0.500				0.283		0.717						1.000	
1.000			1.000							1.000						1.000						1.000	
1.000			0.690		0.310					1.000		0.224		0.052		0.724						1.000	
1.000			0.982		0.018					1.000				0.196		0.804						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						1.000	
1.000			0.907		0.093				0.012	0.988		0.093		0.035		0.872						1.000	
1.000			0.939		0.061				0.015	0.985		0.030				0.970						1.000	
1.000			1.000							1.000						1.000						1.000	
1.000			1.000							1.000						1.000						1.000	
1.000			0.989		0.011				0.011	0.989						1.000						1.000	
1.000			0.900		0.100					1.000				0.050		0.950						1.000	
0.750	0.250		1.000							0.875	0.125					1.000						1.000	
0.875	0.125		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			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	

TABLE 5

Gene frequencies at 25 loci in 70 populations of

Species	Population	Sample size	LDH-B				MDH-A		MDH-B						ME-A			
			a	*b*	*c*	*d*	*a*	*b*	*a*	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*
R. nigroma-	Namioka	19		1.000			1.000			1.000						1.000		
〃culata	Akita	6		1.000			1.000			1.000						1.000		
〃	Sakata	48		1.000			1.000			1.000						1.000		
〃	Shibata	16		0.656	0.344		1.000			0.750	0.250					0.844	0.156	
〃	Niigata	20		1.000			1.000			1.000						1.000		
〃	Kashiwazaki	26		1.000			1.000			1.000						1.000		
〃	Joetsu	36		1.000			1.000			1.000						1.000		
〃	Toyama	37		1.000			1.000			1.000						1.000		
〃	Kanazawa	55		1.000			1.000			1.000						1.000		
〃	Mikuni	25		1.000			1.000			1.000						1.000		
〃	Matsumoto	30		0.950	0.050		1.000		0.817	0.167	0.017					1.000		
〃	Okaya	64		1.000			1.000		0.977	0.023						1.000		
〃	Chino	30		1.000			1.000		0.983	0.017						1.000		
〃	Ina	2		1.000			1.000		1.000							1.000		
〃	Iida	40		1.000			1.000		0.788				0.212			1.000		
〃	Sutama	35		1.000			1.000		1.000							1.000		
〃	Mishima	67		1.000			1.000		1.000							1.000		
〃	Nagoya	30		1.000			1.000		0.533	0.467						1.000		
〃	Inazawa	5		1.000			1.000		0.800				0.200			1.000		
〃	Maibara	21		0.881		0.119	1.000		0.071	0.762	0.119		0.048			0.810	0.190	
〃	Igaueno	48		0.979		0.021	1.000			1.000						1.000		
〃	Shingu	6		1.000			1.000			1.000						1.000		
〃	Higashiosaka	38		0.987		0.013	1.000			1.000						1.000		
〃	Himeji	6		1.000			1.000			1.000						1.000		
〃	Tottori	64		1.000			1.000			0.375		0.625				1.000		
〃	Matsue	75		1.000			1.000			0.153		0.847				1.000		
〃	Gotsu	23		1.000			1.000			0.630		0.370				1.000		
〃	Hagi	66		1.000			1.000			0.886		0.114				1.000		
〃	Yamaguchi	52		1.000			1.000			0.990		0.010				1.000		
〃	Hiroshima	60		1.000			1.000			1.000						1.000		
〃	Kumano	56		1.000			1.000			1.000						1.000		
〃	Hiro	19		1.000			1.000			1.000						1.000		
〃	Miyoshi	19		1.000			1.000			1.000						1.000		
〃	Shobara	19		1.000			1.000			1.000						1.000		
〃	Konko	83		0.994	0.006		1.000			1.000						1.000		
〃	Takamatsu	42		1.000			1.000			1.000						1.000		
〃	Nangoku	32		1.000			1.000			1.000						1.000		
〃	Uwajima	39		1.000			1.000			1.000						1.000		
〃	Matsuyama	23		1.000			1.000			1.000						1.000		
〃	Munakata	58		1.000			0.991	0.009		1.000						1.000		
〃	Sasebo	58		1.000			1.000			1.000						0.550		0.450
〃	Kumamoto	49		1.000			1.000			1.000						0.983		0.017
〃	Kagoshima	60		1.000			1.000			1.000						1.000		
〃	Miyazaki	56		1.000			1.000			0.982				0.018		1.000		
〃	Oita	55		1.000			1.000			1.000						0.983		0.017
〃	Suwon	38	0.013	0.987			1.000					0.987		0.013		1.000		
〃	Beijing	21		1.000			0.929	0.071				0.881		0.119	0.024	0.976		
R. b. porosa	Morioka	26			1.000		1.000				1.000						1.000	
〃	Hiraizumi	9			1.000		1.000				1.000						1.000	
〃	Ichinoseki	39			1.000		1.000				1.000						1.000	
〃	Sukagawa	43			1.000		1.000				1.000						1.000	
〃	Utsunomiya	56			1.000		1.000				1.000						1.000	
〃	Shibata	38		0.259	0.741		1.000			0.293	0.707					0.118	0.882	
〃	Maki	29		0.018	0.982		1.000				1.000						1.000	
〃	Nagano	19			1.000		1.000				1.000						1.000	
〃	Iiyama	30			1.000		1.000				1.000						1.000	
〃	Shinano	8			1.000		1.000				1.000						1.000	
〃	Matsumoto	43		0.116	0.884		1.000		0.128	0.023	0.849						1.000	
R. b. brevi-	Ina	69		0.061		0.939	1.000		0.106		0.894					0.015	0.985	
〃poda	Inazawa	1				1.000	1.000				1.000						1.000	
〃	Gifuhashima	1				1.000	1.000				1.000						1.000	
〃	Maibara	46			0.044	0.956	1.000				1.000					0.044	0.956	
〃	Tsu	30		0.017		0.983	1.000				1.000						1.000	
〃	Igaueno	4				1.000	1.000				1.000						1.000	
〃	Higashiosaka	20				1.000	1.000				1.000						1.000	
〃	Amagasaki	1				1.000	1.000				1.000						1.000	
〃	Tamashima	4			1.000		1.000				1.000						1.000	
〃	Konko	38			1.000		1.000				1.000						1.000	
〃	Shobara	4			1.000		1.000				1.000						1.000	
〃	Kumano	2			1.000		1.000				1.000						1.000	

Rana nigromaculata and *Rana brevipoda* (II)

ME-B											MPI							Pep-A		
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*a*	*b*	*c*
						1.000								1.000					1.000	
						1.000								1.000					1.000	
		0.333				0.667								1.000					1.000	
			0.219			0.781								1.000					0.781	0.219
						1.000								1.000					1.000	
						1.000								1.000					1.000	
		0.067				0.933								1.000					1.000	
		0.333				0.667								1.000					1.000	
		0.438				0.563								1.000					1.000	
		0.200				0.760		0.040						0.920			0.080		1.000	
		1.000											0.133	0.867					1.000	
		0.750				0.250								1.000					1.000	
		0.917				0.083								1.000					1.000	
		1.000												1.000					1.000	
		1.000											0.467	0.533					1.000	
		0.817				0.183								1.000					1.000	
		0.133				0.867								1.000					0.983	0.017
		0.750				0.250							0.033	0.967					1.000	
		0.800				0.200							0.100	0.900					1.000	
		0.905	0.024			0.071					0.024		0.143	0.786	0.048			0.119	0.881	
		0.417			0.010	0.573								1.000				0.010	0.990	
		0.250				0.750								1.000					1.000	
		0.592				0.408								0.961	0.039			0.013	0.882	0.105
		0.083				0.583	0.333							1.000					1.000	
		0.867				0.133								1.000					1.000	
		0.383		0.367		0.250								1.000					1.000	
		0.700				0.300								1.000					1.000	
0.017		0.100				0.333	0.417	0.133				0.100		0.900					1.000	
0.300		0.067				0.283	0.133	0.217						1.000					1.000	
						0.767	0.167	0.067						0.733			0.267		1.000	
						0.816	0.158	0.026						0.803			0.197		1.000	
						0.824	0.147	0.029						0.912			0.088		1.000	
						0.684	0.263	0.053						1.000					1.000	
						0.694	0.278	0.028						1.000					1.000	
		0.283				0.633	0.067	0.017						1.000					1.000	
		0.183				0.817								1.000					1.000	
		0.967				0.033								0.583			0.417		1.000	
						0.883	0.117							1.000					1.000	
		0.413				0.587								1.000					1.000	
0.133		0.517				0.350								1.000					1.000	
		0.983				0.017								1.000					1.000	
		1.000											0.033	0.967					1.000	
	0.133	0.717				0.067		0.083				0.083		0.917					1.000	
	0.383	0.517				0.033		0.067				0.050		0.950					1.000	
		0.967				0.033								1.000					0.983	0.017
		0.639				0.083		0.056	0.167	0.056			0.028	0.917			0.056		0.972	0.028
		0.905				0.095							0.024	0.881		0.024	0.071		0.976	0.024
			1.000								0.115			0.885						1.000
			1.000								0.111			0.889						1.000
			1.000								0.117			0.883						1.000
			1.000								0.117			0.883						1.000
			1.000								0.016			0.875		0.109				1.000
			0.721			0.279								1.000					0.250	0.750
			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
			1.000											1.000					0.955	0.045
			1.000											1.000					1.000	
			1.000											1.000					1.000	
		0.011	0.567		0.367	0.056					0.333		0.022	0.433	0.211				1.000	
			0.883		0.117									1.000					1.000	
			0.750		0.250									0.125	0.875				1.000	
			0.975		0.025						0.100			0.325	0.575				0.875	0.125
			1.000											1.000					1.000	
			1.000								0.500			0.500					1.000	
			1.000								0.340			0.660					0.674	0.326
			1.000								0.875			0.125					1.000	
			1.000								0.750			0.250					1.000	

TABLE 5
Gene frequencies at 25 loci in 70 populations of

Species	Population	Sample size	Pep-B			Pep-C				Pep-D					6-PGD			PGM		
			a	*b*	*c*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*a*	*b*	*c*
R. nigroma-	Namioka	19			1.000		1.000				1.000					1.000			1.000	
〃culata	Akita	6		0.833	0.167		1.000				1.000					1.000			1.000	
〃	Sakata	48		0.867	0.133	0.317	0.683				1.000					1.000			1.000	
〃	Shibata	16		0.900	0.100	0.250	0.719		0.031		0.875		0.125			0.719	0.281		1.000	
〃	Niigata	20		0.825	0.175	0.450	0.550				1.000					1.000			1.000	
〃	Kashiwazaki	26		0.917	0.083	0.104	0.896				1.000					1.000		0.160	0.840	
〃	Joetsu	36		0.700	0.300	0.267	0.733				1.000					1.000			1.000	
〃	Toyama	37		1.000		0.650	0.350				1.000					1.000			1.000	
〃	Kanazawa	55		0.906	0.094	0.719	0.281				1.000					1.000			1.000	
〃	Mikuni	25		1.000		0.750	0.250				1.000					1.000			1.000	
〃	Matsumoto	30		1.000		0.950	0.050				0.900		0.100			1.000			1.000	
〃	Okaya	64		1.000		0.983	0.017				1.000					1.000			1.000	
〃	Chino	30		1.000		0.900	0.100				1.000					1.000		0.017	0.983	
〃	Ina	2		1.000		1.000					1.000					1.000			1.000	
〃	Iida	40		1.000		1.000					0.983			0.017		1.000			0.983	0.017
〃	Sutama	35		1.000		1.000					1.000					1.000			1.000	
〃	Mishima	67		1.000		1.000					1.000					1.000			1.000	
〃	Nagoya	30		1.000		1.000					1.000				0.417	0.467	0.117		1.000	
〃	Inazawa	5		1.000		1.000					1.000					1.000			1.000	
〃	Maibara	21		1.000		0.452	0.548				0.333		0.667		0.024	0.952	0.024		1.000	
〃	Igaueno	48		0.760	0.240	0.917	0.083				0.938		0.063		0.010	0.990			1.000	
〃	Shingu	6		0.083	0.917	0.583	0.417				1.000					1.000			1.000	
〃	Higashiosaka	38		0.803	0.197	0.934	0.066				0.513		0.474	0.013	0.026	0.974			1.000	
〃	Himeji	6		1.000		0.917	0.083			0.333	0.500		0.167			1.000			1.000	
〃	Tottori	64		1.000		0.133	0.867			0.200	0.800					0.050	0.950		1.000	
〃	Matsue	75	0.067	0.933		0.067	0.933				1.000					0.117	0.883		1.000	
〃	Gotsu	23		1.000		0.100	0.900				1.000					1.000			1.000	
〃	Hagi	66		1.000		0.017	0.983			0.133	0.867					0.017	0.983		1.000	
〃	Yamaguchi	52		0.983	0.017	0.450	0.550			0.050	0.950					1.000			1.000	
〃	Hiroshima	60	0.042	0.958		0.458	0.542				1.000					0.033	0.967		1.000	
〃	Kumano	56	0.013	0.987		0.750	0.250				1.000					0.053	0.947		1.000	
〃	Hiro	19	0.118	0.882		0.941	0.059				1.000					0.147	0.853		1.000	
〃	Miyoshi	19	0.026	0.974		0.816	0.184				1.000					0.184	0.816		1.000	
〃	Shobara	19		1.000		0.861	0.139				1.000					0.167	0.833		1.000	
〃	Konko	83	0.117	0.883		0.700	0.300				1.000					1.000			1.000	
〃	Takamatsu	42		1.000		1.000					1.000					1.000			1.000	
〃	Nangoku	32	0.117	0.883		1.000					1.000				0.033	0.967			1.000	
〃	Uwajima	39	0.067	0.933		1.000					1.000					1.000			1.000	
〃	Matsuyama	23		1.000		0.696	0.304				1.000					1.000			1.000	
〃	Munakata	58	0.267	0.733		0.033	0.967			0.083	0.917					1.000			1.000	
〃	Sasebo	58		0.800	0.200	0.100	0.900				0.767	0.233				1.000			1.000	
〃	Kumamoto	49	0.133	0.667	0.200	0.017	0.983				1.000				0.017	0.983			1.000	
〃	Kagoshima	60		0.950	0.050	0.883	0.117				1.000					1.000			1.000	
〃	Miyazaki	56	0.100	0.567	0.333		0.967	0.033			1.000				0.017	0.983			1.000	
〃	Oita	55		1.000		0.350	0.650				0.850	0.150				1.000			1.000	
〃	Suwon	38		0.556	0.444	0.417	0.583				1.000				0.250	0.750			0.972	0.028
〃	Beijing	21		0.857	0.143	0.786	0.214				1.000				0.024	0.976			0.810	0.190
R. b. porosa	Morioka	26	0.173	0.827			1.000				0.173		0.827			0.019	0.981		1.000	
〃	Hiraizumi	9	0.111	0.889			1.000				0.056		0.944				1.000		1.000	
〃	Ichinoseki	39	0.083	0.917			1.000				0.067		0.933			0.017	0.983		1.000	
〃	Sukagawa	43	0.233	0.767			1.000				0.233		0.767			0.050	0.950		0.983	0.017
〃	Utsunomiya	56	0.203	0.797			0.953		0.047		0.234		0.766			0.391	0.609		0.984	0.016
〃	Shibata	38	0.044	0.912	0.044		0.750		0.250		0.250		0.750			0.485	0.515		1.000	
〃	Maki	29	0.022	0.978			0.870		0.130				1.000			0.609	0.391		1.000	
〃	Nagano	19	0.158	0.842			0.895		0.105		0.158		0.842			0.342	0.658		1.000	
〃	Iiyama	30	0.033	0.967			0.817		0.183				1.000			0.617	0.383		1.000	
〃	Shinano	8	0.313	0.688			0.875		0.125		0.250		0.750			1.000			1.000	
〃	Matsumoto	43	0.128	0.872			0.965		0.035		0.174		0.826			0.930	0.070		1.000	
R. b. brevi-	Ina	69	0.394	0.606		0.097	0.565		0.339		0.303		0.576	0.121		0.561	0.439		1.000	
〃 poda	Inazawa	1	0.500	0.500			0.500		0.500				0.500	0.500		1.000			1.000	
〃	Gifuhashima	1	0.500	0.500			1.000				0.500		0.500			0.500	0.500		1.000	
〃	Maibara	46		1.000		0.097	0.806		0.097		0.145		0.855			0.911	0.089		1.000	
〃	Tsu	30	0.267	0.733		0.283	0.367		0.350		0.200		0.633	0.167		0.750	0.250		1.000	
〃	Igaueno	4		1.000			1.000				0.250		0.750		0.375	0.625			1.000	
〃	Higashiosaka	20		1.000			0.975		0.025		0.050		0.750	0.200	0.200	0.650	0.150		1.000	
〃	Amagasaki	1		1.000			0.500		0.500				1.000			0.500	0.500		1.000	
〃	Tamashima	4	0.250	0.750			1.000						1.000			1.000			1.000	
〃	Konko	38	0.065	0.935			1.000				0.174		0.826			1.000			0.940	0.060
〃	Shobara	4	0.125	0.875			1.000						1.000			1.000			1.000	
〃	Kumano	2	1.000				1.000						1.000			1.000			1.000	

Rana nigromaculata and *Rana brevipoda* (III)

SOD-B			Ab							Hb			
a	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*a*	*b*	*c*	*d*
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			0.818		0.182				0.083			0.917
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
0.041	0.959			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			0.967		0.033				0.033			0.967
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000							0.013		0.988
	1.000			1.000									1.000
	1.000		0.054	0.946									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000			0.875			0.125				0.167		0.833
	1.000			0.990			0.010				0.031		0.969
	1.000			1.000									1.000
	1.000			0.986			0.014				0.013		0.987
	1.000			1.000									1.000
	1.000		0.016	0.960	0.024								1.000
	1.000		0.021	0.915	0.063								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
0.042	0.958			1.000									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
0.184	0.816			1.000									1.000
0.096	0.904			1.000									1.000
0.012	0.988			1.000									1.000
	1.000		0.125	0.875									1.000
	1.000		0.097	0.903									1.000
	1.000			0.955	0.045								1.000
	1.000			1.000									1.000
	1.000		0.019	0.981									1.000
	1.000			1.000									1.000
	1.000			1.000									1.000
	1.000		0.059	0.941									1.000
	1.000		0.010	0.990									1.000
0.013	0.987		0.083	0.917									1.000
0.024	0.976			0.154	0.846							0.357	0.643
	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				0.145	0.855		
	1.000			0.276		0.724				0.421	0.329		0.250
	1.000					1.000				0.379	0.621		
	1.000					1.000				0.132	0.868		
	1.000					1.000				0.267	0.733		
	1.000					1.000				0.375	0.625		
	1.000			0.093		0.907				0.616	0.233		0.151
	1.000			0.087				0.913		0.022	0.978		
	1.000						1.000				1.000		
	1.000						1.000				1.000		
	0.900	0.100				0.022	0.978				1.000		
	1.000						1.000				1.000		
	1.000						1.000				1.000		
	1.000			0.033			0.933		0.033		1.000		
	1.000						1.000				1.000		
0.500	0.500						1.000				1.000		
0.487	0.513						0.786	0.214			1.000		
0.125	0.875						1.000				1.000		
	1.000						1.000				1.000		

and 454 frogs of 23 populations belonging to *R. b. brevipoda* and *R. b. porosa*. The results showed that six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*~*c*, were observed. In the 1162 *R. nigromaculata*, homozygous AA, BB and CC bands were found in one, 1112 and six frogs, respectively, and heterozygous AB, AC and BC bands were found in 23, one and 19 frogs, respectively. Alleles *a*, *b* and *c* were 0.011, 0.975 and 0.014 in frequency, respectively. In *R. brevipoda*, BB, AB and BC bands were observed in 450, one and three of the 454 frogs, respectively, and alleles *a*, *b* and *c* were 0.001, 0.996 and 0.003, respectively, in frequency.

In 69 of the 70 populations of *R. nigromaculata* and *R. brevipoda* other than the Shingu population, allele *b* was overwhelmingly high in frequency, being 0.829 ~1.000. In the Shingu population, allele *c* was very high in frequency, being 0.917, while allele *b* was only 0.083 in frequency. In 10 populations, the Matsumoto, Okaya, Inazawa, Hiroshima, Kumano, Hiro, Miyoshi, Shobara, Munakata and Beijing, allele *a* was only 0.017~0.105 in frequency in addition to allele *b*. In the Maibara and Igaueno populations of *R. nigromaculata*, allele *c* was 0.167 and 0.021, respectively, in frequency. In the Higashiosaka population of *R. nigromaculata* and the Tsu population of *R. b. brevipoda*, allele *a* was 0.013 and 0.017, respectively, in frequency and allele *c* was 0.158 and 0.050, respectively, in frequency. All the other populations of *R. nigromaculata*, *R. b. brevipoda* and *R. b. porosa* had only allele *b* (Table 5–I; Fig. 2).

2. ADA locus

Electrophoretic patterns at the ADA locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT–B locus. It was found that there were 11 phenotypes, BB, CC, DD, EE, AB, AC, BC, BD, CD, CE and DE, produced by five alleles, *a*~*e*. In the 1162 *R. nigromaculata*, homozygous BB, CC and DD bands were observed in 29, 682 and 219 frogs, respectively, and heterozygous AB, AC, BC, BD, CD and CE bands were found in 10, nine, 48, four, 159 and two frogs, respectively. Alleles *a*, *b*, *c*, *d* and *e* were 0.008, 0.052, 0.681, 0.259 and 0.001, respectively, in frequency.

In the 454 *R. brevipoda*, DD, EE, CD and DE bands produced by three alleles, *c*, *d* and *e*, were observed in 410, two, 19 and 23 frogs, respectively. Alleles *c*, *d* and *e* were 0.021, 0.949 and 0.030, respectively, in frequency.

In nine populations of *R. nigromaculata*, including three Tohoku populations, the Namioka, Akita and Sakata, five Hokuriku populations, the Shibata, Niigata, Kashiwazaki, Joetsu and Toyama, and one Shikoku population, the Nangoku, and all the 23 populations of *R. brevipoda*, allele *d* was very high in frequency, being 0.750~1.000. In addition to allele *d*, the Toyama and Nangoku of *R. nigromaculata* and the Matsumoto, Ina, Maibara and Higashiosaka of *R. brevipoda* had allele *c* in frequencies of 0.025~0.167. The Morioka, Ichinoseki and Utsunomiya populations of *R. b. porosa* had allele *e* in frequencies of 0.017~0.250. The other seven populations of *R. nigromaculata* and 16 populations of *R. brevipoda* had only allele *d*.

On the other hand, 13 populations of *R. nigromaculata*, the Chino, Sutama, Ina,

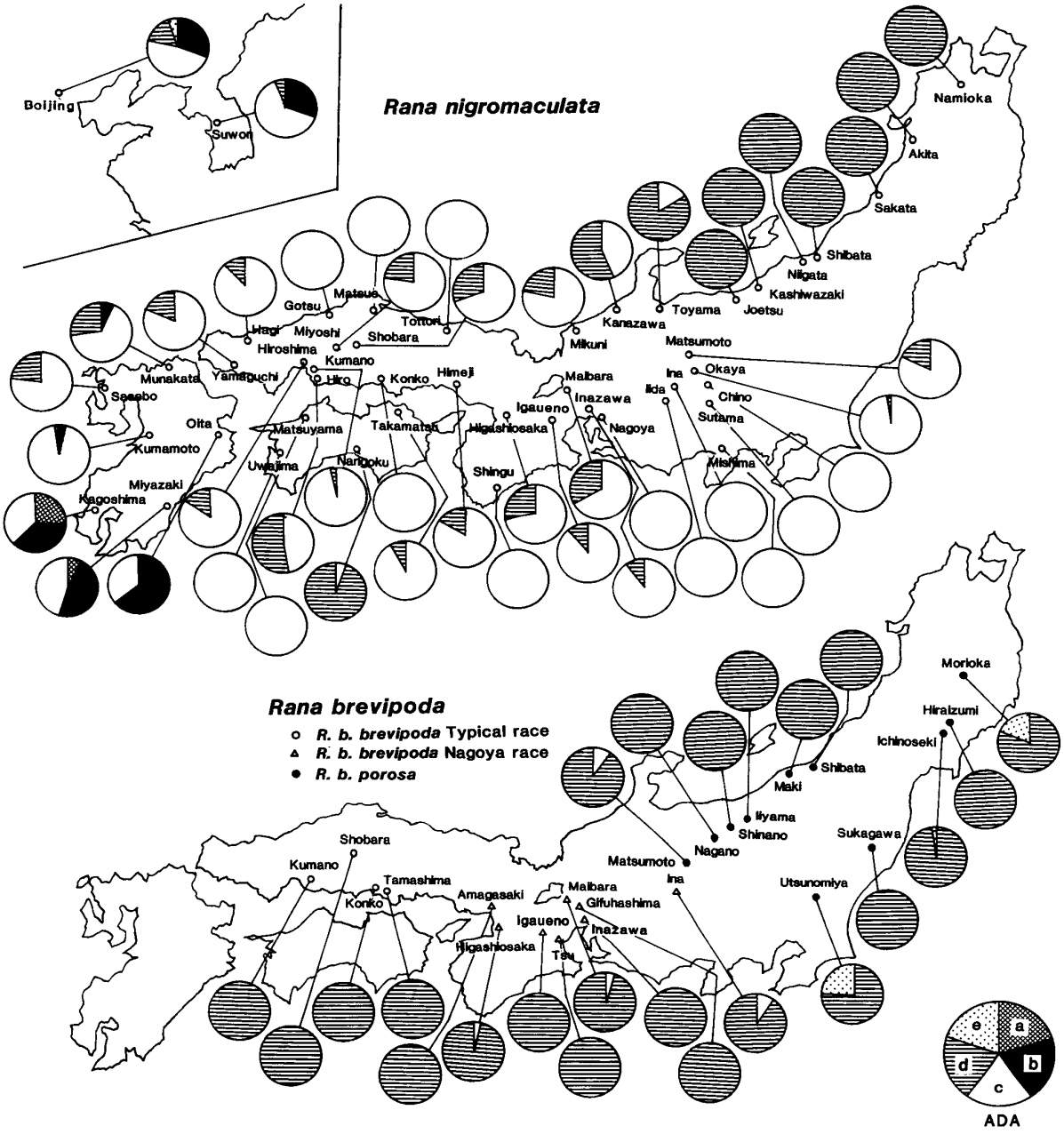

Fig. 3. Geographic distribution of ADA alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

Iida, Mishima, Nagoya, Shingu, Tottori, Matsue, Gotsu, Konko, Uwajima and Matsuyama, had only allele *c*, while 19 populations of *R. nigromaculata*, the Mikuni, Matsumoto, Okaya, Inazawa, Maibara, Igaueno, Higashiosaka, Himeji, Hagi, Yamaguchi, Hiroshima, Kumano, Miyoshi, Shobara, Takamatsu, Munakata, Sasebo, Kumamoto and Suwon, had allele *c* in high frequencies of 0.639~0.983, and had allele *d* in low frequencies of 0.017~0.333. Three of the 19 populations, the Munakata, Kumamoto and Suwon, had allele *b* in frequencies of 0.033~0.306 in addition to alleles *c* and *d*. The Kanazawa and Hiro populations of *R. nigromaculata* had allele *c* in frequencies of 0.438 and 0.471, respectively, and had

allele *d* in frequencies of 0.563 and 0.529, respectively. The Kagoshima and Miyazaki populations had allele *a* in frequencies of 0.250 and 0.067, respectively, allele *b* in frequencies of 0.367 and 0.483, respectively, and allele *c* in frequencies of 0.383 and 0.450, respectively. The Oita population had alleles *b* and *c* in frequencies of 0.650 and 0.350, respectively. The Beijing population of *R. nigromaculata* had alleles *b*, *c*, *d* and *e* in frequencies of 0.310, 0.476, 0.167 and 0.048, respectively (Table 5–I; Fig. 3)

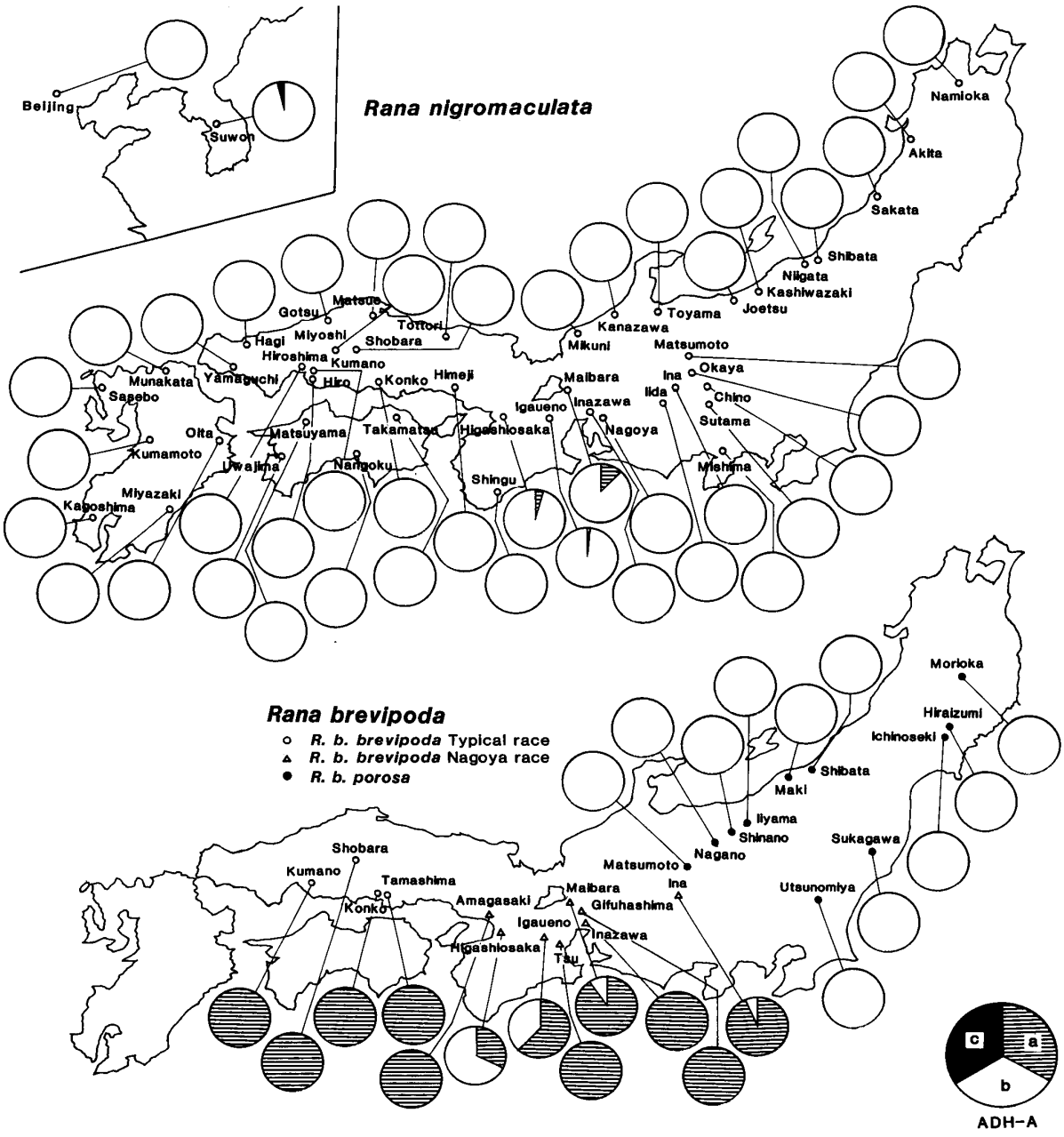

Fig. 4. Geographic distribution of ADH–A alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

3. ADH-A locus

Electrophoretic patterns at the ADH-A locus were analyzed in 1580 frogs of 70 populations including 1144 frogs of 47 populations belonging to *R. nigromaculata* and 436 frogs of 23 populations belonging to *R. brevipoda*. The results showed that there were four phenotypes, AA, BB, AB and BC, produced by alleles *a*, *b* and *c*. In the 1144 *R. nigromaculata*, AA, BB, AB and BC bands produced by alleles *a*, *b* and *c* were found in one, 1135, seven and one frogs, respectively. Alleles *a*, *b* and *c* were 0.004, 0.996 and 0.0004, respectively, in frequency. In the 436 *R. brevipoda*, AA, BB and AB bands were found in 123, 295 and 18 frogs, respectively. Alleles *a* and *b* were 0.303 and 0.697, respectively, in frequency.

