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Genetic Differentiation of 30 Populations of 12 Brown Frog Species Distributed in the Palearctic Region Elucidated by the Electrophoretic Method

By

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ABSTRACT

Genetic differentiation of 30 populations of 12 brown frog species distributed in the Palearctic region was elucidated by the method of starch-gel electrophoresis analyzing enzymes and blood proteins. Eight of these species, *Rana japonica, R. tsushimensis, R. okinavana, R. longicrus, R. temporaria, R. asiatica, R. amurensis* and *R. latouchii,* have 26 chromosomes in diploid number, while the other four, *R. ornativentris, R. chensinensis, R. dybowskii* and *R. arvalis,* have 24 chromosomes.

It was found that 15 kinds of enzymes and three kinds of blood proteins extracted from these frogs were controlled by genes at 25 loci. At each locus, there were 2~55 phenotypes, 13.4 on the average, produced by 2~23 alleles, 8.4 on the average. At the 25 loci in the 30 populations, the mean proportions of heterozygous loci per individual were 1.7~ 22.0%, 10.1% on the average, the mean proportions of polymorphic loci were 8.0~60.0%, 33.2% on the average, and the number of alleles per locus was 1.08~1.92, 1.43 on the average.

The genetic distances among 13 populations of *R. japonica* were 0.006~0.182. That between the Aomori and Hiroshima populations of *R. ornativentris* was 0.160, while those among the three populations, the Mongolia, Siberia and Manchuria, of *R. amurensis* were 0.005~0.008, 0.007 on the average. Of four populations of *R. chensinensis,* those between the Siberia and Manchuria, between the Sapporo and Beijing, between the Sapporo and Manchuria, between the Sapporo and Siberia, between the Beijing and Siberia, and between the Beijing and Manchuria, were 0.254, 0.442, 0.512, 0.638, 0.819 and 0.842, respectively. Among the eight species having 26 chromosomes, the genetic distances were 0.294~2.913, while among the four species having 24 chromosomes, they were 0.260~1.018. Those between the eight species with 26 chromosomes and the four species with 24 chromosomes were 0.410~2.266.

A dendrogram drawn for the brown frogs of 30 populations of 12 species on the basis of the genetic distances by UPGMA clustering method showed that *R. latouchii* (2n=26) first deviated and next *R. tsushimensis* (2n=26) and then *R. arvalis* (2n=24).

The remaining brown frogs, thereafter, were divided into two large groups of species, one having 26 chromosomes and the other having 24 chromosomes.

INTRODUCTION

As the brown frog species found in Japan and adjacent territories closely resemble each other in morphology and ecology, their classification has been confusing for a long time. STEJNEGER (1907) described six species and one subspecies as brown frogs distributed in and around Japan. They were *Rana japonica* GÜNTHER (1858) and R. *japonica ornativentris* WERNER (1904) distributed widely in Japan, *R. tsushimensis* STEJNEGER (1907) a new species in Tsushima Isl., *R. okinavana* BOETTGER (1895) in Okinawa Isl., *R. temporaria* LINNAEUS (1758) in Hokkaido, synonymous with *R. dybowskii* GÜNTHER (1869) in Abrek Bay near Vladivostok, *R. amurensis* BOULENGER (1886) in Amur Province, and *R. longicrus* STEJNEGER (1898) in Taiwan. Okada (1931) described five species and four subspecies. They were *R. japonica, R. temporaria tsushimensis, R. macropus* BOULENGER (1886) in Ryukyu Isls., synonymous with *R. okinavana* BOETTGER (1895), *R. temporaria,* synonymous with *R. dybowskii,* including *R. temporaria temporaria* distributed in Hokkaido, *R. temporaria ornativentris* distributed widely in Japan, *R. temporaria martensi* BOULENGER (1886) which is morphologically and ecologically intermediate between *R. japonica* and *R. temporaria ornativentris,* and *R. temporaria coreana* OKADA (1927), *R. amurensis* and *R. longicrus.* DAVID (1875) described a brown frog collected at an altitude of more than 1000 meters in southern Shensi, China, as *Rana chensinensis.* STEJNEGER (1925) placed *R. amurensis* as a synonym of *R. chensinensis* by re-examining specimens kept in the United States National Museum. POPE and BORING (1940) suggested that the brown frog of North China should be *R. temporaria chensinensis* on account of its close similarity to the European form of *R. temporaria,* except for its smaller body size. LIU (1950) limited the application of this subspecies name to the brown frog in the northern and northwestern China. He preferred to regard the brown frog distributed in the three eastern provinces of China as a distinct subspecies and gave this frog the name, *Rana temporaria amurensis.* According to him, *R. temporaria chensinensis* is distinguishable from *R. temporaria amurensis* in its smaller body size and prominent dorso-lateral glandular folds, which are ungulate near the tympanum. BALCELLS (1956) described the brown frog distributed in Siberia, North China and Hokkaido, Japan, as *R. temporaria chensinensis.*

NAKAMURA and UENO (1963) changed *R. temporaria temporaria* in Hokkaido into *R. temporaria dybowskii* GÜNTHER (1869) and recognized *R. temporaria ornativentris* WERNER (1904), *R. amurensis amurensis* BOULENGER (1886), *R. amurensis coreana* OKADA (1927) and *R. amurensis tsushimensis* STEJNEGER (1907). They changed the name *R. macropus* into *R. okinavana* BOETTGER (1895). OKADA (1966) revised his first classification of brown frogs published in 1931 as the following four species and two subspecies; *R. japonica, R. ornativentris, R. tsushimensis, R. macropus, R. temporaria chensinensis* and *R. temporaria martensi.* KAWAMURA (1962) and NAKA-

MURA and UENO (1963) placed *R. temporaria martensi* as a synonym of *R. japonica* in agreement with STEJNEGER's opinion (1907). In 1972, NAKAMURA and UENO changed *R. temporaria ornativentris* WERNER and *R. temporaria dybowskii* GÜNTHER into *R. ornativentris* WERNER and *R. chensinensis dybowskii* DAVID (1875).

As brown frogs distributed in China, LIU and HU (1961) described four species and one subspecies, *R. japonica japonica* GÜNTHER, *R. japonica chaochiaoensis* LIU (1945), *R. amurensis* BOULENGER (1886), *R. temporaria chensinensis* DAVID (1875) and *R. latouchii* BOULENGER (1899).

KAWAMURA (1962) clarified the taxonomic positions of four brown frog subspecies principally on the basis of reproductive isolation. First of all, KAWAMURA insisted that the name of *Rana temporaria temporaria* L. distributed in Hokkaido should be provisionally changed into *Rana chensinensis* DAVID, as the Japanese *R. temporaria* is completely isolated from the European *R. temporaria* by means of gametic isolation and hybrid inviability (KAWAMURA and KOBAYASHI, 1960; KAWAMURA and NISHIOKA, 1962), in addition to that the European *R. temporaria* is 26 in diploid chromosome number (WITSCHI, 1922a, b, 1924; PROKOFIEVA, 1935; WICKBOM, 1945), while *R. temporaria temporaria* from Sakhalin and Hokkaido are 24 (KAWAMURA, 1943; WITSCHI, KODANI and MIKAMO, 1958; KOBAYASHI, 1962). As there were no reports on the chromosomes of *R. t. chensinensis* and *R. amurensis* distributed in China, as well as on hybridization experiments between them or between these and the Japanese *R. temporaria*, there were no data to ascertain whether the Japanese *R. temporaria* is reproductively isolated from the Chinese brown frogs or not. Accordingly, it seemed not unreasonable that the brown frogs of Hokkaido and Sakhalin is provisionally given the species name, *Rana chensinensis* DAVID.

Secondly, KAWAMURA (1962) gave *R. temporaria ornativentris* the position of a valid species and named *Rana ornativentris* WERNER, as the brown frog named *R. temporaria ornativentris* is morphologically different from the European *R. temporaria temporaria*, the Japanese *R. temporaria* (=*R. chensinensis*), *R. japonica* and *R. arvalis*, and moreover, it is reproductively isolated from each of these four kinds of brown frogs. Thirdly, KAWAMURA (1962) changed the subspecific name, *R. temporaria tsushimensis*, into the original name, *Rana tsushimensis*, which was first described by STEJNEGER (1907). It seemed reasonable that the brown frog distributed in the Island of Tsushima is *R. tsushimensis*, as this brown frog was confirmed to be perfectly isolated by gametic isolation or hybrid inviability from *R. japonica*, *R. ornativentris*, Japanese *R. chensinensis* and European *R. temporaria* (KAWAMURA and NISHIOKA, unpublished data).

KAWAMURA, NISHIOKA and UEDA (1981) made 99 kinds of crossing experiments among 14 brown frog species, *R. amurensis coreana*, *R. arvalis*, *R. chensinensis*, *R. dalmatina*, *R. dybowskii*, *R. japonica*, *R. latouchii*, *R. longicrus*, *R. macrocnemis*, *R. okinavana*, *R. ornativentris*, *R. sylvatica*, *R. temporaria* and *R. tsushimensis*, distributed in Japan, Korea, Taiwan, Europe and North America. It was found that all these species are completely isolated from one another by gametic isolation, hybrid inviability, hybrid sterility or cooperation of these reproductively isolating

mechanisms. Later, KAWAMURA, NISHIOKA, UEDA, BORKIN and WU (1985) again conducted crossing experiments among various brown frog species and populations collected from Japan, Taiwan, China and Russia, mainly to confirm the existence of isolating mechanisms among Japanese, Chinese and Russian populations of *Rana chensinensis* and between these three populations and the other brown frog species distributed in the Far East. The results of these experiments seemed to show that each of the Japanese and Russian *R. chensinensis* is a valid species, distinct from the Chinese *R. chensinensis*. They suggested that the Japanese *R. chensinensis* should be named *Rana ezoensis* at least.

Although all these populations of *R. chensinensis* may be valid species, the genetic relationships among them were obscure. Thus, it was the aim of the present authors to clarify the genetic differentiation of 12 brown frog species including *R. chensinensis* distributed in the Palearctic region by the method of starch-gel electrophoresis analyzing enzymes and blood proteins extracted from these frogs.

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), the *Rana nigromaculata* group by NISHIOKA, SUMIDA and OHTANI (1992) and six pond frog species by NISHIOKA and SUMIDA (1992).

MATERIALS AND METHODS

A total of 468 frogs including 203 females and 265 males belonging to 30 populations of 12 brown frog species distributed in the Palearctic region was used in the present study. Eight of these species, *Rana japonica* GÜNTHER, *R. tsushimensis* STEJNEGER, *R. okinavana* BOETTGER, *R. longicrus* STEJNEGER, *R. temporaria* LINNAEUS, *R. asiatica* BEDRIAGA, *R. amurensis* BOULENGER and *R. latouchii* BOULENGER, are 26 in diploid number, while the other four species, *R. ornativentris* WERNER, *R. chensinensis* DAVID, *R. dybowskii* GÜNTHER and *R. arvalis* NILSSON, are 24. The collecting stations, numbers and sexes of these frogs are shown in Table 1 and Fig. 1.

Of the specimens of the four populations of *R. chensinensis,* those collected from Manchuria were larger than those of the other three populations and are called "hashima" in China.

Fifteen kinds of enzymes extracted from skeletal muscles and livers and three kinds of blood proteins were analyzed by the method of horizontal starch-gel electrophoresis. Shown in Table 2 are the names and abbreviations of analyzed enzymes and blood proteins, the samples from which these enzymes and blood proteins were extracted and the buffer systems.

The method of electrophoresis has been reported in detail by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was performed by the method

TABLE 1
Collecting stations and the number of the frogs examined in the present study

Species	Chr. no. (2n)	Country	District	Station	Population	No. of frogs		
						Total	Female	Male
R. japonica	26	Japan	Iwate	Morioka	Morioka	3	1	2
〃	〃	〃	〃	Ichinoseki	Ichinoseki	37	19	18
〃	〃	〃	Miyagi	Wakuya	Wakuya	24	13	11
〃	〃	〃	Fukushima	Fukushima	Fukushima	28	15	13
〃	〃	〃	〃	Sukagawa	Sukagawa	2	1	1
〃	〃	〃	Tochigi	Utsunomiya	Utsunomiya	29	14	15
〃	〃	〃	Kanagawa	Isehara	Isehara	13	7	6
〃	〃	〃	Shizuoka	Shizuoka	Shizuoka	28	17	11
〃	〃	〃	Toyama	Toyama	Toyama	15	6	9
〃	〃	〃	Fukui	Fukui	Fukui	21	17	4
〃	〃	〃	Hiroshima	Hiroshima	Hiroshima	36	16	20
〃	〃	〃	〃	Geihoku	Geihoku	6	6	0
〃	〃	〃	Shimane	Oki	Oki	5	3	2
R. ornativentris	24	Japan	Aomori	Hirosaki	Aomori	8	4	4
〃	〃	〃	Hiroshima	Hiroshima	Hiroshima	21	4	17
R. chensinensis	24	Japan	Hokkaido	Sapporo	Sapporo	31	4	27
〃	〃	China	Hebei	Beijing	Beijing	14	0	14
〃	〃	Russia	Siberia	Maritime territory	Siberia	3	1	2
〃 (hashima)	〃	China	Manchuria	North-east China	Manchuria	3	1	2
R. dybowskii	24	Japan	Nagasaki	Tsushima	Tsushima	24	10	14
R. tsushimensis	26	Japan	Nagasaki	Tsushima	Tsushima	42	14	28
R. okinavana	26	Japan	Okinawa	Yona	Okinawa	8	3	5
R. longicrus	26	Taiwan		Taipei	Taiwan	24	5	19
R. latouchii	26	Taiwan		Taipei	Taiwan	6	1	5
R. arvalis	24	Luxembourg		Luxembourg	Luxembourg	6	3	3
R. temporaria	26	Russia		Leningrad	Leningrad	6	3	3
R. asiatica	26	Russia		Kirghizia	Kirghizia	3	3	0
R. amurensis	26	Mongolia		North Mongolia	Mongolia	13	9	4
〃	〃	Russia	Siberia	Maritime territory	Siberia	6	2	4
〃	〃	China	Manchuria	North-east China	Manchuria	3	1	2
Total						468	203	265

of BREWER (1970) and HARRIS and HOPKINSON (1976), while that of blood proteins was made by the amido-black staining method.

When each of the multiple alleles at a locus exists in a frequency of more than 1%, this locus is recognized to be polymorphic. The fixation index (Fst) coined by WRIGHT (1978) was utilized as a standard to show the degree of genetic differentiation that was found at a definite locus among local populations. Genetic variations among various populations were shown by the mean proportions of polymorphic loci per population and by the mean proportions of heterozygous loci per individual (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974). The genetic relationships among species and populations were evaluated by calculating the genetic distances (D) by the method of NEI (1975). A dendrogram for these

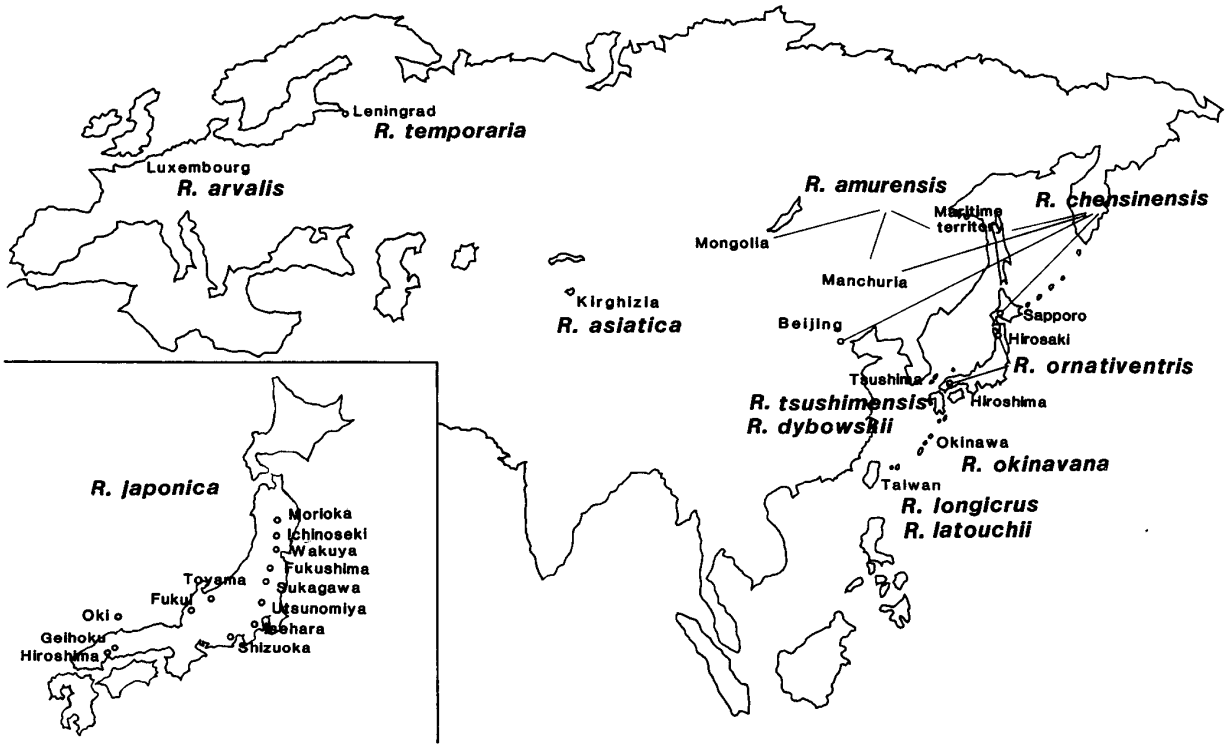

Fig. 1. Map showing localities of 30 populations of brown frogs used in this study.

TABLE 2

Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	Sample	Buffer system
Aspartate aminotransferase	AAT	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	〃	〃
Adenylate kinase	AK	〃	〃
Creatine kinase	CK	〃	T-B-E pH 8.0
Fumarase	Fum	Liver	〃
α-Glycerophosphate dehydrogenase	α-GDH	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	〃	T-B-E pH 8.0
Isocitrate dehydrogenase	IDH	〃	T-C pH 7.0
Lactate dehydrogenase	LDH	〃	T-C pH 6.0
Malate dehydrogenase	MDH	〃	〃
Malic enzyme	ME	〃	T-C pH 7.0
Mannose phosphate isomerase	MPI	〃	〃
Peptidase	Pep	Liver	T-B-E pH 8.0
Phosphoglucomutase	PGM	Skeletal muscle	〃
Superoxide dismutase	SOD	〃	〃
Serum albumin	Ab	Blood serum	〃
Serum protein-C	Prot-C	〃	〃
Hemoglobin	Hb	Erythrocyte	T-B-E pH 8.6

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

species and populations was drawn on the basis of the 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 15 kinds of enzymes extracted from skeletal muscles and livers, and three kinds of blood proteins were analyzed in 468 brown frogs belonging to 30 populations of 12 species distributed in the Palearctic region. It was found that these enzymes and blood proteins were controlled by genes at 25 loci. The electrophoretic bands at each locus were named A, B, C, --- in the order of mobility from fast to slow, and the corresponding alleles were shown by *a*, *b*, *c*, --- (Fig. 2).

At each of the 25 loci, there were 2~55 phenotypes, 13.4 on the average. These phenotypes were controlled by 2~23 multiple alleles, 8.4 on the average. The smallest number of phenotypes was found at the AK locus, which showed two phenotypes controlled by two alleles. At the CK and SOD-A loci, three or four phenotypes controlled by three alleles were found. At the PGM and IDH-A loci, there were six or seven phenotypes controlled by four alleles. At the AAT-A, LDH-A and Pep-A loci, there were five to nine phenotypes controlled by five alleles. At the α-GDH, MDH-A and MDH-B loci, there were seven or eight phenotypes controlled by six alleles. At five loci, the Fum, Hb-I, IDH-B, ME-

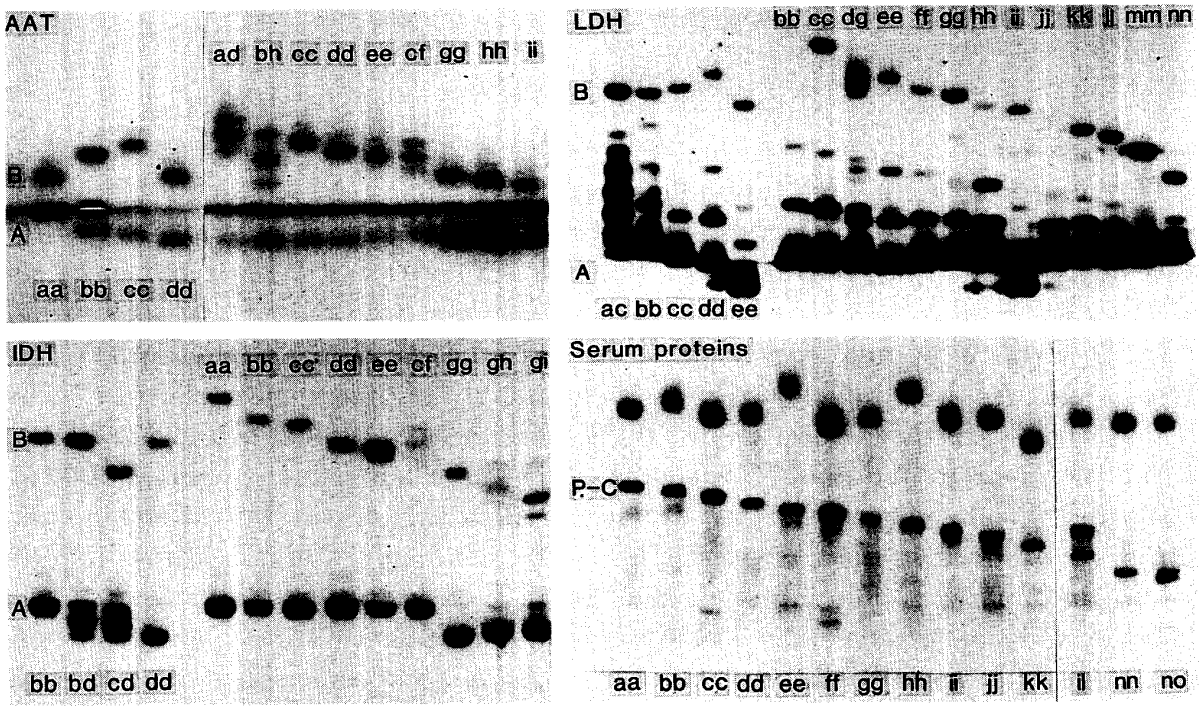

Fig. 2. Electrophoretic patterns of three enzymes, AAT, IDH and LDH, and one blood protein, Prot-C, in 30 populations of brown frogs in the Palearctic region.