Almost all the 47 populations of *R. nigromaculata* and the 11 populations of *R. b. porosa* were occupied by allele *b*, which was 0.881~1.000 in frequency. In addition to allele *b*, the Maibara, Igaueno and Higashiosaka populations of *R. nigromaculata* had allele *a* in frequencies of 0.010~0.119. In the Suwon population of the same species, allele *c* was 0.028 in frequency. All the remaining 43 populations of *R. nigromaculata* and the 11 populations of *R. b. porosa* were occupied by allele *b*. Eight of the 12 populations of *R. b. brevipoda* had only allele *a*, while the other four had allele *b* in frequencies of 0.061~0.675 in addition to allele *a*. Although *R. b. brevipoda* were almost sympatric with *R. nigromaculata*, the Igaueno and Higashiosaka populations of *R. b. brevipoda* had especially high frequencies of allele *b*, being 0.375 and 0.675, respectively. In the Ina and Maibara populations of the same subspecies, allele *b* was very low in frequency, being 0.061 and 0.097, respectively. In the other populations of this subspecies, no invasion of allele *b* was found (Table 5-I; Fig. 4)

4. ADH-B locus

Electrophoretic patterns at the ADH-B locus were analyzed in the same 1580 frogs as those used in the analyses at the ADH-A locus. The results showed that there were six phenotypes, BB, CC, DD, AB, BC and BD, produced by four alleles, *a*~*d*. In the 1144 *R. nigromaculata*, BB, CC, AB and BC bands were found in 931, 111, one and 101 frogs, respectively. Alleles *a*, *b* and *c* were 0.0004, 0.858 and 0.141, respectively, in frequency. On the other hand, in the 436 *R. brevipoda*, BB, DD, AB and BD bands were found in 425, one, two and eight frogs, respectively. Alleles *a*, *b* and *d* were 0.002, 0.986 and 0.011, respectively, in frequency.

In 39 of the 47 populations of *R. nigromaculata* other than eight populations, the Mikuni, Tottori, Matsue, Gotsu, Kumano, Miyoshi, Shobara and Miyazaki, and the 23 populations of *R. brevipoda*, allele *b* was very high in frequency, being 0.750~1.000. In addition to allele *b*, 12 of these populations of *R. nigromaculata*, the Sakata, Joetsu, Toyama, Kanazawa, Himeji, Hiroshima, Hiro, Konko, Munakata, Sasebo, Kumamoto and Kagoshima, had allele *c* in frequencies of 0.017 ~0.250. The Mishima population of *R. nigromaculata* and the Maibara population of *R. b. brevipoda* had allele *a* in frequencies of 0.017 and 0.032, respectively. The

Utsunomiya population of *R. b. porosa* had allele *d* in a frequency of 0.156. All the remaining 47 populations including 26 of *R. nigromaculata,* 10 of *R. b. porosa* and 11 of *R. b. brevipoda* had only allele *b*. In eight populations of *R. nigromaculata,* allele *c* was high in frequency, being 0.528~1.000. Of these populations, the Tottori and Matsue had only allele *c*, while the other six populations, the Mikuni, Kumano, Shobara, Gotsu, Miyoshi and Miyazaki, had allele *b* in frequencies of 0.300~0.472 in addition to allele *c* (Table 5–I; Fig. 5).

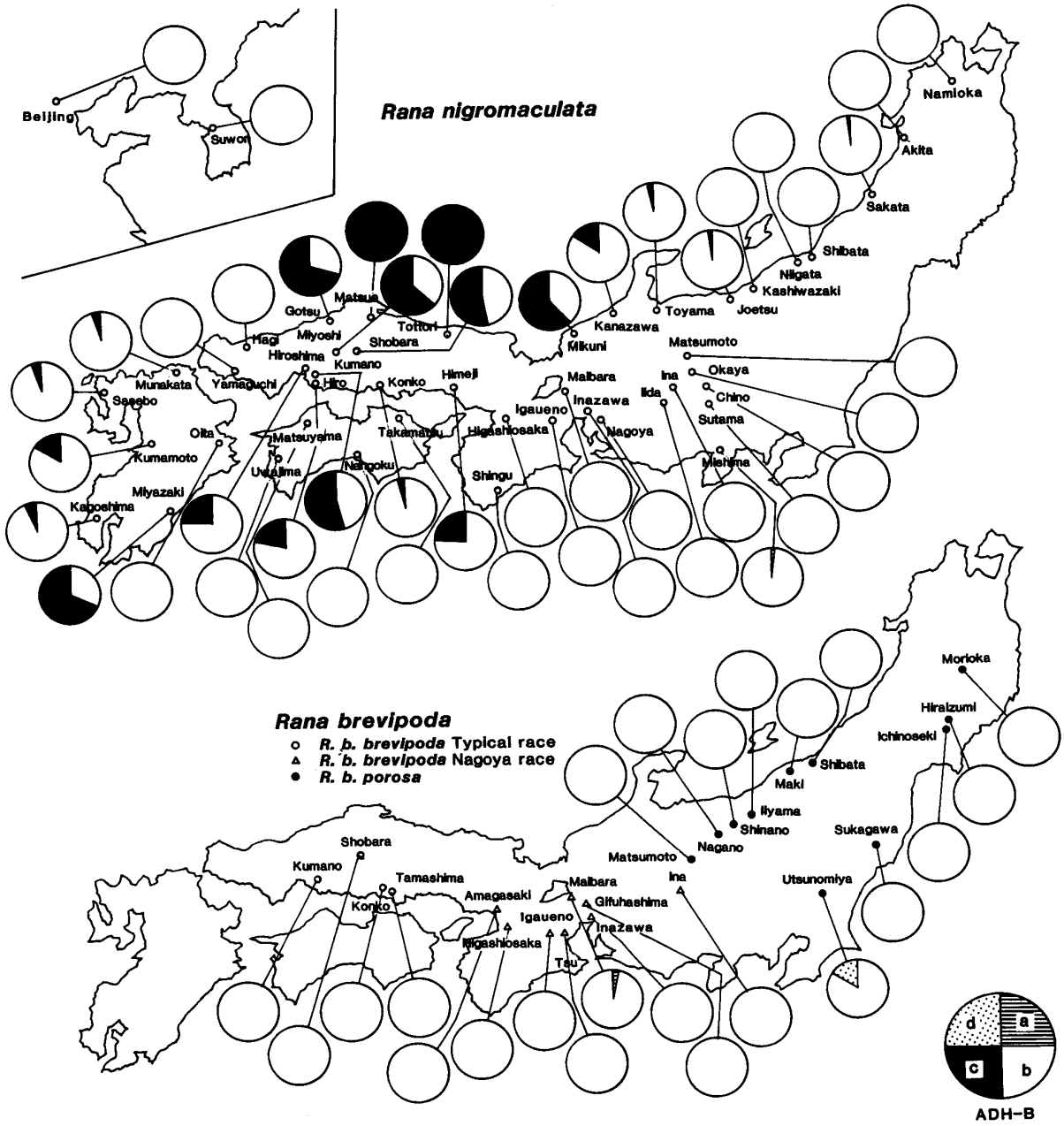

Fig. 5. Geographic distribution of ADH–B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

5. AK locus

Electrophoretic patterns at the AK locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT-B and ADA loci. The results showed that there were four phenotypes, AA, BB, AB and BC, produced by alleles *a*, *b* and *c*. In *R. nigromaculata*, BB and BC bands were found in 1161 and one of the 1162 frogs, respectively, and alleles *b* and *c* were 0.9996 and 0.0004, respectively, in frequency. In *R. brevipoda*, AA, BB and AB bands were found in two, 446 and six of the 454 frogs, respectively. Alleles *a* and *b* were 0.011 and 0.989, respectively, in frequency.

All the 68 populations of *R. nigromaculata* other than the Kumano population, *R. b. porosa* other than the Utsunomiya population and *R. b. brevipoda* had only allele *b*. The Kumano population of *R. nigromaculata* had allele *c* in a frequency of 0.013 and the Utsunomiya population of *R. b. porosa* had allele *a* in a frequency of 0.156, in addition to allele *b* (Table 5-I).

6. Fum locus

Electrophoretic patterns at the Fum locus were analyzed in the same 1580 frogs as those used in the analyses at the ADH-A and ADH-B loci. It was found that there were six phenotypes, AA, BB, CC, AB, AC and BC, produced by alleles *a*, *b* and *c*. In the 1144 *R. nigromaculata*, homozygous AA, BB and CC bands were found in 29, 1050 and seven frogs, respectively, while heterozygous AB, AC and BC bands were found in four, one and 53 frogs, respectively. Alleles *a*, *b* and *c* which produced these bands were 0.028, 0.943 and 0.030, respectively, in frequency. In *R. brevipoda*, BB and BC bands were found in 429 and seven frogs, respectively. Alleles *b* and *c* were 0.992 and 0.008, respectively, in frequency.

In 45 of the 47 populations of *R. nigromaculata* other than the Oita and Yamaguchi and the 23 populations of *R. brevipoda*, allele *b* was very high in frequency, being 0.717~1.000. In addition to allele *b*, seven populations of *R. nigromaculata*, the Nagoya, Matsue, Gotsu, Hagi, Shobara, Munakata and Miyazaki, and two populations of *R. brevipoda*, the Igaueno and Higashiosaka, had allele *c* in frequencies of 0.017~0.283. In the Suwon and Beijing populations of *R. nigromaculata*, allele *a* was found in frequencies of 0.028 and 0.024, respectively, and allele *c* was found in frequencies of 0.028 and 0.024, respectively. In the remaining 57 populations, only allele *b* was found. In the Yamaguchi population of *R. nigromaculata*, alleles *b*, *c* and *a* were found in frequencies of 0.533, 0.433 and 0.033, respectively. In the Oita population of *R. nigromaculata*, allele *a* was high in frequency, being 0.983, while allele *b* was 0.017 (Table 5-I; Fig. 6).

7. α-GDH locus

Electrophoretic patterns at the α-GDH locus were analyzed in 2233 frogs of 70 populations including 1733 frogs of 47 populations belonging to *R. nigromaculata* and 500 frogs of 23 populations belonging to *R. brevipoda*. The results showed that there were seven phenotypes, AA, CC, AC, BC, CD, CE and CF, produced by six

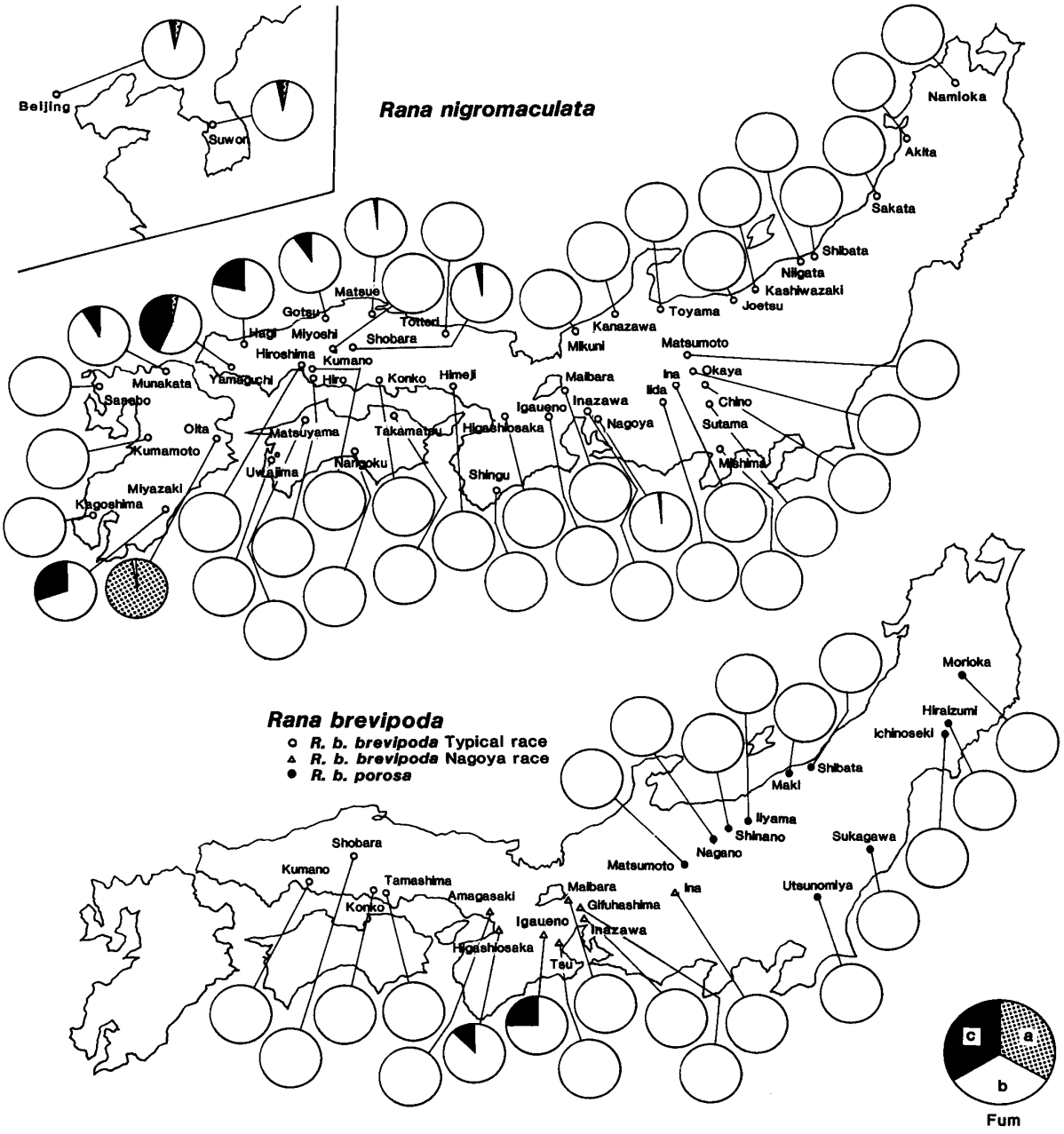

Fig. 6. Geographic distribution of Fum alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

alleles, *a*~*f*. In the 1733 *R. nigromaculata*, homozygous AA and CC bands were found in five and 1697 frogs, respectively, and heterozygous AC, BC, CD, CE and CF bands were found in 17, four, two, two and six frogs, respectively. Alleles *a*, *b*, *c*, *d*, *e* and *f* were 0.008, 0.001, 0.988, 0.001, 0.001 and 0.002, respectively, in frequency. In the 500 *R. brevipoda*, on the other hand, AA, CC and AC bands were found in 466, four and 30 frogs, respectively. Alleles *a* and *c* were 0.962 and 0.038, respectively, in frequency. In *R. nigromaculata* and *R. brevipoda*, alleles *c* and *a* were very high in frequency.

In the 47 populations of *R. nigromaculata*, allele *c* was high in frequency, being

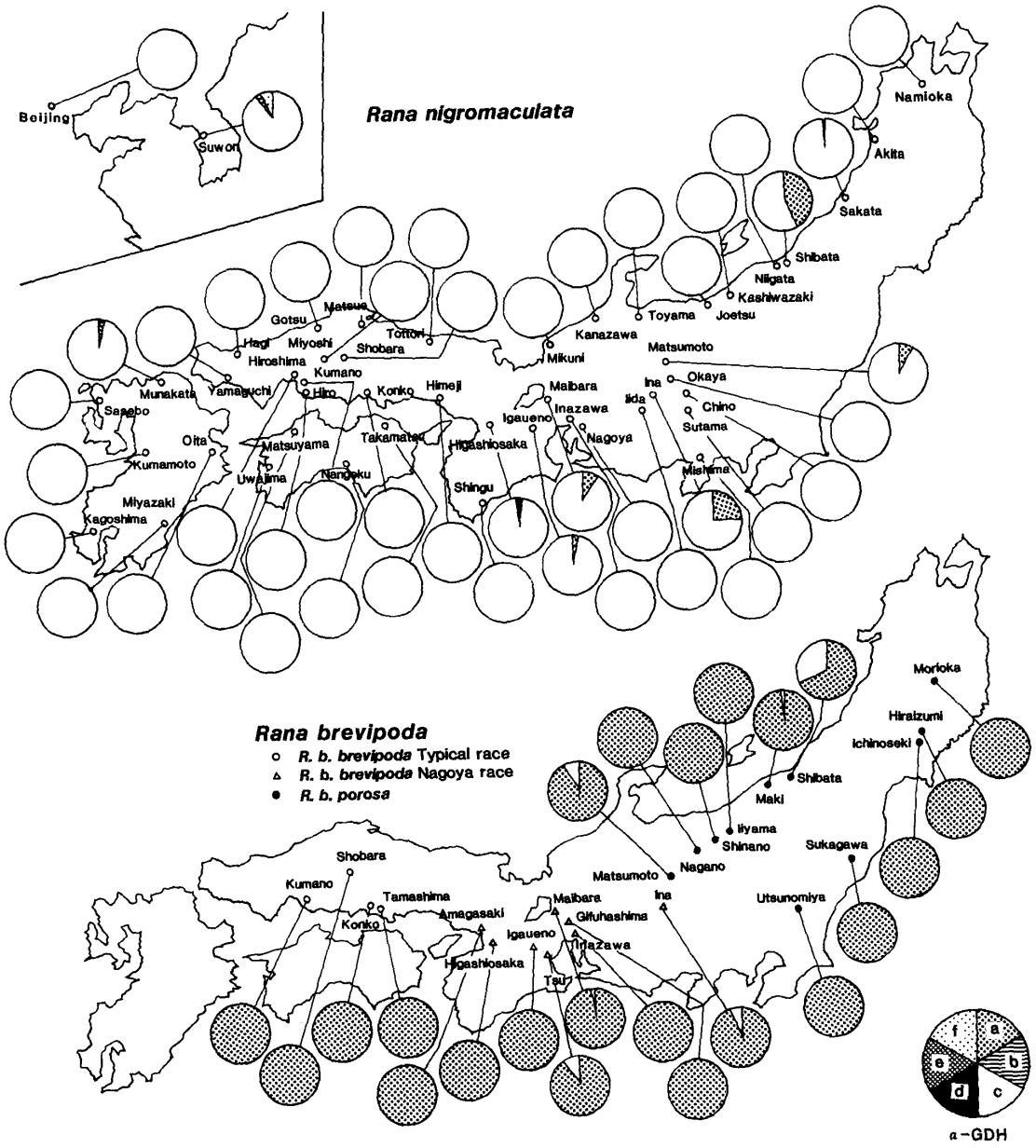

Fig. 7. Geographic distribution of α–GDH alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

0.563~1.000. In addition to allele *c*, in two populations of the Shibata and Ina, allele *a* was 0.438 and 0.250, respectively, in frequency. In four population of the Matsumoto, Maibara, Igaueno and Higashiosaka, allele *a* was 0.013~0.095 in frequency. In addition to alleles *c* and *a*, the Higashiosaka population had allele *d* in a frequency of 0.013. The Sakata population had allele *d* in a frequency of 0.010 in addition to allele *c*, the Munakata population had allele *b* in a frequency of 0.034 in addition to allele *c*, and the Suwon population had alleles *e* and *f* in frequencies of 0.026 and 0.079, respectively, in addition to allele *c*. The remaining 38 populations had allele *c* alone.

In *R. brevipoda,* six populations of the Shibata, Maki, Matsumoto, Ina, Tsu and Maibara had allele *a* in frequencies of 0.690~0.989, while they had allele *c* in frequencies of 0.011~0.310. The remaining 17 populations had only allele *a* (Table 5-I; Fig. 7).

8. GPI locus

Electrophoretic patterns at the GPI locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT-B, ADA and AK loci. The results showed that there were five phenotypes, AA, BB, CC, AB and BC, produced by alleles *a, b* and *c*. In the 1162 *R. nigromaculata,* homozygous AA, BB and CC bands were found in three, 1114 and two frogs, respectively, and heterozygous AB and BC bands were found in 29 and 14 frogs, respectively. Alleles *a, b* and *c* were 0.015, 0.977 and 0.008, respectively, in frequency. In the 453 *R. brevipoda,* AA, BB, AB and BC bands were found in 11, 430, 11 and one frogs, respectively. Alleles *a, b* and *c* were 0.036, 0.962 and 0.001, respectively, in frequency.

Of the 70 populations, 32 of *R. nigromaculata* and 18 of *R. brevipoda* had allele *b* alone. In 19 of the remaining 20 populations other than the Sukagawa of *R. b. porosa,* allele *b* was overwhelmingly high in frequency, being 0.833~0.989. In the Sukagawa population, each of alleles *a* and *b* was 0.500 in frequency. In addition to allele *b*, 10 populations of *R. nigromaculata,* the Namioka, Joetsu, Toyama, Chino, Hiroshima, Hiro, Shobara, Konko, Matsuyama and Sasebo, had allele *a* in frequencies of 0.017~0.150. In the Nagoya population of *R. nigromaculata,* there were alleles *a* and *c* in frequencies of 0.083 and 0.017, respectively. In the Inazawa, Miyazaki, Suwon and Beijing populations of *R. nigromaculata,* allele *c* was 0.071~0.167 in frequency. The Matsumoto population of *R. b. porosa* and the Ina and Maibara populations of *R. b. brevipoda* had allele *a* in frequencies of 0.011 ~0.015, and the Igaueno population of the latter subspecies had allele *c* in a frequency of 0.125 (Table 5-I).

9. IDH-B locus

Electrophoretic patterns at the IDH-B locus were analyzed in the same 2233 frogs as those used in the analyses at the α-GDH locus. It was found that there were 18 phenotypes, BB, CC, DD, EE, FF, GG, AG, BC, BD, BE, BF, CE, CG, CH, DF, EF, EG and EH, produced by eight alleles, *a~h*. In the 1733 *R. nigromaculata,* five homozygous BB, CC, EE, FF and GG bands were found in 752, 165, 400, four and 19 frogs, respectively, and 10 heterozygous AG, BC, BE, BF, CE, CG, CH, EF, EG and EH bands were found in three, 20, 209, 26, 113, seven, two, four, seven and two frogs, respectively. In these frogs, alleles *a, b, c, e, f, g* and *h* were 0.001, 0.508, 0.136, 0.327, 0.011, 0.016 and 0.001, respectively, in frequency. In the 500 *R. brevipoda,* homozygous BB, DD and FF bands were found in three, 15 and 400 frogs, respectively, and heterozygous BD, BF and DF bands were found in one, 16 and 65 frogs, respectively. In these frogs, alleles *b, d* and *f* were 0.023, 0.096 and 0.881, respectively, in frequency.