TABLE 3
Number of phenotypes and alleles at 25 loci in 30 populations of 12 brown frog species distributed in the Palearctic region

Locus	No. of phenotypes	No. of alleles	Locus	No. of phenotypes	No. of alleles
AAT-A	8	5	ME-A	13	9
AAT-B	19	11	ME-B	18	11
ADA	18	11	MPI	55	23
AK	2	2	Pep-A	9	5
CK	3	3	PGM	6	4
Fum	13	7	SOD-A	4	3
α-GDH	8	6	SOD-B	12	9
GPI	20	13	Ab	21	11
IDH-A	7	4	Prot-C	24	15
IDH-B	13	9	Hb-I	9	8
LDH-A	5	5	Hb-II	12	11
LDH-B	20	14			
MDH-A	7	6	Average	13.4	8.4
MDH-B	8	6			

A and SOD-B, there were nine to 13 phenotypes controlled by seven to nine alleles. At the AAT-B, ADA, ME-B, Ab and Hb-II loci, there were 12~21 phenotypes controlled by 11 alleles. At the GPI, LDH-B and Prot-C loci, 20 or 24 phenotypes controlled by 13~15 alleles were observed. Of the 25 loci in total, the MPI locus was the largest in the number of phenotypes. At this locus, 55 phenotypes controlled by 23 alleles were observed (Table 3).

II. Gene frequency

1. AAT-A locus

The analyses of the electrophoretic patterns at the AAT-A locus in the 468 brown frogs belonging to the 30 populations of the 12 species indicated that there were eight phenotypes, AA, BB, CC, DD, EE, AD, BD and DE, produced by five alleles, *a*~*e*.

In 28 of the 30 populations of the 12 species other than two populations of *Rana latouchii* and *R. arvalis*, allele *d* was overwhelmingly high in gene frequency, being 0.500~1.000. In three populations, the Utsunomiya and Shizuoka of *Rana japonica* and the one population of *R. okinavana*, there was allele *b* in frequencies of 0.052, 0.018 and 0.063, respectively, in addition to allele *d*. In the Hiroshima population of *R. ornativentris*, there was allele *a* in a frequency of 0.238 in addition to allele *d*. In the Siberia population of *R. chensinensis*, there was allele *e* in a frequency of 0.500 in addition to allele *d*. The other 23 populations of nine species had only allele *d*. The Taiwan population of *R. latouchii* had only allele *c*, while the Luxembourg population of *R. arvalis* had only allele *b* (Table 4-I; Fig. 3).

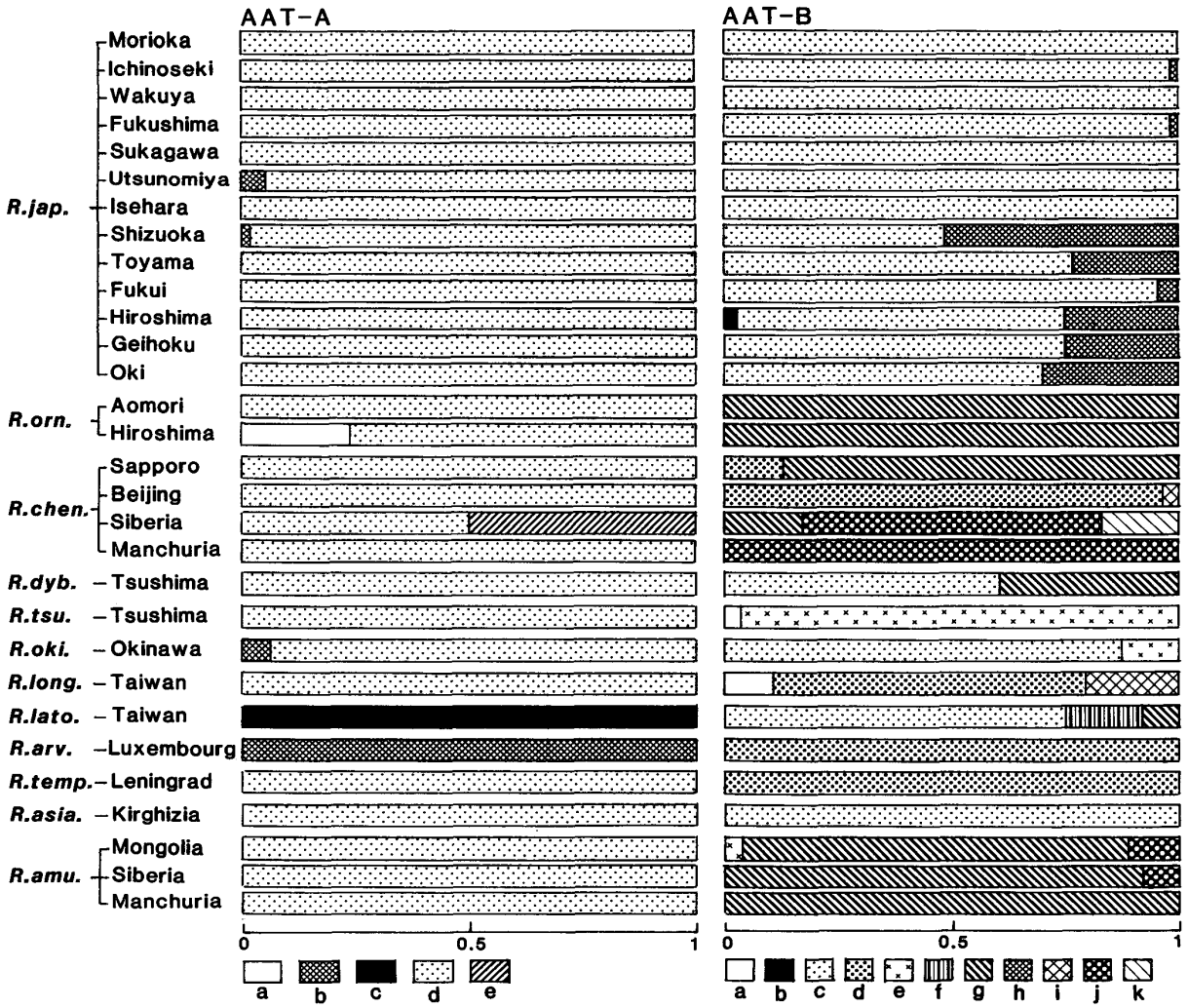

Fig. 3. Gene frequencies at two loci, AAT-A and AAT-B, in 30 populations of brown frogs in the Palearctic region.

2. AAT-B locus

The analyses of the electrophoretic patterns at the AAT-B locus in the 468 brown frogs belonging to the 30 populations of the 12 species showed that there were 19 phenotypes, CC, DD, EE, GG, HH, II, JJ, AD, AE, BH, CE, CF, CG, CH, DG, DI, EJ, GJ and JK, produced by 11 alleles, *a*~*k*.

In 12 of the 13 populations of *R. japonica* other than the Shizuoka and the four populations of *R. dybowskii, R. okinavana, R. latouchii* and *R. asiatica,* allele *c* was high in frequency, being 0.604~1.000. In six populations of *R. japonica,* the Ichinoseki, Fukushima, Toyama, Fukui, Geihoku and Oki, allele *h* was found in frequencies of 0.014~0.300 in addition to allele *c*. In the Hiroshima population, alleles *b* and *h* were found in frequencies of 0.028 and 0.250, respectively. In the Shizuoka population, there were alleles *c* and *h* in frequencies of 0.482 and 0.518, respectively. The remaining five populations of *R. japonica* had only allele *c*. In addition to allele *c*, *R. dybowskii* had allele *g* in a frequency of 0.396, *R. okinavana* had allele *e* in a frequency of 0.125, and *R. latouchii* had alleles *f* and *g* in frequencies of 0.167 and

TABLE 4

Gene frequencies at 25 loci in 30 populations of 12 brown

Species	Population	Sample size	AAT-A					AAT-B										
			a	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*
R. jap.	Morioka	3				1.000				1.000								
〃	Ichinoseki	37				1.000				0.986					0.014			
〃	Wakuya	24				1.000				1.000								
〃	Fukushima	28				1.000				0.982					0.018			
〃	Sukagawa	2				1.000				1.000								
〃	Utsunomiya	29		0.052		0.948				1.000								
〃	Isehara	13				1.000				1.000								
〃	Shizuoka	28		0.018		0.982				0.482					0.518			
〃	Toyama	15				1.000				0.767					0.233			
〃	Fukui	21				1.000				0.952					0.048			
〃	Hiroshima	36				1.000			0.028	0.722					0.250			
〃	Geihoku	6				1.000				0.750					0.250			
〃	Oki	5				1.000				0.700					0.300			
R. orn.	Aomori	8				1.000								1.000				
〃	Hiroshima	21	0.238			0.762								1.000				
R. chen.	Sapporo	31				1.000					0.129			0.871				
〃	Beijing	14				1.000					0.964					0.036		
〃	Siberia	3				0.500	0.500							0.167			0.667	0.167
〃	Manchuria	3				1.000											1.000	
R. dyb.	Tsushima	24				1.000				0.604				0.396				
R. tsu.	Tsushima	42				1.000		0.036				0.964						
R. oki.	Okinawa	8		0.063		0.938				0.875		0.125						
R. long.	Taiwan	24				1.000		0.104			0.688					0.208		
R. lato.	Taiwan	6			1.000					0.750			0.167	0.083				
R. arv.	Luxembourg	6		1.000							1.000							
R. temp.	Leningrad	6				1.000					1.000							
R. asia.	Kirghizia	3				1.000				1.000								
R. amu.	Mongolia	13				1.000						0.038		0.846			0.115	
〃	Siberia	6				1.000								0.917			0.083	
〃	Manchuria	3				1.000								1.000				

TABLE 4

Gene frequencies at 25 loci in 30 populations of 12 brown

Species	Population	Sample size	α-GDH						GPI												
			a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*
R. jap.	Morioka	3				1.000									1.000						
〃	Ichinoseki	37				1.000									1.000						
〃	Wakuya	24				1.000				0.083					0.917						
〃	Fukushima	28				1.000									1.000						
〃	Sukagawa	2		0.250		0.750									1.000						
〃	Utsunomiya	29		0.052		0.948				0.034					0.966						
〃	Isehara	13				1.000				0.269					0.731						
〃	Shizuoka	28				1.000				0.036					0.964						
〃	Toyama	15				1.000									1.000						
〃	Fukui	21		0.167		0.833				0.024					0.976						
〃	Hiroshima	36		0.028		0.972				0.028					0.972						
〃	Geihoku	6		0.083		0.917									1.000						
〃	Oki	5				1.000									1.000						
R. orn.	Aomori	8				1.000					1.000										
〃	Hiroshima	21		0.095		0.905					1.000										
R. chen.	Sapporo	31				0.968		0.032				1.000									
〃	Beijing	14		0.179		0.821						0.929							0.071		
〃	Siberia	3				1.000							1.000								
〃	Manchuria	3				1.000							1.000								
R. dyb.	Tsushima	24		0.458		0.542					0.646					0.354					
R. tsu.	Tsushima	42				1.000														0.964	0.036
R. oki.	Okinawa	8				1.000				0.188					0.813						
R. long.	Taiwan	24				1.000													1.000		
R. lato.	Taiwan	6		1.000															1.000		
R. arv.	Luxembourg	6	1.000									1.000									
R. temp.	Leningrad	6				1.000				0.333							0.250				0.417
R. asia.	Kirghizia	3				1.000												1.000			
R. amu.	Mongolia	13			0.885		0.115		0.038	0.962											
〃	Siberia	6			0.667		0.333		0.250	0.750											
〃	Manchuria	3			1.000				0.333	0.667											

frog species distributed in the Palearctic region (I)

ADA											AK		CK			Fum						
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*a*	*b*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*f*	*g*
			0.333				0.667					1.000		1.000				0.667			0.333	
			0.622		0.081		0.297					1.000		1.000			0.068	0.757			0.176	
			0.333		0.375		0.292					1.000		0.979	0.021		0.146	0.833			0.021	
					0.875		0.125					1.000		1.000			0.196	0.679			0.125	
					1.000							1.000		1.000				0.750			0.250	
					0.621		0.379					1.000		1.000			0.017	0.948			0.034	
					0.692		0.308					1.000		1.000				1.000				
			0.036		0.714		0.232		0.018			1.000		1.000			0.071	0.911			0.018	
							0.967		0.033			1.000		1.000			0.033	0.967				
							0.952		0.048			1.000		1.000				1.000				
					0.181		0.806		0.014			1.000		1.000			0.097	0.903				
					0.250		0.417		0.333			1.000		1.000				1.000				
			0.100		0.100		0.800					1.000		1.000			0.500	0.500				
						1.000						1.000		1.000					0.500	0.500		
						0.833		0.167				1.000		1.000		0.333			0.405	0.262		
					0.919	0.081						1.000		1.000						1.000		
						1.000						1.000		1.000						1.000		
	0.833				0.167							1.000		1.000				1.000				
	1.000											1.000		1.000					1.000			
0.458	0.542											1.000		1.000		0.458			0.542			
		1.000										1.000		1.000				0.905			0.095	
							1.000					1.000		1.000				0.938			0.063	
			0.271		0.729							1.000		1.000			0.333				0.667	
				1.000							1.000			1.000								1.000
					0.083		0.917					1.000		1.000							1.000	
										1.000		1.000		1.000				0.250			0.750	
			0.167		0.833							1.000		1.000							1.000	
							1.000					1.000	1.000								1.000	
					0.083		0.917					1.000	1.000								1.000	
							1.000					1.000	1.000								1.000	

frog species distributed in the Palearctic region (II)

IDH-A				IDH-B									LDH-A					LDH-B					
a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*a*	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*e*	*f*
	1.000						1.000								1.000								
	1.000						1.000								1.000								
	1.000						1.000								1.000								
0.232	0.768						1.000								1.000								
0.250	0.750						1.000								1.000								
0.034	0.966						1.000								1.000								
	1.000				0.154		0.846								1.000								
	1.000				0.179		0.821								1.000								
	1.000						1.000								1.000								
	1.000						1.000								1.000								
	1.000				0.014		0.986								1.000								
	1.000						1.000								1.000								
	1.000						1.000								1.000								
	1.000			0.250	0.750										1.000								1.000
	1.000			1.000											1.000				0.310				0.452
	1.000					0.984			0.016				0.016		0.984						0.016		
	1.000			0.036	0.821					0.143					1.000								
	1.000				0.500		0.500								1.000					0.333			
	1.000				0.500		0.500								1.000								
	1.000				0.771		0.229								1.000					1.000			
		0.012	0.988				0.798			0.202						1.000							1.000
		0.063	0.938				1.000										1.000						
	1.000						1.000								1.000								
			1.000							0.667	0.083	0.250		1.000									
	1.000							1.000							1.000								
0.250	0.167		0.583				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 4

Gene frequencies at 25 loci in 30 populations of 12 brown

Species	Population	Sample size	LDH-B								MDH-A						MDH-B					
			g	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*a*	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*f*
R. jap.	Morioka	3	1.000									1.000										1.000
〃	Ichinoseki	37	1.000									1.000										1.000
〃	Wakuya	24	1.000									1.000							0.042			0.958
〃	Fukushima	28	1.000									1.000							0.036			0.964
〃	Sukagawa	2	1.000									1.000										1.000
〃	Utsunomiya	29	1.000									1.000										1.000
〃	Isehara	13	1.000									1.000										1.000
〃	Shizuoka	28	1.000									1.000							0.036			0.964
〃	Toyama	15	1.000									1.000										1.000
〃	Fukui	21	1.000									1.000										1.000
〃	Hiroshima	36	1.000									1.000							0.181			0.819
〃	Geihoku	6	1.000									1.000							0.250			0.750
〃	Oki	5	1.000									1.000							0.700			0.300
R. orn.	Aomori	8									1.000								1.000			
〃	Hiroshima	21				0.238					1.000								1.000			
R. chen.	Sapporo	31	0.984										1.000						1.000			
〃	Beijing	14		0.393			0.607								1.000				1.000			
〃	Siberia	3		0.667									1.000						1.000			
〃	Manchuria	3	1.000										1.000						1.000			
R. dyb.	Tsushima	24											1.000						1.000			
R. tsu.	Tsushima	42										0.976			0.024			1.000				
R. oki.	Okinawa	8			1.000							1.000										1.000
R. long.	Taiwan	24						0.563		0.438		1.000										1.000
R. lato.	Taiwan	6	1.000													1.000					1.000	
R. arv.	Luxembourg	6							1.000				1.000				0.667		0.333			
R. temp.	Leningrad	6	1.000											1.000					1.000			
R. asia.	Kirghizia	3										1.000							1.000			
R. amu.	Mongolia	13										1.000								1.000		
〃	Siberia	6										1.000								1.000		
〃	Manchuria	3										1.000								1.000		

TABLE 4

Gene frequencies at 25 loci in 30 populations of 12 brown

Species	Population	Sample size	MPI																			
			e	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	*v*	*w*
R. jap.	Morioka	3									1.000										
〃	Ichinoseki	37									0.905			0.054				0.041			
〃	Wakuya	24									0.833			0.146				0.021			
〃	Fukushima	28									0.232			0.625				0.143			
〃	Sukagawa	2												1.000							
〃	Utsunomiya	29						0.052			0.379			0.431				0.138			
〃	Isehara	13									0.500			0.385				0.115			
〃	Shizuoka	28									0.982			0.018							
〃	Toyama	15						0.033			0.800			0.167							
〃	Fukui	21		0.119				0.429			0.429			0.024							
〃	Hiroshima	36						0.028			0.958			0.014							
〃	Geihoku	6						0.083			0.917										
〃	Oki	5		0.100				0.700			0.100								0.100		
R. orn.	Aomori	8			0.313					0.063			0.188								
〃	Hiroshima	21			0.190		0.095			0.190			0.238				0.167				0.095
R. chen.	Sapporo	31						0.871				0.065		0.032							
〃	Beijing	14						0.893													
〃	Siberia	3				0.500															
〃	Manchuria	3			0.333			0.167		0.167			0.167								
R. dyb.	Tsushima	24							0.646				0.354								
R. tsu.	Tsushima	42	0.036		0.869				0.060				0.012								
R. oki.	Okinawa	8									0.313		0.375		0.125			0.188			
R. long.	Taiwan	24					0.083				0.625	0.083			0.208						
R. lato.	Taiwan	6							0.083		0.167					0.583				0.167	
R. arv.	Luxembourg	6										1.000									
R. temp.	Leningrad	6			0.083								0.917								
R. asia.	Kirghizia	3											1.000								
R. amu.	Mongolia	13										1.000									
〃	Siberia	6						0.083				0.917									
〃	Manchuria	3										1.000									

frog species distributed in the Palearctic region (III)

ME-A									ME-B											MPI			
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*a*	*b*	*c*	*d*
				1.000								1.000											
				1.000							0.014	0.986											
				1.000								1.000											
				0.911				0.089			0.143	0.857											
				1.000								1.000											
				0.983				0.017			0.069	0.931											
				1.000								1.000											
				0.554		0.446						1.000											
				1.000								1.000											
				0.905				0.095			0.048	0.952											
				1.000								0.833		0.167									
				1.000							0.083	0.917											
				1.000								1.000											
				0.438		0.563			0.063	0.875						0.063				0.063			0.375
				0.952		0.048			0.286	0.714													0.024
				1.000						0.871		0.081				0.048							0.032
		1.000							0.036	0.964												0.107	
						1.000													1.000	0.333			0.167
						1.000									0.833				0.167				0.167
						1.000									1.000								
					0.750	0.250					1.000										0.024		
	0.625			0.375					0.188	0.813													
0.792	0.208													1.000									
								1.000					0.083				0.917						
				1.000									1.000										
				1.000							0.500		0.500										
			1.000							1.000													
				1.000														1.000					
				1.000														1.000					
				1.000														1.000					

frog species distributed in the Palearctic region (IV)

Pep-A					PGM				SOD-A			SOD-B								
a	*b*	*c*	*d*	*e*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
1.000						1.000				1.000									1.000	
0.946	0.054				0.027	0.973				1.000									1.000	
1.000					0.083	0.917			0.021	0.979									1.000	
0.946	0.054				0.143	0.857				1.000									1.000	
1.000						1.000				1.000									1.000	
0.966	0.034				0.086	0.914			0.017	0.983				0.138					0.862	
1.000					0.038	0.962				1.000									1.000	
0.071	0.893	0.036				1.000				0.929	0.071								1.000	
0.200	0.800				0.167	0.833				1.000				0.433					0.567	
	0.905	0.095			0.405	0.595				1.000				0.286					0.714	
0.153	0.847				0.014	0.986			0.153	0.847									1.000	
	0.917	0.083				1.000			0.417	0.583									1.000	
	0.800	0.200				1.000			0.100	0.900									1.000	
1.000						1.000				1.000				1.000						
0.071	0.929					0.857		0.143		0.643	0.357			0.524					0.476	
0.032	0.968					1.000				1.000					0.532				0.468	
	1.000				1.000					1.000						1.000				
0.833	0.167					0.833	0.167			1.000				1.000						
1.000						1.000				1.000					1.000					
0.188		0.813				1.000				1.000					1.000					
	0.464	0.536			0.988	0.012				1.000								1.000		
	0.500		0.438	0.063		1.000				1.000									1.000	
1.000						1.000				1.000									1.000	
		1.000					1.000			1.000		0.750					0.250			
1.000					0.417	0.583				1.000					1.000					
1.000					1.000					1.000									0.083	0.917
1.000						1.000				1.000				1.000						
1.000						1.000				1.000									1.000	
1.000						1.000				1.000									1.000	
1.000						1.000				1.000		0.167							0.833	

TABLE 4

Gene frequencies at 25 loci in 30 populations of 12 brown

Species	Population	Sample size	Ab											Prot-C								
			a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
R. jap.	Morioka	2		0.250	0.750													0.500			0.500	
〃	Ichinoseki	30		0.533	0.467											0.317		0.517			0.167	
〃	Wakuya	5		0.400	0.600											0.200		0.300			0.500	
〃	Fukushima	24		0.375	0.625											0.042		0.958				
〃	Sukagawa	1		0.500	0.500													1.000				
〃	Utsunomiya	21		0.119	0.881											0.048		0.929			0.024	
〃	Isehara	13			1.000													0.962			0.038	
〃	Shizuoka	17	0.235	0.765														0.971			0.029	
〃	Toyama	15		0.933	0.067													1.000				
〃	Fukui	13	0.154	0.615	0.231											0.154		0.808			0.038	
〃	Hiroshima	34	0.426	0.574														0.632			0.368	
〃	Geihoku	6	0.083	0.917														1.000				
〃	Oki	1		1.000														0.500			0.500	
R. orn.	Aomori	7			0.071			0.643			0.286					0.929				0.071			
〃	Hiroshima	16			0.125			0.750			0.125											0.281
R. chen.	Sapporo	30									0.017	0.900	0.083								0.100	
〃	Beijing	12				0.500			0.500								0.917				0.083	
〃	Siberia	3				0.500					0.500							0.500				
〃	Manchuria	—																				
R. dyb.	Tsushima	23				0.348			0.652													
R. tsu.	Tsushima	40					1.000							1.000								
R. oki.	Okinawa	6			1.000										1.000							
R. long.	Taiwan	22			0.159			0.841								0.682				0.318		
R. lato.	Taiwan	5						0.500		0.500							0.300				0.700	
R. arv.	Luxembourg	6									1.000								1.000			
R. temp.	Leningrad	3						1.000											1.000			
R. asia.	Kirghizia	3	1.000															1.000				
R. amu.	Mongolia	6							1.000									1.000				
〃	Siberia	1							1.000									1.000				
〃	Manchuria	—																				

0.083, respectively. *R. asiatica* had only allele *c*.