R. nigromaculata distinctly differed from *R. brevipoda* in gene frequency. There

were also remarkable differences in gene frequency between different groups of populations in *R. nigromaculata*. In *R. nigromaculata*, 29 populations including 19 populations in the Tohoku, Hokuriku and Chubu districts, three populations in the Kinki district, four populations in Hiroshima Prefecture, the Konko population in Okayama Prefecture and the Takamatsu and Nangoku populations in the Shikoku district had high frequencies of allele *b*, being 0.564~1.000. In 14 populations, the Sakata, Joetsu, Toyama, Kanazawa, Mikuni, Chino, Nagoya, Inazawa, Hiroshima, Kumano, Miyoshi, Shobara, Takamatsu and Nangoku, allele *e* was 0.017~0.436 in frequency, in addition to allele *b*. In the Matsumoto,

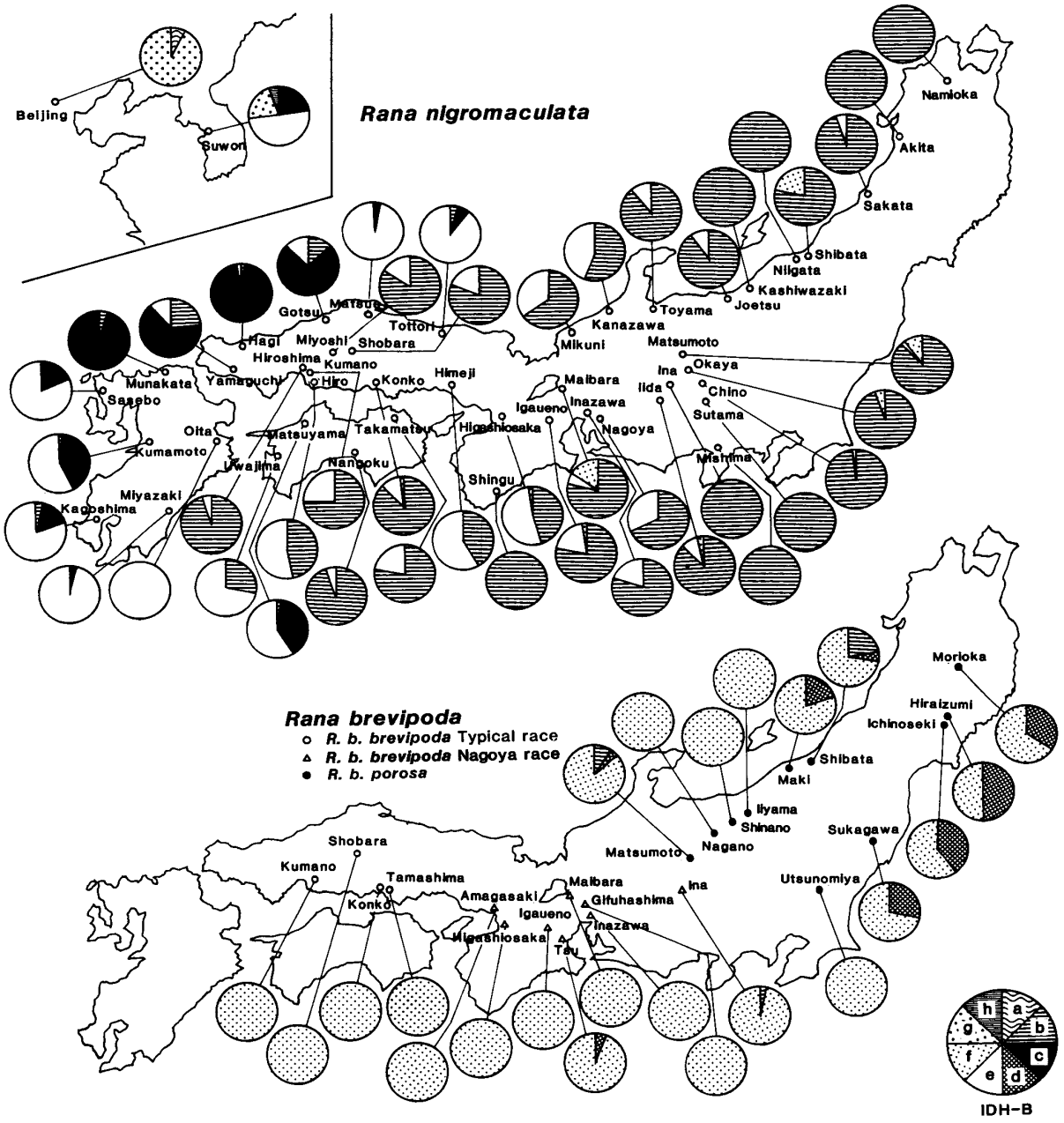

Fig. 8. Geographic distribution of IDH-B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

Iida, Maibara, Igaueno and Konko populations, alleles *e* and *f* were 0.017~0.196 and 0.018~0.167, respectively, in frequency in addition to allele *b*. In the Shibata and Okaya populations, allele *f* was 0.219 and 0.047, respectively, in frequency, in addition to allele *b*. Of the above 29 populations of *R. nigromaculata*, the remaining eight had only allele *b*.

In four populations of *R. nigromaculata*, the Gotsu, Hagi, Yamaguchi and Munakata, allele *c* was very high in frequency, being 0.644~0.970, while these populations had allele *b* in frequencies of 0.015~0.240. The former three of these four populations had allele *e* in frequencies of 0.015~0.130 in addition to alleles *c* and *b*. The Beijing population had alleles *g* and *a* in frequencies of 0.929 and 0.071, respectively.

In the remaining 13 populations of *R. nigromaculata*, allele *e* was high in frequency, being 0.513~1.000. In three populations, the Himeji, Hiro and Uwajima, allele *b* was 0.282~0.474 in frequency in addition to allele *e*. In the Sasebo and Miyazaki populations, allele *c* was 0.181 and 0.036, respectively, in frequency, in addition to allele *e*. In five populations, the Tottori, Matsue, Matsuyama, Kumamoto and Kagoshima, alleles *b* and *c* were 0.007~0.039 and 0.027~0.418, respectively, in frequency in addition to allele *e*. In the Higashiosaka population, alleles *b* and *f* were 0.461 and 0.013, respectively, in frequency in addition to allele *e*. In the Suwon population, alleles *c*, *g* and *h* were 0.224, 0.211 and 0.053, respectively, in frequency in addition to allele *e*. The Oita population had only allele *e*.

In *R. brevipoda*, four populations of *R. b. porosa* and 10 populations of *R. b. brevipoda* all had only allele *f*. In the remaining seven populations of *R. b. porosa*, allele *f* was 0.500~0.872 in frequency. Five of these populations, the Morioka, Hiraizumi, Ichinoseki, Sukagawa and Maki, had allele *d* in frequencies of 0.196 ~0.500 in addition to allele *f*, and the Shibata and Matsumoto populations had allele *b* in frequencies of 0.224 and 0.093, respectively, and allele *d* in frequencies of 0.052 and 0.035, respectively, in addition to allele *f*. The remaining two populations of *R. b. brevipoda*, the Ina and Tsu, had allele *f* in frequencies of 0.970 and 0.950, respectively, while in the Ina population, allele *b* was 0.030, and in the Tsu population, allele *d* was 0.050 in frequency, in addition to allele *f* (Table 5–I; Fig. 8).

10. LDH–A locus

Electrophoretic patterns at the LDH–A locus were analyzed in the same 2233 frogs as those used in the analyses at the α–GDH and IDH–B loci. It was found that there were five phenotypes, CC, DD, AC, BC and CD, produced by four alleles, *a*~*d*. In the 1733 *R. nigromaculata*, these CC, DD, AC, BC and CD bands were observed in 1703, seven, 14, five and four frogs, respectively. Alleles *a*, *b*, *c* and *d* were 0.004, 0.001, 0.989 and 0.005, respectively, in frequency. In the 500 *R. brevipoda*, all the frogs showed a homozygous CC band produced by allele *c*.

Although nearly all the populations of *R. nigromaculata* had only allele *c*, the Mikuni population had allele *b* in a frequency of 0.100, the Sasebo population had

allele *a* in a frequency of 0.121 and the Matsue, Matsuyama and Munakata populations had allele *d* in frequencies of 0.007~0.239, in addition to allele *c*. All the remaining 42 populations of *R. nigromaculata* and the 23 populations of *R. brevipoda* had only allele *c* (Table 5–I).

11. LDH–B locus

Electrophoretic patterns at the LDH–B locus were analyzed in the same 2233 frogs as those used in the analyses at the α–GDH, IDH–B and LDH–A loci. The results showed that there were seven phenotypes, BB, CC, DD, AB, BC, BD and

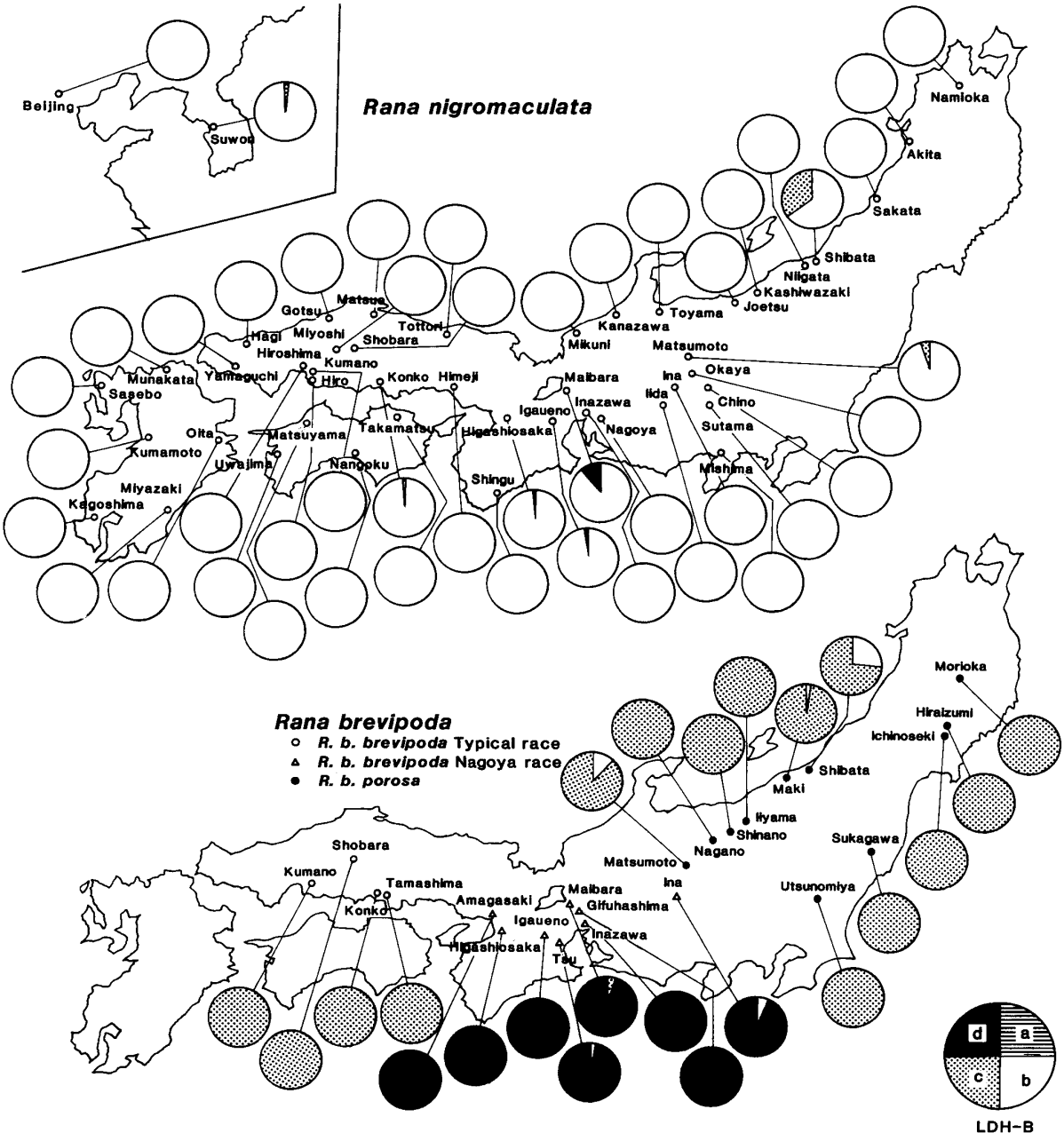

Fig. 9. Geographic distribution of LDH–B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

CD, produced by four alleles, *a*~*d*. In the 1733 *R. nigromaculata,* BB, CC, AB, BC and BD bands produced by four alleles were found in 1710, one, one, 13 and eight frogs, respectively. Alleles *a, b, c* and *d* were 0.0003, 0.993, 0.004 and 0.002, respectively, in frequency. In the 500 *R. brevipoda,* homozygous BB, CC and DD bands were found in four, 343 and 126 frogs, respectively, and heterozygous BC, BD and CD bands were found in 18, five and four frogs, respectively. Alleles *b, c* and *d* were 0.031, 0.708 and 0.261, respectively, in frequency.

In the 47 populations of *R. nigromaculata,* allele *b* was very high in frequency, being 0.656~1.000. In addition to allele *b*, allele *c* was 0.006~0.344 in frequency in the Shibata, Matsumoto and Konko populations, allele *d* was 0.013~0.119 in the Maibara, Igaueno and Higashiosaka populations, and allele *a* was 0.013 in the Suwon population. The other 40 populations had only allele *b*.

In the Shibata, Maki and Matsumoto populations of *R. b. porosa,* allele *c* was high in frequency, being 0.741~0.982, while allele *b* was 0.018~0.259. The remaining eight populations of *R. b. porosa* and the Tamashima, Konko, Shobara and Kumano populations belonging to the Typical race of *R. b. brevipoda* had only allele *c*. In contrast, eight populations belonging to the Nagoya race of *R. b. brevipoda* had allele *d* which was very high in frequency, being 0.939~1.000. In addition to allele *d*, the Ina and Tsu populations had allele *b* in frequencies of 0.061 and 0.017, respectively, and the Maibara population had allele *c* in a frequency of 0.044. The other five populations of *R. b. brevipoda* had only allele *d* (Table 5-II; Fig. 9).

12. MDH-A locus

Electrophoretic patterns at the MDH-A locus were analyzed in the same 2233 frogs as those used in the analyses at the α-GDH, IDH-B, LDH-A and LDH-B loci. It was found that there were two phenotypes, AA and AB, produced by alleles *a* and *b*. In the 1733 *R. nigromaculata,* AA and AB bands were found in 1729 and four frogs, respectively. Alleles *a* and *b* were 0.999 and 0.001, respectively, in frequency. All the frogs belonging to *R. brevipoda* revealed only a homozygous AA band produced by allele *a*.

Of the 70 populations in total, 68 other than the Munakata and Beijing of *R. nigromaculata* had only allele *a*. The Munakata and Beijing populations had allele *b* in frequencies of 0.009 and 0.071, respectively, in addition to allele *a* (Table 5-II).

13. MDH-B locus

Electrophoretic patterns at the MDH-B locus were analyzed in the same 2233 frogs as those used in the analyses at the α-GDH, IDH-B, LDH-A, LDH-B and MDH-A loci. The results showed that there were 12 phenotypes, AA, BB, CC, DD, EE, AB, AC, AE, BC, BD, BF and DF, produced by six alleles *a*~*f*. In the 1733 *R. nigromaculata,* homozygous AA, BB, DD and EE bands were found in 250, 1203, 135 and one frogs, respectively, while heterozygous AB, AC, AE, BC, BD, BF and DF bands were found in 27, one, 19, 13, 76, two and six frogs, respectively.

Alleles *a*, *b*, *c*, *d*, *e* and *f* were 0.158, 0.728, 0.004, 0.102, 0.006 and 0.002, respectively, in frequency. In the 500 *R. brevipoda*, AA, BB, CC, AC and BC bands were found in two, two, 467, 14 and 15 frogs, respectively. Alleles *a*, *b* and *c* were 0.018, 0.019 and 0.963, respectively, in frequency.

In *R. nigromaculata*, 34 populations including 10 in the Tohoku and Hokuriku districts, five in the Kinki district and 19 in the Chugoku, Shikoku and Kyushu districts other than the Tottori and Matsue populations in the Chugoku district had allele *b* in high frequencies of 0.630~1.000. In the Shibata population, allele *c* which was assumed to have invaded by natural hybridization from *R. b. porosa*

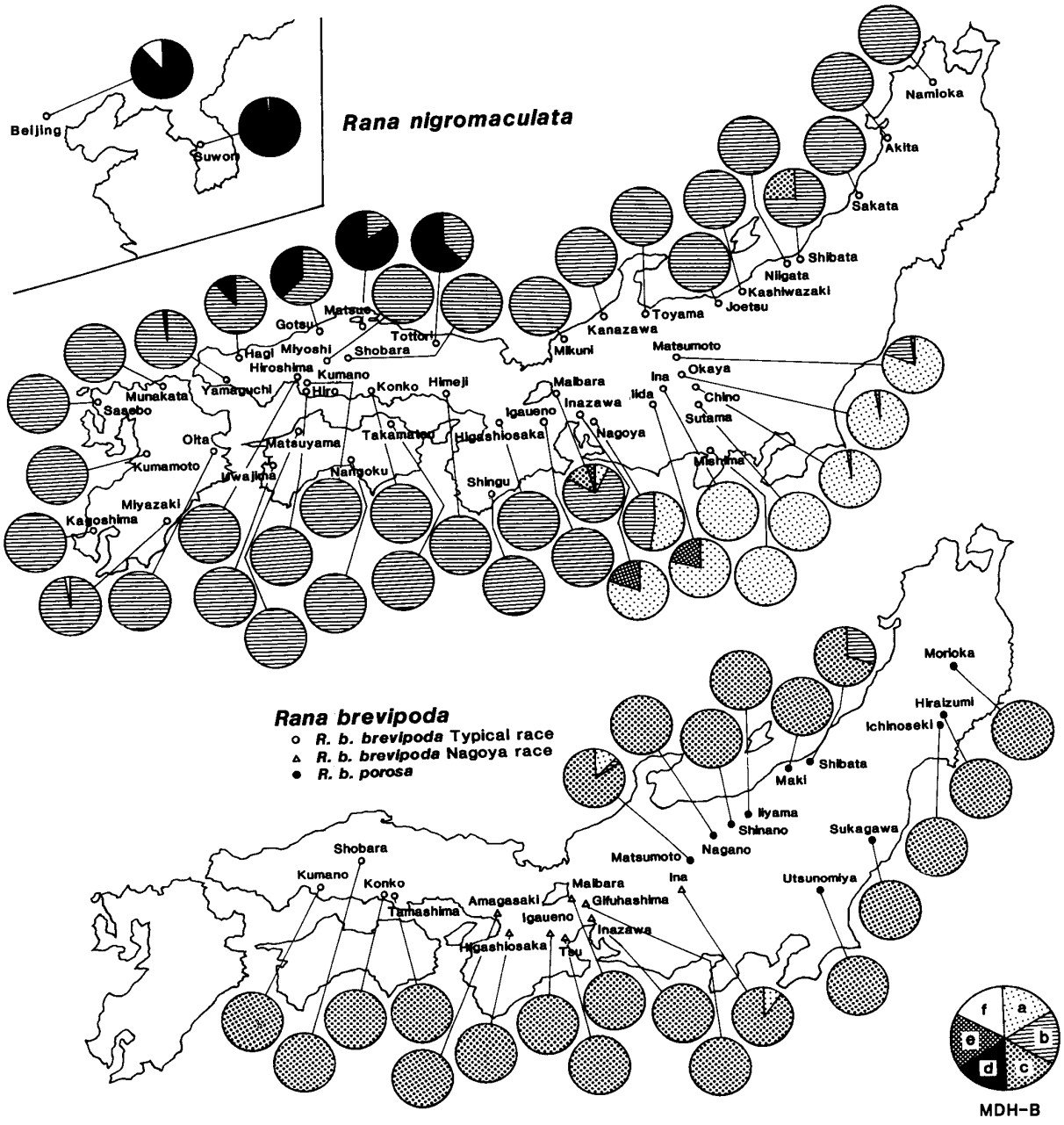

Fig. 10. Geographic distribution of MDH-B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

was found in a frequency of 0.250, in addition to allele *b*. The Gotsu, Hagi and Yamaguchi populations had allele *d* in frequencies of 0.010~0.370, the Miyazaki population had allele *f* in a frequency of 0.018, and the Maibara population had alleles *a*, *c* and *e* in frequencies of 0.071, 0.119 and 0.048, respectively, in addition to allele *b*. The remaining 28 of the 34 populations had only allele *b*. In the Tottori, Matsue, Suwon and Beijing populations, allele *d* was high in frequency, being 0.625~0.987. In addition to allele *d*, the Tottori and Matsue populations had allele *b* in frequencies of 0.375 and 0.153, respectively, while the Suwon and Beijing populations had allele *f* in frequencies of 0.013 and 0.119, respectively. In nine populations distributed in the Chubu district, allele *a* was high in frequency, being 0.533~1.000. In addition to allele *a*, the Okaya, Chino and Nagoya populations had allele *b* in frequencies of 0.023, 0.017 and 0.467, respectively, the Iida and Inazawa populations had allele *e* in frequencies of 0.212 and 0.200, respectively, and the Matsumoto population had alleles *b* and *c* in frequencies of 0.167 and 0.017, respectively. The remaining three populations had only allele *a* (Table 5–II; Fig. 10).

In *R. brevipoda,* allele *c* was high in frequency, being 0.707~1.000. In the Shibata population, allele *b* which was assumed to have invaded by natural hybridization from *R. nigromaculata* was found in a frequency of 0.293. In the Matsumoto population, alleles *a* and *b* which invaded from *R. nigromaculata* were found in frequencies of 0.128 and 0.023, respectively, and in the Ina population, allele *a* which invaded from *R. nigromaculata* was also found in a frequency of 0.106, in addition to allele *c*. All the remaining 20 populations had only allele *c* (Table 5–II; Fig. 10).

14. ME–A locus

Electrophoretic patterns at the ME–A locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT–B, ADA and AK loci. The results showed that there were six phenotypes, BB, CC, DD, AB, BC and BD, produced by four alleles, *a*~*d*. In the 1162 *R. nigromaculata,* homozygous BB, CC and DD bands were found in 1126, two and five frogs, respectively, and heterozygous AB, BC and BD bands were found in one, nine and 19 frogs, respectively. Alleles *a*, *b*, *c* and *d* were 0.0004, 0.981, 0.006 and 0.012, respectively, in frequency. In the 454 *R. brevipoda,* CC and BC bands were found in 441 and 13 frogs, respectively. Alleles *b* and *c* were 0.014 and 0.986, respectively, in frequency.

In all the populations of *R. nigromaculata,* allele *b* was high in frequency, being 0.550~1.000. In the Shibata and Maibara populations, allele *c* which was assumed to have invaded by natural hybridization from *R. brevipoda* was found in frequencies of 0.156 and 0.190, respectively, in addition to allele *b*. In the Beijing population, allele *a* was found in a frequency of 0.024, and in each of the Kumamoto and Oita populations, allele *d* was found in a frequency of 0.017. In the Sasebo population, allele *d* was found in a frequency of 0.450. In this population, there was no great difference in frequency between alleles *b* and *d*. The remaining 41 populations had only allele *b*.

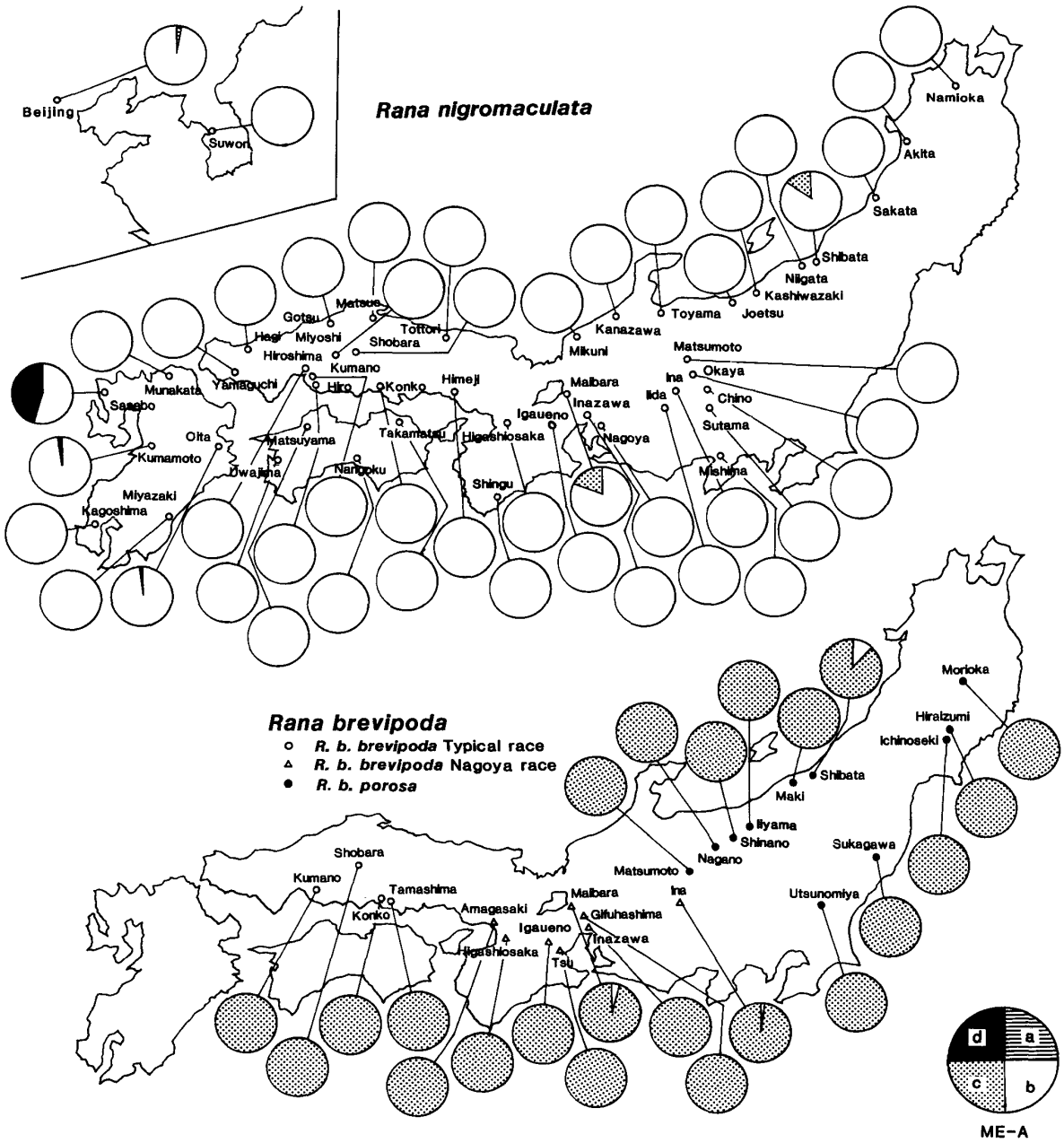

Fig. 11. Geographic distribution of ME–A alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

In *R. brevipoda*, allele *c* was high in frequency, being 0.882~1.000. In the Shibata population, allele *b* which was assumed to have invaded by natural hybridization from *R. nigromaculata* was found in a frequency of 0.118. In the Ina and Maibara populations, allele *b* which invaded from *R. nigromaculata* was also found in frequencies of 0.015 and 0.044, respectively, in addition to allele *c*. All the remaining 20 populations had only allele *c* (Table 5–II; Fig. 11).

15. ME–B locus

Electrophoretic patterns at the ME–B locus were analyzed in the same 1616

frogs as those used in the analyses at the ME–A locus. It was found that there were 31 phenotypes, including homozygous AA, BB, CC, DD, EE, FF, GG, HH, II and JJ bands and heterozygous AC, AG, AI, BC, BG, BI, CD, CE, CG, CH, CI, CJ, CK, DF, DG, EG, FG, GH, GI, GK and HI bands, produced by 11 alleles, *a*~*k*. In the 1162 *R. nigromaculata,* nine homozygous AA, BB, CC, DD, EE, GG, HH, II and JJ bands were found in three, four, 406, one, six, 361, 17, four and one frogs, respectively, and 20 heterozygous AC, AG, AI, BC, BG, BI, CD, CE, CG, CH, CI, CJ, CK, DG, EG, FG, GH, GI, GK and HI bands were found in eight, eight, five, 17, two, four, one, five, 213, two, six, four, one, five, five, one, 45, 12, one and 14 frogs, respectively. In these frogs, 11 alleles, *a, b, c, d, e, f, g, h, i, j* and *k*, were found in frequencies of 0.012, 0.013, 0.460, 0.003, 0.009, 0.0004, 0.436, 0.041, 0.021, 0.003 and 0.001, respectively. In the 454 *R. brevipoda,* homozygous DD, FF and GG bands were found in 396, six and three frogs, respectively, and heterozygous CD, DF, DG and FG bands were found in one, 30, 17 and one frogs, respectively. In these frogs, alleles *c, d, f* and *g* were found in frequencies of 0.001, 0.925, 0.047 and 0.026, respectively.

In 23 populations of *R. nigromaculata* including 10 in the Tohoku and Hokuriku districts, six in the Chugoku district, three in the Shikoku district, one in the Chubu district, and three in the Kinki district, allele *g* was high in frequency, being 0.563~1.000. Eight populations, the Sakata, Joetsu, Toyama, Kanazawa, Mishima, Shingu, Takamatsu and Matsuyama, had allele *c* in frequencies of 0.067~0.438 in addition to allele *g*. The Shibata population had allele *d* which was assumed to have invaded from *R. brevipoda* in a frequency of 0.219 in addition to allele *g*. In addition to allele *g*, the Mikuni population had alleles *c* and *i* in frequencies of 0.200 and 0.040, respectively, the Igaueno population had alleles *c* and *f* in frequencies of 0.417 and 0.010, respectively, the Himeji population had alleles *c* and *h* in frequencies of 0.083 and 0.333, respectively, and six populations, the Hiroshima, Kumano, Hiro, Miyoshi, Shobara and Uwajima, had allele *h* in frequencies of 0.117~0.278. In the former five of these six populations, allele *i* was found in frequencies of 0.026~0.067. The Konko population had alleles *c, h* and *i* in frequencies of 0.283, 0.067 and 0.017, respectively. The remaining four populations, the Namioka, Akita, Niigata and Kashiwazaki, had only allele *g*.