In the two populations of *R. ornativentris*, the three populations of *R. amurensis* and one of the four populations of *R. chensinensis*, allele *g* was high in frequency, being 0.846~1.000. In addition to allele *g*, the Mongolia population of *R. amurensis* had alleles *e* and *j* in frequencies of 0.038 and 0.115, respectively, the Siberia population of the same species had allele *j* in a frequency of 0.083, and the Sapporo population of *R. chensinensis* had allele *d* in a frequency of 0.129. The Aomori and Hiroshima populations of *R. ornativentris* and the Manchuria population of *R. amurensis* had only allele *g*.

In the Beijing population of *R. chensinensis* and the three populations of *R. longicrus*, *R. arvalis* and *R. temporaria*, allele *d* was high in frequency, being 0.688~1.000. In addition to allele *d*, the Beijing population had allele *i* in a frequency of 0.036, and *R. longicrus* had alleles *a* and *i* in frequencies of 0.104 and 0.208, respectively. *R. arvalis* and *R. temporaria* had only allele *d*. The Siberia population of *R. chensinensis* had allele *j* in a frequency of 0.667 and, in addition, it had alleles *g* and *k*, each of which was 0.167 in frequency. In *R. tsushimensis*, there were alleles *e* and *a*, which were 0.964 and 0.036, respectively, in frequency. The Manchuria population of *R. chensinensis* (hashima) had only allele *j* (Table 4–I; Fig. 3).

3. ADA locus

The analyses of the electrophoretic patterns at the ADA locus in the 468 frogs

frog species distributed in the Palearctic region (V)

						Hb-I								Hb-II									
j	*k*	*l*	*m*	*n*	*o*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*
										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						0.118		0.882						
										1.000						0.967		0.033						
										1.000						0.769		0.231						
										1.000								1.000						
										1.000								1.000						
										1.000								1.000						
													1.000		1.000									
0.656		0.063											1.000						1.000					
	0.900								1.000								1.000							
											1.000													1.000
			0.500								1.000				1.000									
				0.978	0.022						1.000				1.000									
										1.000												1.000		
												1.000		0.083								0.917		
										1.000						1.000								
								1.000							1.000									
						1.000																	1.000	
							1.000														1.000			
							0.667				0.333										1.000			
									1.000															1.000
									1.000															1.000

belonging to the 30 populations of the 12 species showed that there were 18 phenotypes, AA, BB, CC, DD, EE, FF, GG, HH, KK, AB, BF, DF, DH, FG, FH, FJ, GI and HJ, produced by 11 alleles, *a*~*k*.

In the Ichinoseki population of *R. japonica,* there were alleles *d*, *f* and *h*, which were 0.622, 0.081 and 0.297, respectively, in frequency. In five populations of *R. japonica,* the Fukushima, Sukagawa, Utsunomiya, Isehara and Shizuoka, the Sapporo population of *R. chensinensis* and two populations of *R. longicrus* and *R. asiatica,* allele *f* was high in frequency, being 0.621~1.000. Of these populations, the Sukagawa had only allele *f*, while in three populations, the Fukushima, Utsunomiya and Isehara, there was allele *h* in frequencies of 0.125~0.379 in addition to allele *f*. The Shizuoka population had alleles *d*, *h* and *j* in frequencies of 0.036, 0.232 and 0.018, respectively, in addition to allele *f*. The Sapporo population of *R. chensinensis* had allele *g* in a frequency of 0.081 and the two populations of *R. longicrus* and *R. asiatica* had allele *d* in frequencies of 0.271 and 0.167, respectively, in addition to allele *f*.

In five populations of *R. japonica,* the Morioka, Toyama, Fukui, Hiroshima and Oki, two populations of *R. okinavana* and *R. arvalis,* and three populations of *R. amurensis,* allele *h* was high in frequency, being 0.667~1.000. In addition to allele *h*, the Morioka population of *R. japonica* had allele *d* in a frequency of 0.333, the Toyama and Fukui populations had allele *j* in frequencies of 0.033 and 0.048, respectively, the Hiroshima population had alleles *f* and *j* in frequencies of 0.181 and 0.014, respectively, and the Oki population had alleles *d* and *f* each in a

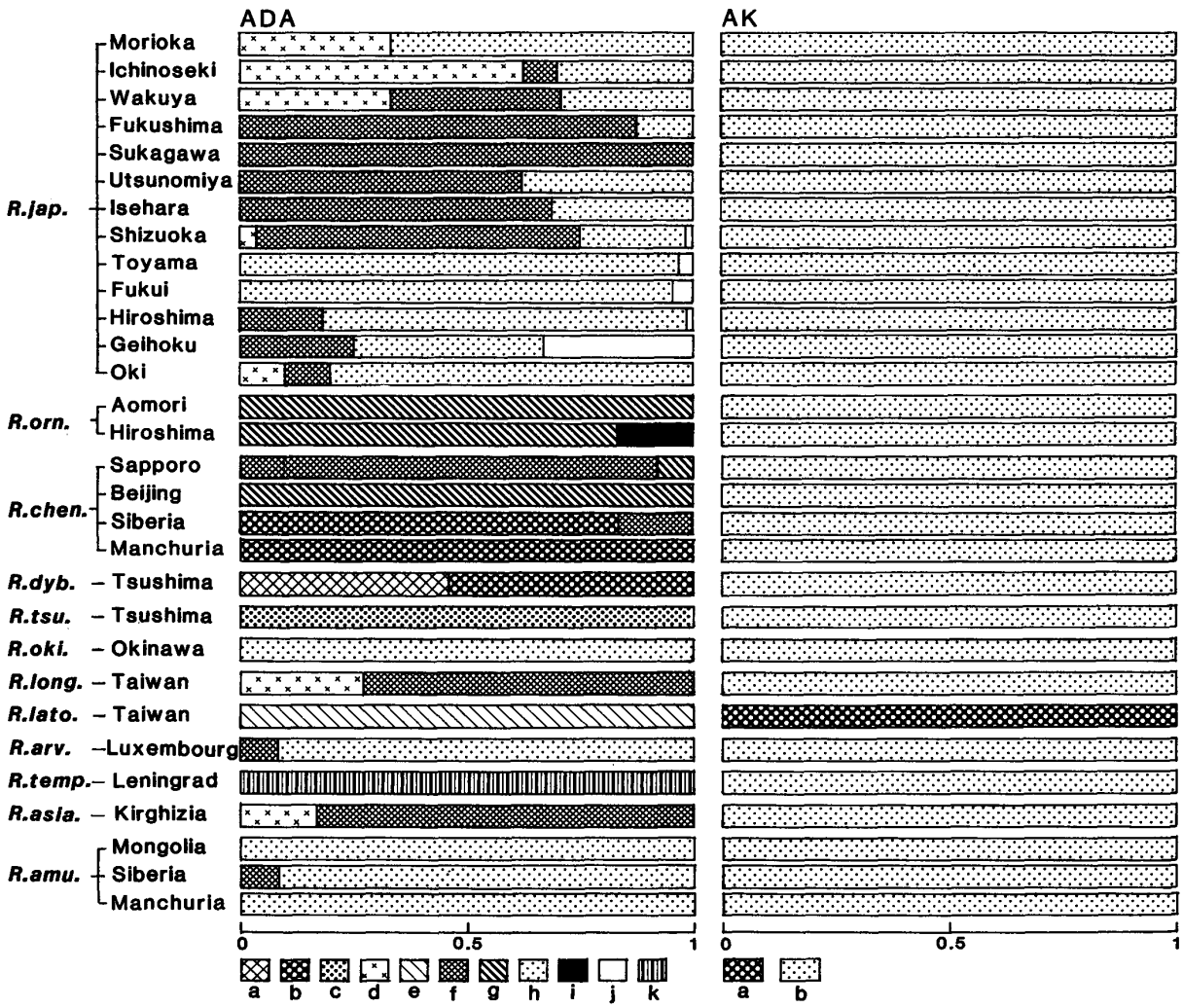

Fig. 4. Gene frequencies at two loci, ADA and AK, in 30 populations of brown frogs in the Palearctic region.

frequency of 0.100. While the population of *R. arvalis* and the Siberia population of *R. amurensis* had allele *f* in a frequency of 0.083 in addition to allele *h*, the Mongolia and Manchuria populations of *R. amurensis* and the population of *R. okinavana* had only allele *h*. While the Wakuya population of *R. japonica* had alleles *f*, *d* and *h* in frequencies of 0.375, 0.333 and 0.292, respectively, the Geihoku population had alleles *h*, *j* and *f* in frequencies of 0.417, 0.333 and 0.250, respectively (Table 4–I; Fig. 4).

In the two populations of *R. ornativentris* and the Beijing population of *R. chensinensis*, allele *g* was high in frequency, being 0.833~1.000. While the Hiroshima population of *R. ornativentris* had allele *i* in a frequency of 0.167 in addition to allele *g*, the Aomori population had only allele *g*. In the Siberia and Manchuria populations of *R. chensinensis* and the population of *R. dybowskii*, allele *b* was high in frequency, being 0.542~1.000. In addition to allele *b*, the Siberia population had allele *f* in a frequency of 0.167, and the population of *R. dybowskii* had allele *a* in a frequency of 0.458. The Manchuria population of *R. chensinensis* (hashima), and

the populations of *R. tsushimensis*, *R. latouchii* and *R. temporaria* had only alleles *b*, *c*, *e* and *k*, respectively (Table 4–I; Fig. 4).

4. AK locus

By analyses of the electrophoretic patterns at the AK locus in the 468 brown frogs belonging to the 30 populations of the 12 species, it was found that all the 29 populations other than that of *R. latouchii* showed phenotype BB controlled by allele *b*, while the population of *R. latouchii* showed only phenotype AA controlled by allele *a* (Table 4–I; Fig. 4).

5. CK locus

The electrophoretic patterns at the CK locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were three phenotypes, AA, BB and BC, controlled by three alleles, *a*~*c*.

Of these 30 populations, 27 other than the three populations of *R. amurensis*

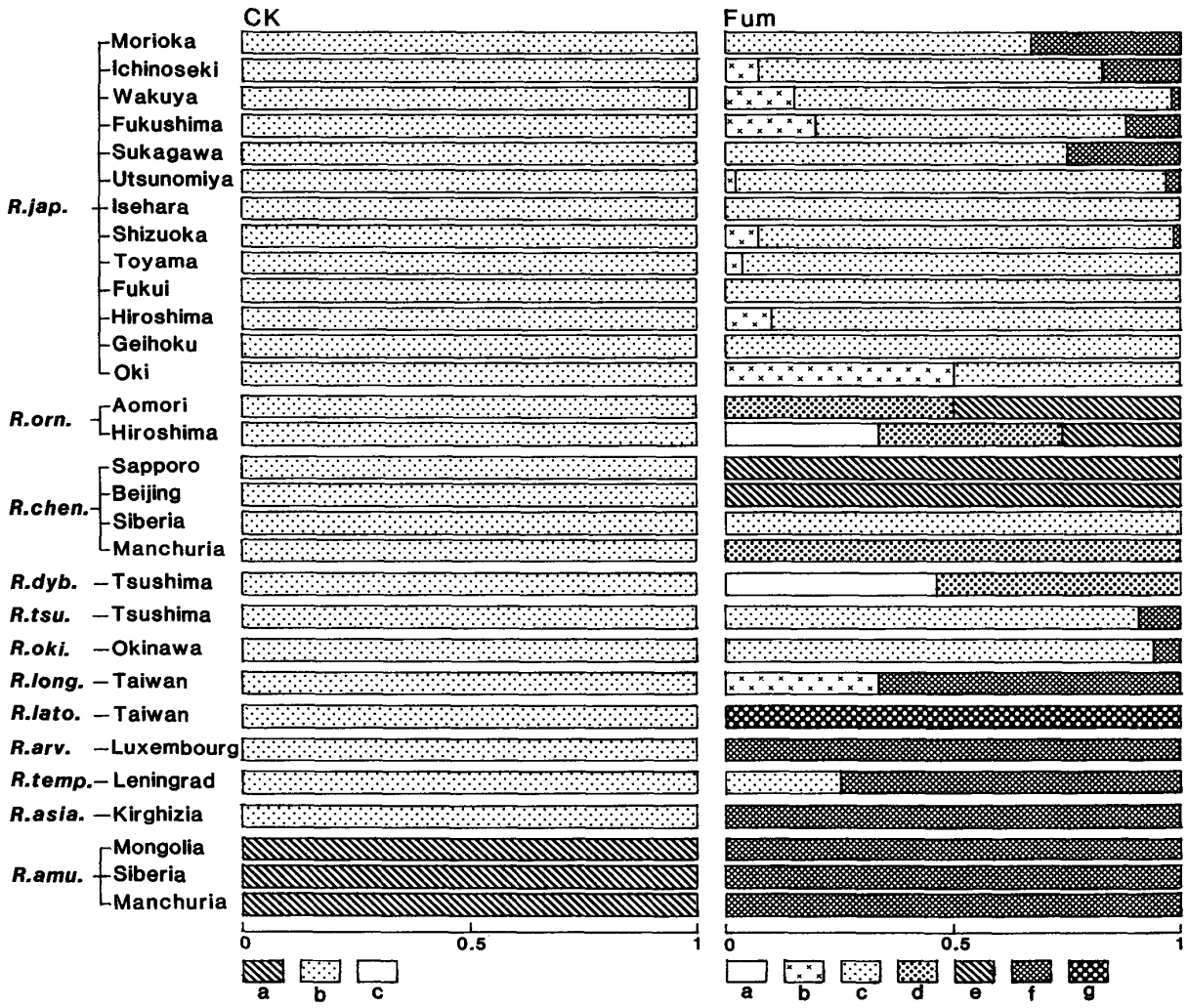

Fig. 5. Gene frequencies at two loci, CK and Fum, in 30 populations of brown frogs in the Palearctic region.

almost all showed phenotype BB controlled by allele *b*, except that the Wakuya population of *R. japonica* which had allele *c* in a frequency of 0.021. The three populations of *R. amurensis* had only allele *a* (Table 4–I; Fig. 5).

6. Fum locus

By analyses of the electrophoretic patterns at the Fum locus in the 468 brown frogs belonging to the 30 populations of the 12 species, 13 phenotypes, AA, BB, CC, DD, EE, FF, GG, AD, AE, BC, BF, CF and DE, produced by seven alleles, *a*~*g*, were observed.

In the 15 populations of four species including the 12 populations of *R. japonica* other than the Oki, the Siberia population of *R. chensinensis*, and the populations of *R. tsushimensis* and *R. okinavana*, allele *c* was high in frequency, being 0.667~1.000. Of these 15 populations, the Morioka and Sukagawa populations of *R. japonica* and the two populations of *R. tsushimensis* and *R. okinavana* had allele *f* in frequencies of 0.063~0.333 in addition to allele *c*, and the Toyama and Hiroshima populations of *R. japonica* had allele *b* in frequencies of 0.033 and 0.097, respectively, in addition to allele *c*. In five populations of *R. japonica*, the Ichinoseki, Wakuya, Fukushima, Utsunomiya and Shizuoka, there were alleles *b* and *f* in frequencies of 0.017~0.196 and 0.018~0.176, respectively, in addition to allele *c*. The other three populations of *R. japonica*, the Isehara, Fukui and Geihoku, and the Siberia population of *R. chensinensis* had only allele *c*. The Oki population of *R. japonica* had alleles *b* and *c* each in a frequency of 0.500 (Table 4–I; Fig. 5).

The Aomori population of *R. ornativentris* had alleles *d* and *e* each in a frequency of 0.500, while the Hiroshima population of the same species had alleles *d*, *a* and *e* in frequencies of 0.405, 0.333 and 0.262, respectively. The population of *R. dybowskii* had alleles *d* and *a* in frequencies of 0.542 and 0.458, respectively. Seven populations of five species, including the four populations of *R. longicrus*, *R. arvalis*, *R. temporaria* and *R. asiatica*, and the three populations of *R. amurensis*, the Mongolia, Siberia and Manchuria, had allele *f* in high frequency, being 0.667 ~1.000. Of these populations, that of *R. longicrus* had allele *b* in a frequency of 0.333, and that of *R. temporaria* had allele *c* in a frequency of 0.250, in addition to allele *f*. All the remaining five populations of *R. arvalis*, *R. asiatica* and *R. amurensis* had only allele *f*.

The Sapporo and Beijing populations of *R. chensinensis* had only allele *e*, the population of *R. latouchii* had only allele *g*, and the Manchuria population of *R. chensinensis* (hasima) had only allele *d* (Table 4–I; Fig. 5).

7. α–GDH locus

The electrophoretic patterns at the α–GDH locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were eight phenotypes, AA, BB, CC, DD, EE, BD, CE and DF, produced by six alleles, *a*~*f*.

In the 24 populations of eight species including 13 populations of *R. japonica*, two populations of *R. ornativentris*, four populations of *R. chensinensis* and five populations of *R. tsushimensis*, *R. okinavana*, *R. longicrus*, *R. temporaria* and *R. asiatica*, allele *d*

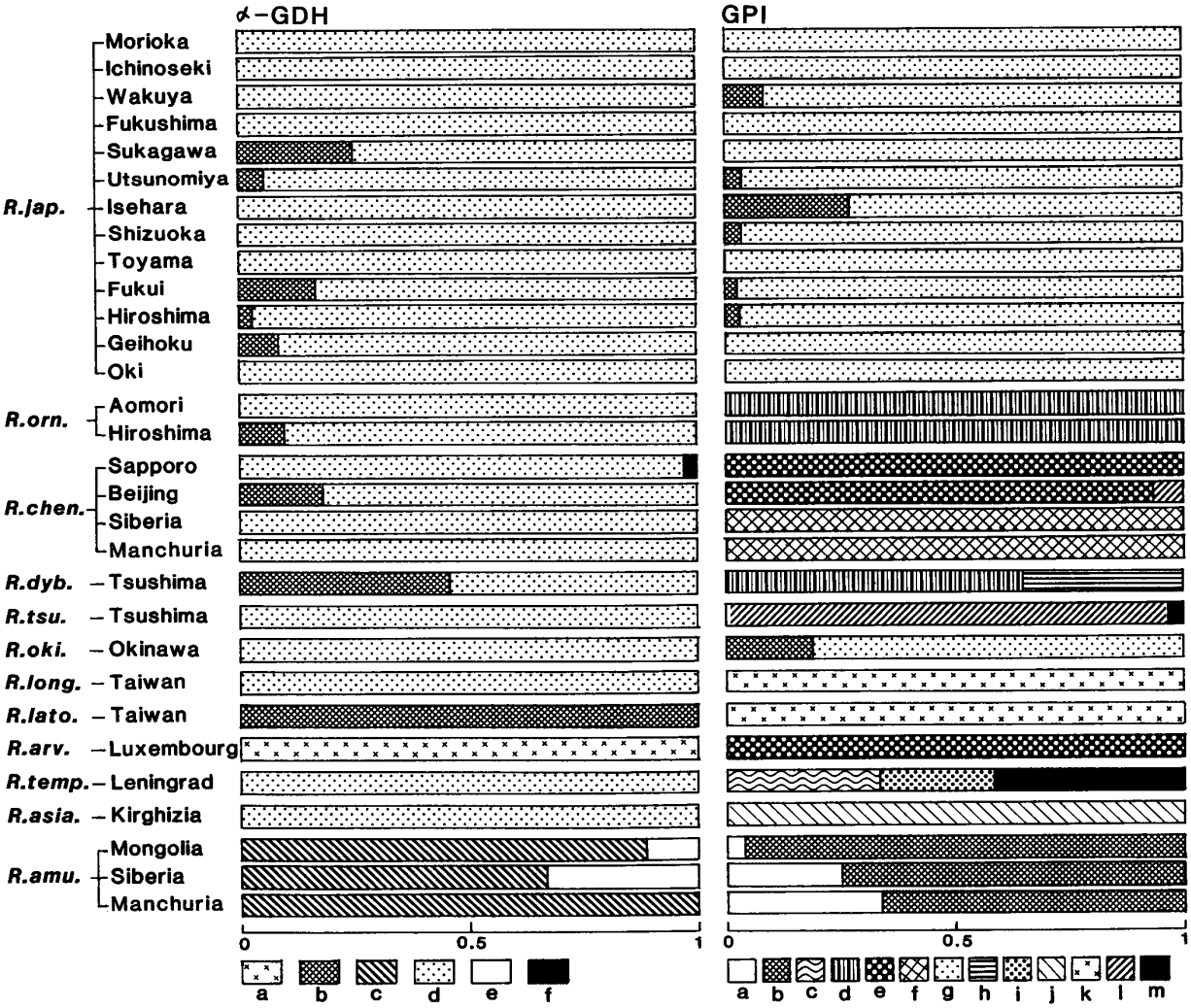

Fig. 6. Gene frequencies at two loci, α-GDH and GPI, in 30 populations of brown frogs in the Palearctic region.

was very high in frequency, being 0.750~1.000. In the Sukagawa, Utsunomiya, Fukui, Hiroshima and Geihoku populations of *R. japonica*, the Hiroshima population of *R. ornativentris*, and the Beijing population of *R. chensinensis*, there was allele *b* in frequencies of 0.028~0.250 in addition to allele *d*. The Sapporo population of *R. chensinensis* had allele *f* in a frequency of 0.032 in addition to allele *d*. The other eight populations of *R. japonica*, the Aomori population of *R. ornativentris*, the Siberia and Manchuria (hashima) populations of *R. chensinensis* and five populations of *R. tsushimensis*, *R. okinavana*, *R. longicrus*, *R. temporaria* and *R. asiatica* had only allele *d*. The population of *R. dybowskii* had alleles *d* and *b* in frequencies of 0.542 and 0.458, respectively.

In the three populations of *R. amurensis*, allele *c* was high in frequency, being 0.667~1.000. The Mongolia and Siberia populations had allele *e* in frequencies of 0.115 and 0.333, respectively, in addition to allele *c*. The Manchuria population had only allele *c*. The population of *R. arvalis* had allele *a*, while *R. latouchii* had only allele *b* (Table 4–II; Fig. 6).

8. GPI locus

The analyses of the electrophoretic patterns at the GPI locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were 20 phenotypes, AA, BB, CC, DD, EE, FF, GG, HH, JJ, KK, LL, MM, AB, BG, CI, CM, DH, EL, IM and LM, produced by 13 alleles, *a*~*m*.