In 21 populations of *R. nigromaculata,* including eight populations in the Chubu district other than the Mishima population, the Higashiosaka and Maibara populations in the Kinki district, the Gotsu and Tottori in the Chugoku district, the Nangoku in the Shikoku district, six populations in the Kyushu district, and the Suwon and Beijing populations, allele *c* was high in frequency, being 0.517 ~1.000. Of these populations, 12 populations, the Okaya, Chino, Sutama, Nagoya, Inazawa, Higashiosaka, Tottori, Gotsu, Nangoku, Sasebo, Oita and Beijing, had allele *g* in frequencies of 0.017~0.408, the Maibara population had alleles *d* and *g* in frequencies of 0.024 and 0.071, respectively, the Munakata population had alleles *a* and *g* in frequencies of 0.133 and 0.350, respectively, the Kagoshima population had alleles *b, g* and *i* in frequencies of 0.133, 0.067 and 0.083, respectively, the Miyazaki population had alleles *b, g* and *i* in frequencies of

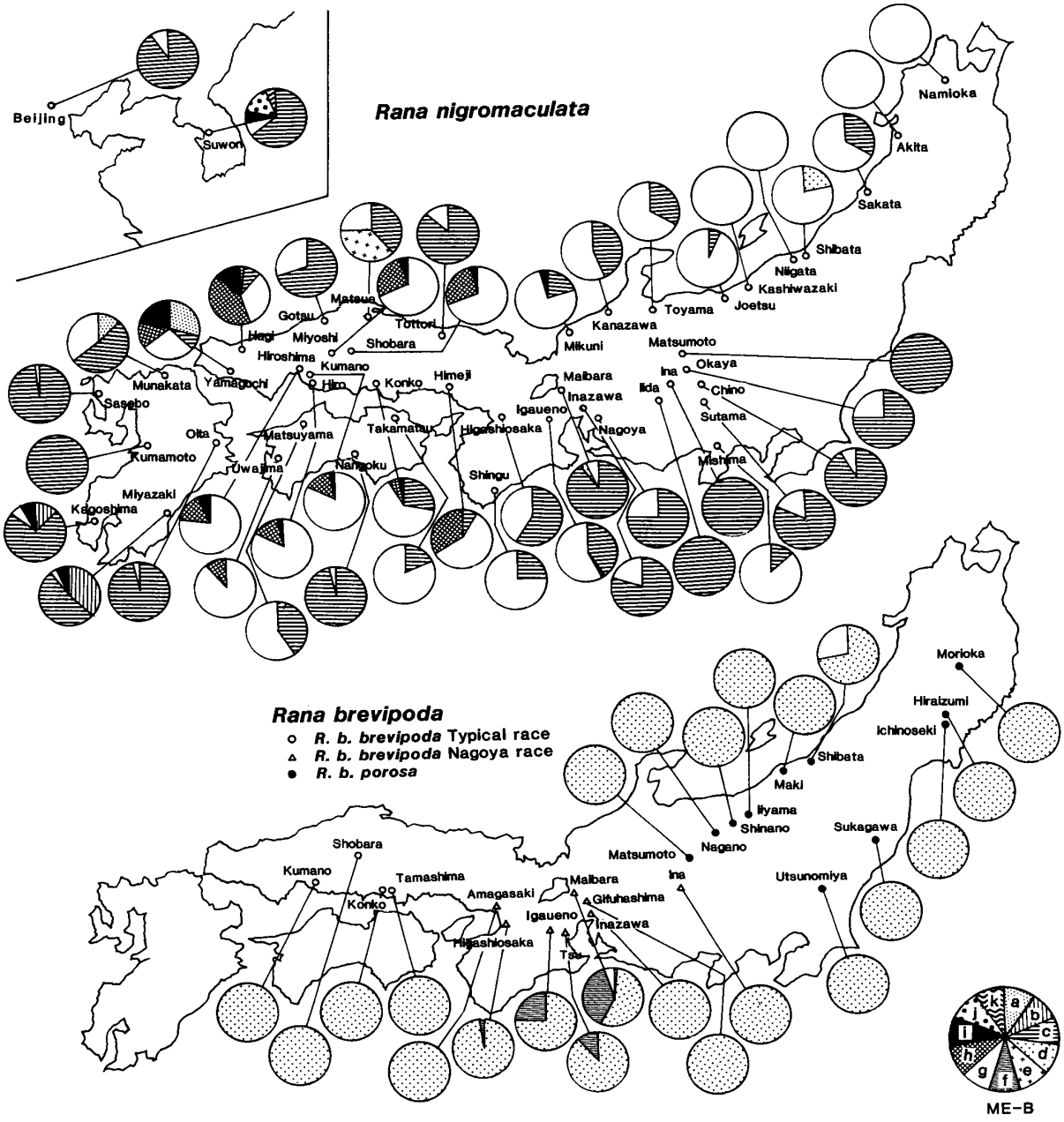

Fig. 12. Geographic distribution of ME-B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

0.383, 0.033 and 0.067, respectively, and the Suwon population had alleles *g*, *i*, *j* and *k* in frequencies of 0.056~0.167, in addition to allele *c*. The remaining four populations, the Matsumoto, Ina, Iida and Kumamoto, had only allele *c*.

The Hagi population in Yamaguchi Prefecture had alleles *a*, *c*, *g*, *h* and *i* in frequencies of 0.017, 0.100, 0.333, 0.417 and 0.133, respectively, and the Yamaguchi population had alleles *a*, *c*, *g*, *h* and *i* in frequencies of 0.300, 0.067, 0.283, 0.133 and 0.217, respectively. The Matsue population had alleles *c*, *e* and *g* in frequencies of 0.383, 0.367 and 0.250, respectively.

In *R. brevipoda*, allele *d* was high in frequency, being 0.567~1.000. In addition

to allele *d*, the Maibara population had alleles *c*, *f* and *g* in frequencies of 0.011, 0.367 and 0.056, respectively, and the Shibata population had allele *g* derived from *R. nigromaculata* in a frequency of 0.279. Three populations, the Tsu, Igaueno and Higashiosaka, had allele *f* in frequencies of 0.025~0.250. The remaining 18 populations had only allele *d* (Table 5–II; Fig. 12).

16. MPI locus

Electrophoretic patterns at the MPI locus were analyzed in the same 1616 frogs as those used in the analyses at the ME–A and ME–B loci. The results showed that there were 14 phenotypes, AA, BB, CC, DD, EE, GG, AD, AE, BD, CD, CE, DE, DF and DG, produced by seven alleles, *a*~*g*. In the 1162 *R. nigromaculata*, homozygous BB, CC, DD and GG bands were found in one, eight, 1045 and 12 frogs, respectively, and heterozygous AD, BD, CD, DE, DF and DG bands were found in one, 12, 33, five, one and 44 frogs, respectively. Alleles *a*, *b*, *c*, *d*, *e*, *f* and *g* were found in frequencies of 0.0004, 0.006, 0.021, 0.941, 0.002, 0.0004 and 0.029, respectively. In the 454 *R. brevipoda*, homozygous AA, DD and EE bands were found in 12, 344 and 11 frogs, respectively, and heterozygous AD, AE, CD, CE, DE and DF bands were found in 52, 12, one, one, 14 and seven frogs, respectively. Alleles *a*, *c*, *d*, *e* and *f* were found in frequencies of 0.097, 0.002, 0.839, 0.054 and 0.008, respectively.

In 64 populations including 47 populations of *R. nigromaculata*, 11 populations of *R. brevipoda porosa*, five populations of the Nagoya race of *R. b. brevipoda* other than the Maibara, Igaueno and Higashiosaka, and the Konko population of the Typical race of *R. b. brevipoda*, allele *d* was high in frequency, being 0.533~1.000. In *R. nigromaculata*, five populations, the Matsumoto, Iida, Nagoya, Inazawa and Kumamoto, had allele *c* in frequencies of 0.033~0.467, three populations, the Hagi, Kagoshima and Miyazaki, had allele *b* in frequencies of 0.050~0.100, five populations, the Mikuni, Hiroshima, Kumano, Hiro and Nangoku, had allele *g* in frequencies of 0.080~0.417, the Higashiosaka population had allele *e* in a frequency of 0.039, the Maibara population had alleles *a*, *c* and *e* in frequencies of 0.024, 0.143 and 0.048, respectively, the Suwon population had alleles *c* and *g* in frequencies of 0.028 and 0.056, respectively, and the Beijing population had alleles *c*, *f* and *g* in frequencies of 0.024, 0.024 and 0.071, respectively, in addition to allele *d*. In four populations of *R. b. porosa*, the Morioka, Hiraizumi, Ichinoseki and Sukagawa, and the Konko population of *R. b. brevipoda*, allele *a* was found in frequencies of 0.111~0.340, and in the Utsunomiya population, alleles *a* and *f* were found in frequencies of 0.016 and 0.109, respectively, in addition to allele *d*. The remaining 41 populations had only allele *d*.

The Maibara population of the Nagoya race of *R. b. brevipoda* had alleles *a*, *c*, *d* and *e* in frequencies of 0.022~0.433. In the Igaueno and Higashiosaka populations, allele *e* was found in frequencies of 0.875 and 0.575, respectively, and allele *d* was 0.125 and 0.325, respectively, in frequency. The Higashiosaka had allele *a* in a frequency of 0.100 in addition. In the Tamashima population of the Typical race of *R. b. brevipoda*, there were alleles *a* and *d*, each of which was 0.500 in

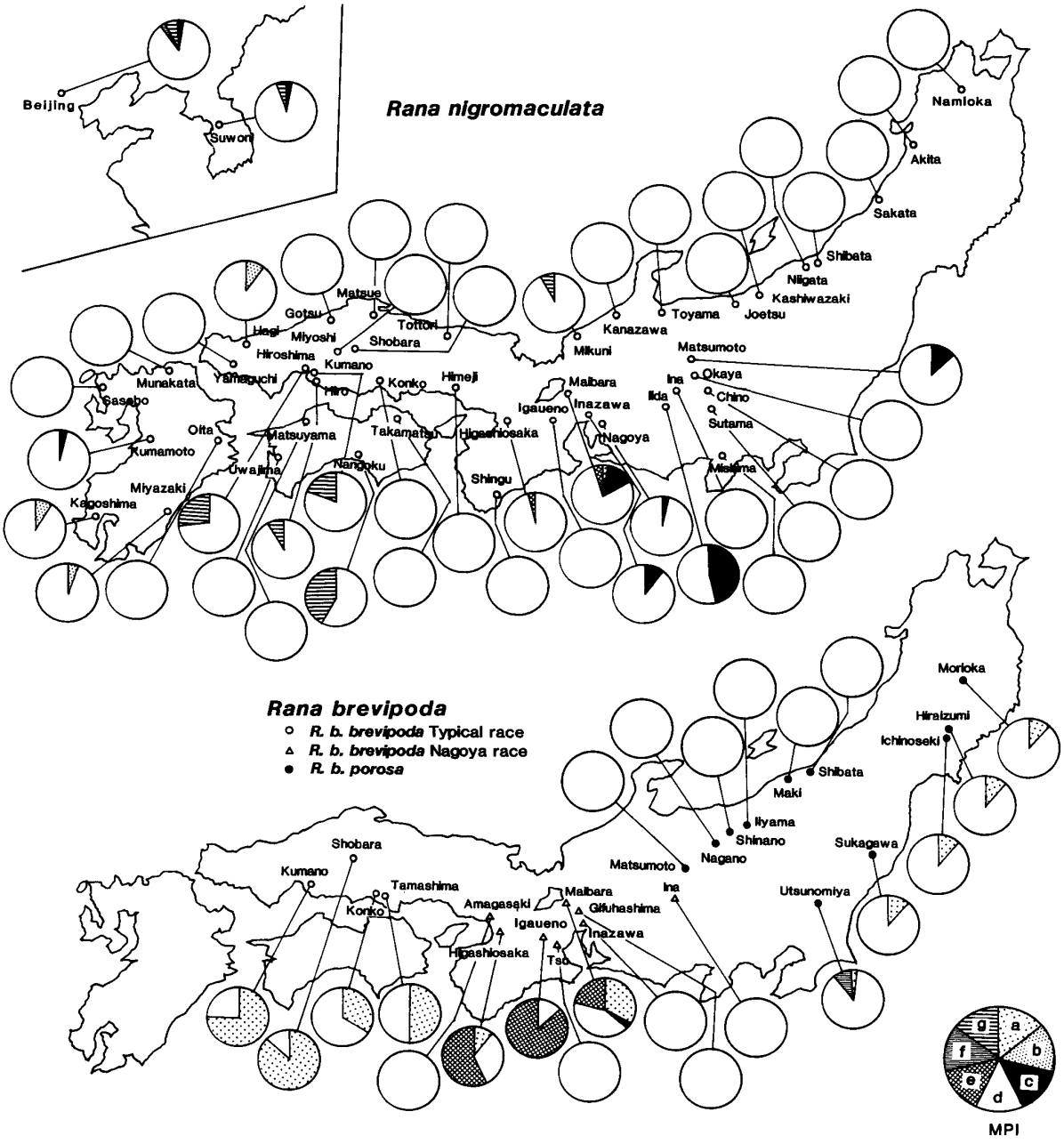

Fig. 13. Geographic distribution of MPI alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

frequency. In the Shobara and Kumano populations, allele *a* was found in frequencies of 0.875 and 0.750, respectively, and allele *d* was 0.125 and 0.250, respectively, in frequency (Table 5–II; Fig. 13).

17. Pep–A locus

Electrophoretic patterns at the Pep–A locus were analyzed in the same 1580 frogs as those used in the analyses at the ADH–A, ADH–B and Fum loci. The results showed that there were five phenotypes, AA, BB, CC, AB and BC, produced by three alleles, *a*~*c*. In the 1144 *R. nigromaculata*, five bands, AA, BB,

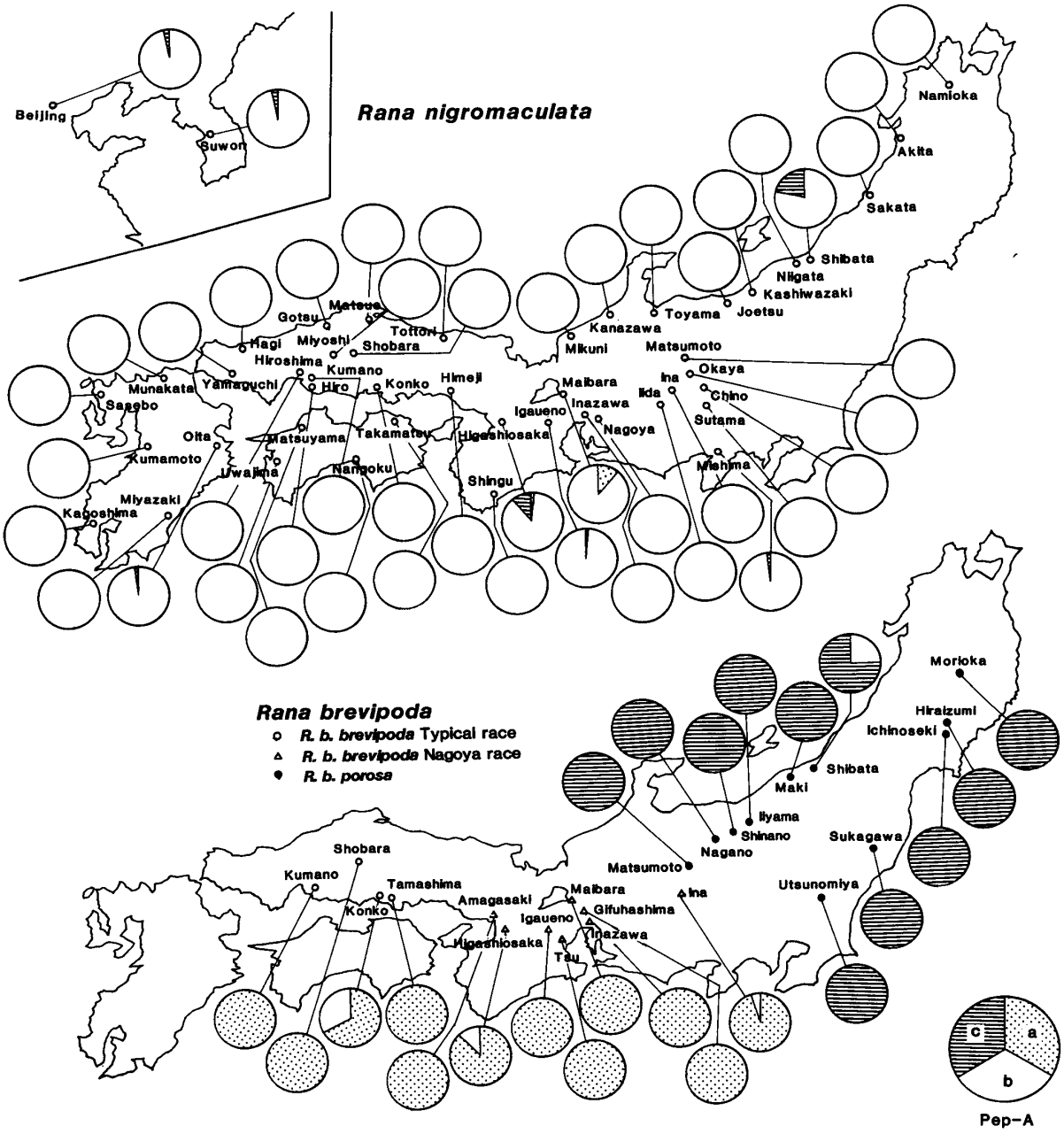

Fig. 14. Geographic distribution of Pep-A alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

CC, AB and BC, were observed in one, 1120, one, five and 17 frogs, respectively. Alleles *a*, *b* and *c* were 0.003, 0.989 and 0.008, respectively, in frequency. In the 436 *R. brevipoda*, five bands, AA, BB, CC, AB and BC, were observed in 132, seven, 271, 17 and nine frogs, respectively. Alleles *a*, *b* and *c* were 0.322, 0.046 and 0.632, respectively, in frequency.

In *R. nigromaculata*, allele *b* was high in frequency, being 0.781~1.000. In addition to allele *b*, allele *c* was 0.017~0.219 in frequency in the Shibata, Mishima, Oita, Suwon and Beijing populations, and allele *a* was 0.119 and 0.010 in frequency in the Maibara and Igaueno populations, respectively. In the

Higashiosaka population, alleles *a* and *c* were found in frequencies of 0.013 and 0.105, respectively, in addition to allele *b*. All the remaining 39 populations had only allele *b*.

In *R. b. porosa,* allele *c* was high in frequency, being 0.750~1.000. In addition to allele *c*, the Shibata population had allele *b* derived from *R. nigromaculata* in a frequency of 0.250. The remaining 10 populations had only allele *c*.

In *R. b. brevipoda,* allele *a* was high in frequency being 0.674~1.000. In the Ina, Higashiosaka and Konko populations, allele *b* was 0.045~0.326 in frequency in addition to allele *a*. All the remaining nine populations had only allele *a* (Table 5–II; Fig. 14).

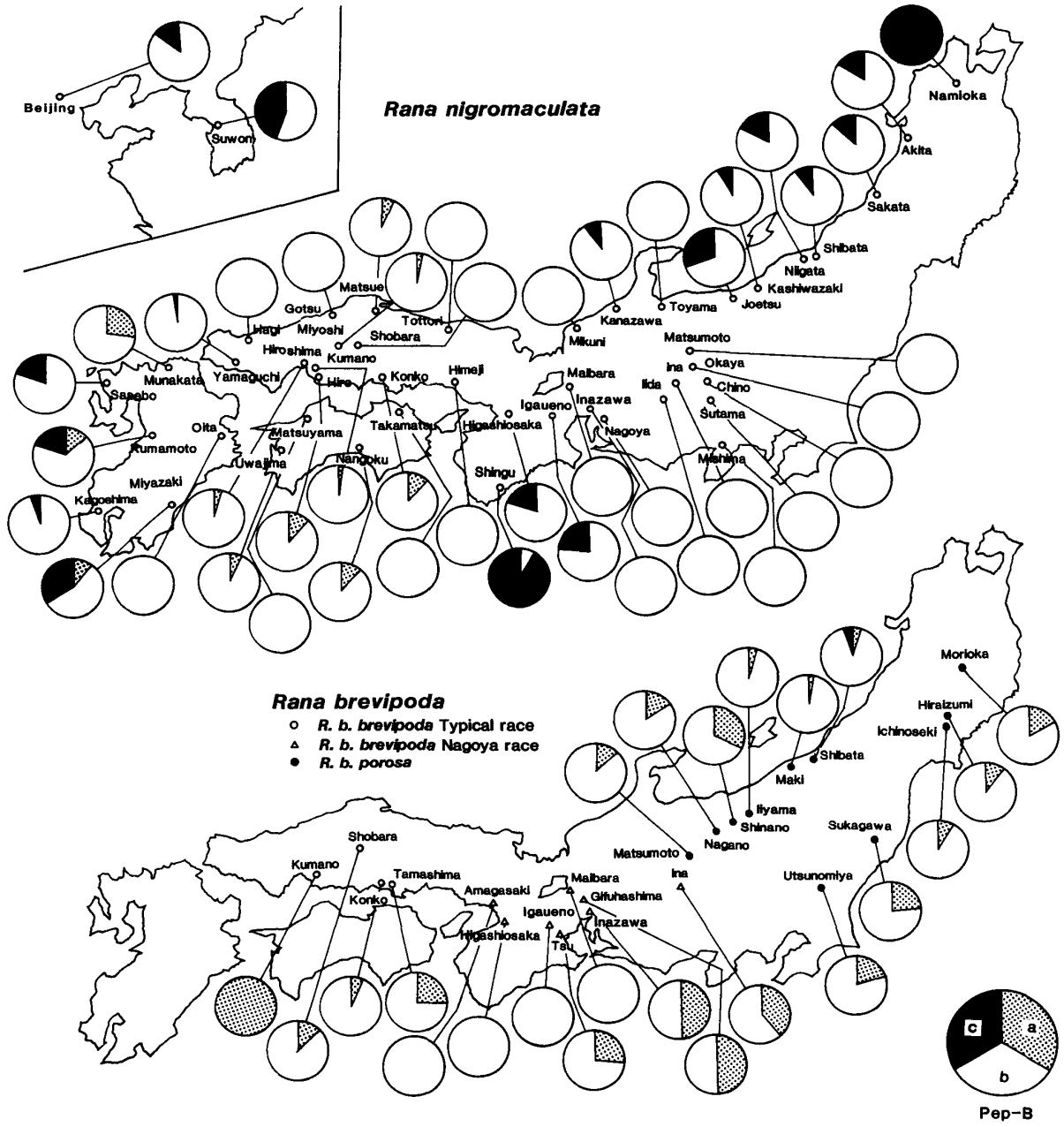

Fig. 15. Geographic distribution of Pep–B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

18. Pep-B locus

Electrophoretic patterns at the Pep-B locus were analyzed in the same 1580 frogs as those used in the analyses at the Pep-A locus. The results showed that there were six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*~*c*. In the 1144 *R. nigromaculata,* homozygous AA, BB and CC bands were found in two, 931 and 43 frogs, respectively, and heterozygous AB, AC and BC bands were in 52, four and 112 frogs, respectively. Alleles *a, b* and *c* were 0.026, 0.885 and 0.088, respectively, in frequency. In the 436 *R. brevipoda,* AA, BB, AB and BC bands were found in 15, 328, 90 and three frogs, respectively. Alleles *a, b* and *c* were 0.138, 0.859 and 0.003, respectively, in frequency.

In 45 populations of *R. nigromaculata* other than the Namioka and Shingu and in 20 populations of *R. brevipoda* other than the Inazawa, Gifuhashima and Kumano populations, allele *b* was high in frequency, being 0.556~1.000. The Namioka population had only allele *c*, while in the Shingu population, allele *c* was 0.917 in frequency and allele *b* was 0.083. The Kumano population of the *R. b. brevipoda* had only allele *a*, while in the Inazawa and Gifuhashima populations alleles *a* and *b* were each 0.500 in frequency. In 14 populations of *R. nigromaculata,* the Akita, Sakata, Shibata, Niigata, Kashiwazaki, Joetsu, Kanazawa, Igaueno, Higashiosaka, Yamaguchi, Sasebo, Kagoshima, Suwon and Beijing, allele *c* was 0.017~0.444 in frequency, in addition to allele *b*. In 24 populations including nine populations of *R. nigromaculata,* the Matsue, Hiroshima, Kumano, Hiro, Miyoshi, Konko, Nangoku, Uwajima and Munakata, and 10 populations of *R. b. porosa* other than the Shibata population and five populations of *R. b. brevipoda,* the Ina, Tsu, Tamashima, Konko and Shobara, allele *a* was 0.013~0.394 in frequency in addition to allele *b*. In the Kumamoto and Miyazaki populations of *R. nigromaculata* and the Shibata population of *R. b. porosa,* alleles *a* and *c* were 0.044~0.133 and 0.044~0.333, respectively, in frequency in addition to allele *b*. The remaining 20 populations of *R. nigromaculata* and four populations of *R. b. brevipoda* had only allele *b* (Table 5-III; Fig. 15).

19. Pep-C locus

Electrophoretic patterns at the Pep-C locus were analyzed in 1578 frogs of 70 populations including 1144 frogs of 47 populations belonging to *R. nigromaculata* and 434 frogs of 23 populations belonging to *R. brevipoda*. The results showed that there were seven phenotypes, AA, BB, DD, AB, AD, BC and BD, produced by four alleles, *a*~*d*. In the 1144 *R. nigromaculata,* AA, BB, AB, BC and BD bands were found in 572, 345, 224, two and one frogs, respectively. Alleles *a, b, c* and *d* were 0.598, 0.401, 0.001 and 0.0004, respectively, in frequency. In the 434 *R. brevipoda,* AA, BB, DD, AB, AD and BD bands were found in six, 338, 20, 13, four and 53 frogs, respectively. Alleles *a, b* and *d* were 0.033, 0.855 and 0.112, respectively, in frequency.

In 27 populations of *R. nigromaculata,* including three of the seven in the Hokuriku district, nine in the Chubu district, four of the five in the Kinki district,

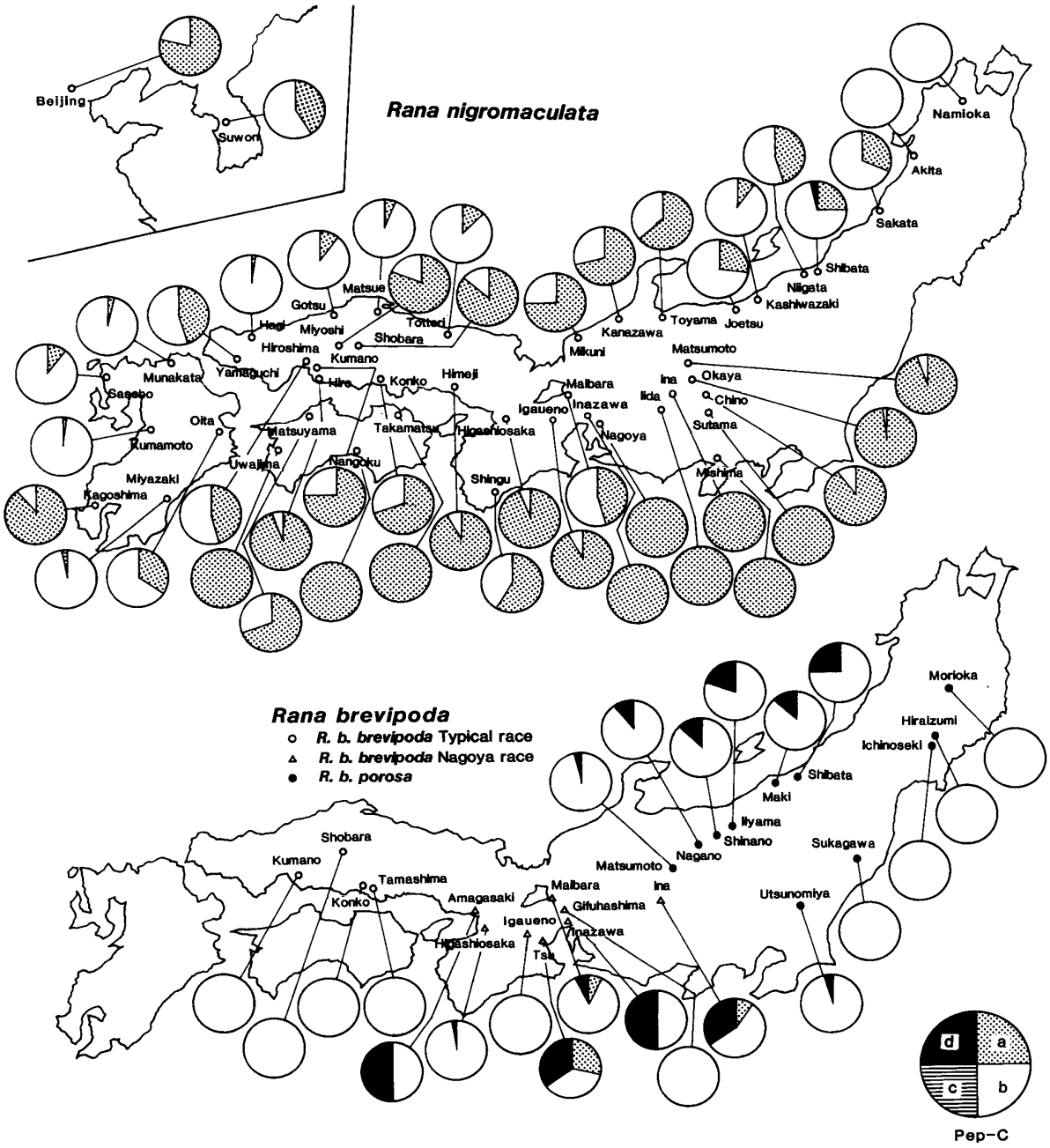

Fig. 16. Geographic distribution of Pep-C alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

five of the 11 in the Chugoku district, four in the Shikoku district, one in the Kyushu district and the Beijing population, allele *a* was found in high frequency, being 0.583~1.000. Nine of these populations, the Sutama, Mishima, Ina, Iida, Nagoya, Inazawa, Takamatsu, Nangoku and Uwajima, had only allele *a*. In the remaining 18 populations, there was allele *b* in frequencies of 0.017~0.417 in addition to allele *a*. On the other hand, in 20 populations including three in the Tohoku district, four in the Hokuriku district, one in the Kinki district, six in the Chugoku district, five in the Kyushu district and the Suwon population, allele *b* was high in frequency, being 0.542~1.000. The Namioka and Akita populations

had only allele *b*, while the Miyazaki population had allele *c* in a frequency of 0.033 in addition to allele *b*. In the remaining 17 populations, allele *a* was 0.017~0.458 in frequency in addition to allele *b*.