In the 13 populations of *R. japonica* and the population of *R. okinavana,* allele *g* was very high in frequency, being 0.731~1.000. While six populations of *R. japonica,* the Wakuya, Utsunomiya, Isehara, Shizuoka, Fukui and Hiroshima, and the population of *R. okinavana* had allele *b* in frequencies of 0.024~0.269 in addition to allele *g*, the other seven populations of *R. japonica* had only allele *g*. The two populations of *R. ornativentris* had only allele *d*, while the population of *R. dybowskii* had alleles *d* and *h* in frequencies of 0.646 and 0.354, respectively. In the Sapporo and Beijing populations of *R. chensinensis* and the population of *R. arvalis,* allele *e* was high in frequency, being 0.929~1.000. Of these three populations,the Beijing had allele *l* in a frequency of 0.071 in addition to allele *e*, and the other two populations had only allele *e*. The Siberia and Manchuria (hashima) populations of *R. chensinensis* had only allele *f*, the population of *R. asiatica* had only allele *j* and the two populations of *R. longicrus* and *R. latouchii* had only allele *k*. In the population of *R. tsushimensis*, allele *l* was 0.964 in frequency, while allele *m* was 0.036. In the population of *R. temporaria*, there were alleles *c*, *i* and *m* in frequencies of 0.333, 0.250 and 0.417, respectively. In the three populations of *R. amurensis,* allele *b* was high in frequency, being 0.667~0.962, while allele *a* was 0.038~0.333 (Table 4–II; Fig. 6).

9. IDH–A locus

The analyses of the electrophoretic patterns at the IDH–A locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were seven phenotypes, AA, BB, DD, AB, AD, BD and CD, produced by four alleles, *a*~*d*.

In 23 populations of seven species including the 13 of *R. japonica,* the two of *R. ornativentris,* the four of *R. chensinensis* and the four of *R. dybowskii, R. longicrus, R. arvalis* and *R. asiatica,* allele *b* was high in frequency, being 0.750~1.000. Of these populations, the Fukushima, Sukagawa and Utsunomiya of *R. japonica* had allele *a* in frequencies of 0.034~0.250 in addition to allele *b*. All the other 20 populations had only allele *b*. In the population of *R. temporaria,* alleles *d, a* and *b* were 0.583, 0.250 and 0.167 in frequency, respectively. In the populations of *R. tsushimensis, R. okinavana* and *R. latouchii,* allele *d* was very high in frequency, being 0.938 ~1.000. In addition to allele *d*, the populations of *R. tsushimensis* and *R. okinavana* had allele *c* in frequencies of 0.012 and 0.063, respectively. The population of *R. latouchii* had only allele *d*. The three populations of *R. amurensis* had only allele *a* (Table 4–II; Fig. 7).

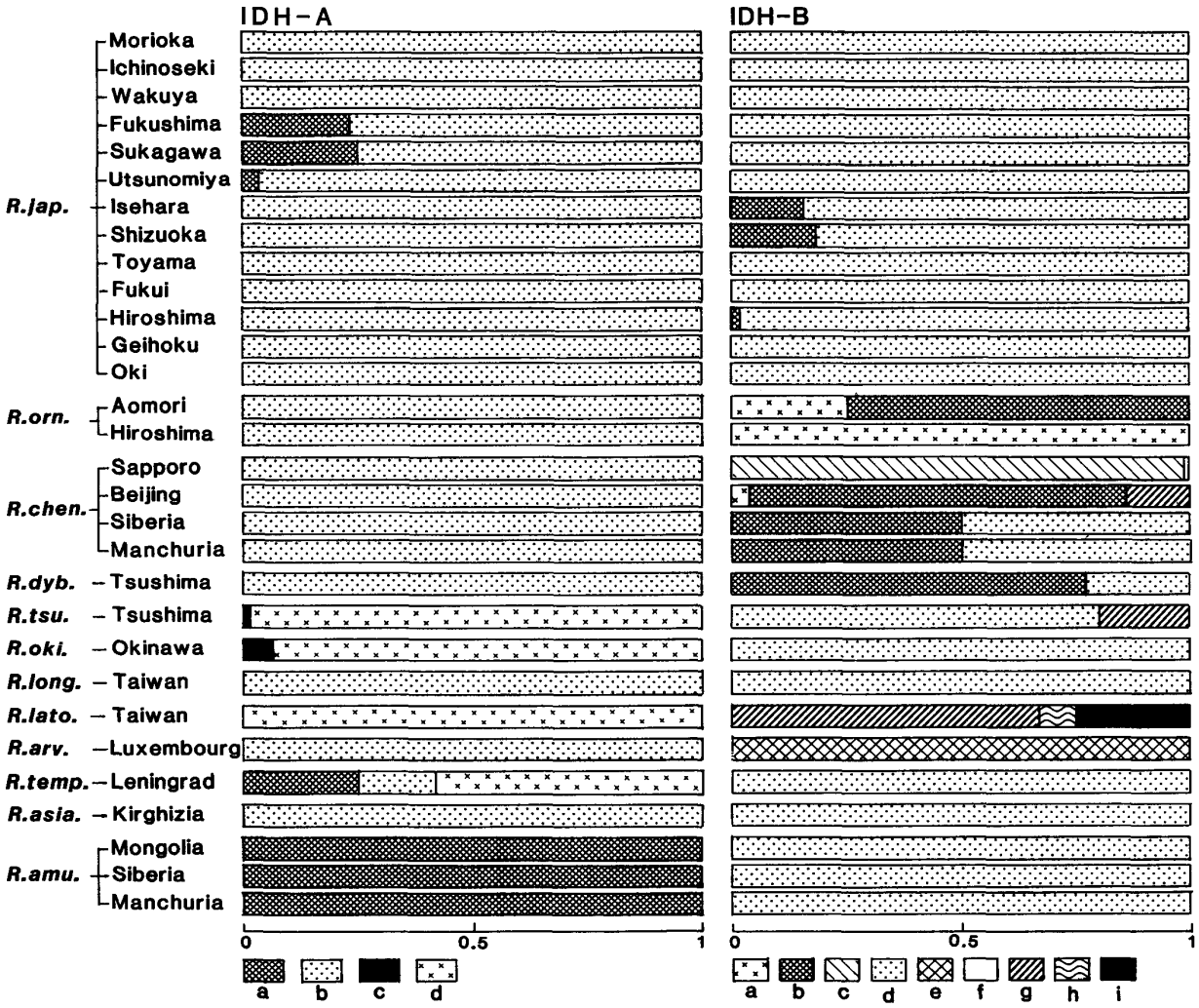

Fig. 7. Gene frequencies at two loci, IDH-A and IDH-B, in 30 populations of brown frogs in the Palearctic region.

10. IDH-B locus

By analyses of the electrophoretic patterns at the IDH-B locus in the 468 brown frogs of the 30 populations of the 12 species, it was found that there were 13 phenotypes, AA, BB, CC, DD, EE, GG, AB, BD, BG, CF, DG, GH and GI, produced by nine alleles, *a*~*i*.

In 21 populations of seven species, including the 13 of *R. japonica*, the five of *R. tsushimensis*, *R. okinavana*, *R. longicrus*, *R. temporaria* and *R. asiatica*, and the three of *R. amurensis*, allele *d* was very high in frequency, being 0.798~1.000. Of these populations, the Isehara, Shizuoka and Hiroshima of *R. japonica* had allele *b* in frequencies of 0.014~0.179, and the population of *R. tsushimensis* had allele *g* in a frequency of 0.202 in addition to allele *d*. The other 17 populations of six species had only allele *d*.

The Hiroshima population of *R. ornativentris* had only allele *a*, while the Aomori population had alleles *b* and *a* in frequencies of 0.750 and 0.250, respectively.

The Sapporo population of *R. chensinensis* had alleles *c* and *f* in frequencies of 0.984 and 0.016, respectively, while the Beijing population had alleles *b*, *a* and *g* in frequencies of 0.821, 0.036 and 0.143, respectively. The Siberia and Manchuria populations of the same species had alleles *b* and *d* each in a frequency of 0.500. The population of *R. dybowskii* had also alleles *b* and *d* in frequencies of 0.771 and 0.229, respectively. In the population of *R. latouchii*, alleles *g*, *h* and *i* were 0.667, 0.083 and 0.250 in frequency, respectively. The population of *R. arvalis* had only allele *e* (Table 4–II; Fig. 7).

11. LDH–A locus

The analyses of the electrophoretic patterns at the LDH–A locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were five phenotypes, BB, CC, DD, EE and AC, produced by five alleles, *a*~*e*.

Of the 30 populations, that of *R. tsushimensis* had only allele *d*, that of *R. okinavana* had only allele *e* and that of *R. latouchii* had only allele *b*. In the other 27

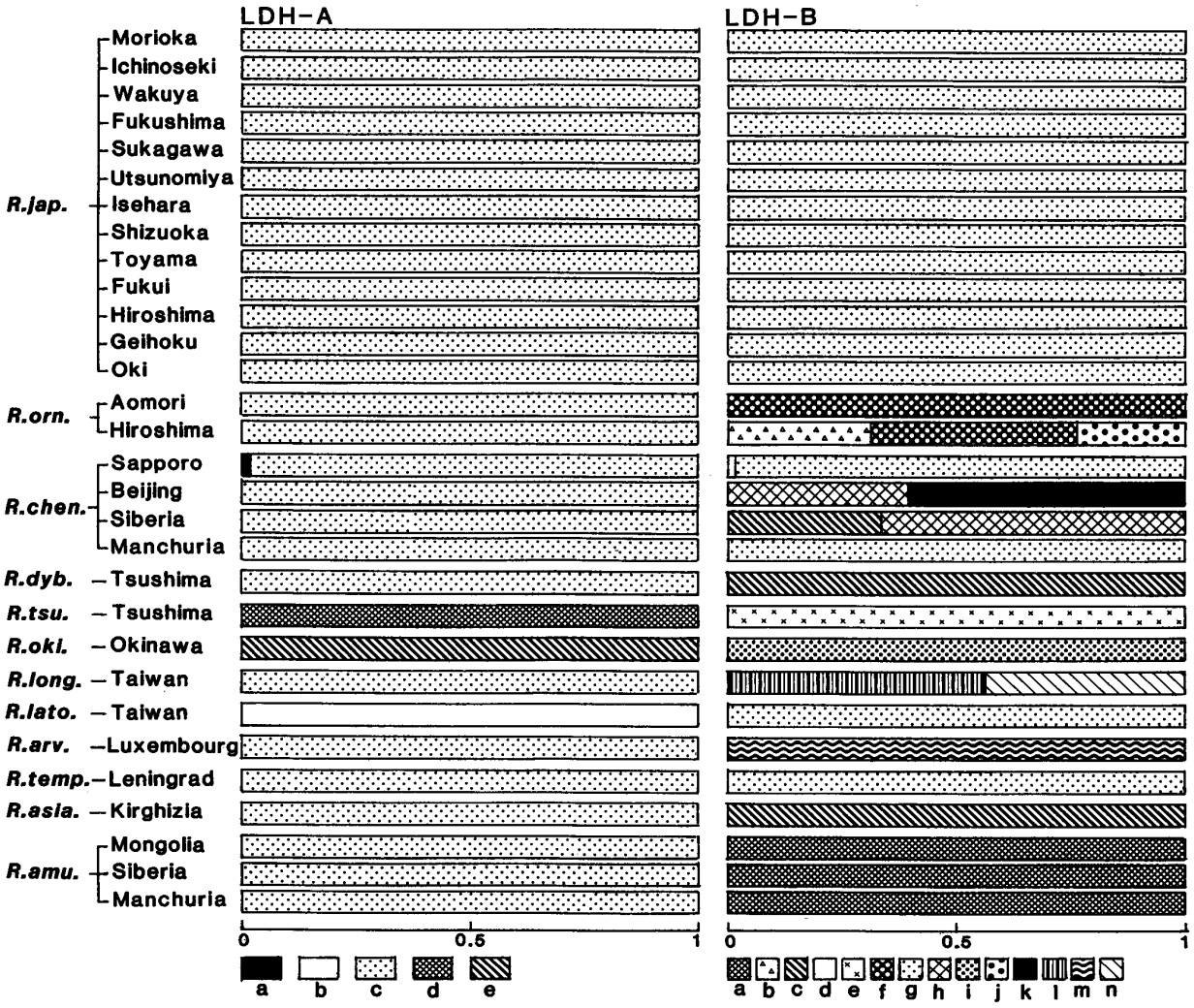

Fig. 8. Gene frequencies at two loci, LDH–A and LDH–B, in 30 populations of brown frogs in the Palearctic region.

populations of nine species, there was only allele *c*, except that the Sapporo population of *R. chensinensis* had allele *a* in a frequency of 0.016 in addition to allele *c* (Table 4–II; Fig. 8).

12. LDH–B locus

By analyses of the electrophoretic patterns at the LDH–B locus in the 468 brown frogs of the 30 populations of the 12 species, it was found that there were 20 phenotypes, AA, BB, CC, EE, FF, GG, HH, II, JJ, KK, LL, MM, NN, BF, BJ, CH, DG, FJ, HK and LN, produced by 14 alleles, *a*~*n*.

The 13 populations of *R. japonica*, the two populations of *R. latouchii* and *R. temporaria*, and the Manchuria population of *R. chensinensis* (hashima) had only allele *g*. The Sapporo population of *R. chensinensis* had alleles *g* and *d* in frequencies of 0.984 and 0.016, respectively, while the Beijing population of the same species had alleles *k* and *h* in frequencies of 0.607 and 0.393, respectively, and the Siberia population had alleles *h* and *c* in frequencies of 0.667 and 0.333, respectively. While the Aomori population of *R. ornativentris* had only allele *f*, the Hiroshima population had alleles *f*, *b* and *j* in frequencies of 0.452, 0.310 and 0.238, respectively. The population of *R. longicrus* had alleles *l* and *n* in frequencies of 0.563 and 0.438, respectively.

The populations of *R. dybowskii* and *R. asiatica* had only allele *c*, while those of *R. tsushimensis*, *R. okinavana* and *R. arvalis* had only alleles *e*, *i* and *m*, respectively. The three populations of *R. amurensis* had only allele *a* (Table 4–II, III; Fig. 8).

13. MDH–A locus

The analyses of the electrophoretic patterns at the MDH–A locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were seven phenotypes, AA, BB, CC, DD, EE, FF and BE, produced by six alleles, *a*~*f*.

Nineteen populations of five species, including the 13 populations of *R. japonica*, the three populations of *R. amurensis* and the three populations of *R. okinavana*, *R. longicrus* and *R. asiatica*, had only allele *b*. The population of *R. tsushimensis* had allele *e* in a frequency of 0.024 in addition to allele *b*. The two populations of *R. ornativentris* had only allele *a*, and five populations including two of the Sapporo and Siberia of *R. chensinensis*, and three of *R. dybowskii*, *R. arvalis* and the Manchuria of *R. chensinensis* (hashima) had only allele *c*. The Beijing population of *R. chensinensis* had only allele *e*, the population of *R. latouchii* had only allele *f*, and that of *R. temporaria* had only allele *d* (Table 4–III; Fig. 9).

14. MDH–B locus

The analyses of the electrophoretic patterns at the MDH–B locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were eight phenotypes, AA, BB, CC, DD, EE, FF, AC and CF, produced by six alleles, *a*~*f*.

In the 12 populations of *R. japonica* other than the Oki, and two populations of *R. okinavana* and *R. longicrus*, allele *f* was high in frequency, being 0.750~1.000. Five populations of *R. japonica*, the Wakuya, Fukushima, Shizuoka, Hiroshima and

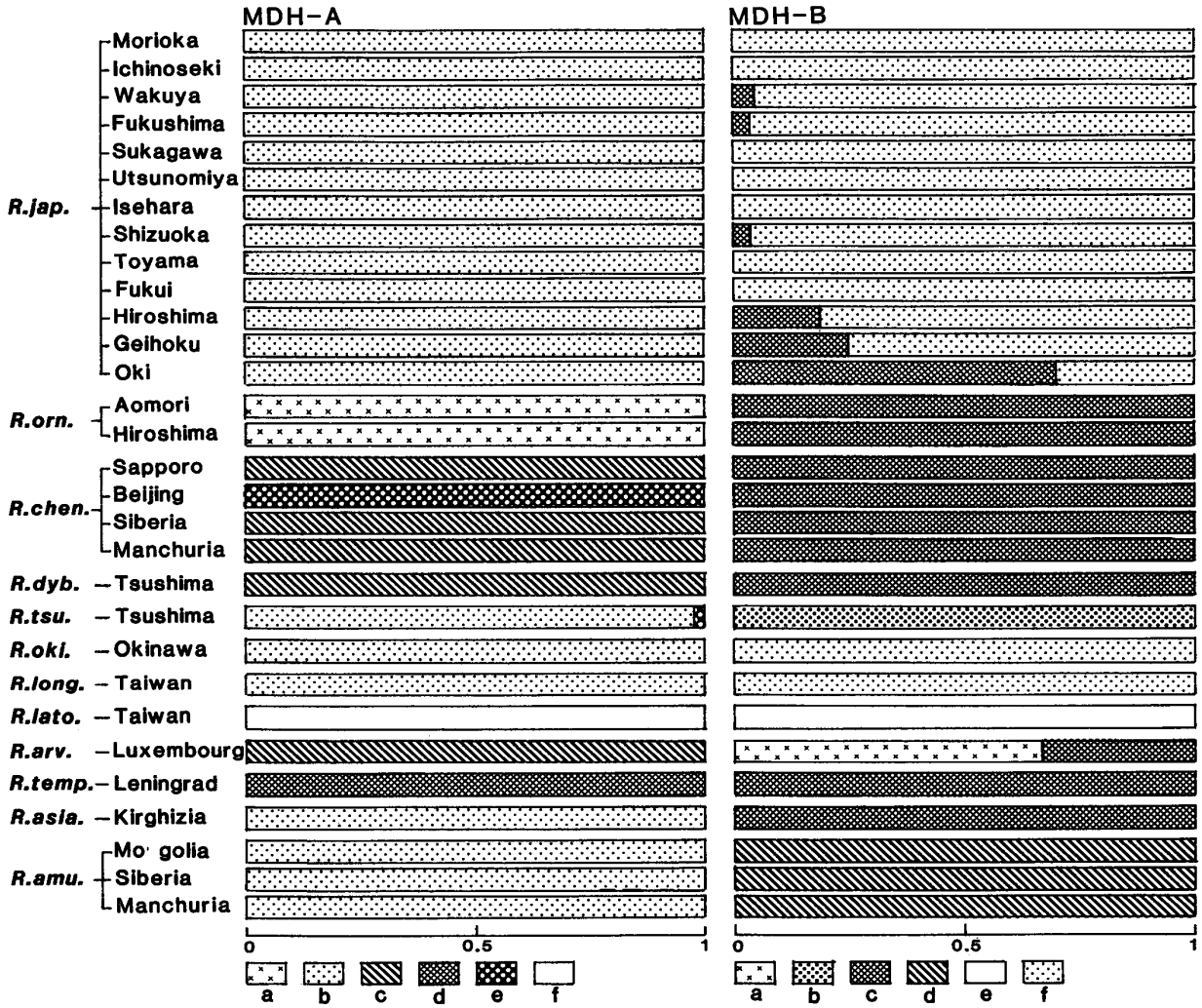

Fig. 9. Gene frequencies at two loci, MDH-A and MDH-B, in 30 populations of brown frogs in the Palearctic region.

Geihoku, had allele *c*, which was 0.036~0.250 in frequency in addition to allele *f*. The other seven populations of *R. japonica* and two populations of *R. okinavana* and *R. longicrus* had only allele *f*. The Oki population of *R. japonica* had alleles *c* and *f* in frequencies of 0.700 and 0.300, respectively.

Nine populations of five species, including the two of *R. ornativentris*, the four of *R. chensinensis* and the three populations of *R. dybowskii*, *R. temporaria* and *R. asiatica*, had only allele *c*. In the population of *R. arvalis*, alleles *a* and *c* were in frequencies of 0.667 and 0.333, respectively. The populations of *R. tsushimensis* and *R. latouchii* had only alleles *b* and *e*, respectively, while the three populations of *R. amurensis* had only allele *d* (Table 4–III; Fig. 9).

15. ME-A locus

The analyses of the electrophoretic patterns at the ME-A locus in the 468 brown frogs of the 30 populations of the 12 species showed that there were 13 phenotypes, AA, BB, CC, DD, EE, FF, GG, HH, AB, BE, EG, EI and FG, produced by nine alleles, *a*~*i*.

In 19 populations of six species, including the 12 populations of *R. japonica* other than the Shizuoka population, the Hiroshima population of *R. ornativentris*, the Sapporo population of *R. chensinensis*, the populations of *R. arvalis* and *R. temporaria* and the three populations of *R. amurensis*, allele *e* was very high in frequency, being 0.905~1.000. Of these populations, the Fukushima, Utsunomiya and Fukui of *R. japonica* had allele *i* in frequencies of 0.017~0.095 in addition to allele *e*. The Hiroshima population of *R. ornativentris* had allele *g* in a frequency of 0.048 in addition to allele *e*. The remaining 15 populations of the five species had only allele *e*. The Shizuoka population of *R. japonica* had alleles *e* and *g* in frequencies of 0.554 and 0.446, respectively, while the Aomori population of *R. ornativentris* had alleles *e* and *g* in frequencies of 0.438 and 0.563, respectively. The Siberia and Manchuria populations of *R. chensinensis* and the population of *R. dybowskii* had only allele *g*, while the Beijing population of *R. chensinensis* had only allele *c*.

The population of *R. tsushimensis* had alleles *f* and *g* in frequencies of 0.750 and 0.250, respectively, while the population of *R. okinavana* had alleles *b* and *e* in

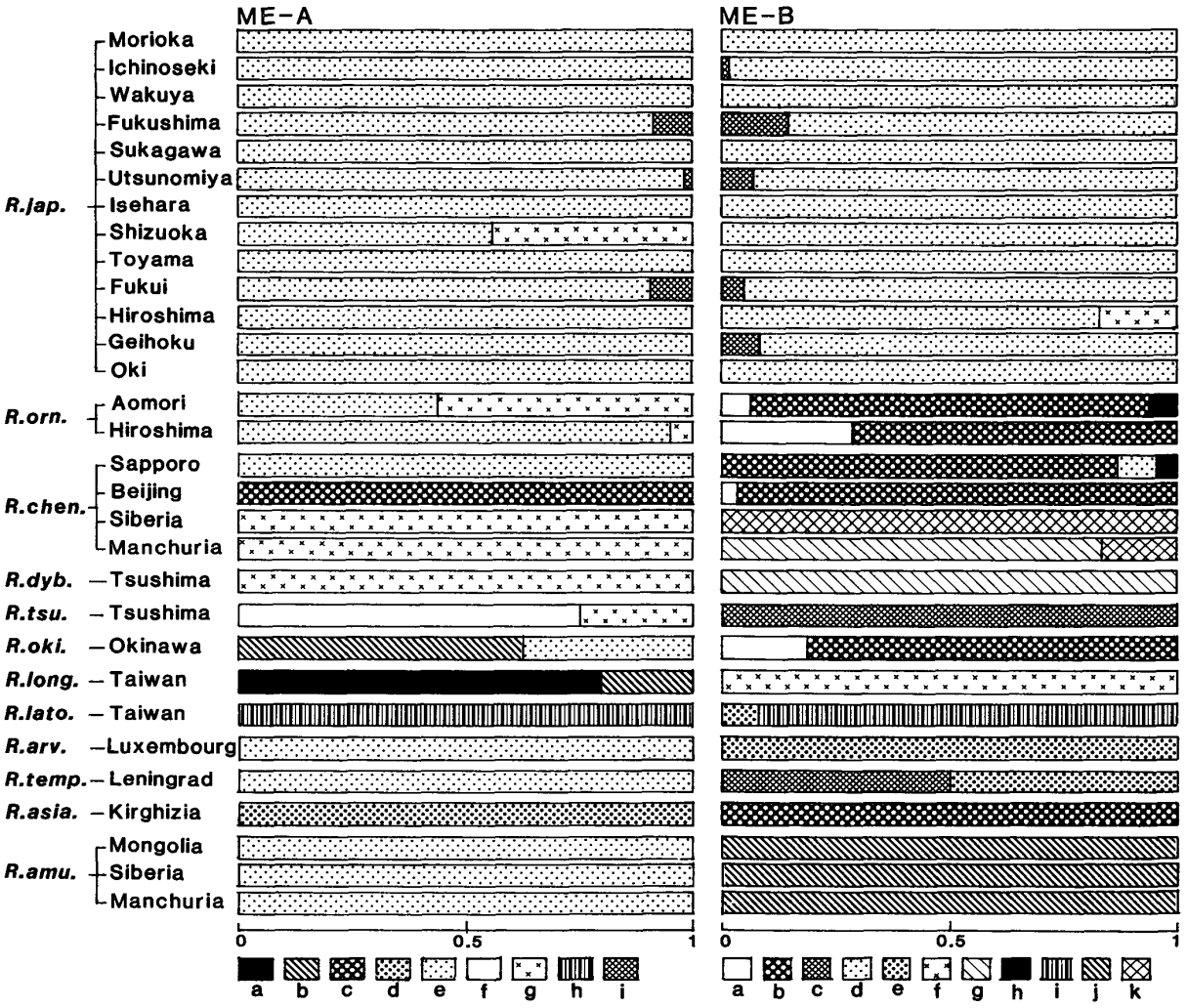

Fig. 10. Gene frequencies at two loci, ME-A and ME-B, in 30 populations of brown frogs in the Palearctic region.

frequencies of 0.625 and 0.375, respectively. The population of *R. longicrus* had alleles *a* and *b* in frequencies of 0.792 and 0.208, respectively. The populations of *R. latouchii* and *R. asiatica* had only alleles *h* and *d*, respectively (Table 4–III; Fig. 10).

16. ME–B locus

The analyses of the electrophoretic patterns at the ME–B locus in the 468 brown frogs belonging to the 30 populations of the 12 species showed that there were 18 phenotypes, AA, BB, CC, DD, EE, FF, GG, II, JJ, KK, AB, BD, BH, CD, CE, DF, EI and GK, produced by 11 alleles, *a*~*k*.

In the 13 populations of *R. japonica,* allele *d* was high in frequency, being 0.833~1.000. Of these populations, the Ichinoseki, Fukushima, Utsunomiya, Fukui and Geihoku had allele *c* in frequencies of 0.014~0.143, and the Hiroshima population had allele *f* in a frequency of 0.167 in addition to allele *d*. In the two populations of *R. ornativentris*, the Sapporo and Beijing populations of *R. chensinensis*, and the two populations of *R. okinavana* and *R. asiatica,* allele *b* was high in frequency, being 0.714~1.000. Of these populations, the Hiroshima of *R. ornativentris,* the Beijing of *R. chensinensis* and the population of *R. okinavana* had allele *a* in frequencies of 0.036~0.286, in addition to allele *b*. The Aomori population of *R. ornativentris* had alleles *a* and *h* each in a frequency of 0.063, and the Sapporo population of *R. chensinensis* had alleles *d* and *h* in frequencies of 0.081 and 0.048, respectively, in addition to allele *b*. The population of *R. asiatica* had only allele *b*, while the Siberia population of *R. chensinensis* had only allele *k*.

The population of *R. dybowskii* had only allele *g*, while the Manchuria population of *R. chensinensis* (hashima) had alleles *g* and *k* in frequencies of 0.833 and 0.167, respectively. The populations of *R. tsushimensis, R. longicrus* and *R. arvalis* had only alleles *c, f* and *e*, respectively, while the population of *R. temporaria* had alleles *c* and *e* each in a frequency of 0.500. The population of *R. latouchii* had alleles *i* and *e* in frequencies of 0.917 and 0.083, respectively. The three populations of *R. amurensis* had only allele *j* (Table 4–III; Fig. 10).

17. MPI locus

By analyzing the electrophoretic patterns at the MPI locus in the 468 brown frogs belonging to the 30 populations of the 12 species, it was found that there were 55 phenotypes, DD, GG, JJ, KK, LL, MM, NN, OO, PP, QQ, RR, SS, TT, WW, AG, AH, BG, CJ, DG, DH, DJ, DO, DW, EG, FJ, FM, GI, GJ, GK, GL, GO, GS, IM, IO, IQ, JM, JN, JP, KO, KR, LO, LS, MN, MO, MP, MQ, MR, MT, MU,NQ, OQ, OT, PT, RV and SW, produced by 23 alleles, *a*~*w*.

In seven populations, the Morioka, Ichinoseki, Wakuya, Shizuoka, Toyama, Hiroshima and Geihoku, of the 13 populations of *R. japonica*, allele *m* was very high in frequency, being 0.800~1.000, while the Morioka population had only allele *m*. In addition to allele *m*, the Ichinoseki and Wakuya populations had allele *p* in frequencies of 0.054 and 0.146, respectively, and allele *t* in frequencies of 0.041 and 0.021, respectively. The Shizuoka population had allele *p* in a frequency of 0.018,

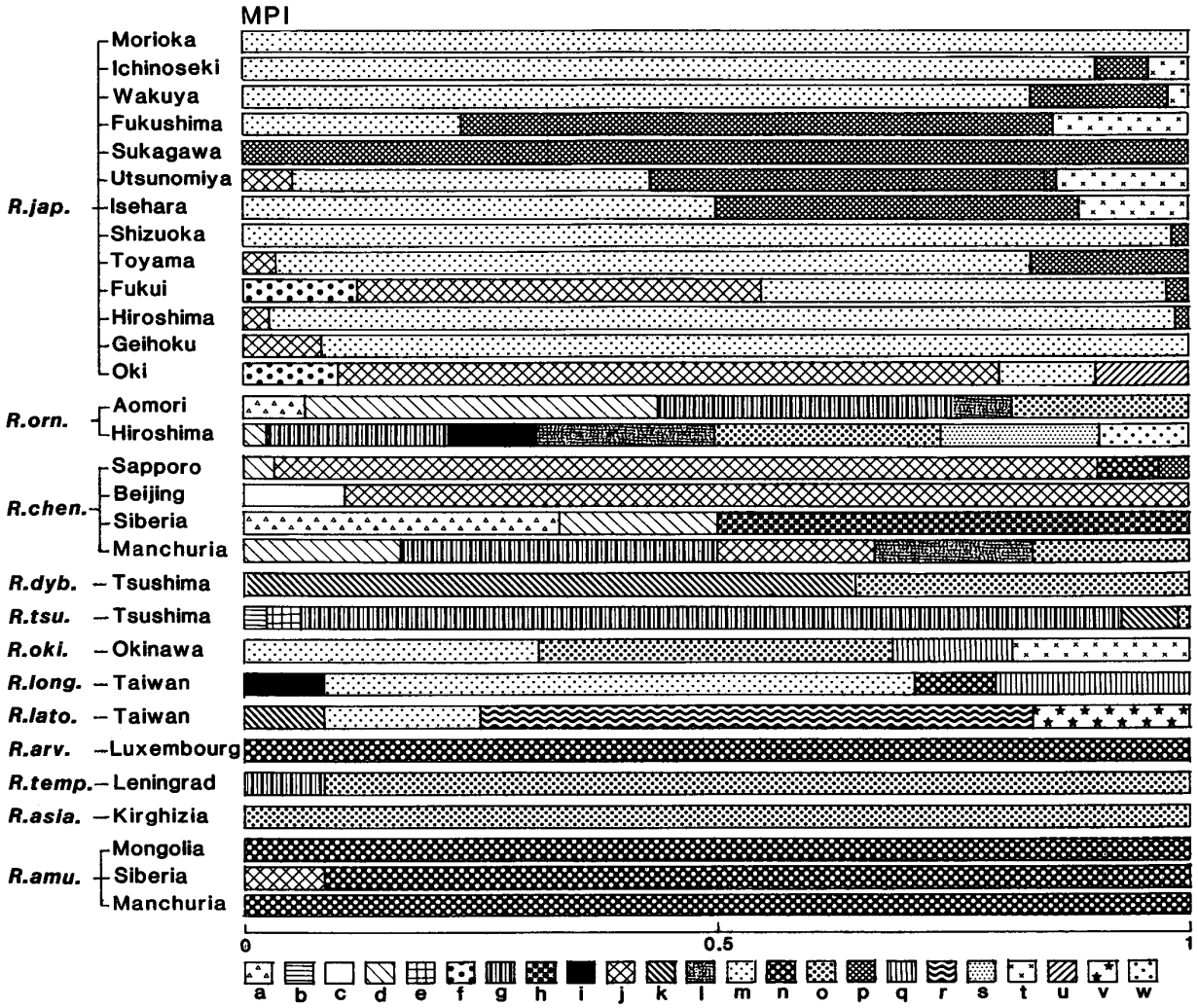

Fig. 11. Gene frequencies at MPI locus in 30 populations of brown frogs in the Palearctic region.

the Toyama and Hiroshima populations had allele *j* in frequencies of 0.033 and 0.028, respectively, and allele *p* in frequencies of 0.167 and 0.014, respectively, and the Geihoku population had allele *j* in a frequency of 0.083, in addition to allele *m*. The Sukagawa population had only allele *p*, while the Fukushima population had allele *p* in a frequency of 0.625 and alleles *m* and *t* in frequencies of 0.232 and 0.143, respectively. The Utsunomiya population had alleles *p*, *m*, *t* and *j* in frequencies of 0.431, 0.379, 0.138 and 0.052, respectively. In the Isehara population, alleles *m*, *p* and *t* were in frequencies of 0.500, 0.385 and 0.115, respectively, and in the Fukui population, alleles *j* and *m* were each in a frequency of 0.429 and alleles *f* and *p* were in frequencies of 0.119 and 0.024, respectively. In the Oki population, allele *j* was high in frequency, being 0.700, and each of alleles *f*, *m* and *u* was in a frequency of 0.100 (Table 4–III, IV; Fig. 11).

In the Aomori population of *R. ornativentris*, alleles *d* and *g* were contained in frequencies of 0.375 and 0.313, respectively, and alleles *a*, *l* and *o* were in frequencies of 0.063, 0.063 and 0.188, respectively. In the Hiroshima population, alleles *d*, *g*, *i*, *l*, *o*, *s* and *w* were contained in frequencies of 0.024~0.238. In the

Sapporo and Beijing populations of *R. chensinensis*, allele *j* was very high in frequency, being 0.871 and 0.893, respectively, while the Sapporo population had alleles *d*, *n* and *p* in frequencies of 0.032~0.065, and the Beijing population had allele *c* in a frequency of 0.107 in addition to allele *j*. The Siberia population of *R. chensinensis* had alleles *h*, *a* and *d* in frequencies of 0.500, 0.333 and 0.167, respectively (Table 4–III, IV; Fig. 11).

The population of *R. dybowskii* had alleles *k* and *o* in frequencies of 0.646 and 0.354, respectively, while the population of *R. tsushimensis* had allele *g* in a high frequency, being 0.869, and moreover, had alleles *b*, *e*, *k* and *o* in frequencies of 0.012~0.060. In the population of *R. okinavana*, there were alleles *m*, *o*, *q* and *t* in frequencies of 0.125~0.375. The population of *R. longicrus* had allele *m* in a high frequency, being 0.625, and moreover, had alleles *i*, *n* and *q* in frequencies of 0.083, 0.083 and 0.208, respectively. In the population of *R. latouchii*, allele *r* was high in frequency, being 0.583, and in addition to this allele, there were alleles *k*, *m* and *v* in frequencies of 0.083, 0.167 and 0.167, respectively. The Manchuria population of *R. chensinensis* (hashima) had allele *g* in a frequency of 0.333, and moreover had alleles *d*, *j*, *l* and *o*, each of which was 0.167 in frequency. The population of *R. arvalis* and the three populations of *R. amurensis* had only allele *n*, except that the Siberia population of *R. amurensis* had allele *j* in a frequency of 0.083 in addition to allele *n*. The two populations of *R. temporaria* and *R. asiatica* had only allele *o*, except that the population of *R. temporaria* had allele *g* in a frequency of 0.083 in addition to allele *o* (Table 4–III, IV; Fig. 11).

18. Pep–A locus

The analyses of the electrophoretic patterns at the Pep–A locus in the 468 brown frogs belonging to the 30 populations of the 12 species showed that there were nine phenotypes, AA, BB, CC, DD, AB, AC, BC, BD and BE, produced by five alleles, *a*~*e*.

In the seven populations of *R. japonica* distributed east from Isehara, allele *a* was very high in frequency, being 0.946~1.000, while the Ichinoseki, Fukushima and Utsunomiya populations had allele *b* in a frequency of 0.034 or 0.054 in addition to allele *a*. The remaining four populations had only allele *a*. On the other hand, in the six populations of *R. japonica* distributed west from Shizuoka, allele *b* was high in frequency, being 0.800~0.917, while the Shizuoka had alleles *a* and *c* in frequencies of 0.071 and 0.036, respectively, the Toyama and Hiroshima populations had allele *a* in frequencies of 0.200 and 0.153, respectively, and the Fukui, Geihoku and Oki populations had allele *c* in frequencies of 0.083~0.200, in addition to allele *b*.

The 10 populations of seven species, including the Aomori population of *R. ornativentris*, the Siberia and Manchuria populations of *R. chensinensis*, the populations of *R. longicrus*, *R. arvalis*, *R. temporaria* and *R. asiatica*, and three populations of *R. amurensis*, had only allele *a*, except that the Siberia population of *R. chensinensis* had allele *b* in a frequency of 0.167 in addition to allele *a*. In the Hiroshima population of *R. ornativentris* and the Sapporo and Beijing populations of *R.*

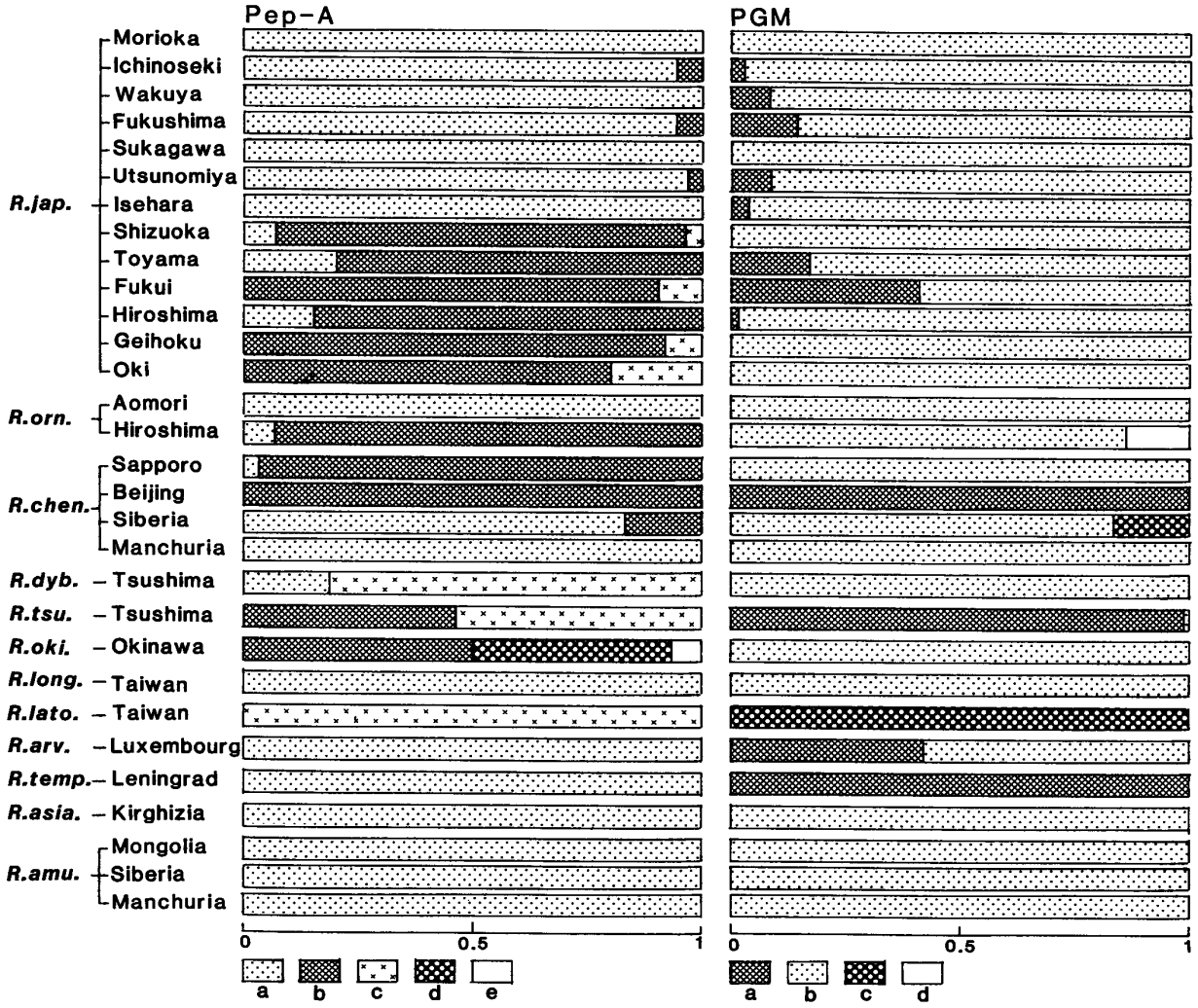

Fig. 12. Gene frequencies at two loci, Pep-A and PGM, in 30 populations of brown frogs in the Palearctic region.

chensinensis, the allele *b* was very high in frequency, being 0.929~1.000, while these Hiroshima and Sapporo populations had allele *a* in frequencies of 0.071 and 0.032, respectively. The Beijing population had only allele *b*.

The population of *R. dybowskii* had alleles *c* and *a* in frequencies of 0.813 and 0.188, respectively, while that of *R. tsushimensis* had alleles *c* and *b* in frequencies of 0.536 and 0.464, respectively. The population of *R. okinavana* had alleles *b*, *d* and *e* in frequencies of 0.500, 0.438 and 0.063, respectively. The population of *R. latouchii* had only allele *c* (Table 4-IV; Fig. 12).

19. PGM locus

By analyses of the electrophoretic patterns at the PGM locus in the 468 brown frogs belonging to the 30 populations of the 12 species, it was found that there were six phenotypes, AA, BB, CC, AB, BC and BD, produced by four alleles, *a*~*d*.

In 24 populations of eight species, including the 12 populations of *R. japonica* other than the Fukui population, the two populations of *R. ornativentris*, the

Sapporo, Siberia and Manchuria populations of *R. chensinensis*, the four populations of *R. dybowskii, R. okinavana, R. longicrus* and *R. asiatica*, and the three populations of *R. amurensis*, allele *b* was very high in frequency, being 0.833~1.000. Of these populations, the Ichinoseki, Wakuya, Fukushima, Utsunomiya, Isehara, Toyama and Hiroshima populations of *R. japonica* had allele *a* in frequencies of 0.014~0.167 in addition to allele *b*. The Hiroshima population of *R. ornativentris* had allele *d* in a frequency of 0.143, and the Siberia population of *R. chensinensis* had allele *c* in a frequency of 0.167 in addition to allele *b*. The other 15 populations of eight species had only allele *b*. The Fukui population of *R. japonica* had alleles *b* and *a* in frequencies of 0.595 and 0.405, respectively. The population of *R. arvalis* had alleles *b* and *a* in frequencies of 0.583 and 0.417, respectively.

The Beijing population of *R. chensinensis* and the population of *R. temporaria* had only allele *a*. In the population of *R. tsushimensis*, allele *a* was very high in frequency, being 0.988, and there was allele *b* in a frequency of 0.012. The population of *R. latouchii* had only allele *c* (Table 4–IV; Fig. 12).

20. SOD–A locus

The analyses of the electrophoretic patterns at the SOD–A locus in the 468 brown frogs belonging to the 30 populations of the 12 species showed four phenotypes, BB, CC, AB and BC, produced by three alleles, *a~c*.

Most of the 30 populations of the 12 species were occupied by allele *b*. While the Geihoku population of *R. japonica* and the Hiroshima population of *R. ornativentris* had allele *b* in frequencies of 0.583 and 0.643, respectively, the other 28 populations had allele *b* in frequencies of 0.847~1.000. In addition to allele *b*, there was allele *a* in a frequency of 0.417 in the Geihoku population and allele *c* in a frequency of 0.357 in the Hiroshima population of *R. ornativentris*. In the Wakuya, Utsunomiya, Hiroshima and Oki populations of *R. japonica*, there was allele *a* in frequencies of 0.017~0.153 in addition to allele *b* (Table 4–IV; Fig. 13).

21. SOD–B locus

The analyses of the electrophoretic patterns at the SOD–B locus in the 468 brown frogs belonging to the 30 populations of the 12 species showed that there were 12 phenotypes, AA, CC, DD, EE, GG, HH, II, AF, BH, CH, DH and HI, produced by nine alleles, *a~i*.

In 18 populations of four species, including the 13 populations of *R. japonica*, the three populations of *R. amurensis*, and the two populations of *R. okinavana* and *R. longicrus*, allele *h* was high in frequency, being 0.567~1.000. Of these populations, the Toyama, Fukui and Utsunomiya of *R. japonica* had allele *c* in frequencies of 0.138~0.433, and the Manchuria population of *R. amurensis* had allele *b* in a frequency of 0.167 in addition to allele *h*. The remaining 14 populations of the four species had only allele *h*.