In 20 populations including 11 of *R. b. porosa*, five of the Nagoya race of *R. b. brevipoda*, the Ina, Gifuhashima, Maibara, Igaueno and Higashiosaka, and four of the Typical race of *R. b. brevipoda*, allele *b* was high in frequency, being 0.565 ~1.000. While 10 of these populations, including the Morioka, Hiraizumi, Ichinoseki and Sukagawa of *R. b. porosa*, the Gifuhashima and Igaueno of the Nagoya race of *R. b. brevipoda*, and the Tamashima, Konko, Shobara and Kumano of the Typical race of *R. b. brevipoda*, had only allele *b*, the remaining seven populations of *R. b. porosa* and the Higashiosaka population of *R. b. brevipoda* had allele *d* in frequencies of 0.025~0.250 in addition to allele *b*. The Maibara population had alleles *a* and *d* each in a frequency of 0.097 in addition to allele *b*. The Ina population had alleles *a* and *d* in frequencies of 0.097 and 0.339, respectively.

The Inazawa and Amagasaki populations of *R. b. brevipoda* had alleles *b* and *d* each in a frequency of 0.500. The Tsu population had alleles *b*, *d* and *a* in frequencies of 0.367, 0.350 and 0.283, respectively (Table 5–III; Fig. 16).

20. Pep–D locus

Electrophoretic patterns at the Pep–D locus were analyzed in the same 1580 frogs as those used in the analyses at the Pep–A and Pep–B loci. It was found that there were 11 phenotypes, AA, BB, CC, DD, EE, AB, AD, BC, BD, BE and DE, produced by five alleles, *a*~*e*. In the 1144 *R. nigromaculata*, homozygous AA, BB, CC and DD bands were found in one, 1028, three and 18 frogs, respectively, and heterozygous AB, AD, BC, BD and BE bands were found in 29, one, 17, 45 and two frogs, respectively. Alleles *a*, *b*, *c*, *d* and *e* were 0.014, 0.939, 0.010, 0.036 and 0.001, respectively, in frequency. In the 436 *R. brevipoda*, homozygous BB, DD and EE bands were found in 17, 293 and two frogs, respectively, and heterozygous BD, BE and DE bands were found in 101, four and 19 frogs, respectively. Alleles *b*, *d* and *e* were 0.159, 0.810 and 0.031, respectively, in frequency.

In 44 of the 47 populations of *R. nigromaculata* other than the Maibara, Higashiosaka and Himeji, allele *b* was high in frequency, being 0.767~1.000. In addition to allele *b*, four populations, the Tottori, Hagi, Yamaguchi and Munakata, had allele *a* in frequencies of 0.050~0.200, the Shibata, Matsumoto and Igaueno populations had allele *d* in frequencies of 0.063~0.125, the Sasebo and Oita populations had allele *c* in frequencies of 0.233 and 0.150, respectively, and the Iida population had allele *e* in a frequency of 0.017. All the remaining 34 populations had only allele *b*. In the Maibara population, alleles *d* and *b* were 0.667 and 0.333, respectively, in frequency. In the Higashiosaka population, alleles *b*, *d* and *e* were 0.513, 0.474 and 0.013, respectively, and in the Himeji population, alleles *b*, *a* and *d* were 0.500, 0.333 and 0.167, respectively, in frequency.

Of the 23 populations of *R. brevipoda*, the Inazawa had alleles *d* and *e* each of

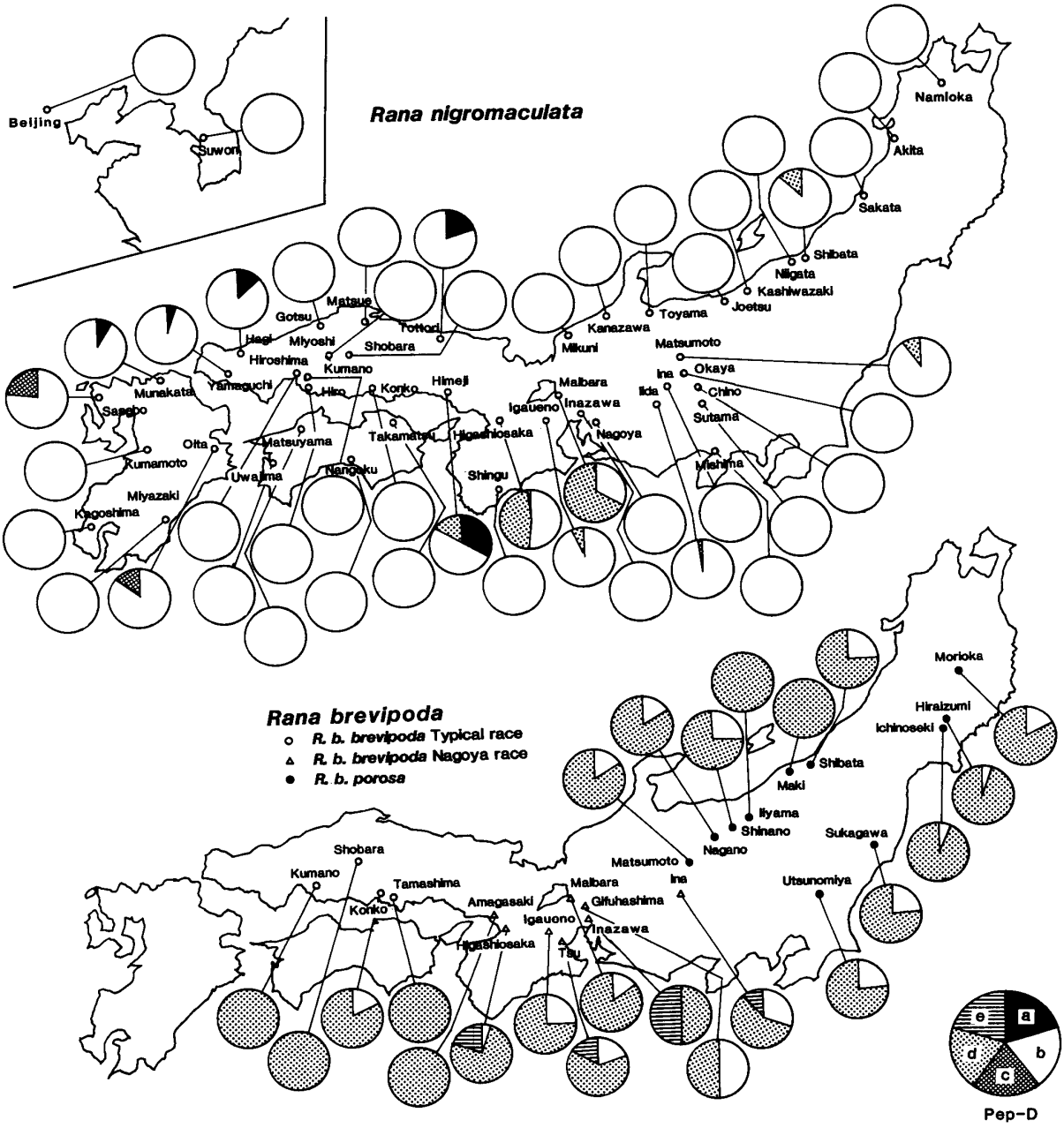

Fig. 17. Geographic distribution of Pep-D alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

which was 0.500 in frequency, and the Gifuhashima had alleles *b* and *d* each of which was 0.500 in frequency. The Ina population had alleles *d*, *b* and *e* in frequencies of 0.576, 0.303 and 0.121, respectively. In the remaining 20 populations, allele *d* was high in frequency, being 0.633~1.000. Six of these populations, the Maki, Iiyama, Amagasaki, Tamashima, Shobara and Kumano, had only allele *d*. In addition to allele *d*, the Tsu population had alleles *b* and *e* in frequencies of 0.200 and 0.167, respectively, and the Higashiosaka had alleles *b* and *e* in frequencies of 0.050 and 0.200, respectively. In the remaining 12 populations, allele *b* was 0.056~0.250 in frequency, in addition to allele *d* (Table 5–III; Fig. 17).

21. 6-PGD locus

Electrophoretic patterns at the 6-PGD locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT-B and ADA loci. It was found that there were six phenotypes, AA, BB, CC, AB, AC and BC, produced by three alleles, *a*~*c*. In the 1162 *R. nigromaculata*, homozygous AA, BB and CC bands were observed in four, 1077 and one frogs, respectively, while heterozygous AB, AC and BC bands were found in 65, five and 10 frogs, respectively. Alleles *a*, *b* and *c* were 0.034, 0.959 and 0.007, respectively, in frequency. In the 454 *R.*

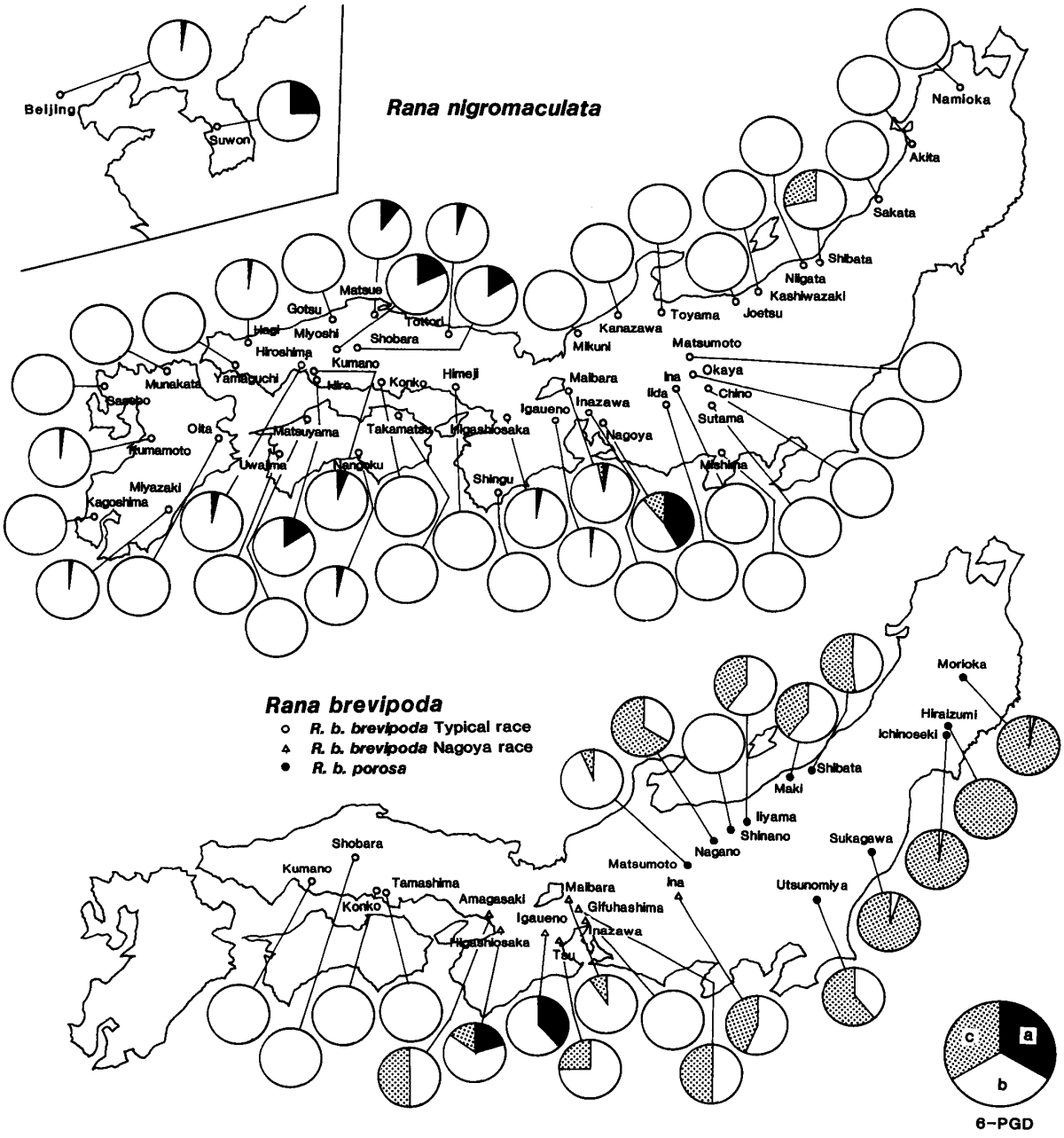

Fig. 18. Geographic distribution of 6-PGD alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

brevipoda, AA, BB, CC, AB and BC bands were found in one, 197, 138, nine and 109 frogs, respectively. Alleles *a, b* and *c* were 0.012, 0.564 and 0.424, respectively, in frequency.

In all the 46 populations of *R. nigromaculata* other than the Nagoya, allele *b* was high in frequency, being 0.719~1.000. In 15 of these populations, the Igaueno, Higashiosaka, Tottori, Matsue, Hagi, Hiroshima, Kumano, Hiro, Miyoshi, Shobara, Nangoku, Kumamoto, Miyazaki, Suwon and Beijing, allele *a* was 0.010 ~0.250 in frequency, in addition to allele *b*. In addition to allele *b*, the Shibata population had allele *c* in a frequency of 0.281 and the Maibara population had alleles *a* and *c*, each of which was 0.024 in frequency. The remaining 29 populations had only allele *b*. The Nagoya population had alleles *a, b* and *c* in frequencies of 0.417, 0.467 and 0.117, respectively.

In six of the 11 populations of *R. b. porosa,* the Morioka, Hiraizumi, Ichinoseki, Sukagawa, Utsunomiya and Nagano, allele *c* was high in frequency, being 0.609~1.000, while allele *b* was 0~0.391. In the Shibata population, alleles *c* and *b* were 0.515 and 0.485, respectively, in frequency. In the remaining four populations, allele *b* was 0.609~1.000, and allele *c* was 0~0.391 in frequency.

In the Gifuhashima and Amagasaki of the eight populations of the Nagoya race of *R. b. brevipoda,* alleles *b* and *c* were 0.500 each in frequency, in the Ina, Tsu and Maibara, allele *b* was 0.561~0.911, and allele *c* was 0.089~0.439 in frequency, and in the Igaueno and Higashiosaka, allele *b* was 0.625 and 0.650, respectively. In addition, the Igaueno had allele *a* in a frequency of 0.375, while the Higashiosaka population had alleles *a* and *c* in frequencies of 0.200 and 0.150, respectively. The Inazawa population had only allele *b*. Four populations of the Typical race of *R. b. brevipoda* also had only allele *b* (Table 5–III; Fig. 18).

22. PGM locus

Electrophoretic patterns at the PGM locus were analyzed in the same 1616 frogs as those used in the analyses at the AAT-B, ADA, AK and 6-PGD loci. The results showed that there were four phenotypes, BB, CC, AB and BC, produced by three alleles, *a~c*. In the 1162 *R. nigromaculata,* BB, CC, AB and BC bands were found in 1145, two, nine and six frogs, respectively. Alleles *a, b* and *c* were 0.004, 0.992 and 0.004, respectively, in frequency. In the 454 *R. brevipoda,* BB and BC bands were found in 449 and five frogs, respectively. Alleles *b* and *c* were 0.994 and 0.006, respectively, in frequency.

In all the populations of *R. nigromaculata* and *R. brevipoda,* allele *b* was high in frequency, being 0.810~1.000. In addition to allele *b*, the Kashiwazaki and Chino populations of *R. nigromaculata* had allele *a* in frequencies of 0.160 and 0.017, respectively. In the Iida, Suwon and Beijing populations of *R. nigromaculata* and the Sukagawa, Utsunomiya and Konko populations of *R. brevipoda,* allele *c* was observed in frequencies of 0.016~0.190 in addition to allele *b*. All the remaining 62 populations had only allele *b* (Table 5–III).

23. SOD-B locus

Electrophoretic patterns at the SOD-B locus were analyzed in the same 2233 frogs as those used in the analyses at the α-GDH, IDH-B, LDH-A, LDH-B, MDH-A and MDH-B loci. The results showed that there were five phenotypes, AA, BB, CC, AB and BC, produced by three alleles, a~c. In the 1733 *R. nigromaculata,* AA, BB and AB bands were found in three, 1706 and 24 frogs, respectively. Alleles a and b were 0.009 and 0.991, respectively, in frequency. In the 500 *R. brevipoda,* AA, BB, CC, AB and BC bands were found in nine, 460, two,

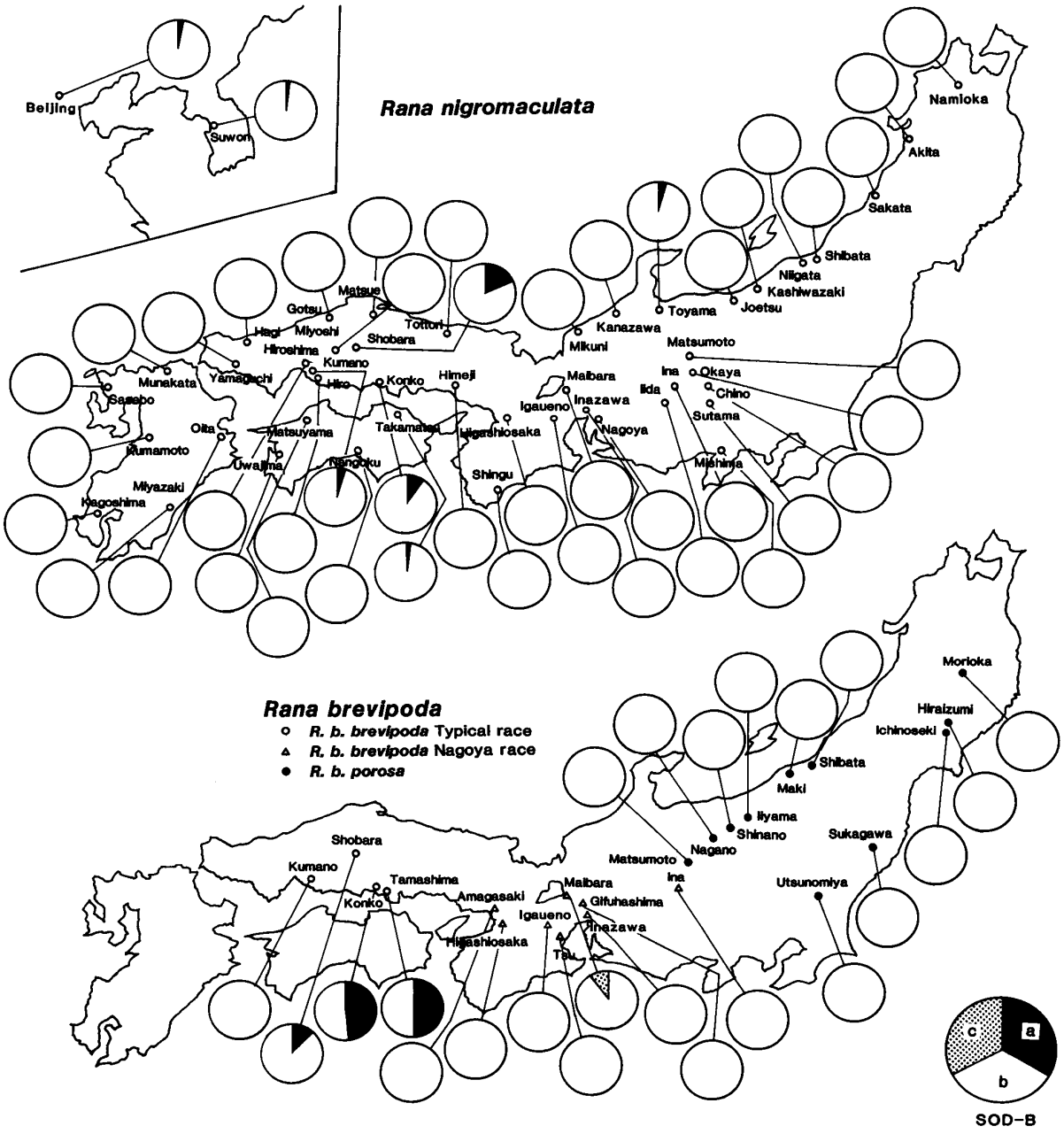

Fig. 19. Geographic distribution of SOD-B alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda.*

24 and five frogs, respectively. Alleles *a*, *b* and *c* were 0.042, 0.949 and 0.009, respectively, in frequency.

In 68 of the 70 populations of *R. nigromaculata* and *R. brevipoda* other than the Tamashima and Konko of *R. b. brevipoda*, allele *b* was very high in frequency, being 0.816~1.000. In addition to allele *b*, seven populations of *R. nigromaculata*, the Toyama, Kumano, Shobara, Konko, Takamatsu, Suwon and Beijing, and the Shobara population of *R. brevipoda* had allele *a* in frequencies of 0.012~0.184, and the Maibara population of *R. brevipoda* had allele *c* in a frequency of 0.100. The remaining 59 populations had only allele *b*. In the Tamashima population of *R. brevipoda*, alleles *a* and *b* were each 0.500 in frequency, and in the Konko population of *R. brevipoda*, alleles *a* and *b* were 0.487 and 0.513, respectively (Table 5-III; Fig. 19).

24. Ab locus

Electrophoretic patterns at the Ab locus were analyzed in 2123 frogs of 70 populations including 1589 of 47 populations belonging to *R. nigromaculata* and 534 of 23 populations belonging to *R. brevipoda*. The results showed that there were 14 phenotypes, AA, BB, CC, DD, EE, FF, AB, BC, BD, BE, BF, DE, EF and EG, produced by seven alleles, *a*~*g*. In the 1589 *R. nigromaculata*, homozygous AA, BB and CC bands were found in four, 1513 and nine frogs, respectively, and heterozygous AB, BC, BD and BE bands were found in 32, 18, six and seven frogs, respectively. Alleles *a*, *b*, *c*, *d* and *e* were 0.013, 0.972, 0.011, 0.002 and 0.002, respectively, in frequency. In the 534 *R. brevipoda*, homozygous BB, DD, EE and FF bands were found in one, 308, 116 and 57 frogs, respectively, and heterozygous BD, BE, BF, DE, EF and EG bands were found in 27, one, 12, two, nine and one frogs, respectively. Alleles *b*, *d*, *e*, *f* and *g* were 0.039, 0.604, 0.229, 0.126 and 0.001, respectively, in frequency.

In 46 of the 47 populations of *R. nigromaculata* other than the Beijing, allele *b* was very high in frequency, being 0.818~1.000. In addition to allele *b*, seven populations, the Mishima, Nangoku, Uwajima, Sasebo, Miyazaki, Oita and Suwon, had allele *a* in frequencies of 0.010~0.125, the Shibata and Matsumoto populations had allele *d* in frequencies of 0.182 and 0.033, respectively, the Maibara, Igaueno and Higashiosaka populations had allele *e* in frequencies of 0.010~0.125, the Matsuyama population had allele *c* in a frequency of 0.045, the Tottori population had alleles *a* and *c* in frequencies of 0.016 and 0.024, respectively, and the Matsue population had alleles *a* and *c* in frequencies of 0.021 and 0.063, respectively. All the remaining 31 populations had only allele *b*. In the Beijing population, allele *c* was high in frequency, being 0.846, while allele *b* was 0.154.

In the 11 populations of *R. b. porosa*, allele *d* was very high in frequency, being 0.724~1.000. In the Shibata and Matsumoto populations, allele *b* was found in frequencies of 0.276 and 0.093, respectively, in addition to allele *d*. All the remaining nine populations had only allele *d*.

In 11 of the 12 populations of *R. b. brevipoda* other than the Ina, allele *e* was very high in frequency, being 0.786~1.000. In addition to allele *e*, the Maibara

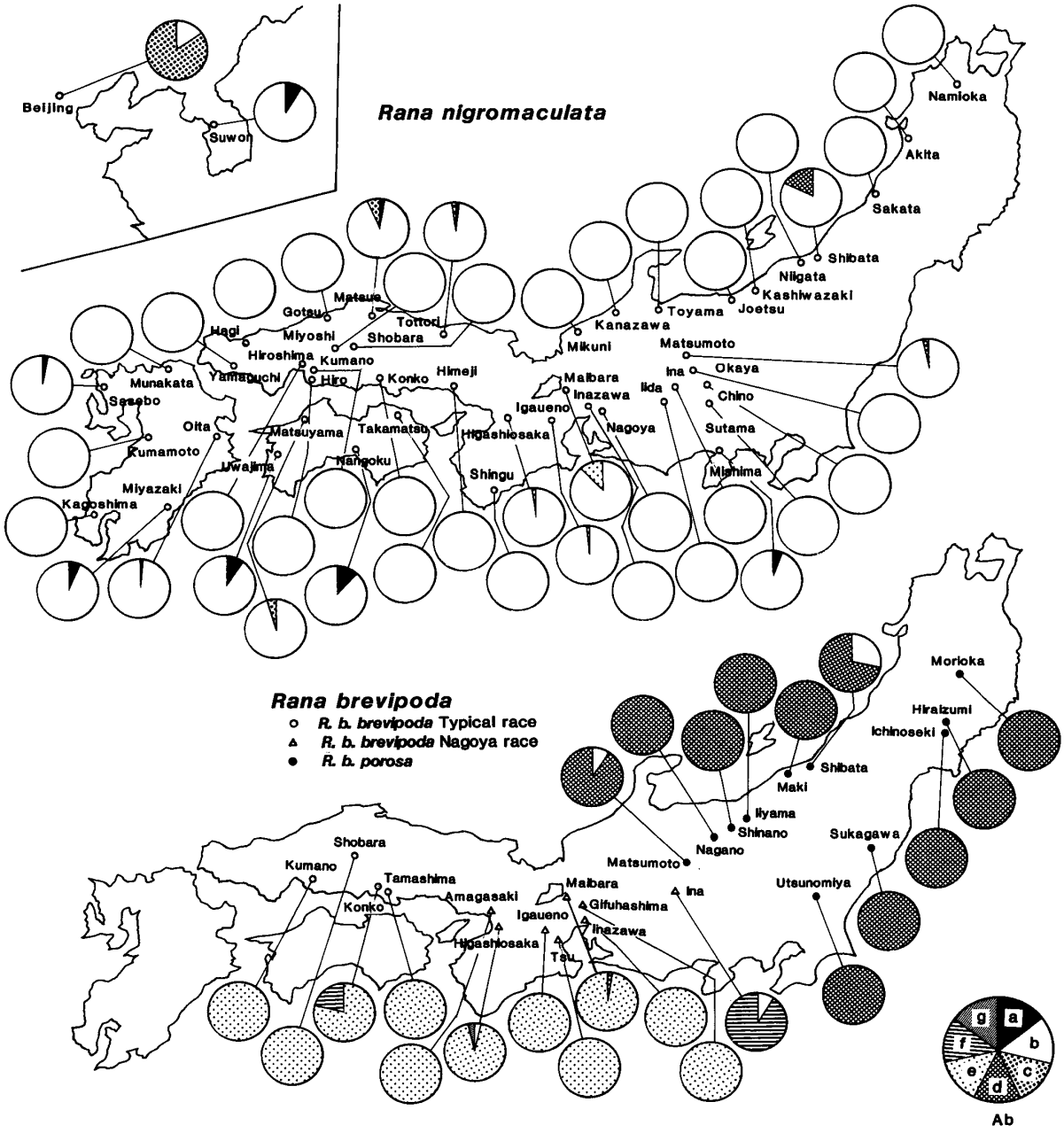

Fig. 20. Geographic distribution of Ab alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

population had allele *d* in a frequency of 0.022, the Konko population had allele *f* in a frequency of 0.214, and the Higashiosaka population had alleles *b* and *g* in a frequency of 0.033 each. All the remaining eight populations had only allele *e*. In the Ina population, allele *f* was very high in frequency, being 0.913, while allele *b* was 0.087 (Table 5–III; Fig. 20).