The Hiroshima population of *R. ornativentris* had alleles *c* and *h* in frequencies of 0.524 and 0.476, respectively, while the Aomori population of *R. ornativentris*, the Siberia population of *R. chensinensis* and the population of *R. asiatica* had only allele

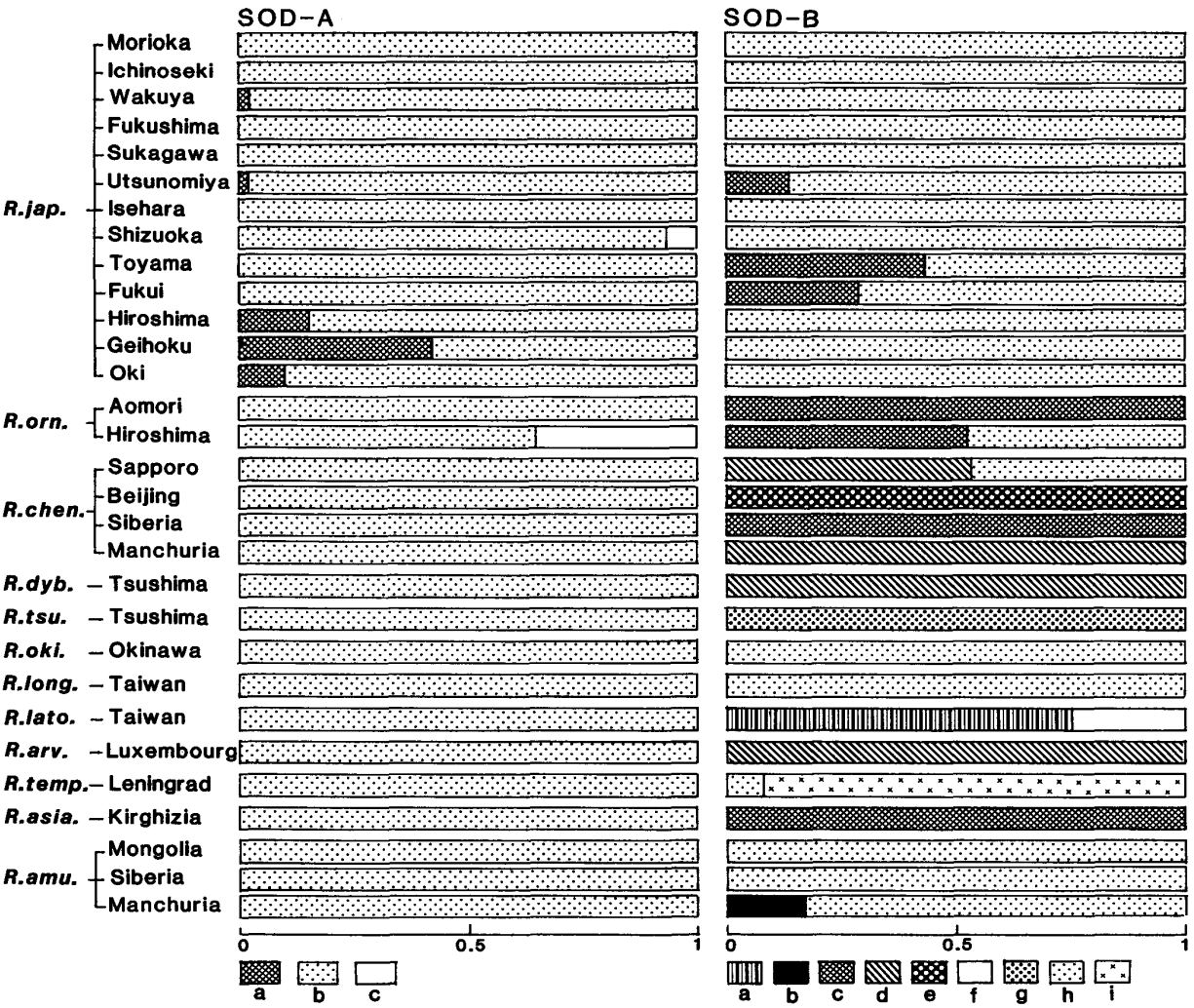

Fig. 13. Gene frequencies at two loci, SOD-A and SOD-B, in 30 populations of brown frogs in the Palearctic region.

c. The populations of *R. dybowskii* and *R. arvalis* and the Manchuria population of *R. chensinensis* (hashima) had only allele *d*, while the Sapporo population of *R. chensinensis* had alleles *d* and *h* in frequencies of 0.532 and 0.468, respectively. The Beijing population of *R. chensinensis* and the population of *R. tsushimensis* had only alleles *e* and *g*, respectively. The population of *R. latouchii* had alleles *a* and *f* in frequencies of 0.750 and 0.250, respectively, while that of *R. temporaria* had alleles *i* and *h* in frequencies of 0.917 and 0.083, respectively (Table 4–IV; Fig. 13).

22. Ab locus

The analyses of the electrophoretic patterns at the Ab locus in the 365 brown frogs belonging to the 28 populations of the 12 species showed that there were 21 phenotypes, AA, BB, CC, DD, EE, FF, GG, HH, II, JJ, AB, AC, BC, CF, CI, DG, DI, FH, FI, IK and JK, produced by 11 alleles, *a*~*k*.

In five populations, the Morioka, Wakuya, Fukushima, Utsunomiya and Isehara of *R. japonica* and the population of *R. okinavana,* allele *c* was high in

frequency, being 0.600~1.000. Of these populations, the Isehara and that of *R. okinavana* had only allele *c*, while the other four populations had allele *b* in frequencies of 0.119~0.400 in addition to allele *c*. In the Sukagawa population of *R. japonica*, alleles *b* and *c* were each found in a frequency of 0.500, while in the Ichinoseki population, alleles *b* and *c* were found in frequencies of 0.533 and 0.467, respectively. In the remaining six populations of *R. japonica*, allele *b* was high in frequency, being 0.574~1.000. Of these populations, the Shizuoka, Hiroshima and Geihoku had allele *a* in frequencies of 0.083~0.426, the Toyama had allele *c* in a frequency of 0.067 and the Fukui had alleles *a* and *c* in frequencies of 0.154 and 0.231, respectively, in addition to allele *b*. The Oki population had only allele *b*.

The two populations of *R. ornativentris* and the two populations of *R. longicrus* and *R. temporaria* had allele *f* in high frequencies, being 0.643~1.000. In addition to allele *f*, the two populations of *R. ornativentris* had allele *c* in frequencies of 0.071 and 0.125, and allele *i* in frequencies of 0.286 and 0.125, respectively, and the population of *R. longicrus* had allele *c* in a frequency of 0.159. The population of *R. temporaria* had only allele *f*. The Sapporo population of *R. chensinensis* had

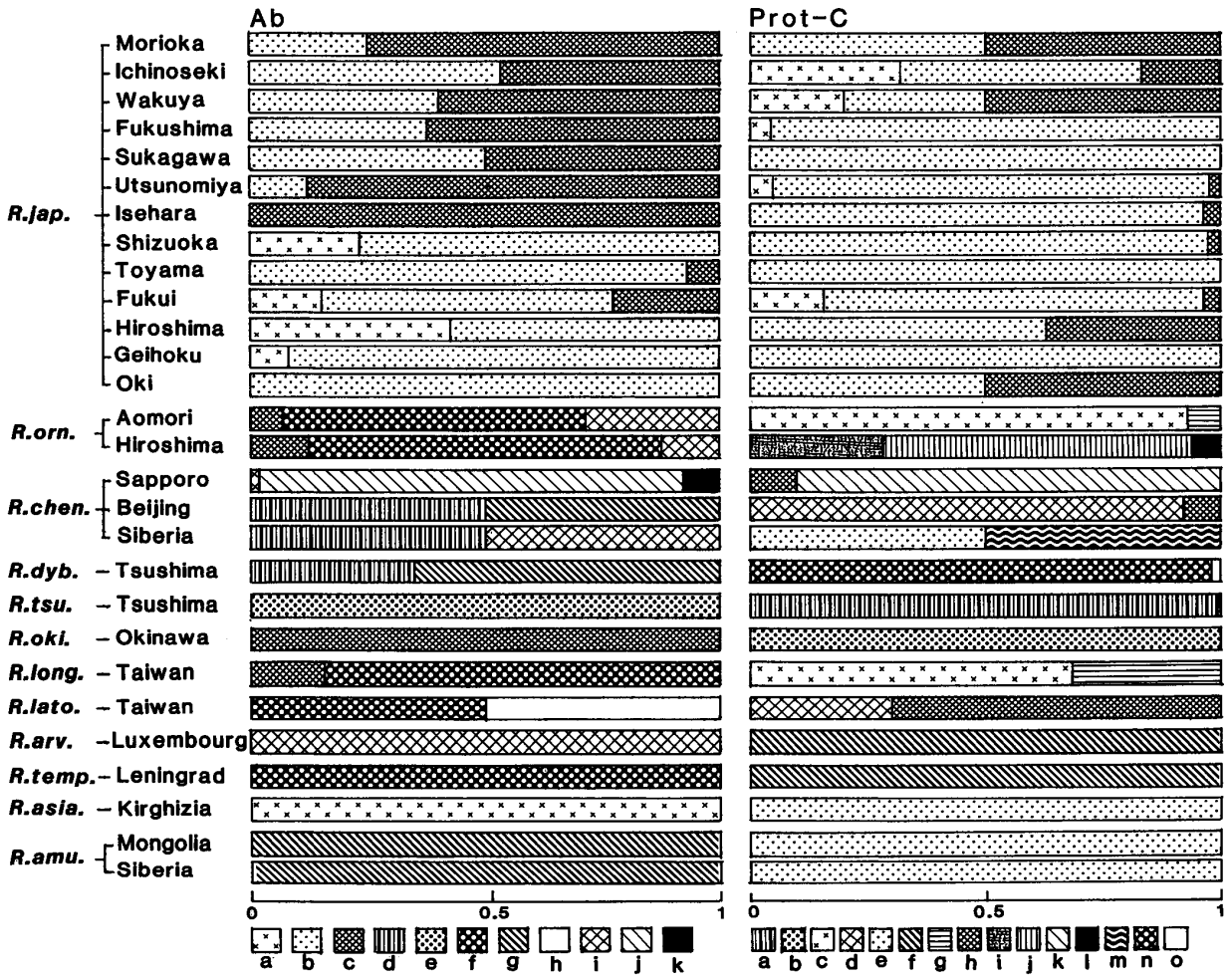

Fig. 14. Gene frequencies at two loci, Ab and Prot-C, in 30 populations of brown frogs in the Palearctic region.

alleles *j*, *i* and *k* in frequencies of 0.900, 0.017 and 0.083, respectively, while the Beijing population of the same species had alleles *d* and *g* each in a frequency of 0.500, and the Siberia population had alleles *d* and *i* each in a frequency of 0.500. The population of *R. dybowskii* had alleles *g* and *d* in frequencies of 0.652 and 0.348, respectively. The population of *R. latouchii* had alleles *f* and *h* each in a frequency of 0.500. The populations of *R. tsushimensis*, *R. arvalis* and *R. asiatica* had only alleles *e*, *i* and *a*, respectively. The Mongolia and Siberia populations of *R. amurensis* had only allele *g* (Table 4–V; Fig. 14).

23. Prot–C locus

The analyses of the electrophoretic patterns at the Prot–C locus in the 365 brown frogs belonging to the 28 populations of the 12 species showed that there were 24 phenotypes, AA, BB, CC, DD, EE, FF, GG, HH, II, JJ, KK, MM, NN, CE, CG, CH, DH, EH, EM, HK, IJ, IL, JL and NO, produced by 15 alleles, *a*~*o*.

The Morioka and Oki populations of *R. japonica* had alleles *e* and *h* each in a frequency of 0.500, while the Wakuya population had alleles *h*, *e* and *c* in frequencies of 0.500, 0.300 and 0.200, respectively. The Ichinoseki population had alleles *e*, *c* and *h* in frequencies of 0.517, 0.317 and 0.167, respectively. In the other nine populations of *R. japonica* other than these four populations, the population of *R. asiatica*, and the Mongolia and Siberia populations of *R. amurensis*, allele *e* was high in frequencies of 0.632~1.000. In addition to allele *e*, the Fukushima population of *R. japonica* had allele *c* in a frequency of 0.042, the Isehara, Shizuoka and Hiroshima populations had allele *h* in frequencies of 0.029~0.368, the Utsunomiya and Fukui populations had allele *c* in frequencies of 0.048 and 0.154, respectively, and allele *h* in frequencies of 0.024 and 0.038, respectively. All the remaining three populations of *R. japonica*, the population of *R. asiatica* and the two populations of *R. amurensis* had only allele *e*.

The Aomori population of *R. ornativentris* had allele *c* in a frequency of 0.929 and allele *g* in a frequency of 0.071. The Hiroshima population of the same species had alleles *j*, *i* and *l* in frequencies of 0.656, 0.281 and 0.063, respectively. The Sapporo population of *R. chensinensis* had alleles *k* and *h* in frequencies of 0.900 and 0.100, respectively, the Beijing population had alleles *d* and *h* in frequencies of 0.917 and 0.083, respectively, and the Siberia population had alleles *e* and *m* each in a frequency of 0.500. The population of *R. dybowskii* had alleles *n* and *o* in frequencies of 0.978 and 0.022, respectively. The population of *R. longicrus* had alleles *c* and *g* in frequencies of 0.682 and 0.318, respectively. The population of *R. latouchii* had alleles *h* and *d* in frequencies of 0.700 and 0.300, respectively. The populations of *R. tsushimensis*, *R. okinavana* and *R. arvalis* had only alleles *a*, *b* and *f*, respectively. That of *R. temporaria* had also only allele *f* (Table 4–V; Fig. 14).

24. Hb–I locus

By analyses of the electrophoretic patterns at the Hb–I locus in the 365 brown frogs belonging to the 28 populations of the 12 species, it was found that there were nine phenotypes, AA, BB, CC, DD, EE, FF, GG, HH and BE, produced by

eight alleles, *a*~*h*.

The 13 populations of *R. japonica* and the two populations of *R. tsushimensis* and *R. longicrus* had only allele *e*, the two populations of *R. ornativentris* had only allele *h*, the Sapporo population of *R. chensinensis* had only allele *d*, and the Beijing and Siberia populations of *R. chensinensis* and the population of *R. dybowskii* had only allele *f*. The four populations of *R. okinavana*, *R. latouchii*, *R. arvalis* and *R. temporaria* had only alleles *g*, *c*, *a* and *b*, respectively. The Mongolia and Siberia populations of *R. amurensis* had only allele *d*. In the population of *R. asiatica*, allele *b* was 0.667 and allele *e* was 0.333 in frequency, differing from the other species which had only species-specific alleles (Table 4–V; Fig. 15).

25. Hb–II locus

The analyses of the electrophoretic patterns at the Hb–II locus in the 365 brown frogs belonging to the 28 populations of the 12 species showed that there were 12 phenotypes, BB, CC, DD, EE, FF, GG, HH, II, JJ, KK, AI and CE, produced by 11 alleles, *a*~*k*.

The seven populations of *R. japonica* distributed in eastern Japan from Morioka

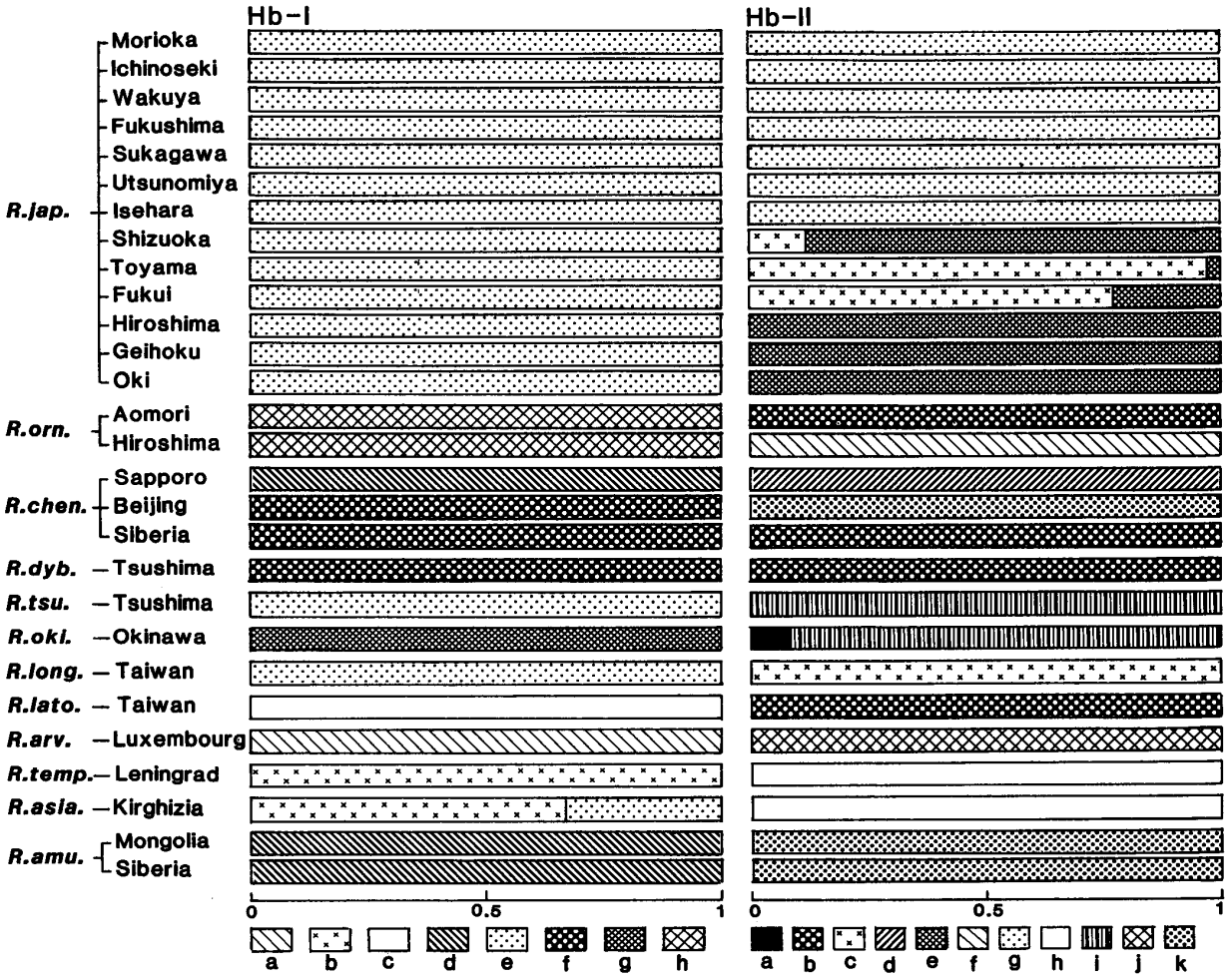

Fig. 15. Gene frequencies at two loci, Hb–I and Hb–II, in 30 populations of brown frogs in the Palearctic region.

to Isehara had only allele *g*, while the Toyama and Fukui populations had allele *c* in frequencies of 0.967 and 0.769, respectively and, moreover, had allele *e* in frequencies of 0.033 and 0.231, respectively. The Hiroshima, Geihoku and Oki populations of *R. japonica* had only allele *e*, and the Shizuoka population of the same species had allele *c* in a frequency of 0.118 in addition to allele *e*. The population of *R. longicrus* had only allele *c*.

While the Hiroshima population of *R. ornativentris* had only allele *f*, the Aomori population of the same species, the Siberia population of *R. chensinensis* and the two populations of *R. dybowskii* and *R. latouchii* had only allele *b*. The Sapporo population of *R. chensinensis* had only allele *d*, while the Beijing population of the same species and the Mongolia and Siberia populations of *R. amurensis* had only allele *k*. The two populations of *R. tsushimensis* and *R. okinavana* had only allele *i*, except that *R. okinavana* had allele *a* in a frequency of 0.083 in addition to allele *i*. The population of *R. arvalis* had only allele *j*, while those of *R. temporaria* and *R. asiatica* had only allele *h* (Table 4–V; Fig. 15).

III. Genetic variation

1. Fixation indexes in the 13 populations of *R. japonica*

At the 25 loci analyzed in 247 frogs belonging to the 13 populations of *R. japonica,* the fixation indexes (Fst) were calculated according to WRIGHT (1978). When the gene frequencies at a definite locus are the same in all the 13 populations, the fixation index is zero, while this is 1.000 when there is a population-specific allele in one or more. When the values in fixation index were arranged in decreasing order, the Hb–II locus was 0.925 and showed that the genetic differentiation was nearly complete in this locus. The Pep–A locus was

TABLE 5
Fixation index at 25 loci in 13 populations of *R. japonica*

Locus	Fixation index	Locus	Fixation index
AAT–A	0.038	ME–A	0.298
AAT–B	0.234	ME–B	0.086
ADA	0.419	MPI	0.457
AK	0	Pep–A	0.747
CK	0.019	PGM	0.177
Fum	0.186	SOD–A	0.231
α–GDH	0.135	SOD–B	0.285
GPI	0.144	Ab	0.381
IDH–A	0.196	Prot–C	0.298
IDH–B	0.138	Hb–I	0
LDH–A	0	Hb–II	0.925
LDH–B	0		
MDH–A	0		
MDH–B	0.394		

0.747 and somewhat lower in genetic differentiation. Four loci of MPI, ADA, MDH-B and Ab were 0.457~0.381, five loci of ME-A, Prot-C, SOD-B, AAT-B and SOD-A were 0.298~0.231, and six loci of IDH-A, Fum, PGM, GPI, IDH-B and α-GDH were 0.196~0.135. Three loci of ME-B, AAT-A and CK were 0.086~0.019 and showed that the genetic differentiation was very slight. Five loci of AK, LDH-A, LDH-B, MDH-A and Hb-I were zero in fixation index and indicated that these loci were not genetically differentiated (Table 5).

2. Proportion of heterozygous loci

The mean proportion of heterozygous loci per individual was examined at the 25 loci controlling 15 enzymes and three blood proteins in 468 brown frogs belonging to 30 populations of 12 species distributed in the Palearctic region. The results showed that the values in the 30 populations were 1.7~22.0%, 10.1% on the average. The expected values were 2.2~22.7%, 10.1% on the average. Of these values, those of the 13 populations of *R. japonica* were 7.0~14.0%, 10.2% on the average. The expected values were 6.5~14.7%, 10.5% on the average.