25. Hb locus

Electrophoretic patterns at the Hb locus were analyzed in 2237 frogs of 70 populations, including 1696 of 47 populations belonging to *R. nigromaculata* and 541

of 23 populations belonging to *R. brevipoda*. It was found that there were eight phenotypes, AA, BB, CC, DD, AB, AD, BD and CD, produced by four alleles, *a*~*d*. In the 1696 *R. nigromaculata*, homozygous BB, CC and DD bands were found in two, two and 1674, respectively, and heterozygous AD, BD and CD bands were found in four, eight and six frogs, respectively. Alleles *a*, *b*, *c* and *d* were 0.001, 0.004, 0.003 and 0.992, respectively, in frequency. In the 541 *R. brevipoda*, homozygous AA, BB and DD bands were found in 32, 407 and four frogs, respectively, and heterozygous AB, AD and BD bands were found in 74, 15 and nine frogs, respectively. Alleles *a*, *b* and *d* were 0.141, 0.829 and 0.030,

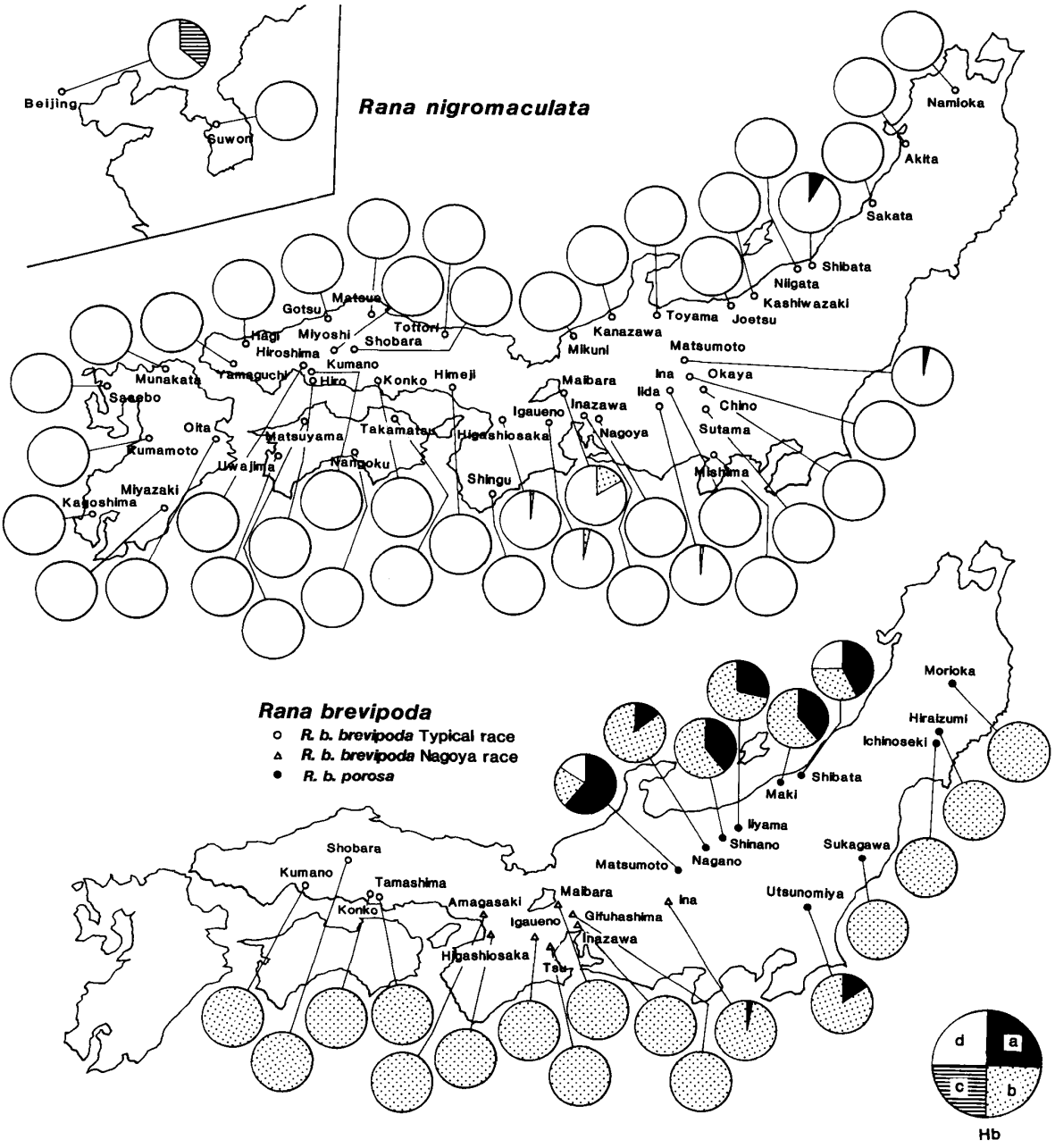

Fig. 21. Geographic distribution of Hb alleles among 70 populations of *Rana nigromaculata* and *Rana brevipoda*.

respectively, in frequency.

In the 47 populations of *R. nigromaculata,* allele *d* was high in frequency, being 0.643~1.000. In addition to allele *d*, the Shibata and Matsumoto populations had allele *a* in frequencies of 0.083 and 0.033, respectively. The Iida, Maibara, Igaueno and Higashiosaka populations had allele *b* in frequencies of 0.013~0.167, and the Beijing population had allele *c* in a frequency of 0.357. The remaining 40 populations had only allele *d*.

In nine of the 11 populations of *R. b. porosa* other than the Shibata and Matsumoto, allele *b* was high in frequency, being 0.621~1.000. In addition to allele *b*, the Utsunomiya, Maki, Nagano, Iiyama and Shinano populations had allele *a* in frequencies of 0.132~0.379. The remaining four populations had only allele *b*. The Shibata and Matsumoto populations had allele *a* in frequencies of 0.421 and 0.616, respectively, allele *b* in frequencies of 0.329 and 0.233, respectively, and allele *d* in frequencies of 0.250 and 0.151, respectively.

In the 12 populations of *R. b. brevipoda,* allele *b* was overwhelmingly high in frequency, being 0.978~1.000. In addition to allele *b*, the Ina population had allele *a* in a frequency of 0.022. All the remaining 11 populations had only allele *b* (Table 5–III; Fig. 21).

III. Genetic differentiation

1. Fixation index (Fst)

The fixation index (Fst) was calculated by the method of WRIGHT (1978) at 28 loci in 1777 frogs of 47 populations belonging to *R. nigromaculata* and 560 frogs of 23 populations belonging to *R. brevipoda.* When the gene frequencies at a definite locus are the same in all the populations, the fixation index is zero, as no differentiation has occurred. In contrast, when there is a characteristic allele at a definite locus in one or more populations, the fixation index is 1.000. The higher the fixation index, the more advanced is the differentiation in the locus.

a. *Rana nigromaculata*

The results of examination of fixation indexes in the 47 populations of *Rana nigromaculata* showed that the MDH–B locus was the most advanced one in differentiation, being 0.805 in Fst. The ADA, IDH–B, ADH–B, Pep–C and Fum loci were gradually lower, being from 0.595 to 0.555 in Fst. The AAT–B, ME–B, Ab and Pep–B loci were further lower, being from 0.497 to 0.407 in Fst. The Pep–D and ME–A loci were 0.344 and 0.300, respectively, the α–GDH, LDH–B, Hb, MPI and 6–PGD loci were from 0.250 to 0.214, the LDH–A, PGM, Pep–A and SOD–B loci were from 0.156 to 0.107 and the GPI, ADH–A, MDH–A and AK loci were from 0.082 to 0.013 in Fst. The other three loci, the AAT–A, CK and IDH–A loci, were zero in Fst, that is, these loci were not differentiated (Table 6).

TABLE 6
Fixation index at 28 loci in *Rana nigromaculata* and *R. brevipoda*

Locus	Fixation index (Fst)		Locus	Fixation index (Fst)	
	R. nigro.	*R. b. bre., R. b. por.*		*R. nigro.*	*R. b. bre., R. b. por.*
AAT-A	0	0	MDH-A	0.062	0
AAT-B	0.497	0.052	MDH-B	0.805	0.198
ADA	0.595	0.149	ME-A	0.300	0.084
ADH-A	0.081	0.895	ME-B	0.472	0.260
ADH-B	0.583	0.129	MPI	0.218	0.532
AK	0.013	0.150	Pep-A	0.131	0.908
CK	0	0	Pep-B	0.407	0.315
Fum	0.555	0.195	Pep-C	0.556	0.270
α-GDH	0.250	0.179	Pep-D	0.344	0.170
GPI	0.082	0.386	6-PGD	0.214	0.501
IDH-A	0	0	PGM	0.147	0.041
IDH-B	0.585	0.271	SOD-B	0.107	0.394
LDH-A	0.156	0	Ab	0.430	0.905
LDH-B	0.233	0.922	Hb	0.228	0.383

b. *Rana brevipoda*

In the 23 populations of *Rana brevipoda*, the most advanced locus in differentiation was the LDH-B locus, being 0.922. The differentiation was most advanced in the Nagoya race. The Pep-A, Ab and ADH-A loci were 0.908, 0.905 and 0.895, respectively, in Fst and there were distinct differences in every loci between *R. b. brevipoda* and *R. b. porosa*. The MPI and 6-PGD loci were 0.532 and 0.501, respectively, the SOD-B, GPI, Hb and Pep-B loci were from 0.394 to 0.315, the IDH-B, Pep-C and ME-B loci were from 0.271 to 0.260, the MDH-B, Fum, α-GDH and Pep-D loci were from 0.198 to 0.170, the AK, ADA and ADH-B loci were from 0.150 to 0.129, and the ME-A, AAT-B and PGM loci were from 0.084 to 0.041 in Fst. The other five loci, the AAT-A, CK, IDH-A, LDH-A and MDH-A loci, were zero in Fst and were not differentiated (Table 6).

2. Average heterozygosity

a. *Rana nigromaculata*

The average heterozygosity of each of the 47 populations was calculated on the 28 loci analyzed in 1144~1733 frogs. It was found that the highest rate was 13.8% and 13.5% in the Maibara and Suwon populations, respectively, followed by 11.9% and 11.5% in the Higashiosaka and Beijing populations, respectively. Four populations, the Shobara, Shibata, Himeji and Mikuni, were 10.4~9.5%, six populations, the Hiro, Miyazaki, Yamaguchi, Sasebo, Nagoya and Miyoshi, were 8.9~8.1%, four populations, the Gotsu, Kumano, Igaueno and Inazawa, were 7.5~7.1%, four populations, the Kanazawa, Kumamoto, Munakata and Matsumoto, were 6.8~6.0%, eight populations, the Kagoshima, Matsuyama, Toyama, Tottori, Hiroshima, Hagi, Matsue and Konko, were 5.9~5.4%, four populations,

TABLE 7

Genetic variabilities at 28 loci in *Rana nigromaculata*

Population	Sample size	Mean proportion of heterozygous loci per individual (%)		Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
Namioka	19	0.2	(0.2)	3.6	1.04
Akita	6	1.2	(1.0)	3.6	1.04
Sakata	48	3.3	(4.5)	21.4	1.21
Shibata	16	10.3	(15.3)	46.4	1.50
Niigata	20	3.0	(2.8)	7.1	1.07
Kashiwazaki	26	2.1	(2.2)	10.7	1.11
Joetsu	36	4.7	(4.5)	21.4	1.21
Toyama	37	5.8	(6.3)	25.0	1.25
Kanazawa	55	6.8	(8.3)	21.4	1.21
Mikuni	25	9.5	(8.2)	25.0	1.29
Matsumoto	30	6.0	(6.4)	39.3	1.46
Okaya	64	2.1	(2.4)	21.4	1.21
Chino	30	1.8	(1.7)	21.4	1.21
Ina	2	1.8	(1.3)	3.6	1.04
Iida	40	4.1	(4.1)	21.4	1.25
Sutama	35	1.0	(1.1)	3.6	1.04
Mishima	67	1.1	(1.4)	14.3	1.14
Nagoya	30	8.3	(7.8)	25.0	1.32
Inazawa	5	7.1	(6.0)	25.0	1.25
Maibara	21	13.8	(16.6)	57.1	1.82
Igaueno	48	7.1	(6.9)	50.0	1.57
Shingu	6	4.8	(4.2)	14.3	1.14
Higashiosaka	38	11.9	(11.3)	53.6	1.71
Himeji	6	10.1	(8.7)	21.4	1.29
Tottori	64	5.8	(5.8)	25.0	1.32
Matsue	75	5.5	(5.9)	32.1	1.43
Gotsu	23	7.5	(7.5)	21.4	1.25
Hagi	66	5.5	(7.0)	32.1	1.46
Yamaguchi	52	8.6	(9.9)	28.6	1.46
Hiroshima	60	5.6	(8.4)	35.7	1.39
Kumano	56	7.2	(8.4)	39.3	1.43
Hiro	19	8.9	(8.9)	35.7	1.39
Miyoshi	19	8.1	(8.5)	28.6	1.32
Shobara	19	10.4	(9.9)	35.7	1.39
Konko	83	5.4	(6.0)	28.6	1.39
Takamatsu	42	2.8	(2.9)	14.3	1.14
Nangoku	32	4.1	(4.4)	25.0	1.25
Uwajima	39	3.9	(3.3)	14.3	1.14
Matsuyama	23	5.9	(7.1)	21.4	1.25
Munakata	58	6.6	(8.0)	42.9	1.50
Sasebo	58	8.3	(8.6)	39.3	1.39
Kumamoto	49	6.7	(5.5)	28.6	1.39
Kagoshima	60	5.9	(7.2)	25.0	1.39
Miyazaki	56	8.6	(10.8)	42.9	1.57
Oita	55	3.8	(4.8)	28.6	1.29
Suwon	38	13.5	(14.4)	60.7	1.93
Beijing	21	11.5	(15.3)	64.3	1.82
Average	37.8	6.1	(6.6)	27.8	1.33

Parentheses show an expected value.

the Shingu, Joetsu, Nangoku and Iida, were 4.8~4.1%, five populations, the Uwajima, Oita, Sakata, Niigata and Takamatsu, were 3.9~2.8%, four populations, the Okaya, Kashiwazaki, Chino and Ina, were 2.1~1.8%, and three populations, the Akita, Mishima and Sutama, were 1.2~1.0% in average heterozygosity. The lowest was 0.2% in the Namioka population. The average heterozygosity in the 47 populations of *R. nigromaculata* was 6.1%. There were no remarkable differences between these actual rates and the expected values in each of the 47 populations, except that slight differences were found in a few sympatric populations such as the Shibata in which *R. nigromaculata* were mixed with *R. brevipoda* (Table 7).

b. *Rana brevipoda*

The average heterozygosity of each of the 23 populations was calculated on the 28 loci analyzed in 434~541 frogs. The results were as follows. The highest rate

TABLE 8
Genetic variabilities at 28 loci in *Rana brevipoda*

Species	Population	Sample size	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
R. b. porosa	Morioka	26	4.3 (5.4)	21.4	1.21
〃	Hiraizumi	9	4.0 (3.6)	14.3	1.14
〃	Ichinoseki	39	3.7 (3.7)	21.4	1.21
〃	Sukagawa	43	6.3 (7.0)	25.0	1.25
〃	Utsunomiya	56	8.0 (9.5)	35.7	1.39
〃	Shibata	38	18.0 (18.2)	46.4	1.57
〃	Maki	29	6.0 (5.7)	25.0	1.25
〃	Nagano	19	4.9 (5.0)	17.9	1.18
〃	Iiyama	30	3.8 (4.4)	14.3	1.14
〃	Shinano	8	5.8 (5.3)	14.3	1.14
〃	Matsumoto	43	8.8 (8.9)	42.9	1.54
R. b. brevipoda	Ina	69	10.4 (11.4)	53.4	1.61
〃	Inazawa	1	10.7 (5.4)	10.7	1.11
〃	Gifuhashima	1	10.7 (5.4)	10.7	1.11
〃	Maibara	46	9.0 (9.6)	50.0	1.64
〃	Tsu	30	8.3 (9.3)	32.1	1.43
〃	Igaueno	4	10.7 (8.9)	25.0	1.25
〃	Higashiosaka	20	9.4 (9.5)	35.7	1.50
〃	Amagasaki	1	7.1 (3.4)	7.1	1.07
〃	Tamashima	4	9.2 (4.9)	10.7	1.11
〃	Konko	38	8.2 (8.0)	25.0	1.25
〃	Shobara	4	2.7 (2.3)	10.7	1.11
〃	Kumano	2	1.8 (1.3)	3.6	1.04
Average		24.3	7.5 (6.8)	24.1	1.27

Parentheses show an expected value.

was 18.0% in the Shibata population, followed by 10.7% in three populations, the Igaueno, Inazawa and Gifuhashima. Four populations, the Ina, Higashiosaka, Tamashima and Maibara, were 10.4~9.0% in average heterozygosity, four populations, the Matsumoto, Tsu, Konko and Utsunomiya, were 8.8~8.0%, the Amagasaki population was 7.1%, three populations, the Sukagawa, Maki and Shinano, were 6.3~5.8%, five populations, the Nagano, Morioka, Hiraizumi, Iiyama and Ichinoseki, were 4.9~3.7%, the Shobara population was 2.7% and the Kumano population was 1.8%. In four populations including the Inazawa, Gifuhashima and Amagasaki, each of which contained a single frog and the Tamashima population had only four frogs, there were large differences between the foregoing actual rates and the expected values, but no remarkable differences were found in the other 19 populations (Table 8).

3. Proportion of polymorphic loci

The proportion of polymorphic loci which contain plural alleles at the rates of more than 1% was estimated in each population of *Rana nigromaculata* and *R. brevipoda*.

a. *Rana nigromaculata*

The highest in the proportion of polymorphic loci among the 47 populations of *R. nigromaculata* was 64.3% in the Beijing population, followed by 60.7% in the Suwon population. In three populations, the Maibara, Higashiosaka and Igaueno, the proportions of polymorphic loci were 57.1~50.0%. It was 46.4% in the Shibata population, 42.9% in each of the Munakata and Miyazaki populations, 39.3% in each of three populations, the Matsumoto, Kumano and Sasebo, 35.7% in each of three populations, the Hiroshima, Hiro and Shobara, 32.1% in each of two populations, the Matsue and Hagi, 28.6% in each of five populations, the Yamaguchi, Miyoshi, Konko, Kumamoto and Oita, 25.0% in each of seven populations, the Toyama, Mikuni, Nagoya, Inazawa, Tottori, Nangoku and Kagoshima, 21.4% in each of nine populations, the Sakata, Joetsu, Kanazawa, Okaya, Chino, Iida, Himeji, Gotsu and Matsuyama, 14.3% in each of four populations, the Mishima, Shingu, Takamatsu and Uwajima, 10.7% and 7.1% in the Kashiwazaki and Niigata populations, respectively, and 3.6% in each of four populations, the Namioka, Akita, Sutama and Ina. The proportions of polymorphic loci in the 47 populations of *R. nigromaculata* were 27.8% on the average (Table 7).

b. *Rana brevipoda*

The highest in the proportion of polymorphic loci among the 23 populations of *Rana brevipoda* was 53.4% in the Ina population. This was followed by 50.0%, 46.4% and 42.9% in the Maibara, Shibata and Matsumoto populations, respectively. In each of the Utsunomiya and Higashiosaka populations, the proportion of polymorphic loci was 35.7%. It was 32.1% in the Tsu population, 25.0% in each of the Maki, Igaueno, Konko and Sukagawa populations, 21.4% in each of

the Ichinoseki and Morioka populations, 17.9% in the Nagano population, 14.3% in each of the Hiraizumi, Iiyama and Shinano populations, 10.7% in each of the Inazawa, Gifuhashima, Tamashima and Shobara populations, and 7.1% and 3.6% in the Amagasaki and Kumano populations, respectively. The proportions of polymorphic loci in the 23 populations of *R. brevipoda* were 24.1% on the average (Table 8).

4. Mean number of alleles per locus

a. *Rana nigromaculata*

The largest mean number of alleles per locus among 47 populations was 1.93 in the Suwon population, followed by 1.82 in each of the Beijing and Maibara populations. It was 1.71 in the Higashiosaka population, 1.57 in each of the Igaueno and Miyazaki populations, 1.50 in each of the Shibata and Munakata populations, 1.46 in each of the Matsumoto, Hagi and Yamaguchi populations, 1.43 in each of the Matsue and Kumano populations, 1.39 in each of the Hiroshima, Hiro, Shobara, Konko, Sasebo, Kumamoto and Kagoshima populations, 1.32 in each of the Nagoya, Tottori and Miyoshi populations, 1.29 in each of the Mikuni, Himeji and Oita populations, 1.25 in each of the Toyama, Iida, Inazawa, Gotsu, Nangoku and Matsuyama populations, 1.21 in each of the Sakata, Joetsu, Kanazawa, Okaya and Chino populations, 1.14 in each of the Mishima, Shingu, Takamatsu and Uwajima populations, 1.11 and 1.07 in the Kashiwazaki and Niigata populations, respectively, and 1.04 in each of the Namioka, Akita, Sutama and Ina populations. The mean numbers of alleles per locus in the 47 populations of *R. nigromaculata* were 1.33 on the average (Table 7).

b. *Rana brevipoda*

The largest mean number of alleles per locus among 23 populations was 1.64 in the Maibara population, followed by 1.61, 1.57, 1.54, 1.50, 1.43 and 1.39 in the Ina, Shibata, Matsumoto, Higashiosaka, Tsu and Utsunomiya populations, respectively. The mean number of alleles per locus was 1.25 in each of the Sukagawa, Maki, Igaueno and Konko populations, 1.21 in each of the Morioka and Ichinoseki populations, 1.18 in the Nagano population, 1.14 in each of the Hiraizumi, Iiyama and Shinano populations, 1.11 in each of the Inazawa, Gifuhashima, Tamashima and Shobara populations, and 1.07 and 1.04 in the Amagasaki and Kumano populations, respectively. The mean numbers of alleles per locus in the 23 populations of *R. brevipoda* were 1.27 on the average (Table 8).

IV. Genetic distance

On the basis of the gene frequencies at 28 loci controlling 17 enzymes and two blood proteins, genetic distances were estimated by the method of NEI (1975) (Table 9).

1. Populational difference

a. *Rana nigromaculata*

i) Of the 47 populations of *R. nigromaculata*, 24 (1~24) distributed in eastern Japan were separated from one another by 0.000~0.204, 0.071 on the average, in genetic distance. The genetic distances among 10 (1~10) populations in the Tohoku and Hokuriku districts were 0.002~0.106, 0.030 on the average, those among nine (11~19) populations in the Chubu district were 0.000~0.039, 0.014 on the average, and those among five (20~24) populations in the Kinki district were 0.014~0.110, 0.054 on the average. In contrast, the genetic distances between the group of the nine populations in the Chubu district and the group of the 10 populations in the Tohoku and Hokuriku districts were 0.042~0.204, 0.113 on the average, and those between the group of the five populations in the Kinki district and the group of the 19 (1~19) populations in the Tohoku, Hokuriku, and Chubu districts were 0.013~0.139, 0.074 on the average. In eastern Japan, comparatively large genetic distances were found among the Namioka(1) population in the Tohoku district and the group of the nine populations in the Chubu district, being 0.156~0.204, 0.180 on the average. Those among two(14, 15) populations, the Ina and Iida, and three(2, 4, 6) populations, the Akita, Shibata and Kashiwazaki, were 0.148~0.162, 0.156 on the average.

ii) In western Japan, the genetic distances among the 21(25~45) populations were 0.002~0.200, 0.069 on the average. The genetic distances among the 11(25~35) populations in the Chugoku district were 0.002~0.120, 0.059 on the average, those among the four(36~39) populations in the Shikoku district were 0.011~0.092, 0.049 on the average, and those among the six(40~45) populations in the Kyushu district were 0.015~0.098, 0.055 on the average. The genetic distances among the four(25~28) populations in the San-in region of the Chugoku district were 0.011~0.111, 0.056 on the average, while those among the seven(29~35) populations in the Sanyo region of the Chugoku district were 0.002~0.053, 0.024 on the average. Those between the group of the four populations in the San-in region and the group of the seven populations in the Sanyo region were 0.048~0.120, 0.088 on the average, except the genetic distance between the Hagi (28) and Yamaguchi(29) populations was 0.017. On the other hand, the genetic distances between the group of the four (36~39) populations in the Shikoku district and the group of the seven (29~35) populations in the Sanyo region of the Chugoku district were 0.006~0.090, 0.040 on the average, while those between the former group and the group of the four(25~28) populations in the San-in region were 0.045~0.200, 0.109 on the average. Especially, those between the Nangoku(37) population in the Shikoku district and the Tottori(25) and Matsue (26) populations in the San-in region were large, being 0.166 and 0.200, respectively. Those between the group of the five(40~44) populations in the Kyushu district other than the Oita(45) population and the group of the 11 (25~35) populations in the Chugoku district were 0.013~0.113, 0.073 on the average, while those between the Oita population and the group of the 11 populations in the