The largest in the mean proportion of heterozygous loci per individual was 22.0% in the Hiroshima population of *R. ornativentris*. This was followed by 18.7% in the Siberia population of *R. chensinensis*, 17.4% in the population of *R. dybowskii*, 14.0% and 13.8% in the Geihoku and Oki populations of *R. japonica*, respectively, 13.7% in the population of *R. latouchii*, 12.4~12.0% in the Fukui and Hiroshima populations of *R. japonica*, the population of *R. okinavana* and the population of *R. longicrus*, 11.7% in the Aomori population of *R. ornativentris*, 11.5% in the Fukushima population of *R. japonica*, 11.1% in the Manchuria population of *R. chensinensis* (hashima), and 10.1% in the Utsunomiya population of *R. japonica* and the population of *R. temporaria*. The values in the mean proportion of heterozygous loci per individual were 9.8~7.0% in 10 populations including the Shizuoka, Wakuya, Sukagawa, Toyama, Isehara, Ichinoseki and Morioka populations of *R. japonica*, the Beijing and Sapporo populations of *R. chensinensis* and the population of *R. tsushimensis*, and 5.3~4.0% in the population of *R. arvalis*, the Manchuria and Siberia populations of *R. amurensis* and the population of *R. asiatica*. The smallest value was 1.7% in the Mongolia population of *R. amurensis* (Table 6).

The population in which there was the largest difference between actual and expected values in the mean proportion of heterozygous loci per individual was found in the Shizuoka population of *R. japonica*, where the actual value was 9.8%, while the expected value was 13.5%. The actual and expected values were 11.1% and 7.4%, respectively, in the Manchuria population of *R. chensinensis* (hashima) and these two values were 14.0% and 10.6% in the Geihoku population of *R. japonica*. The other remaining populations in which there were differences more than 2% between actual and expected values were the Fukui, Wakuya, Sukagawa and Ichinoseki of *R. japonica* and the population of *R. latouchii* (Table 6).

TABLE 6
Genetic variabilities at 25 loci in 30 populations of 12 brown frog species distributed in the Palearctic region

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. japonica	Morioka	3	7.0 (7.1)	16.0	1.16
〃	Ichinoseki	37	7.6 (9.6)	36.0	1.52
〃	Wakuya	24	9.2 (11.6)	40.0	1.56
〃	Fukushima	28	11.5 (12.0)	48.0	1.56
〃	Sukagawa	2	8.7 (6.5)	16.0	1.16
〃	Utsunomiya	29	10.1 (10.2)	60.0	1.76
〃	Isehara	13	7.7 (7.3)	24.0	1.28
〃	Shizuoka	28	9.8 (13.5)	56.0	1.72
〃	Toyama	15	8.3 (8.4)	36.0	1.40
〃	Fukui	21	12.4 (14.7)	52.0	1.68
〃	Hiroshima	36	12.2 (13.2)	56.0	1.68
〃	Geihoku	6	14.0 (10.6)	36.0	1.40
〃	Oki	5	13.8 (12.7)	32.0	1.44
R. ornativentris	Aomori	8	11.7 (11.8)	28.0	1.48
〃	Hiroshima	21	22.0 (22.7)	56.0	1.92
R. chensinensis	Sapporo	31	7.5 (7.7)	48.0	1.64
〃	Beijing	14	7.6 (8.8)	36.0	1.40
〃	Siberia	3	18.7 (17.6)	40.0	1.48
〃 (hashima)	Manchuria	3	*11.1 (*7.4)	*14.3	*1.24
R. dybowskii	Tsushima	24	17.4 (16.1)	40.0	1.40
R. tsushimensis	Tsushima	42	7.3 (7.4)	40.0	1.52
R. okinavana	Okinawa	8	12.0 (12.2)	40.0	1.52
R. longicrus	Taiwan	24	12.2 (13.5)	32.0	1.44
R. latouchii	Taiwan	6	13.7 (11.7)	28.0	1.44
R. arvalis	Luxembourg	6	5.3 (4.3)	12.0	1.12
R. temporaria	Leningrad	6	10.1 (9.6)	24.0	1.32
R. asiatica	Kirghizia	3	4.0 (2.9)	8.0	1.08
R. amurensis	Mongolia	13	1.7 (2.2)	12.0	1.16
〃	Siberia	6	4.6 (5.1)	20.0	1.20
〃	Manchuria	3	*4.8 (*3.4)	*9.5	*1.10
Average		15.6	10.1 (10.1)	33.2	1.43

Parentheses show an expected value. * values at 21 loci

3. Proportion of polymorphic loci

At the 25 loci controlling the enzymes and blood proteins in the 468 brown frogs belonging to the 30 population of the 12 species, the mean proportion of polymorphic loci which contained each allele at the rate of more than 1% was estimated in each population. It was found that the values in all the 30 populations were 8.0~60.0%, 33.2% on the average. The highest value was 60.0% in the Utsunomiya population of *R. japonica*. This was followed by 56.0% in the Shizuoka and

Hiroshima populations of *R. japonica* and the Hiroshima population of *R. ornativentris*, 52.0% in the Fukui population of *R. japonica*, 48.0% in the Fukushima population of *R. japonica* and in the Sapporo population of *R. chensinensis*, 40.0% in the Wakuya population of *R. japonica*, the Siberia population of *R. chensinensis* and the three populations of *R. dybowskii*, *R. tsushimensis* and *R. okinavana*, 36.0% in the Ichinoseki, Toyama and Geihoku populations of *R. japonica* and the Beijing population of *R. chensinensis*, 32.0% in the Oki population of *R. japonica* and the population of *R. longicrus*, 28.0% in the Aomori population of *R. ornativentris* and the population of *R. latouchii*, 24.0% in the Isehara population of *R. japonica* and the population of *R. temporaria*, 20.0% in the Siberia population of *R. amurensis*,

TABLE 7

Genetic identity(I) and genetic distance(D) among 30 populations of

Species	Population	*jap*												
		Mori.	Ichi.	Waku.	Fuku.	Suka.	Utsu.	Iseh.	Shiz.	Toya.	Fukui.	Hiro.	Geih.	Oki
jap.	Morioka	—	0.993	0.987	0.937	0.904	0.964	0.961	0.907	0.942	0.914	0.947	0.922	0.882
〃	Ichinoseki	0.007	—	0.994	0.947	0.917	0.968	0.966	0.915	0.934	0.909	0.941	0.924	0.881
〃	Wakuya	0.013	0.006	—	0.968	0.940	0.985	0.985	0.922	0.934	0.910	0.943	0.927	0.884
〃	Fukushima	0.065	0.055	0.033	—	0.987	0.988	0.981	0.902	0.896	0.883	0.903	0.892	0.869
〃	Sukagawa	0.100	0.087	0.062	0.013	—	0.972	0.964	0.868	0.860	0.848	0.864	0.858	0.834
〃	Utsunomiya	0.037	0.033	0.015	0.013	0.029	—	0.993	0.914	0.934	0.908	0.931	0.917	0.883
〃	Isehara	0.040	0.035	0.015	0.020	0.036	0.007	—	0.918	0.920	0.901	0.927	0.912	0.870
〃	Shizuoka	0.098	0.089	0.081	0.103	0.141	0.089	0.086	—	0.941	0.920	0.964	0.962	0.898
〃	Toyama	0.060	0.068	0.068	0.109	0.150	0.068	0.083	0.060	—	0.982	0.981	0.960	0.923
〃	Fukui	0.090	0.096	0.094	0.125	0.165	0.086	0.104	0.083	0.019	—	0.964	0.949	0.936
〃	Hiroshima	0.055	0.060	0.059	0.102	0.146	0.071	0.076	0.037	0.019	0.037	—	0.986	0.942
〃	Geihoku	0.082	0.079	0.076	0.115	0.153	0.087	0.092	0.039	0.040	0.052	0.014	—	0.933
〃	Oki	0.125	0.126	0.123	0.140	0.182	0.124	0.139	0.107	0.080	0.066	0.060	0.069	—
tsu.	Tsushima	0.973	0.945	0.931	0.893	0.992	0.903	0.927	0.867	0.848	0.804	0.886	0.888	0.900
oki.	Okinawa	0.336	0.351	0.354	0.369	0.419	0.344	0.357	0.352	0.303	0.303	0.294	0.332	0.362
long.	Taiwan	0.320	0.316	0.311	0.314	0.354	0.333	0.321	0.354	0.434	0.480	0.379	0.427	0.460
temp.	Leningrad	0.595	0.596	0.585	0.557	0.615	0.582	0.600	0.738	0.652	0.655	0.662	0.691	0.619
asia.	Kirghizia	0.499	0.500	0.486	0.455	0.478	0.464	0.478	0.584	0.571	0.609	0.584	0.604	0.539
amu.	Mongolia	0.612	0.658	0.658	0.647	0.652	0.660	0.649	0.813	0.725	0.751	0.692	0.771	0.691
〃	Siberia	0.599	0.642	0.641	0.623	0.626	0.640	0.633	0.792	0.715	0.738	0.679	0.753	0.673
〃	Manchuria	0.623	0.670	0.673	0.659	0.664	0.671	0.668	0.830	0.731	0.761	0.706	0.785	0.704
lato.	Taiwan	1.607	1.604	1.605	1.604	1.580	1.591	1.607	1.683	1.636	1.534	1.663	1.688	1.633
orn.	Aomori	0.720	0.718	0.708	0.726	0.768	0.701	0.690	0.768	0.752	0.831	0.779	0.820	0.720
〃	Hiroshima	0.735	0.722	0.721	0.736	0.782	0.722	0.717	0.654	0.646	0.670	0.614	0.616	0.551
chen.	Sapporo	0.631	0.612	0.586	0.550	0.583	0.566	0.553	0.501	0.567	0.552	0.509	0.507	0.410
〃	Beijing	1.065	1.043	1.032	1.024	1.121	1.027	1.019	0.890	0.903	0.815	0.905	0.916	0.762
〃	Siberia	0.701	0.686	0.664	0.682	0.723	0.636	0.636	0.647	0.669	0.724	0.700	0.721	0.685
〃	Manchuria	0.633	0.631	0.620	0.631	0.676	0.625	0.615	0.646	0.703	0.747	0.683	0.717	0.617
dyb.	Tsushima	0.783	0.778	0.772	0.786	0.809	0.773	0.756	0.740	0.812	0.810	0.785	0.795	0.695
arv.	Luxembourg	0.853	0.909	0.914	0.940	0.954	0.889	0.906	1.092	0.937	0.969	0.961	1.060	0.942

Genetic identity(I) is given above the diagonal

16.0% in the Morioka and Sukagawa populations of *R. japonica*, 14.3% in the Manchuria population of *R. chensinensis* (hashima), 12.0% in the population of *R. arvalis* and the Mongolia population of *R. amurensis*, 9.5% in the Manchuria population of *R. amurensis* and 8.0% in the population of *R. asiatica* (Table 6).

4. Number of alleles per locus

The number of alleles per locus was counted at the 25 loci in the 468 brown frogs belonging to the 30 populations of the 12 species distributed in the Palearctic region. It was 1.08~1.92, 1.43 on the average, in the 30 populations of the 12

12 brown frog species distributed in the Palearctic region

tsu.	*oki.*	*long.*	*temp.*	*asia.*	*amu.*			*lato.*	*orn.*		*chen.*				*dyb.*	*arv.*
Tsus.	Okin.	Taiw.	Lenin.	Kirg.	Mong.	Sibe.	Manch.	Taiw.	Aomo.	Hiro.	Sapp.	Beij.	Sibe.	Manch.	Tsus.	Luxem.
0.378	0.714	0.726	0.552	0.607	0.542	0.550	0.536	0.200	0.487	0.480	0.532	0.345	0.496	0.531	0.457	0.426
0.389	0.704	0.729	0.551	0.606	0.518	0.526	0.512	0.201	0.488	0.486	0.542	0.352	0.503	0.532	0.459	0.403
0.394	0.702	0.733	0.557	0.615	0.518	0.527	0.510	0.201	0.493	0.486	0.557	0.356	0.515	0.538	0.462	0.401
0.410	0.692	0.731	0.573	0.635	0.524	0.536	0.517	0.201	0.484	0.479	0.577	0.359	0.506	0.532	0.456	0.391
0.371	0.658	0.702	0.541	0.620	0.521	0.535	0.515	0.206	0.464	0.457	0.558	0.326	0.486	0.509	0.445	0.385
0.405	0.709	0.717	0.559	0.629	0.517	0.527	0.511	0.204	0.496	0.486	0.568	0.358	0.529	0.535	0.462	0.411
0.396	0.700	0.725	0.549	0.620	0.523	0.531	0.513	0.200	0.502	0.488	0.575	0.361	0.529	0.541	0.469	0.404
0.420	0.704	0.702	0.478	0.558	0.443	0.453	0.436	0.186	0.464	0.520	0.606	0.411	0.524	0.524	0.477	0.336
0.428	0.738	0.648	0.521	0.565	0.484	0.489	0.481	0.195	0.471	0.524	0.567	0.406	0.512	0.495	0.444	0.392
0.448	0.738	0.619	0.519	0.544	0.472	0.478	0.467	0.216	0.436	0.512	0.576	0.442	0.485	0.474	0.445	0.379
0.412	0.745	0.685	0.516	0.558	0.501	0.507	0.494	0.190	0.459	0.541	0.601	0.405	0.497	0.505	0.456	0.383
0.411	0.718	0.652	0.501	0.547	0.463	0.471	0.456	0.185	0.440	0.540	0.602	0.400	0.486	0.488	0.452	0.347
0.407	0.696	0.631	0.539	0.583	0.501	0.510	0.495	0.195	0.487	0.577	0.664	0.467	0.504	0.540	0.499	0.390
—	0.476	0.366	0.437	0.346	0.248	0.251	0.248	0.192	0.287	0.280	0.281	0.331	0.334	0.308	0.299	0.180
0.742	—	0.568	0.413	0.520	0.445	0.446	0.435	0.197	0.375	0.417	0.423	0.322	0.382	0.347	0.358	0.263
1.006	0.566	—	0.511	0.632	0.504	0.516	0.497	0.166	0.486	0.448	0.488	0.397	0.489	0.503	0.445	0.423
0.827	0.885	0.671	—	0.552	0.414	0.422	0.416	0.193	0.472	0.447	0.489	0.471	0.456	0.518	0.413	0.476
1.062	0.654	0.458	0.594	—	0.437	0.449	0.439	0.139	0.613	0.530	0.529	0.437	0.583	0.531	0.574	0.393
1.393	0.810	0.686	0.881	0.827	—	0.995	0.993	0.054	0.373	0.363	0.365	0.200	0.310	0.332	0.304	0.421
1.382	0.806	0.662	0.863	0.802	0.005	—	0.992	0.056	0.383	0.373	0.383	0.208	0.316	0.337	0.311	0.421
1.396	0.832	0.699	0.878	0.823	0.007	0.008	—	0.055	0.382	0.368	0.370	0.201	0.309	0.327	0.309	0.423
1.650	1.626	1.793	1.646	1.976	2.913	2.889	2.897	—	0.111	0.104	0.159	0.118	0.119	0.156	0.209	0.107
1.249	0.981	0.721	0.750	0.489	0.987	0.960	0.962	2.202	—	0.852	0.611	0.566	0.626	0.611	0.623	0.381
1.274	0.875	0.803	0.804	0.635	1.014	0.985	0.999	2.266	0.160	—	0.677	0.558	0.509	0.497	0.556	0.361
1.268	0.859	0.717	0.715	0.637	1.007	0.961	0.995	1.838	0.492	0.390	—	0.643	0.528	0.599	0.564	0.495
1.105	1.132	0.924	0.754	0.827	1.607	1.571	1.603	2.134	0.569	0.583	0.442	—	0.441	0.431	0.443	0.388
1.096	0.962	0.715	0.786	0.539	1.171	1.152	1.175	2.126	0.469	0.676	0.638	0.819	—	0.776	0.649	0.409
1.176	1.060	0.686	0.658	0.632	1.104	1.088	1.117	1.857	0.493	0.699	0.512	0.842	0.254	—	0.771	0.453
1.208	1.026	0.810	0.883	0.555	1.190	1.166	1.175	1.564	0.474	0.587	0.573	0.815	0.432	0.260	—	0.435
1.715	1.334	0.860	0.742	0.934	0.865	0.865	0.860	2.234	0.965	1.018	0.704	0.946	0.893	0.792	0.833	—

and genetic distance(D) is given below.

species. The largest number was 1.92 in the Hiroshima population of *R. ornativentris*. The second was 1.76 in the Utsunomiya population of *R. japonica*. This was followed by 1.72 in the Shizuoka population of *R. japonica*, 1.68 in the Fukui and Hiroshima populations of *R. japonica*, 1.64 in the Sapporo population of *R. chensinensis*, 1.56 in the Wakuya and Fukushima populations of *R. japonica*, 1.52 in the Ichinoseki population of *R. japonica* and the populations of *R. tsushimensis* and *R. okinavana*, 1.48 in the Aomori population of *R. ornativentris* and the Siberia population of *R. chensinensis*, 1.44 in the Oki population of *R. japonica* and the populations of *R. longicrus* and *R. latouchii*, 1.40 in the Toyama and Geihoku populations of *R. japonica*, the Beijing population of *R. chensinensis* and the population of *R. dybowskii*, 1.32 in the population of *R. temporaria*, 1.28 in the Isehara population of *R. japonica*, 1.24 in the Manchuria population of *R. chensinensis* (hashima), 1.20 in the Siberia population of *R. amurensis*, 1.16 in the Morioka and Sukagawa populations of *R. japonica* and the Mongolia population of *R. amurensis*, 1.12 in the population of *R. arvalis* and 1.10 in the Manchuria population of *R. amurensis*. The smallest number of alleles was 1.08 in the population of *R. asiatica* (Table 6).

IV. Genetic distances

Genetic distances among different populations were estimated from gene frequencies at the 25 loci examined in the 468 brown frogs belonging to the 30 populations of the 12 species. Of these 12 species, eight including *R. japonica, R. tsushimensis, R. okinavana, R. longicrus, R. temporaria, R. asiatica, R. amurensis* and *R. latouchii* had 26 chromosomes in diploid number, while the other four species including *R. ornativentris, R. chensinensis, R. dybowskii* and *R. arvalis* had 24 chromosomes in diploid number.

1. Genetic distance between different populations of the same species

The genetic distances among the 13 populations of *R. japonica* were 0.006~0.182. It was found that these populations were divided into the northern group including seven populations, the Morioka, Ichinoseki, Wakuya, Fukushima, Sukagawa, Utsunomiya and Isehara, and the southern group including six populations, the Shizuoka, Toyama, Fukui, Hiroshima, Geihoku and Oki. The genetic distances among seven northern populations were 0.006~0.100, 0.034 on the average, while those among six southern populations were 0.014~0.107, 0.052 on the average. The genetic distances between the northern and southern populations were 0.055~0.182, 0.102 on the average (Table 7).

The genetic distance between the Aomori and Hiroshima populations of *R. ornativentris* was 0.160, while those among the three populations, the Mongolia, Siberia and Manchuria, of *R. amurensis* were 0.005~0.008, 0.007 on the average.

Among the four populations, the Sapporo, Beijing, Siberia and Manchuria, of *R. chensinensis*, the genetic distances were 0.254~0.842, 0.585 on the average. The largest was 0.842 between the Beijing and Manchuria populations. This was

followed by 0.819 between the Beijing and Siberia populations, 0.638 between the Sapporo and Siberia populations, 0.512 between the Sapporo and Manchuria populations, and 0.442 between the Sapporo and Beijing populations. The smallest was 0.254 between the Siberia and Manchuria populations (Table 7).

2. Genetic distance between different species

a. Among the eight species having 26 chromosomes

Among the eight species having 26 chromosomes, *R. japonica*, *R. tsushimensis*, *R. okinavana*, *R. longicrus*, *R. temporaria*, *R. asiatica*, *R. amurensis* and *R. latouchii*, the smallest genetic distance was 0.294 between the Hiroshima population of *R. japonica* and the population of *R. okinavana*. The largest genetic distance was 2.913 between the Mongolia population of *R. amurensis* and the population of *R. latouchii* (Table 7).

i) The genetic distances between *R. japonica* (13 populations) and *R. okinavana* were 0.294~0.419, 0.344 on the average, those between *R. japonica* (13 populations) and *R. longicrus* were 0.311~0.480, 0.369 on the average, those between *R. japonica* (13 populations) and *R. asiatica* were 0.455~0.609, 0.527 on the average, and those between *R. japonica* (13 populations) and *R. amurensis* (three populations) were 0.599~0.830, 0.689 on the average. The genetic distances between *R. japonica* (13 populations) and *R. temporaria* were 0.557~0.738, 0.627 on the average, those between *R. japonica* (13 populations) and *R. tsushimensis* were 0.804~0.992, 0.904 on the average, and those between *R. japonica* (13 populations) and *R. latouchii* were 1.534~1.688, 1.618 on the average (Table 7).

ii) The genetic distance between *R. okinavana* and *R. longicrus* was 0.566, that between *R. okinavana* and *R. asiatica* was 0.654, that between *R. okinavana* and *R. tsushimensis* was 0.742, and those between *R. okinavana* and *R. amurensis* (three populations) were 0.806~0.832, 0.816 on the average. The genetic distance between *R. okinavana* and *R. temporaria* was 0.885, and that between *R. okinavana* and *R. latouchii* was 1.626 (Table 7).

iii) The genetic distance between *R. longicrus* and *R. asiatica* was 0.458, that between *R. longicrus* and *R. okinavana* was 0.566, that between *R. longicrus* and *R. temporaria* was 0.671, and those between *R. longicrus* and *R. amurensis* (three populations) were 0.662~0.699, 0.682 on the average. The genetic distance between *R. longicrus* and *R. tsushimensis* was 1.006, and that between *R. longicrus* and *R. latouchii* was 1.793 (Table 7).

iv) The genetic distances between *R. asiatica* and *R. amurensis* (three populations) were 0.802~0.827, 0.817 on the average, that between *R. asiatica* and *R. temporaria* was 0.594, that between *R. asiatica* and *R. tsushimensis* was 1.062, and that between *R. asiatica* and *R. latouchii* was 1.976 (Table 7).

v) The genetic distances between *R. amurensis* (three populations) and *R. temporaria* were 0.863~0.881, 0.874 on the average, those between *R. amurensis* (three populations) and *R. tsushimensis* were 1.382~1.396, 1.390 on the average, and those between *R. amurensis* (three populations) and *R. latouchii* were 2.889 ~2.913, 2.900 on the average.

vi) The genetic distance between *R. temporaria* and *R. tsushimensis* was 0.827 and that between *R. temporaria* and *R. latouchii* was 1.646.

vii) The genetic distance between *R. tsushimensis* and *R. latouchii* was 1.650 (Table 7).

b. Among the four species having 24 chromosomes

Among the four species having 24 chromosomes, *R. chensinensis, R. ornativentris, R. dybowskii* and *R. arvalis*, the smallest genetic distance was 0.260 between the Manchuria population of *R. chensinensis* (hashima) and the population of *R. dybowskii*. The largest genetic distance was 1.018 between the Hiroshima population of *R. ornativentris* and the population of *R. arvalis*.

i) The genetic distances between *R. chensinensis* (Sapporo population) and *R. ornativentris* (two populations) were 0.390 and 0.492, 0.441 on the average, that between *R. chensinensis* (Sapporo population) and *R. dybowskii* was 0.573, and that between *R. chensinensis* (Sapporo population) and *R. arvalis* was 0.704.