Species																								*Rana ni*
Area		Tohoku			Hokuriku							Chubu									K			
Population	(No.)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(	
Namioka	(1)																							
Akita	(2)	.025																						
Sakata	(3)	.036	.008																					
Shibata	(4)	.059	.027	.027																				
Niigata	(5)	.032	.007	.005	.026																			
Kashiwazaki	(6)	.032	.002	.007	.026	.006																		
Joetsu	(7)	.021	.004	.004	.026	.002	.004																	
Toyama	(8)	.060	.023	.007	.036	.009	.019	.013																
Kanazawa	(9)	.074	.042	.020	.053	.025	.038	.027	.009															
Mikuni	(10)	.106	.067	.050	.082	.049	.062	.054	.032	.016														
Matsumoto	(11)	.173	.132	.089	.123	.106	.125	.113	.068	.056	.073													
Okaya	(12)	.176	.135	.099	.134	.108	.128	.118	.075	.063	.071	.006												
Chino	(13)	.182	.142	.103	.142	.118	.135	.125	.081	.068	.078	.004	.001											
Ina	(14)	.201	.160	.116	.148	.132	.153	.141	.091	.077	.089	.005	.005	.003										
Iida	(15)	.204	.162	.118	.160	.134	.155	.142	.092	.075	.086	.008	.012	.011	.012									
Sutama	(16)	.184	.143	.105	.142	.116	.136	.125	.080	.068	.076	.005	.000	.001	.003	.011								
Mishima	(17)	.156	.117	.098	.119	.090	.110	.102	.073	.066	.062	.033	.014	.023	.030	.039	.017							
Nagoya	(18)	.169	.128	.090	.122	.100	.120	.107	.062	.042	.052	.021	.020	.021	.026	.027	.021	.036						
Inazawa	(19)	.175	.134	.095	.132	.106	.126	.114	.070	.055	.067	.005	.004	.005	.008	.008	.004	.021	.017					
Maibara	(20)	.130	.088	.061	.085	.081	.086	.081	.055	.048	.071	.048	.069	.065	.071	.067	.071	.097	.061	.064				
Igaueno	(21)	.094	.071	.046	.080	.047	.066	.053	.027	.013	.021	.044	.045	.050	.058	.057	.048	.046	.028	.042	.046			
Shingu	(22)	.086	.108	.099	.138	.096	.111	.093	.096	.083	.090	.124	.118	.125	.137	.139	.124	.111	.110	.119	.110	.057		
Higashiosaka	(23)	.117	.090	.059	.091	.065	.085	.069	.039	.019	.037	.053	.063	.067	.074	.070	.067	.073	.039	.054	.032	.014	.0	
Himeji	(24)	.130	.090	.067	.095	.066	.083	.072	.043	.022	.020	.073	.074	.082	.091	.088	.079	.066	.049	.066	.062	.021	.1	
Tottori	(25)	.206	.164	.142	.180	.170	.165	.157	.135	.090	.072	.125	.133	.127	.139	.133	.135	.158	.110	.120	.113	.114	.1	
Matsue	(26)	.206	.166	.154	.182	.174	.168	.161	.149	.106	.082	.152	.151	.148	.164	.157	.155	.160	.129	.139	.146	.132	.1	
Gotsu	(27)	.151	.111	.096	.131	.117	.111	.108	.093	.065	.052	.099	.104	.101	.114	.111	.108	.121	.090	.099	.084	.078	.1	
Hagi	(28)	.123	.084	.081	.104	.093	.085	.086	.082	.068	.078	.121	.123	.124	.141	.136	.129	.125	.104	.119	.087	.076	.1	
Yamaguchi	(29)	.112	.074	.060	.088	.067	.071	.066	.051	.037	.048	.087	.089	.092	.105	.103	.094	.090	.069	.085	.071	.041	.1	
Hiroshima	(30)	.080	.043	.038	.065	.036	.041	.039	.028	.024	.015	.076	.071	.078	.091	.087	.077	.057	.057	.071	.062	.022	.0	
Kumano	(31)	.115	.076	.063	.092	.058	.071	.065	.043	.027	.005	.079	.072	.081	.093	.089	.078	.057	.053	.070	.079	.022	.0	
Hiro	(32)	.093	.058	.039	.065	.033	.052	.040	.021	.010	.015	.080	.079	.091	.101	.097	.086	.065	.051	.071	.080	.021	.0	
Miyoshi	(33)	.108	.070	.053	.082	.050	.065	.057	.035	.024	.006	.079	.074	.083	.093	.094	.079	.062	.053	.073	.079	.025	.0	
Shobara	(34)	.105	.066	.049	.078	.044	.060	.052	.029	.020	.008	.076	.072	.081	.092	.092	.078	.060	.051	.070	.078	.023	.0	
Konko	(35)	.097	.062	.046	.077	.047	.058	.052	.028	.018	.019	.051	.048	.053	.063	.063	.052	.044	.034	.048	.050	.005	.0	
Takamatsu	(36)	.112	.074	.052	.082	.047	.067	.057	.028	.017	.018	.055	.050	.059	.067	.067	.055	.040	.033	.048	.063	.005	.0	
Nangoku	(37)	.117	.082	.042	.085	.055	.075	.062	.029	.034	.069	.057	.080	.080	.083	.076	.081	.108	.067	.070	.059	.049	.1	
Uwajima	(38)	.135	.098	.078	.104	.071	.091	.078	.050	.025	.026	.082	.074	.085	.095	.091	.080	.058	.046	.067	.089	.018	.0	
Matsuyama	(39)	.136	.096	.074	.106	.080	.092	.080	.053	.025	.032	.073	.074	.078	.089	.083	.079	.076	.044	.064	.069	.025	.1	
Munakata	(40)	.101	.071	.062	.092	.079	.073	.070	.067	.054	.072	.108	.117	.116	.131	.128	.123	.130	.098	.112	.073	.068	.1	
Sasebo	(41)	.132	.106	.083	.122	.112	.108	.097	.084	.053	.081	.100	.118	.111	.123	.117	.121	.151	.087	.104	.063	.071	.1	
Kumamoto	(42)	.127	.106	.086	.128	.115	.109	.099	.087	.054	.072	.093	.108	.100	.113	.106	.111	.142	.080	.098	.065	.065	.1	
Kagoshima	(43)	.155	.117	.078	.120	.094	.112	.094	.057	.027	.049	.067	.080	.080	.087	.079	.083	.103	.048	.065	.069	.037	.1	
Miyazaki	(44)	.133	.120	.106	.147	.129	.124	.111	.110	.073	.075	.149	.162	.157	.172	.165	.166	.183	.127	.146	.117	.102	.1	
Oita	(45)	.194	.152	.123	.168	.149	.151	.140	.115	.085	.116	.132	.150	.145	.155	.148	.153	.183	.115	.134	.111	.106	.1	
Suwon	(46)	.146	.136	.112	.139	.130	.138	.120	.107	.078	.104	.089	.094	.092	.101	.095	.097	.115	.075	.082	.101	.083	.1	
Beijing	(47)	.229	.196	.157	.189	.175	.190	.176	.141	.121	.149	.109	.119	.118	.124	.118	.121	.145	.116	.109	.134	.123	.2	
Morioka	(48)	.539	.490	.491	.294	.509	.496	.500	.517	.525	.582	.546	.589	.589	.578	.608	.596	.594	.564	.585	.415	.562	.6	
Hiraizumi	(49)	.546	.494	.495	.299	.513	.500	.505	.522	.534	.594	.558	.604	.604	.594	.624	.612	.610	.579	.599	.418	.576	.6	
Ichinoseki	(50)	.545	.492	.492	.296	.511	.497	.503	.519	.531	.590	.554	.600	.600	.590	.619	.608	.606	.575	.595	.414	.573	.6	
Sukagawa	(51)	.545	.501	.502	.304	.520	.507	.506	.521	.543	.605	.569	.614	.614	.604	.634	.622	.620	.585	.606	.438	.586	.6	
Utsunomiya	(52)	.518	.472	.471	.282	.489	.477	.481	.496	.502	.551	.520	.563	.564	.553	.581	.571	.569	.555	.560	.397	.538	.6	
Shibata	(53)	.299	.256	.255	.133	.264	.258	.260	.273	.286	.332	.330	.362	.367	.367	.387	.371	.359	.351	.359	.228	.321	.4	
Maki	(54)	.499	.445	.442	.265	.459	.449	.453	.465	.476	.533	.495	.541	.542	.532	.562	.549	.547	.541	.536	.365	.516	.6	
Nagano	(55)	.510	.463	.461	.273	.478	.467	.470	.485	.496	.555	.517	.562	.564	.553	.584	.571	.569	.552	.558	.391	.536	.6	
Iiyama	(56)	.511	.458	.454	.273	.470	.461	.465	.475	.486	.543	.503	.550	.552	.541	.571	.558	.556	.551	.546	.373	.525	.6	
Shinano	(57)	.465	.426	.424	.260	.440	.431	.432	.448	.457	.514	.479	.522	.524	.514	.543	.531	.529	.539	.518	.367	.495	.5	
Matsumoto	(58)	.423	.376	.375	.224	.392	.381	.384	.396	.405	.454	.417	.455	.456	.449	.476	.462	.461	.471	.452	.314	.439	.5	
Ina	(59)	.520	.482	.474	.341	.487	.486	.482	.490	.496	.553	.512	.543	.547	.535	.567	.551	.551	.546	.542	.387	.522	.6	
Inazawa	(60)	.565	.532	.524	.395	.538	.537	.532	.545	.554	.618	.579	.617	.621	.607	.639	.625	.625	.638	.613	.426	.583	.6	
Gifuhashima	(61)	.533	.501	.503	.362	.520	.509	.509	.534	.544	.609	.580	.616	.618	.607	.641	.625	.625	.615	.613	.427	.582	.6	
Maibara	(62)	.553	.494	.490	.363	.506	.498	.502	.510	.521	.576	.540	.584	.587	.575	.588	.592	.590	.599	.577	.375	.556	.6	
Tsu	(63)	.552	.507	.490	.363	.501	.508	.502	.500	.507	.567	.526	.561	.568	.554	.583	.569	.568	.569	.556	.389	.534	.6	
Igaueno	(64)	.578	.518	.519	.385	.539	.524	.531	.546	.562	.620	.584	.632	.633	.622	.628	.641	.640	.626	.622	.410	.604	.7	
Higashiosaka	(65)	.531	.474	.474	.338	.492	.479	.485	.499	.511	.567	.532	.577	.579	.568	.578	.586	.586	.576	.570	.366	.550	.6	
Amagasaki	(66)	.607	.547	.538	.387	.553	.549	.551	.555	.566	.627	.585	.626	.630	.616	.649	.635	.634	.624	.623	.412	.598	.7	
Tamashima	(67)	.600	.553	.555	.399	.574	.560	.564	.583	.599	.662	.620	.672	.673	.661	.680	.681	.680	.695	.666	.437	.639	.7	
Konko	(68)	.543	.489	.489	.344	.508	.493	.500	.514	.529	.589	.552	.599	.600	.589	.611	.608	.607	.620	.593	.394	.572	.6	
Shobara	(69)	.613	.560	.561	.406	.580	.566	.572	.590	.604	.664	.621	.676	.677	.666	.671	.685	.684	.698	.667	.437	.645	.7	
Kumano	(70)	.602	.596	.600	.443	.617	.607	.601	.639	.648	.717	.671	.727	.728	.716	.727	.736	.736	.752	.720	.483	.681	.7	

TABLE 9

Genetic distance among 70 populations of *Rana nigromaculata* and *Rana brevipoda*

Rana nigromaculata																														
bu					Kinki					Chugoku											Shikoku				Kyushu					
)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)	(39)	(40)	(41)	(42)	(43)	(44)	(4!
I																														
9	.017																													
7	.021	.036																												
3	.004	.021	.017																											
7	.071	.097	.061	.064																										
7	.048	.046	.028	.042	.046																									
9	.124	.111	.110	.119	.110	.057																								
)	.067	.073	.039	.054	.032	.014	.077																							
3	.079	.066	.049	.066	.062	.021	.100	.018																						
3	.135	.158	.110	.120	.113	.114	.188	.107	.091																					
7	.155	.160	.129	.139	.146	.132	.199	.132	.107	.011																				
l	.108	.121	.090	.099	.084	.078	.138	.088	.078	.030	.042																			
5	.129	.125	.104	.119	.087	.076	.131	.087	.072	.103	.111	.037																		
3	.094	.090	.069	.085	.071	.041	.106	.052	.045	.108	.118	.048	.017																	
	.077	.057	.057	.071	.062	.022	.073	.047	.032	.104	.110	.063	.057	.040																
)	.078	.057	.053	.070	.079	.022	.081	.043	.021	.087	.093	.061	.076	.049	.010															
	.086	.065	.051	.071	.080	.021	.095	.029	.017	.113	.118	.089	.084	.048	.026	.020														
	.079	.062	.053	.073	.079	.025	.089	.046	.023	.091	.098	.066	.084	.053	.016	.005	.017													
	.078	.060	.051	.070	.078	.023	.089	.042	.021	.100	.107	.072	.084	.051	.017	.007	.013	.002												
	.052	.044	.034	.048	.050	.005	.063	.027	.024	.107	.120	.066	.062	.035	.011	.016	.025	.021	.019											
	.055	.040	.033	.048	.063	.005	.074	.023	.018	.125	.137	.086	.080	.042	.019	.016	.016	.021	.018	.006										
	.081	.108	.067	.070	.059	.049	.134	.051	.078	.166	.200	.131	.135	.090	.071	.084	.055	.075	.068	.063	.061									
	.080	.058	.046	.067	.089	.018	.092	.027	.015	.115	.120	.092	.080	.046	.035	.024	.016	.034	.030	.021	.011	.092								
	.079	.076	.044	.064	.069	.025	.100	.028	.026	.084	.098	.052	.045	.028	.041	.037	.032	.048	.045	.025	.024	.088	.016							
	.123	.130	.098	.112	.073	.068	.119	.075	.079	.096	.113	.032	.013	.024	.061	.080	.079	.085	.085	.059	.078	.105	.084	.041						
	.121	.151	.087	.104	.063	.071	.135	.059	.075	.067	.092	.057	.062	.063	.086	.096	.088	.104	.104	.073	.090	.104	.083	.045	.046					
	.111	.142	.080	.098	.065	.065	.117	.063	.077	.053	.076	.030	.042	.049	.073	.082	.089	.092	.095	.062	.085	.108	.081	.038	.025	.015				
	.083	.103	.048	.065	.069	.037	.127	.029	.038	.088	.111	.076	.081	.052	.070	.062	.041	.065	.060	.049	.045	.061	.038	.025	.068	.046	.046			
	.166	.183	.127	.146	.117	.102	.151	.097	.092	.048	.060	.055	.086	.079	.093	.090	.095	.094	.099	.099	.119	.146	.104	.077	.071	.050	.038	.059		
	.153	.183	.115	.134	.111	.106	.192	.091	.105	.115	.141	.110	.112	.087	.128	.133	.114	.139	.135	.110	.118	.129	.108	.082	.098	.065	.069	.056	.070	
	.097	.115	.075	.082	.101	.083	.137	.084	.099	.073	.071	.068	.085	.083	.105	.112	.100	.117	.116	.090	.100	.123	.095	.071	.080	.072	.061	.066	.086	.104
	.121	.145	.116	.109	.134	.123	.207	.127	.146	.147	.155	.135	.160	.143	.158	.161	.146	.162	.159	.134	.135	.133	.142	.123	.150	.146	.138	.112	.178	.167
	.596	.594	.564	.585	.415	.562	.669	.520	.555	.583	.581	.556	.516	.535	.556	.600	.552	.582	.583	.566	.583	.558	.588	.565	.510	.511	.537	.569	.578	.600
	.612	.610	.579	.599	.418	.576	.689	.525	.563	.597	.597	.572	.529	.547	.570	.615	.562	.594	.594	.582	.597	.562	.604	.580	.523	.522	.554	.584	.596	.615
	.608	.606	.575	.595	.414	.573	.686	.522	.559	.593	.593	.568	.525	.544	.566	.611	.559	.591	.591	.579	.593	.560	.600	.576	.521	.519	.551	.580	.593	.611
	.622	.620	.585	.606	.438	.586	.694	.544	.581	.611	.607	.582	.540	.558	.572	.627	.569	.606	.602	.588	.608	.570	.614	.586	.529	.532	.560	.595	.597	.628
	.571	.569	.555	.560	.397	.538	.642	.500	.531	.552	.551	.527	.495	.512	.531	.571	.530	.556	.558	.540	.558	.537	.563	.541	.487	.490	.513	.543	.548	.578
	.371	.359	.351	.359	.228	.321	.411	.292	.316	.379	.382	.343	.307	.309	.314	.350	.308	.335	.332	.324	.333	.319	.346	.334	.299	.305	.332	.342	.364	.381
	.549	.547	.541	.536	.365	.516	.630	.463	.498	.542	.546	.516	.477	.490	.515	.556	.507	.539	.538	.523	.535	.506	.542	.520	.473	.469	.504	.523	.544	.555
	.571	.569	.552	.558	.391	.536	.644	.492	.527	.564	.564	.537	.498	.511	.535	.577	.525	.558	.557	.542	.556	.525	.562	.541	.489	.490	.520	.544	.560	.578
	.558	.556	.551	.546	.373	.525	.641	.473	.508	.555	.559	.529	.490	.501	.526	.567	.517	.550	.548	.533	.544	.516	.551	.530	.485	.481	.517	.533	.556	.567
	.531	.529	.539	.518	.367	.495	.592	.458	.491	.528	.528	.498	.462	.472	.496	.538	.489	.525	.523	.500	.517	.486	.521	.502	.447	.452	.479	.504	.515	.539
	.462	.461	.471	.452	.314	.439	.535	.401	431	.466	.468	.436	.402	.414	.433	.474	.438	.464	.463	.440	.457	.440	.465	.443	.396	.396	.423	.451	.461	.481
	.551	.551	.546	.542	.387	.522	.631	.496	.528	.583	.581	.552	.516	.518	.539	.574	.520	.557	.556	.535	.545	.521	.550	.540	.500	.505	.530	.540	.575	.595
	.625	.625	.638	.613	.426	.583	.693	.547	.582	.644	.648	.616	.575	.579	.604	.643	.582	.627	.625	.599	.611	.574	.614	.604	.553	.558	.589	.601	.632	.652
	.625	.625	.615	.613	.427	.582	.678	.561	.596	.619	.611	.585	.546	.561	.584	.632	.575	.613	.614	.589	.611	.573	.614	.594	.524	.536	.556	.597	.597	.634
	.592	.590	.599	.577	.375	.556	.686	.513	.548	.598	.603	.568	.527	.540	.554	.596	.552	.590	.586	.568	.576	.538	.584	.566	.527	.525	.557	.567	.602	.613
	.569	.568	.569	.556	.389	.534	.654	.498	.533	.614	.618	.585	.548	.539	.563	.591	.528	.571	.568	.555	.555	.522	.560	.556	.534	.534	.568	.549	.612	.616
	.641	.640	.626	.622	.410	.604	.732	.564	.601	.631	.629	.597	.545	.561	.585	.637	.590	.629	.627	.613	.626	.570	.635	.609	.551	.553	.584	.610	.616	.631
	.586	.586	.576	.570	.366	.550	.672	.509	.540	.575	.577	.546	.498	.516	.537	.584	.541	.576	.575	.559	.572	.527	.580	.555	.502	.500	.533	.557	.571	.584
	.635	.634	.624	.623	.412	.598	.738	.547	.586	.652	.658	.626	.584	.588	.615	.652	.594	.631	.629	.617	.620	.590	.628	.614	.580	.575	.614	.612	.661	.662
	.681	.680	.695	.666	.437	.639	.755	.586	.628	.670	.673	.640	.592	.615	.632	.682	.636	.678	.664	.641	.665	.617	.672	.649	.583	.585	.616	.651	.662	.686
	.608	.607	.620	.593	.394	.572	.690	.532	.564	.598	.601	.567	.524	.543	.564	.610	.567	.604	.590	.572	.592	.554	.600	.576	.520	.520	.552	.579	.595	.614
	.685	.684	.698	.667	.437	.645	.768	.591	.633	.674	.679	.644	.594	.619	.630	.683	.640	.683	.679	.652	.670	.613	.677	.654	.592	.591	.624	.653	.670	.690
	.736	.736	.752	.720	.483	.681	.759	.629	.683	.726	.722	.695	.643	.668	.678	.737	.676	.732	.735	.690	.721	.651	.720	.705	.608	.627	.645	.702	.688	.742

vipoda

							R. brevipoda porosa										*R. b. brevipoda*										
Kyushu					Kor. Chi.												Nagoya race								Typical race		
(41)	(42)	(43)	(44)	(45)	(46)	(47)	(48)	(49)	(50)	(51)	(52)	(53)	(54)	(55)	(56)	(57)	(58)	(59)	(60)	(61)	(62)	(63)	(64)	(65)	(66)	(67)	(68)	(69)
.015																												
.046	.046																											
.050	.038	.059																										
.065	.069	.056	.070																									
.072	.061	.066	.086	.104																								
.146	.138	.112	.178	.167	.069																							
.511	.537	.569	.578	.600	.524	.552																						
.522	.554	.584	.596	.615	.540	.566	.003																					
.519	.551	.580	.593	.611	.537	.562	.002	.000																				
.532	.560	.595	.597	.628	.545	.573	.011	.013	.012																			
.490	.513	.543	.548	.578	.506	.527	.013	.022	.018	.023																		
.305	.332	.342	.364	.381	.333	.362	.049	.051	.048	.058	.039																	
.469	.504	.523	.544	.555	.495	.507	.024	.025	.022	.033	.014	.025																
.490	.520	.544	.560	.578	.508	.527	.011	.016	.012	.018	.005	.031	.008															
.481	.517	.533	.556	.567	.506	.517	.026	.029	.025	.033	.011	.028	.002	.005														
.452	.479	.504	.515	.539	.474	.486	.051	.058	.053	.055	.022	.037	.013	.020	.011													
.396	.423	.451	.461	.481	.423	.443	.059	.064	.060	.066	.032	.025	.013	.029	.016	.009												
.505	.530	.540	.575	.595	.514	.532	.189	.196	.192	.195	.177	.174	.180	.168	.173	.176	.190											
.558	.589	.601	.632	.652	.580	.588	.242	.249	.244	.247	.214	.214	.206	.205	.198	.186	.209	.051										
.536	.556	.597	.597	.634	.551	.580	.192	.200	.197	.197	.182	.194	.194	.176	.189	.186	.204	.046	.029									
.525	.557	.567	.602	.613	.550	.554	.215	.218	.213	.224	.195	.194	.186	.189	.180	.184	.195	.072	.040	.039								
.534	.568	.549	.612	.616	.543	.539	.215	.220	.216	.223	.199	.192	.192	.188	.185	.187	.204	.043	.011	.020	.026							
.553	.584	.610	.616	.631	.570	.591	.220	.224	.218	.226	.202	.210	.199	.198	.194	.199	.208	.103	.075	.062	.027	.062						
.500	.533	.557	.571	.584	.525	.542	.170	.173	.168	.179	.155	.160	.151	.150	.146	.155	.162	.087	.061	.055	.029	.053	.013					
.575	.614	.612	.661	.662	.596	.601	.194	.195	.190	.204	.186	.183	.176	.173	.169	.193	.202	.050	.028	.028	.029	.012	.061	.049				
.585	.616	.651	.662	.686	.619	.633	.188	.192	.187	.196	.167	.187	.159	.163	.153	.151	.168	.119	.081	.081	.058	.079	.095	.089	.080			
.520	.552	.579	.595	.614	.553	.562	.168	.172	.167	.175	.146	.158	.138	.141	.132	.130	.145	.103	.085	.082	.062	.080	.099	.089	.080	.009		
.591	.624	.653	.670	.690	.626	.637	.194	.197	.191	.202	.175	.195	.167	.172	.162	.162	.177	.132	.094	.094	.054	.090	.090	.085	.088	.011	.023	
.627	.645	.702	.688	.742	.649	.681	.215	.223	.219	.218	.193	.226	.199	.193	.192	.171	.200	.134	.089	.089	.089	.101	.126	.120	.119	.033	.056	.029

Chugoku district were 0.087~0.141, 0.120 on the average. The genetic distances between the group of the four (40~43) populations in the Kyushu district other than the Miyazaki(44) and Oita(45) populations and the group of the four (36~39) populations in the Shikoku district were 0.025~0.108, 0.069 on the average. Those between the two populations, the Oita and Miyazaki, in the Kyushu district and the three populations, the Matsuyama (39), Uwajima(38), and Takamatsu(36), in the Shikoku district were 0.077~0.119, 0.101 on the average, while those between the former two populations in the Kyushu district and the Nangoku(37) population in the Shikoku district were large, being 0.129 and 0.146, 0.138 on the average.

iii) The genetic distances between the group of the 24(1~24) populations in eastern Japan and the group of the 21 (25~45) populations in western Japan were 0.005~0.206, 0.086 on the average. Those between the Tottori(25) population in the San-in region of the Chugoku district and the group of the eight populations in eastern Japan, the Namioka(1), Akita(2), Shibata(4), Niigata(5), Kashiwazaki(6), Joetsu(7), Mishima(17) and Shingu(22), were 0.157~0.206, 0.174 on the average. Those between the Matsue(26) population in the San-in region of the Chugoku district and the group of the 14 populations including the foregoing eight populations and six other populations, the Sakata(3), Matsumoto(11), Okaya(12), Ina(14), Iida(15) and Sutama(16), were 0.151~0.206, 0.168 on the average. Those between the group of the two populations, the Gotsu(27) and Hagi(28), in the San-in region of the Chugoku district and the group of the 24(1~24) populations in eastern Japan were 0.052~0.151, 0.102 on the average. The genetic distances between the group of the seven (29~35) populations in the Sanyo region of the Chugoku district and the Namioka (1) population in the Tohoku district were 0.080~0.115, 0.101 on the average, those between the former seven populations and the group of the three populations including the Ina(14) and Iida(15) in the Chubu district and the Shingu(22) in the Kinki district were 0.063~0.106, 0.089 on the average, those between the former seven populations and the group of the 14 populations including the Akita(2) and Sakata(3) in the Tohoku district, the Shibata(4), Niigata(5), Kashiwazaki(6) and Joetsu(7) in the Hokuriku district, the Matsumoto(11), Okaya(12), Chino(13), Sutama(16), Mishima(17), Nagoya(18) and Inazawa(19) in the Chubu district and the Maibara(20) in the Kinki district were 0.033~0.094, 0.065 on the average, and those between the former seven populations and the group of the six populations including the Toyama(8), Kanazawa(9) and Mikuni(10) in the Hokuriku district, and the Igaueno(21), Higashiosaka(23) and Himeji(24) in the Kinki district were 0.005 ~0.052, 0.027 on the average. The genetic distances between the Miyazaki population(44) in the Kyushu district and the group of the 24 (1~24) populations in eastern Japan were 0.073~0.183, 0.130 on the average, and those between the Oita population(45) in the Kyushu district and the latter 24 populations were 0.085~0.194, 0.138 on the average. The genetic distances between the group of the remaining four populations, the Munakata(40), Sasebo(41), Kumamoto(42) and Kagoshima(43), in the Kyushu district, and the Namioka(1) population in the

Tohoku district were 0.101~0.155, 0.129 on the average, while those between the group of the former four populations and the group of the 23 (2~24) populations in eastern Japan other than the Namioka were 0.027~0.151, 0.091 on the average.

iv) The genetic distances among the 45 (1~45) populations of *R. nigromaculata* were 0.000~0.206, 0.078 on the average. While the genetic distances between the Suwon (46) population in Korea and the group of the 45 (1~45) populations in Japan were 0.061~0.146, 0.098 on the average, and those between the Beijing (47) population in China and the group of the 45 populations in Japan were 0.109 ~0.229, 0.147 on the average. The genetic distance between the Suwon and Beijing populations was comparatively small, being 0.069.

b. *Rana brevipoda*

The genetic distances among the 11(48~58) populations of *R. b. porosa* were 0.000~0.066, 0.027 on the average. Those among the 12 (59~70) populations of *R. b. brevipoda* were 0.009~0.134, 0.066 on the average, while those among the eight (59~66) populations of the Nagoya race of *R. b. brevipoda* were 0.011~0.103, 0.045 on the average, and those among the four (67~70) populations of the Typical race of *R. b. brevipoda* were 0.009~0.056, 0.027 on the average. Those between the group of the eight populations of the Nagoya race and the group of the four populations of the Typical race were 0.054~0.134, 0.092 on the average.

2. Subspecific difference

The genetic distances between the group of the 11 (48~58) populations of *Rana b. porosa* and the group of the 12 (59~70) populations of *R. b. brevipoda* were 0.130~0.249, 0.189 on the average. Those between the group of the 11 populations of *R. b. porosa* and the group of the eight populations of the Nagoya race of *R. b. brevipoda* were 0.146~0.249, 0.194 on the average, while those between the group of the 11 populations of *R. b. porosa* and the group of the four populations of the Typical race of *R. b. brevipoda* were 0.130~0.226, 0.178 on the average.

3. Specific difference

The genetic distances between the 47(1~47) populations of *R. nigromaculata* and the 23 (48~70) populations of *R. brevipoda* were 0.133~0.768, 0.546 on the average. Those between the former and the 11(48~58) populations of *R. b. porosa* were 0.133~0.694, 0.504 on the average, while those between the former and the 12 (59~70) populations of *R. b. brevipoda* were 0.338~0.768, 0.584 on the average. The genetic distances between the 45 populations of *R. nigromaculata* other than the Shibata(4) and Maibara(20), in which a distinct invasion of genes from *R. b. porosa* or *R. b. brevipoda* was found, and the 21 populations of *R. brevipoda* other than the Shibata(53) and Matsumoto(58), in which a distinct invasion of genes from *R. nigromaculata* was found, were 0.424~0.768, 0.571 on the average. The genetic distances between the 45 populations of *R. nigromaculata* other than the Shibata and Maibara, and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto were 0.424~0.694, 0.541 on the average, while those between the

former and the 12 populations of *R. b. brevipoda* were 0.474~0.768, 0.593 on the average.