ii) The genetic distances between *R. chensinensis* (Beijing population) and *R. ornativentris* (two populations) were 0.569 and 0.583, 0.576 on the average, that between *R. chensinensis* (Beijing population) and *R. dybowskii* was 0.815, and that between *R. chensinensis* (Beijing population) and *R. arvalis* was 0.946.

iii) The genetic distances between *R. chensinensis* (Siberia population) and *R. ornativentris* (two populations) were 0.469 and 0.676, 0.573 on the average, that between *R. chensinensis* (Siberia population) and *R. dybowskii* was 0.432, and that between *R. chensinensis* (Siberia population) and *R. arvalis* was 0.893.

iv) The genetic distances between *R. chensinensis* (Manchuria population) and *R. ornativentris* (two populations) were 0.493 and 0.699, 0.596 on the average, that between *R. chensinensis* (Manchuria population) and *R. dybowskii* was 0.260, and that between *R. chensinensis* (Manchuria population) and *R. arvalis* was 0.792 (Table 7).

v) The genetic distances between *R. dybowskii* and *R. ornativentris* (two populations) were 0.474 and 0.587, 0.531 on the average, and that between *R. dybowskii* and *R. arvalis* was 0.833 (Table 7).

vi) The genetic distances between *R. arvalis* and *R. ornativentris* (two populations) were 0.965 and 1.018, 0.992 on the average.

c. Between the eight species with 26 chromosomes and the four species with 24 chromosomes

Of the genetic distances between the eight species having 26 chromosomes and the four species having 24 chromosomes, the smallest was 0.410 between the Oki population of *R. japonica* and the Sapporo population of *R. chensinensis*, while the largest was 2.266 between the Hiroshima population of *R. ornativentris* and the population of *R. latouchii* (Table 7).

i) The genetic distances between *R. japonica* (13 populations, 2n=26) and *R. ornativentris* (Aomori population, 2n=24) were 0.690~0.831, 0.746 on the average, and those between *R. japonica* (13 populations, 2n=26) and *R. ornativentris*

(Hiroshima population, 2n=24) were 0.551~0.782, 0.684 on the average. The genetic distances between *R. japonica* (13 populations, 2n=26) and *R. chensinensis* (Sapporo population, 2n=24) were 0.410~0.631, 0.548 on the average, those between *R. japonica* (13 populations, 2n=26) and *R. chensinensis* (Beijing population, 2n=24) were 0.762~1.121, 0.963 on the average, those between *R. japonica* (13 populations, 2n=26) and *R. chensinensis* (Siberia population, 2n=24) were 0.636~0.724, 0.683 on the average, and those between *R. japonica* (13 populations, 2n=26) and *R. chensinensis* (Manchuria population, 2n=24) were 0.615~0.747, 0.657 on the average. The genetic distances between *R. japonica* (13 populations, 2n=26) and *R. dybowskii* (2n=24) were 0.695~0.812, 0.776 on the average, and those between *R. japonica* (13 populations, 2n=26) and *R. arvalis* (2n=24) were 0.853~1.092, 0.948 on the average (Table 7).

ii) The genetic distances between *R. ornativentris* (two populations, 2n=24) and *R. asiatica* (one population, 2n=26) were 0.489 and 0.635, 0.562 on the average, those between *R. ornativentris* (two populations, 2n=24) and *R. longicrus* (one population, 2n=26) were 0.721 and 0.803, 0.762 on the average, those between *R. ornativentris* (two populations, 2n=24) and *R. temporaria* (one population, 2n=26) were 0.750 and 0.804, 0.777 on the average, those between *R. ornativentris* (two populations, 2n=24) and *R. okinavana* (one population, 2n=26) were 0.875 and 0.981, 0.928 on the average, those between *R. ornativentris* (two populations, 2n=24) and *R. amurensis* (three populations, 2n=26) were 0.960~1.014, 0.985 on the average, those between *R. ornativentris* (two populations, 2n=24) and *R. tsushimensis* (one population, 2n=26) were 1.249 and 1.274, 1.262 on the average, and those between *R. ornativentris* (two populations, 2n=24) and *R. latouchii* (one population, 2n=26) were 2.202 and 2.266, 2.234 on the average.

iii) The genetic distance between the Sapporo population of *R. chensinensis* (2n=24) and *R. asiatica* (2n=26) was the smallest, being 0.637, among those between the Sapporo population and the species having 26 chromosomes, except 0.410~0.631, 0.548 on the average, between the Sapporo population of *R. chensinensis* and 13 populations of *R. japonica*. Those between the Sapporo population (2n=24) and *R. temporaria* (2n=26), between the Sapporo population and *R. longicrus* (2n=26), between the Sapporo population and *R. okinavana* (2n=26), between the Sapporo population and *R. amurensis* (three populations, 2n=26), between the Sapporo population and *R. tsushimensis* (2n=26), and between the Sapporo population and *R. latouchii* (2n=26) were 0.715, 0.717, 0.859, 0.988, 1.268 and 1.838, respectively (Table 7).

The genetic distances between the Beijing population of *R. chensinensis* and *R. temporaria*, between the Beijing population and *R. asiatica*, between the Beijing population and *R. longicrus*, between the Beijing population and *R. japonica* (13 populations), between the Beijing population and *R. tsushimensis*, between the Beijing population and *R. okinavana*, between the Beijing population and *R. amurensis* (three populations), and between the Beijing population and *R. latouchii* were 0.754, 0.827, 0.924, 0.963, 1.105, 1.132, 1.594 and 2.134, respectively (Table 7).

The genetic distances between the Siberia population of *R. chensinensis* and *R. asiatica* (2n=26), between the Siberia population and *R. japonica* (13 populations, 2n=26), between the Siberia population and *R. longicrus* (2n=26), between the Siberia population and *R. temporaria* (2n=26), between the Siberia population and *R. okinavana* (2n=26), between the Siberia population and *R. tsushimensis* (2n=26), between the Siberia population and *R. amurensis* (three populations, 2n=26), and between the Siberia population and *R. latouchii* (2n=26) were 0.539, 0.683, 0.715, 0.786, 0.962, 1.096, 1.166 and 2.126, respectively (Table 7).

The genetic distances between the Manchuria population (2n=24) of *R. chensinensis* (hashima) and *R. asiatica* (2n=26), between the Manchuria population and *R. japonica* (13 populations, 2n=26), between the Manchuria population and *R. temporaria* (2n=26), between the Manchuria population and *R. longicrus* (2n=26), between the Manchuria population and *R. okinavana* (2n=26), between the Manchuria population and *R. amurensis* (three populations, 2n=26), between the Manchuria population and *R. tsushimensis* (2n=26), and between the Manchuria population and *R. latouchii* (2n=26) were 0.632, 0.657, 0.658, 0.686, 1.060, 1.103, 1.176 and 1.857, respectively (Table 7).

iv) The genetic distances between *R. dybowskii* (2n=24) and *R. asiatica* (2n=26), between *R. dybowskii* and *R. japonica* (13 populations, 2n=26), between *R. dybowskii* and *R. longicrus* (2n=26), between *R. dybowskii* and *R. temporaria* (2n=26), between *R. dybowskii* and *R. okinavana* (2n=26), between *R. dybowskii* and *R. amurensis* (three populations, 2n=26), between *R. dybowskii* and *R. tsushimensis* (2n=26), and between *R. dybowskii* and *R. latouchii* (2n=26) were 0.555, 0.776, 0.810, 0.883, 1.026, 1.177, 1.208 and 1.564, respectively.

v) The genetic distances between *R. arvalis* (2n=24) and *R. temporaria* (2n=26), between *R. arvalis* and *R. longicrus* (2n=26), between *R. arvalis* and *R. amurensis* (three populations, 2n=26), between *R. arvalis* and *R. asiatica* (2n=26), between *R. arvalis* and *R. japonica* (13 populations, 2n=26), between *R. arvalis* and *R. okinavana* (2n=26), between *R. arvalis* and *R. tsushimensis* (2n=26), and between *R. arvalis* and *R. latouchii* (2n=26) were 0.742, 0.860, 0.863, 0.934, 0.948, 1.334, 1.715 and 2.234, respectively (Table 7).

V. Dendrogram

A dendrogram for the brown frogs of 30 populations of 12 species distributed in the Palearctic region was drawn on the basis of the genetic distances among them by unweighted pair-group arithmetic average (UPGMA) clustering method (Sneath and Sokal, 1973; Nei, 1975). The results showed that *R. latouchii* (2n=26) first deviated from the other brown frogs. The deviation of this species was followed by *R. tsushimensis* (2n=26) and then by *R. arvalis* (2n=24). Thereafter, the brown frogs belonging to 27 populations of nine species were divided into two large groups, one having 26 chromosomes and the other having 24 chromosomes in diploid number. The former group including 20 populations of six species was divided in order into three populations of *R. amurensis*, *R. temporaria*, *R.*

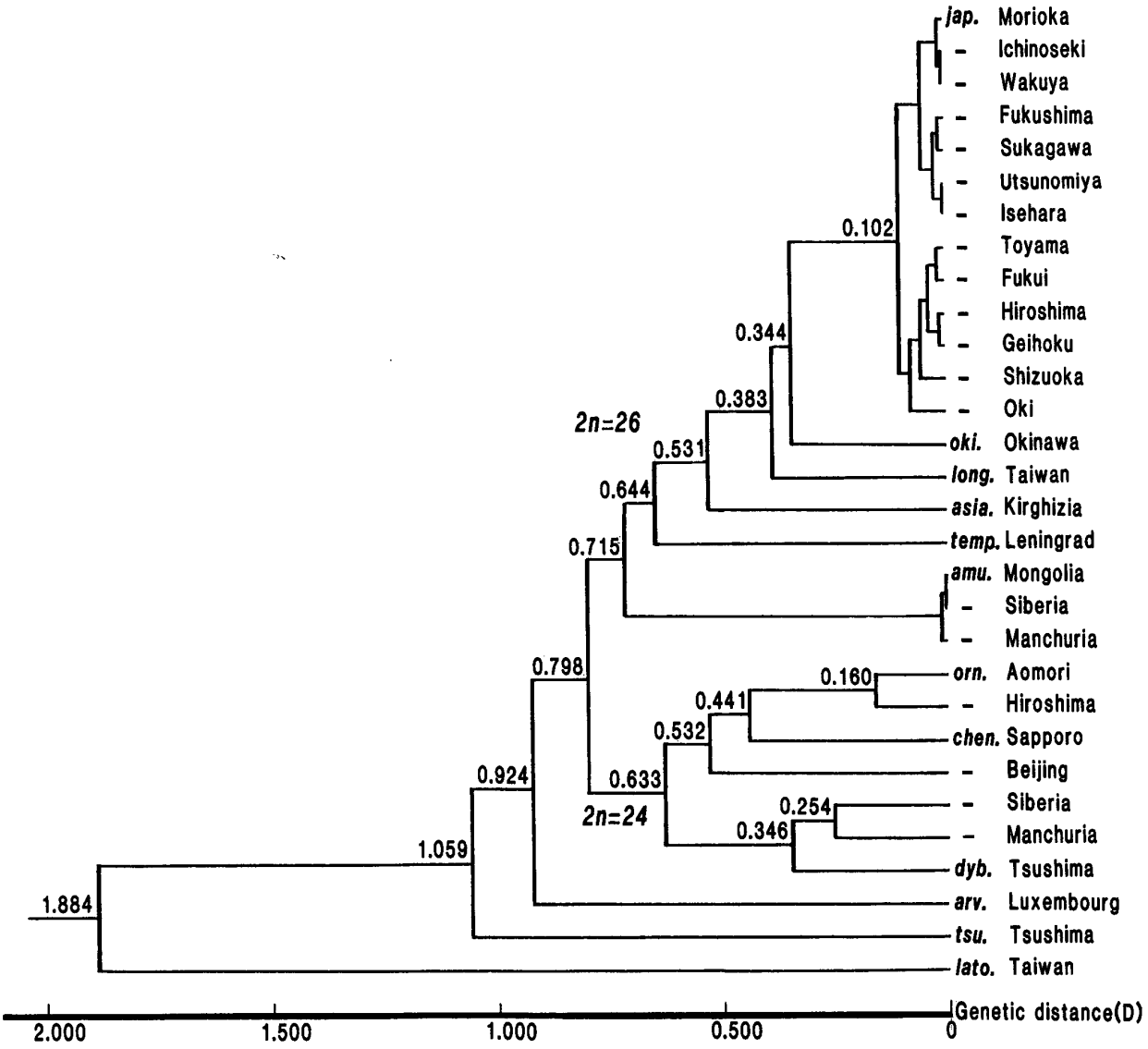

Fig. 16. Dendrogram for 30 populations of brown frogs in the Palearctic region based on genetic distances.

asiatica, R. longicrus, R. okinavana and *R. japonica.* It was found that *R. japonica* was then divided into 13 populations consisting of northern seven populations and southern six populations (Fig. 16).

The group having 24 chromosomes in diploid number was divided into two subgroups. One of the latter was divided in order into *R. dybowskii* and the Manchuria (hashima) and Siberia populations of *R. chensinensis.* The other subgroup was divided in order into the Beijing and Sapporo populations of *R. chensinensis* and the two populations of *R. ornativentris* (Fig. 16).

DISCUSSION

In the present study, 15 kinds of enzymes and three kinds of blood proteins were analyzed in 30 populations of 12 brown frog species distributed in the Palearctic

region by the method of starch-gel electrophoresis in order to elucidate the genetic differentiation of these populations. These populations contain 13 of *R. japonica*, two of *R. ornativentris*, four (the Sapporo, Beijing, Siberia and Manchuria) of *R. chensinensis*, one (the Tsushima) of *R. dybowskii*, one (the Tsushima) of *R. tsushimensis*, one (the Okinawa) of *R. okinavana*, one (the Taiwan) of *R. longicrus*, one (the Taiwan) of *R. latouchii*, one (the Luxembourg) of *R. arvalis*, one (the Leningrad) of *R. temporaria*, one (the Kirghizia) of *R. asiatica*, and three (the Mongolia, Siberia and Manchuria) of *R. amurensis*. It has been reported by many investigators that there are two kinds of brown frog species in diploid chromosome number, one being 26 and the other being 24. The chromosome number of 26 was ascertained in the following eight species, *R. japonica* (KAWAMURA, 1939; KOBAYASHI, 1962; SETO, 1965; KURAMOTO *et al.*, 1974; SUMIDA, 1981; WANG *et al.*, 1983; HENG, 1984; TAN *et al.*, 1986; NISHIOKA *et al.*, 1987; IIZUKA, 1989), *R. tsushimensis* (DAITO, 1967; KAWAMURA and NISHIOKA, 1973; NISHIOKA *et al.*, 1987), *R. okinavana* (KURAMOTO, 1972), *R. longicrus* (KURAMOTO *et al.*, 1974), *R. latouchii* (KURAMOTO, 1980; SETO *et al.*, 1982), *R. temporaria* (WITSCHI, 1924; GUILLEMIN, 1967; MORESCALCHI, 1967; ULLERICH, 1967; IVANOV and MADYANOV, 1973; SCHMID, 1978; SCHEMPP and SCHMID, 1981; NISHIOKA *et al.*, 1987), *R. asiatica* (MASIK *et al.*, 1976; ORLOVA *et al.*, 1977) and *R. amurensis* (ORLOVA *et al.*, 1977; KAWAMURA and NISHIOKA, 1973; KURAMOTO, 1980; WU and YIN, 1983; NISHIOKA *et al.*, 1987).

In contrast, the diploid number of 24 was counted in the remaining four species, *R. ornativentris* (KOBAYASHI, 1962; SETO, 1965; GREEN, 1983; NISHIOKA *et al.*, 1987; IIZUKA, 1989), *R. chensinensis* (KOBAYASHI, 1962; SETO, 1965; ORLOVA *et al.*, 1977; WU, 1981; GREEN, 1983; JIANG *et al.*, 1984; LUO and LI, 1985; TAN *et al.*, 1986; NISHIOKA *et al.*, 1987), *R. dybowskii* (DAITO, 1967; KAWAMURA and NISHIOKA, 1973; GREEN, 1983; NISHIOKA *et al.*, 1987), and *R. arvalis* (WITSCHI *et al.*, 1958; MORESCALCHI, 1967; ULLERICH, 1967; IVANOV and MANDYANOV, 1973; MÉSZÁROS, 1973; GRAFODATSKY *et al.*, 1978; GREEN, 1983).

When karyotypes of *R. japonica, R. tsushimensis, R. amurensis coreana, R. temporaria* and *R. sylvatica*, which have 26 chromosomes in diploid number, were compared with those of *R. ornativentris, R. chensinensis* and *R. dybowskii* from Tsushima and Korea, which have 24 chromosomes in diploid number with Giemsa staining (NISHIOKA, OKUMOTO, UEDA and RYUZAKI, 1987), it was found that chromosomes Nos. 1~10 and 12 in the frogs having 26 chromosomes were nearly the same as chromosomes Nos. 1~5 and 7~12 in the frogs having 24 chromosomes. Chromosome No. 6 in the latter species seemed to have been formed by fusion of chromosomes Nos. 11 and 13 in the former species. Later, NISHIOKA, MIURA, BORKIN and WU (1986) also reported on *R. japonica* from Hiroshima and Miyagi, *R. tsushimensis* and *R. temporaria*, which have 26 chromosomes in diploid number, *R. ornativentris, R. dybowskii* from Tsushima and *R. chensinensis* from Hokkaido, Beijing and Siberia, which have 24 chromosomes in diploid number, that chromosomes Nos. 1~11 in the former species were nearly the same as chromosomes Nos. 1~5 and 7~12 in the latter species by the analyses of C-banding and late replication

banding patterns. Chromosome No. 6 in the frogs with 24 chromosomes seemed to have been formed by fusion of chromosomes Nos. 12 and 13 of the frogs having 26 chromosomes.

A dendrogram drawn for the brown frogs of the 30 populations of the 12 species distributed in the Palearctic region on the basis of the genetic distances by UPGMA clustering method seems to show the origin and differentiation of these brown frogs. Their ancestor seems to have been 26 in diploid chromosome number. From this ancestor, *R. latouchii* was first differentiated in a very old age, next *R. tsushimensis* and thereafter *R. arvalis* were derived from the remaining stock. The former two species had 26 and the latter one species had 24 chromosomes in diploid number. The remaining stock was divided into two groups, one of which had still 26 chromosomes, while the other had 24 chromosomes by fusion of chromosomes Nos. 12 and 13. The first population of the group having 24 chromosomes was *R. arvalis* distributed in Europe. *R. latouchii, R. dybowskii* and *R. arvalis* have 14, nine and seven unique genes at 25 loci, respectively. This seems to show that these three species are very old in origin. The group having 24 chromosomes other than *R. arvalis* was differentiated into three species including *R. ornativentris* in Japan, *R. chensinensis* distributed in Hokkaido (the Sapporo population), China (the Beijing population), Siberia and Manchuria, and *R. dybowskii* distributed in Tsushima.

The present study shows that the three populations, the Sapporo, Beijing and Siberia, of *R. chensinensis* are far separated from each other by large genetic distances. The genetic distances between the Sapporo and Beijing populations, between the Sapporo and Siberia populations, and between the Beijing and Siberia populations were 0.442, 0.638 and 0.819, respectively, while that the Siberia and Manchuria populations was 0.254. KALEZIĆ and HEDGECOCK (1979) showed mean values of genetic distance for three levels of comparison in three species of *Triturus* as follows. Genetic distances between local populations, between subspecies and between species were 0.031 ± 0.017, 0.347 and 0.906 ± 0.058, respectively. According to HEDGECOCK (1976), the genetic distances between two subspecies, *Taricha t. torosa* and *T. t. sierrae,* were 0.162~0.309. When the genetic distances between the populations of *R. chensinensis* are compared with those of *Triturus* and *Taricha,* the genetic distances among the three populations, the Sapporo, Beijing and Siberia, of *R. chensinensis* seem to be larger than those between the different subspecies of *Triturus* and *Taricha,* except that between the Siberia and Manchuria populations, both of which are geographically near to each other. The genetic distance between the latter two populations seems to correspond to a subspecific difference.

KAWAMURA, NISHIOKA, UEDA, BORKIN and WU (1985) preliminarily reported from the results of their crossing experiments that the Hokkaido, Beijing and Siberia populations of *R. chensinensis* are reproductively isolated from each other. While the hybrids between the different populations usually showed incomplete gametic isolation and hybrid inviability, a small number of hybrids attained sexual maturity. This seems to show that the three populations are somewhat nearly

related to one another. However, all the hybrids became completely sterile males. As these populations are reproductively isolated, each of them seems to be a different species.

The genetic distances between *R. chensinensis* (four populations) and *R. ornativentris* (two populations) were 0.390~0.699. While those between three populations, the Sapporo, Beijing and Siberia, of *R. chensinensis* and *R. dybowskii* were 0.432~0.815, that between the Manchuria population of *R. chensinensis* and *R. dybowskii* was 0.260, which seems to be a subspecific size.

Of the group having 26 chromosomes, *R. amurensis* was first differentiated in Mongolia, Siberia and Manchuria, after *R. tsushimensis*. The differentiation of *R. amurensis* was followed one after another by *R. temporaria* in Europe, *R. asiatica* in Kirghizia, *R. longicrus* in Taiwan, *R. okinavana* in Okinawa, and *R. japonica* in Japan. The genetic distances among the three populations of *R. amurensis* were 0.005~0.008, and those between *R. temporaria* and the three populations of *R. amurensis* were 0.863~0.881, while those between *R. asiatica* and the latter were 0.802~0.827. The genetic distances between *R. temporaria* and *R. asiatica*, between *R. longicrus* and *R. temporaria*, between *R. okinavana* and *R. longicrus*, and between *R. tsushimensis* and *R. okinavana* were 0.594, 0.671, 0.566 and 0.742, respectively.

The genetic distances among 13 populations of *R. japonica* were 0.006~0.182. While the seven populations east of Isehara were 0.006~0.900 in genetic distance from one another, the genetic distances between these seven populations and the five west of Shizuoka except the Oki were 0.055~0.165. In accordance with such differences in genetic distance, there were remarkable differences in the morphology of chromosomes between the eastern and western populations of *R. japonica*. SUMIDA (1981) ascertained that there were distinct differences between the eastern and western populations in the lengths of the short arms of chromosomes Nos. 6 and 9, when the karyotypes were analyzed by the method of Giemsa staining. Moreover, SUMIDA and NISHIOKA (1991) found that the eastern populations were clearly isolated from the western ones by various isolating mechanisms such as male preponderance in hybrids and hybrid sterility. In accordance with such differences, the present study clarified that the eastern populations remarkably differed from the western ones in the kind of alleles at the Ab, Hb–II and Pep–A loci.

The genetic distances between the Oki population and the other 12 populations of *R. japonica* were comparatively large, being 0.060~0.182. It seems to be clear that the Oki population is genetically separated to some degree from the other populations in accordance with the geographical isolation of Oki Islands.

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