Rana nigromaculata and *R. brevipoda* were found to be sympatric in the following 10 stations: Shibata(4 and 53) in the Hokuriku district, Matsumoto(11 and 58), Ina(14 and 59) and Inazawa(19 and 60) in the Chubu district, Maibara(20 and 62), Igaueno(21 and 64) and Higashiosaka(23 and 65) in the Kinki district and Konko(35 and 68), Shobara(34 and 69) and Kumano(31 and 70) in the Sanyo region of the Chugoku district (Table 9).

a. Shibata populations (4 and 53)

The genetic distance between the Shibata(4) population of *R. nigromaculata* and the Shibata(53) population of *R. b. porosa* was 0.133. This is very small when compared with those (0.260~0.304, 0.283 on the average) between the former(4) and the group of the nine populations of *R. b. porosa* other than the Shibata(53) and Matsumoto(58) populations. The genetic distances between the Shibata(4) population of *R. nigromaculata* and the group of the 12 (59~70) populations of *R. b. brevipoda* were 0.338~0.443, 0.377 on the average. On the other hand, those between the Shibata(53) population of *R. b. porosa* and the group of the 46 populations of *R. nigromaculata* other than the Shibata(4) were 0.228~0.411, 0.327 on the average (Table 9).

b. Matsumoto populations (11 and 58)

The genetic distance between the Matsumoto(11) population of *R. nigromaculata* and the Matsumoto(58) population of *R. b. porosa* was 0.417, and that between the former(11) and the Shibata(53) population of *R. b. porosa* was 0.330. The genetic distances between the Matsumoto(11) population of *R. nigromaculata* and the group of the nine populations of *R. b. porosa* other than the Shibata(53) and Matsumoto(58) were 0.479~0.569, 0.527 on the average. Those between the former(11) and the group of the 12 (59~70) populations of *R. b. brevipoda* were 0.512~0.671, 0.575 on the average. On the other hand, the genetic distances between the Matsumoto(58) population of *R. b. porosa* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.224 and 0.314, respectively, while those between the former(58) and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Matsumoto populations were 0.375~0.535, 0.438 on the average (Table 9).

c. Maibara populations (20 and 62)

The genetic distance between the Maibara(20) population of *R. nigromaculata* and the Maibara(62) population of *R. b. brevipoda* was 0.375. The genetic distances between the former(20) and the group of the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.228 and 0.314, respectively, while those between the former and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.365~0.438, 0.398 on the average. The genetic distances between the former(20) and the 11 populations of *R. b.*

brevipoda other than the Maibara (62) were 0.366~0.483, 0.415 on the average. On the other hand, the genetic distances between the Maibara(62) population of *R. b. brevipoda* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.363 and 0.375, respectively, while those between the former(62) and the 45 populations of *R. nigromaculata* other than the Shibata and Maibara populations were 0.490~0.686, 0.561 on the average (Table 9).

d. Higashiosaka populations (23 and 65)

The genetic distance between the Higashiosaka(23) population of *R. nigromaculata* and the Higashiosaka(65) population of *R. b. brevipoda* was 0.509. Those between the former(23) and the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.292 and 0.401, respectively, while those between the former(23) and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.458~0.544, 0.500 on the average. The genetic distances between the Higashiosaka(23) population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Higashiosaka(65) were 0.496~0.629, 0.551 on the average. On the other hand, the genetic distances between the Higashiosaka(65) population of *R. b. brevipoda* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.338 and 0.366, respectively, while those between the former(65) and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Higashiosaka populations were 0.474~0.672, 0.547 on the average (Table 9).

e. Ina populations (14 and 59)

The genetic distance between the Ina(14) population of *R. nigromaculata* and the Ina(59) population of *R. b. brevipoda* was 0.535. Those between the former(14) and the Shibata(53) and Matsumoto (58) populations of *R. b. porosa* were 0.367 and 0.449, respectively, while those between the former(14) and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.514~0.604, 0.562 on the average. The genetic distances between the Ina (14) population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Ina were 0.554~0.716, 0.616 on the average. On the other hand, the genetic distances between the Ina(59) population of *R. b. brevipoda* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.341 and 0.387, respectively, while those between the former(59) and the 44 populations of *R. nigromaculata* other than the Shibata, Ina and Maibara populations were 0.474 ~0.631, 0.534 on the average (Table 9).

f. Konko populations (35 and 68)

The genetic distance between the Konko (35) population of *R. nigromaculata* and the Konko (68) population of *R. b. brevipoda* was 0.572, while those between the former(35) and the 11 populations of *R. b. brevipoda* other than the Konko were 0.535~0.690, 0.602 on the average. Those between the former(35) and the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.324 and 0.440,

respectively, while those between the former(35) and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.500~0.588, 0.550 on the average. On the other hand, the genetic distances between the Konko(68) population of *R. b. brevipoda* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.344 and 0.394, respectively, while those between the former(68) and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Konko populations were somewhat large, being 0.489 ~0.690, 0.568 on the average (Table 9).

g. Igaueno populations (21 and 64)

The genetic distance between the Igaueno(21) population of *R. nigromaculata* and the Igaueno(64) population of *R. b. brevipoda* was 0.604, and those between the former(21) and the group of the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.321 and 0.439, respectively, while those between the former(21) and the nine populations of *R. b. porosa* other than the Shibata(53) and Matsumoto(58) populations were 0.495~0.586, 0.545 on the average. The genetic distances between the Igaueno(21) population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Igaueno population were 0.522~0.681, 0.587 on the average. On the other hand, the genetic distances between the Igaueno(64) population of *R. b. brevipoda* and the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.385 and 0.410, respectively, while those between the former(64) and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Igaueno populations were 0.518~0.732, 0.597 on the average (Table 9).

h. Inazawa populations (19 and 60)

The genetic distance between the Inazawa(19) population of *R. nigromaculata* and the Inazawa(60) population of *R. b. brevipoda* was 0.613, while those between the former(19) and the 11 populations of *R. b. brevipoda* other than the Inazawa population were 0.542~0.720, 0.614 on the average. The genetic distances between the former(19) and the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.359 and 0.452, respectively, while those between the former(19) and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.518~0.606, 0.567 on the average. On the other hand, the genetic distances between the Inazawa(60) population of *R. b. brevipoda* and the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.395 and 0.426, respectively, while those between the former(60) and the 44 populations of *R. nigromaculata* other than the Shibata, Inazawa and Maibara populations were 0.524~0.693, 0.595 on the average (Table 9).

i. Shobara populations (34 and 69)

The genetic distance between the Shobara(34) population of *R. nigromaculata* and the Shobara(69) population of *R. b. brevipoda* was 0.679, while those between the former(34) and the 11 populations of *R. b. brevipoda* other than the Shobara

population were 0.556~0.735, 0.615 on the average. The genetic distances between the Shobara(34) population of *R. nigromaculata* and the group of the Shibata(53) and Matsumoto(58) populations of *R. b. porosa* were 0.332 and 0.463, respectively, while those between the former(34) and the nine populations of *R. b. porosa* other than the Shibata and Matsumoto populations were 0.523~0.602, 0.566 on the average. On the other hand, the genetic distances between the Shobara(69) population of *R. b. brevipoda* and the group of the Shibata(4) and Maibara(20) populations of *R. nigromaculata* were 0.406 and 0.437, respectively, while those between the former(69) and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Shobara populations were 0.560~0.768, 0.641 on the average (Table 9).

j. Kumano populations (31 and 70)

The genetic distance between the Kumano (31) population of *R. nigromaculata* and the Kumano (70) population of *R. b. brevipoda* was 0.737, while those between the former(31) and the 11 populations of *R. b. brevipoda* other than the Kumano population were 0.574~0.683, 0.626 on the average. The genetic distances between the Kumano (31) population of *Rana nigromaculata* and the group of the Shibata (53) and Matsumoto (58) populations of *R. b. porosa* were 0.350 and 0.474, respectively, while those between the former (31) and the nine populations of *R. b. porosa* other than the Shibata (53) and Matsumoto (58) were 0.538~0.627, 0.585 on the average. On the other hand, the genetic distances between the Kumano (70) population of *R. b. brevipoda* and the group of the Shibata (4) and Maibara (20) populations of *R. nigromaculata* were 0.443 and 0.483, respectively, while those between the former and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Kumano populations were 0.596~0.759, 0.682 on the average (Table 9).

V. Dendrogram

A dendrogram was drawn for the 47 populations of *R. nigromaculata* and the 23 populations of the two subspecies of *R. brevipoda* on the basis of the genetic distances among them by the UPGMA method (SNEATH and SOKAL, 1973; NEI, 1975). The dendrogram showed that *R. nigromaculata* and *R. brevipoda* were first differentiated. *R. nigromaculata* was divided into a group of the Beijing and Suwon populations and a group of Japanese 45 populations. While *R. brevipoda* was differentiated into two subspecies, *R. b. porosa* and *R. b. brevipoda*, the Japanese *R. nigromaculata* was differentiated into several groups without dividing into the western and eastern groups. *R. b. brevipoda* was differentiated into the Nagoya race and the Typical race (Fig. 22).

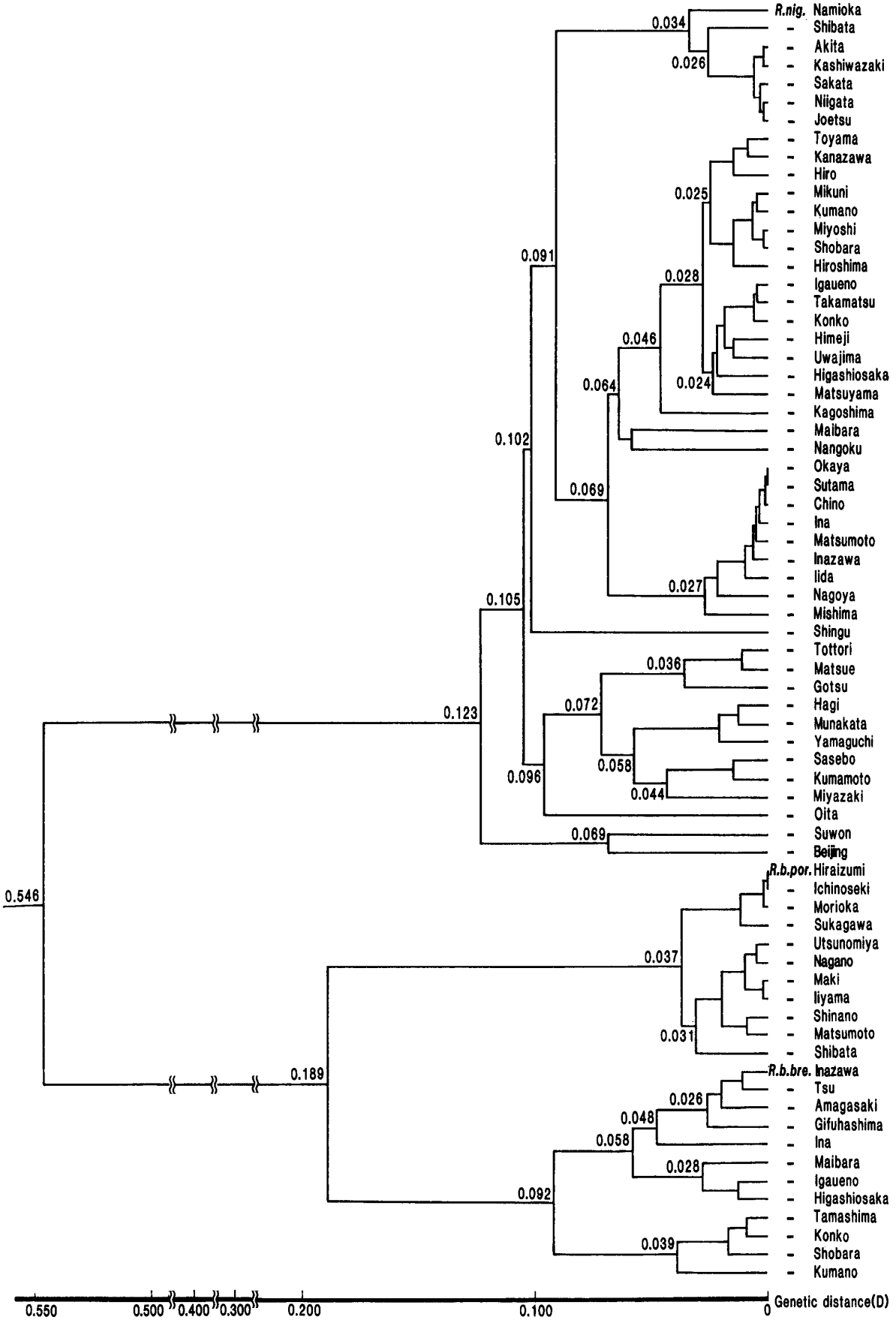

Fig. 22. Dendrogram for 70 populations of *Rana nigromaculata* and *Rana brevipoda* based on genetic distances.

DISCUSSION

A natural hybrid between *Rana nigromaculata* and *Rana brevipoda* was first discovered by MORIYA (1959) in the suburbs of Okayama city, while such a hybrid had not been found for a long time despite the many efforts that were made. These two species are sympatric in this district and according to MORIYA (1951), they seemed to be isolated from each other mainly by ecological factors, such as differences in breeding season and sexual reaction. KAWAMURA and NISHIOKA (1977) confirmed that natural hybridization between the two species was rather frequent in local areas near the lower and upper reaches of the Shinano River. These localities are peculiar in that they are coldest among the districts where *R. brevipoda* live. While *R. nigromaculata* usually spawns earlier than *R. brevipoda* in other sympatric areas, the two species spawn almost simultaneously and are apt to produce natural hybrids due to the powerlessness of ecological isolating mechanisms.

In the present study, 47 populations of *Rana nigromaculata,* 12 populations of *R. brevipoda brevipoda* and 11 populations of *R. brevipoda porosa* were examined by the method of electrophoretic analyses in order to clarify the relationships of these species and subspecies.

1. Sympatric districts of *R. nigromaculata* and *R. brevipoda porosa*

The two species are sympatric in Shibata and Matsumoto which are situated along the upper and lower reaches of the Shinano River, respectively. These are only sympatric districts of the two species in the Tohoku, Hokuriku, Kanto and the northern part of the Chubu areas. Shibata belongs to Niigata Prefecture of the Hokuriku area and Matsumoto belongs to Nagano Prefecture of the Chubu area. When gene invasions were examined on 15 loci which showed distinct differences between the two species in 10 sympatric districts, the most remarkable one was found between *R. nigromaculata* and *R. brevipoda porosa* collected from Shibata, where natural hybridization occurs most frequently in Japan. In 16 specimens of *R. nigromaculata* examined, small percentages of alleles had invaded from *R. brevipoda porosa* into 12 loci, while in 38 specimens of *R. brevipoda porosa,* small percentages of alleles which invaded from *R. nigromaculata* were found at 10 loci. Owing to such reciprocal invasions, it is assumed that the affinity of the two species has become very close. In fact, the two Shibata populations of *R. nigromaculata* and *R. brevipoda porosa* were very small, being 0.133, in genetic distance.

The Shibata populations of *R. nigromaculata* and *R. brevipoda porosa* are peculiar in that they are genetically very close to the other populations of *R. brevipoda porosa* or *R. b. brevipoda* and *R. nigromaculata,* respectively. The Shibata population of *R. brevipoda porosa* was remarkably close to the 46 populations of *R. nigromaculata* other than the Shibata, being 0.228~0.411, 0.327 on the average, in genetic distance. The Shibata population of *R. nigromaculata* was also distinctly close to the nine populations of *R. brevipoda porosa* other than the Shibata and Matsumoto, being

0.260~0.304, 0.283 on the average, in genetic distance, and was somewhat close to the 12 populations of *R. b. brevipoda,* being 0.338~0.443, 0.377 on the average.

In Matsumoto, *R. nigromaculata* and *R. brevipoda porosa* are sympatric, and reciprocal invasions of genes were found between these two species. In 30 specimens of *R. nigromaculata* which were examined, small or minimal percentages of alleles which invaded from *R. brevipoda porosa* were found in seven loci, while in 43 specimens of *R. brevipoda porosa* which were examined, small percentages of alleles which invaded from *R. nigromaculata* were found in seven loci. The genetic distance between *R. nigromaculata* and *R. brevipoda porosa* collected from Matsumoto was comparatively large, being 0.417. Those between *R. brevipoda porosa* from Matsumoto and the 45 populations of *R. nigromaculata* other than the Shibata and Maibara were 0.375~0.535, 0.437 on the average, while those between the Matsumoto population of *R. nigromaculata* and the nine populations of *R. brevipoda porosa* other than the Shibata and Matsumoto were 0.479~0.569, 0.527 on the average, and those between the former and the 12 populations of *R. b. brevipoda* were 0.512~0.671, 0.575 on the average. The comparatively large value in genetic distance between *R. nigromaculata* and *R. brevipoda porosa* in Matsumoto district seems to show that natural invasion of genes was not so distinct as found in the two species of the Shibata district. The genetic distance between the Matsumoto population of *R. brevipoda porosa* and the Shibata population of *R. nigromaculata* was very small, being 0.224.

In the Tohoku, Hokuriku, Kanto and the northern part of the Chubu areas, the genetic distances between the nine populations (1~3, 5~10) of *R. nigromaculata* and the nine populations of *R. brevipoda porosa* other than the Shibata and Matsumoto were 0.424~0.605, 0.496 on the average. Those between the Utsunomiya population in the Kanto area of *R. brevipoda porosa* and the nine populations (1~3, 5~10) of *R. nigromaculata* in the Tohoku and Hokuriku areas were 0.471~0.551, 0.495 on the average.

2. Sympatric districts of *R. nigromaculata* and *R. b. brevipoda*

In the Kinki, Chugoku and the southern part of the Chubu areas, there are eight districts where *R. nigromaculata* and *R. b. brevipoda* are sympatric. Natural hybridization between the two species seems to have most frequently occurred in Maibara in the Kinki area. In five other districts, Higashiosaka, Ina, Konko, Igaueno and Inazawa, the natural hybridization of the two species has occurred, although not so frequently as found in Maibara. In contrast with these six districts, this seems to have scarcely occurred in Shobara and Kumano, since they landed in Japan.

In Maibara, Shiga Prefecture, 21 specimens of *R. nigromaculata* which were examined had large, small or minimal percentages of alleles which invaded from *R. b. brevipoda* in 12 loci, while in 46 specimens of *R. b. brevipoda,* very small or minimal percentages of alleles which invaded from *R. nigromaculata* were observed in five loci. The genetic distance between *R. nigromaculata* and *R. b. brevipoda* collected from Maibara was comparatively small, being 0.375. Those between

the Maibara population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Maibara were 0.366~0.483, 0.415 on the average, while those between the Maibara population of *R. b. brevipoda* and the 45 populations of *R. nigromaculata* other than the Shibata and Maibara were 0.490~0.686, 0.561 on the average.

In Higashiosaka, Osaka Prefecture, *R. nigromaculata* and *R. b. brevipoda* are sympatric and reciprocal invasions of genes were found in the loci of these two species. In 38 specimens of *R. nigromaculata,* small or minimal percentages of alleles which invaded from *R. b. brevipoda* were found in nine loci, while in 20 specimens of *R. b. brevipoda,* small or minimal percentages of alleles which invaded from *R. nigromaculata* were found in only three loci. The genetic distance between the two populations of *R. nigromaculata* and *R. b. brevipoda* was 0.509. Those between the Higashiosaka population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Higashiosaka were 0.496~0.629, 0.551 on the average, while those between the Higashiosaka population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Higashiosaka were 0.474~0.672, 0.547 on the average.

In Ina, Nagano Prefecture, *R. nigromaculata* and *R. b. brevipoda* are sympatric. When only two specimens of *R. nigromaculata* were analyzed, a small percentage of an allele which invaded from *R. b. brevipoda* was found in the α–GDH locus, while in 69 specimens of *R. b. brevipoda,* very small or minimal percentages of alleles which invaded from *R. nigromaculata* were found in nine loci. The genetic distance between the two sympatric species was 0.535. Those between the Ina population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Ina were 0.554~0.716, 0.616 on the average, while those between the Ina population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Ina and Maibara were 0.474~0.631, 0.534 on the average.

In Konko, Okayama Prefecture, *R. nigromaculata* and *R. b. brevipoda* are sympatric. In 83 specimens of *R. nigromaculata,* minimal percentages of alleles which ivaded from *Rana brevipoda* were found in two loci, while an invasion of one allele from *R. nigromaculata* was found in one locus of 38 specimens of *R. b. brevipoda.* The genetic distance between these two sympatric species was 0.572. Those between the Konko population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Konko were 0.535~0.690, 0.602 on the average, while those between the Konko population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Konko were 0.489~0.690, 0.568 on the average.

In Igaueno, Mie Prefecture, *R. nigromaculata* and *R. b. brevipoda* are sympatric. In 48 specimens of *R. nigromaculata,* minimal percentages of alleles which invaded from *R. b. brevipoda* were found in eight loci, while in four specimens of *R. b. brevipoda,* no invasion from *R. nigromaculata* was found at any locus. The genetic distance between these two sympatric species was 0.604. Those between the Igaueno population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Igaueno were 0.522~0.681, 0.588 on the average, while those

between the Igaueno population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Igaueno were 0.518~0.732, 0.597 on the average.

In Inazawa, near Nagoya city, *R. nigromaculata* and *R. b. brevipoda* are sympatric. The specimens of both species which were examined were very small in number. In five specimens of *R. nigromaculata* and one specimen of *R. b. brevipoda,* no invasion was found at any locus. The genetic distance between the two sympatric species was 0.613. Those between the Inazawa population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Inazawa were 0.542~0.720, 0.614 on the average, while those between the Inazawa population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Inazawa and Maibara were 0.524~0.693, 0.595 on the average.

In Shobara, Hiroshima Prefecture, *R. nigromaculata* and *R. b. brevipoda* are sympatric. In 19 specimens of *R. nigromaculata* and four specimens of *R. b. brevipoda* which were examined, no invasion of genes from *R. b. brevipoda* and *R. nigromaculata,* respectively, was found at any locus. The genetic distance between the two sympatric species was 0.679. Those between the Shobara population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Shobara were 0.556~0.735, 0.615 on the average, while those between the Shobara population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Shobara were 0.560~0.768, 0.641 on the average.

In the Kumano district, *R. nigromaculata* and *R. b. brevipoda* are sympatric. In 56 specimens of *R. nigromaculata* and two specimens of *R. b. brevipoda* examined, no invasion of genes from *R. b. brevipoda* and *R. nigromaculata*, respectively, was found at any locus. The genetic distance between the two sympatric species was 0.737. Those between the Kumano population of *R. nigromaculata* and the 11 populations of *R. b. brevipoda* other than the Kumano were 0.574~0.683, 0.626 on the average, while those between the Kumano population of *R. b. brevipoda* and the 44 populations of *R. nigromaculata* other than the Shibata, Maibara and Kumano were 0.596~0.759, 0.683 on the average.

In the Kinki and Chugoku areas and the southern part of the Chubu area, the genetic distances between the 20 populations (12, 13, 15~19, 21, 22, 24~34) of *R. nigromaculata* and the eight populations of *R. b. brevipoda* other than the Ina, Maibara, Higashiosaka and Konko populations were 0.528~0.768, 0.636 on the average.

3. The largest and smallest genetic distances

The largest genetic distances were found between the Shobara and Kumano populations of *R. b. brevipoda* and Shingu population of *R. nigromaculata.* They were 0.768 and 0.759, respectively. The genetic distance between the sympatric *R. nigromaculata* and *R. b. brevipoda* in Kumano was 0.737. These large genetic distances seem to show that the *R. b. brevipoda* and *R. nigromaculata* have preserved nearly similar genetic characters as those which they had at the time of invasion into Japan.

The smallest genetic distance, that is, 0.133, was found between the sympatric *R. nigromaculata* and *R. brevipoda porosa* in Shibata where natural hybridization and mutual exchanges of genes have most frequently occurred. Fairly distinct natural hybridization and mutual exchanges of genes have also occurred in Matsumoto where the two species are sympatric. However, the genetic distance between them was 0.417 which is somewhat larger than 0.375 found between sympatric *R. nigromaculata* and *R. b. brevipoda* collected from Maibara.

In the Tohoku, Hokuriku and Chubu areas, active introgression seems to have occurred from Shibata and Matsumoto into the other districts. The genetic distances between *R. nigromaculata* and *R. b. porosa* in these areas, except the Shibata and Matsumoto populations of the two species, were 0.424~0.634, 0.532 on the average. Those between the Utsunomiya population of *R. b. porosa* and the 17 populations (1~3, 5~10, 12~19) of *R. nigromaculata* except the Shibata and Matsumoto were 0.471~0.581, 0.528 on the average. The specimens described by COPE (1868) as *Tomoptera porosa* were those similar to the Utsunomiya population of *R. b. porosa,* which was considered to have derived from *R. brevipoda* by introgression of genes from *R. nigromaculata.*

In the southern part of the Chubu area and the Kinki and Sanyo areas (12, 13, 15~19, 21, 22, 24, 30~34), the genetic distances between *R. nigromaculata* and *R. b. brevipoda,* except the Ina, Maibara, Higashiosaka and Konko populations, were 0.533~0.768, 0.636 on the average. It was found that there are fairly large differences in the genetic distances of the two species between the eastern and western populations. This seems to show that natural hybridization and introgression have most actively occurred in the eastern areas of Japan.

The genetic distances between the Shingu population of *R. nigromaculata* and the 12 populations of *R. b. brevipoda* were the largest among those between the 24 populations of *R. nigromaculata* in the Tohoku, Hokuriku, Chubu and Kinki areas and the latter 12 populations of *R. b. brevipoda.* They were 0.631~0.768, 0.705 on the average. Such large genetic distances seem attributable to the fact that the introgression has scarcely occurred between the Shingu population and the neighboring populations of *R. nigromaculata,* as the Shingu population has been geographically isolated from the latter.

4. Dendrogram

The dendrogram drawn for *R. nigromaculata, R. brevipoda porosa* and *R. b. brevipoda* exactly seems to show the differentiation of the *R. nigromaculata* group in Japan, Korea and China. According to the dendrogram, *R. brevipoda* seems to be a conservative species in contrast to *R. nigromaculata* which seems to be a progressive one. It is unquestionable that *R. brevipoda* entered Japan earlier than *R. nigromaculata* and was divided into two groups, while *R. nigromaculata* entered later and were divided into more than two groups. The wide distribution of *R. nigromaculata* over Korea and the eastern part of China in contrast to the distribution of *R. brevipoda* limited to the Mainland of Japan and northwestern portion of Shikoku, seems to show that *R. brevipoda* was an earlier invader in Japan than *R. nigromaculata.*

While *R. brevipoda* was found in comparatively mild lowlands, *R. nigromaculata* was distributed all over Japan. Thus, in the Mainland, *R. brevipoda* was surrounded by the newcomer. Although these two species were reproductively isolated from each other by various isolating mechanisms such as seasonal, ecological and sexual ones, these mechanisms were incomplete and natural hybrids and their offspring were produced in many sympatric districts.

In the eastern part of the Chugoku area including Kumano, Shobara and Konko, *R. brevipoda* and *R. nigromaculata* were sympatric and natural hybridization between them has scarcely occurred owing to the presence of reproductively isolating mechanisms (KAWAMURA and NISHIOKA, 1973, 1977, 1978, 1979). Accordingly, there are many *R. brevipoda* specimens which have maintained their own features since their entrance into Japan. These specimens belong to the Typical race of *R. brevipoda*. In the Kinki area including Higashiosaka, Igaueno and Maibara and southern part of the Chubu area including Ina and Inazawa, natural hybridization between the two species has often occurred. As a matter of course, there are many specimens which are considered to be natural hybrids or their offspring in the two areas, although there are some specimens which appear to be almost typical *R. brevipoda*. The dendrogram shows the presence of the group of *R. brevipoda* which was named the Nagoya race by MORIYA (1954).

Another group of *R. brevipoda* was named *R. brevipoda porosa*. This group was divided into two subgroups. One subgroup includes seven populations, the Shibata, Matsumoto, Shinano, Iiyama, Maki, Nagano and Utsunomiya, in the Hokuriku, Chubu and Kanto areas. The other subgroup includes four populations, the Sukagawa, Morioka, Ichinoseki and Hiraizumi, in the Tohoku area. The first subgroup seems to have been produced firstly by natural hybridization between the two species in Shibata and secondly by similar hybridization in Matsumoto. Distinct introgression occurred in the populations of the first subgroup. In the populations of the second subgroup, fairly large introgression seems to have occurred from Shibata and Matsumoto.

The dendrogram shows that *R. nigromaculata* was first divided into two parts, one of which included the populations of Korea and China, while the other included many populations which were differentiated in Japan, where *R. nigromaculata* were locally divided into two groups. One group including 11 populations was independently differentiated, as the two species were allopatric, while the other group including numerous populations was differentiated under some effects from *R. brevipoda,* as the two species were sympatric and natural hybridization and introgression occurred between them. The latter group was divided into three subgroups which consisted of seven populations in the Tohoku area, 18 populations in the Hokuriku, Chugoku, Kinki, Shikoku and Kyushu areas and nine populations in the Chubu area. The first subgroup contains the Shibata population in which the most active hybridization occurred with *R. brevipoda porosa*. The Shibata population of *R. nigromaculata* seemed to be genetically very close to *R. brevipoda porosa* and *R. b, brevipoda,* as the genetic distances between the former and 23 populations of *R. brevipoda* were remarkably small. The second subgroup

contains the Maibara population in which fairly active hybridization occurred with *R. b. brevipoda*. The Maibara population of *R. nigromaculata* seemed to be genetically close to the 23 populations of *R. brevipoda,* as the genetic distances between them were fairly small. The third subgroup contains the Matsumoto population in which somewhat active hybridization occurred with *R. brevipoda porosa,* although the genetic distance was not remarkably small between the Matsumoto population of *R. nigromaculata* and the 23 populations of *R. brevipoda*. This may indicate that the natural hybridization between the two species in the Matsumoto district is a comparatively recent occurrence.

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