

Biochemical Differentiation of Pond Frogs Distributed in the Palearctic Region

By

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ABSTRACT

Eighteen kinds of enzymes and two kinds of blood proteins extracted from 350 frogs belonging to three populations (Hiroshima, Suwon and Beijing) of *Rana nigromaculata* HALLOWELL, two populations (Konko and Maibara) of *R. b. brevipoda* ITO and one population (Utsunomiya) of *R. brevipoda porosa* (COPE), two populations (Beijing and Shandong) of *R. p. plancyi* LATASTE, one population (Suwon) of *R. plancyi chosenica* OKADA, one population (Chiayi) of *R. plancyi fukienensis* POPE, three populations (Luxembourg, Poland and Firenze) of *R. lessonae* CAMERANO, three populations (Wien, Ukraine and Heidelberg) of *R. esculenta* LINNAEUS and three populations (Belgorod, Roscoff and Adana) of *R. ridibunda* PALLAS distributed in the Palearctic region were analyzed by the method of horizontal starch-gel electrophoresis. These enzymes and blood proteins were controlled by 1~15 (5.3) alleles which produced 1~25 (8.5) phenotypes. The mean proportions of heterozygous loci per individual were 2.2~57.7% (14.8%) and the mean proportions of polymorphic loci were 3.9~61.5% (30.6%) in the 19 populations belonging to the six species and three subspecies. The mean numbers of alleles per locus were 1.04~1.88 (1.36) at the 26 loci of the 19 populations.

In the East, the genetic distances between *R. nigromaculata* and *R. brevipoda* and between *R. nigromaculata* and the three populations (Beijing, Shandong and Suwon) of *R. plancyi* were 0.431~0.684 (0.523) and 0.500~0.838 (0.669), respectively, while those between *R. brevipoda* and the latter were 0.638~0.772 (0.717). In contrast, the genetic distances between *R. plancyi fukienensis* and *R. nigromaculata* and between the former and *R. brevipoda* were 0.439~0.571 (0.498) and 0.293~0.381 (0.344), respectively. It was especially interesting that *R. brevipoda* is nearly related to *R. plancyi fukienensis*.

In Europe, the genetic distances between *R. esculenta* and *R. lessonae*, between *R. esculenta* and *R. ridibunda*, and between *R. lessonae* and *R. ridibunda* were 0.164~0.245 (0.197), 0.165~0.289 (0.231) and 0.537~0.688 (0.609), respectively. The genetic distances between the three species in the Far East and the three species in Europe were 1.183~2.173 (1.647).

The dendrogram drawn for the pond frogs distributed in the Palearctic region shows that it agrees well on the whole with the prevailing classification.

INTRODUCTION

In the Palearctic region, it has been recently recognized that there are two

groups, European and Far Eastern, of pond, water or green frogs. The European group contains five species: *Rana esculenta* LINNAEUS, *R. lessonae* CAMERANO, *R. ridibunda* PALLAS, *R. perezi* SEOANE and *R. saharica* BOULENGER. Of the five species, *R. esculenta* has been generally recognized to be a hybrid between *R. lessonae* and *R. ridibunda* since BERGER (1966). *R. perezi* was often being treated as a subspecies of *R. ridibunda*, and *R. saharica* was considered to be a synonym of *R. perezi*, prior to UZZELL (1982). The Far Eastern group of pond frogs contains three species and three subspecies, *R. nigromaculata* HALLOWELL, *R. b. brevipoda* ITO and *R. brevipoda porosa* (COPE) in Japan, *R. plancyi chosenica* OKADA in Korea, *R. plancyi fukienensis* POPE in Taiwan and *R. p. plancyi* LATASTE in China.

NISHIOKA, SUMIDA and OHTANI (1992) have reported on the differentiation of 47 populations of *R. nigromaculata* and 23 populations of *R. brevipoda* distributed in Japan clarified by the method of electrophoretic analyses of enzymes and blood proteins. They presumed that *R. brevipoda* invaded Japan earlier than *R. nigromaculata* and produced *R. brevipoda porosa* by frequent hybridization with *R. nigromaculata* mainly at the Shibata district situated in the most snowy area along the Sea of Japan and introgression of *R. nigromaculata* genes for a long period.

In the present study, the genetic relationships among the pond frogs distributed in the Far East and Europe were clarified by analyzing the enzymes and blood proteins extracted from 350 frogs. These frogs belonging to *R. nigromaculata* were collected from Japan (Hiroshima), Korea (Suwon) and China (Beijing), *R. brevipoda* were from Japan (Konko, Maibara and Utsunomiya), *R. plancyi* were from China (Beijing and Shandong), Korea (Suwon) and Taiwan (Chiayi), *R. lessonae* were from Luxembourg, Poland and Italy (Firenze), *R. esculenta* were from Austria (Wien), Ukraine and Germany (Heidelberg) and *R. ridibunda* were from Ukraine (Belgorod), France (Roscoff) and Turkey (Adana).

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 12 brown frog species by NISHIOKA, SUMIDA, BORKIN and WU (1992).

MATERIALS AND METHODS

A total of 350 pond frogs belonging to 19 populations of six species and three subspecies was utilized in electrophoretic analyses (Table 1; Fig. 1). The species or subspecies, collecting stations, and collectors of frogs are as follows.

1. *Rana nigromaculata* HALLOWELL, from the suburbs of Hiroshima, Japan.
2. *Rana nigromaculata* HALLOWELL, from the suburbs of Suwon, Korea, collected by Dr. M. KURAMOTO in 1980.

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

Species	Country	Station	No. of frogs
<i>Rana nigromaculata</i>	Japan	Hiroshima	38
"	Korea	Suwon	38
"	China	Beijing	21
<i>Rana brevipoda brevipoda</i>	Japan	Konko	38
"	"	Maibara	46
<i>Rana brevipoda porosa</i>	"	Utsunomiya	56
<i>Rana plancyi chosenica</i>	Korea	Suwon	33
<i>Rana plancyi plancyi</i>	China	Beijing	24
"	"	Shandong	12
<i>Rana plancyi fukienensis</i>	Taiwan	Chiayi	7
<i>Rana lessonae</i>	Luxembourg		10
"	Poland		9
"	Italy	Firenze	6
<i>Rana esculenta</i>	Austria	Wien	1
"	Ukraine		1
"	Germany	Heidelberg	2
<i>Rana ridibunda</i>	Ukraine	Belgorod	2
"	France	Roscoff	3
"	Turkey	Adana	3
Total			350

3. *Rana nigromaculata* HALLOWELL, from the suburbs of Beijing, China, collected by Dr. Chih-Ye CHANG in 1979.
4. *Rana brevipoda brevipoda* ITO (Typical race), from Konko, Japan.
5. *Rana brevipoda brevipoda* ITO (Nagoya race), from Maibara, Japan.
6. *Rana brevipoda porosa* (COPE), from Utsunomiya, Japan.
7. *Rana plancyi chosenica* OKADA, from Suwon, Korea, collected by Dr. M. KURAMOTO in 1980.
8. *Rana plancyi plancyi* LATASTE, from the suburbs of Beijing, China, collected by Dr. Chih-Ye CHANG in 1979.
9. *Rana plancyi plancyi* LATASTE, from the suburbs of Shandong, China, collected by Dr. Zheng'an WU in 1980.
10. *Rana plancyi fukienensis* POPE, from the suburbs of Chiayi, Taiwan, collected by Mr. S. LIM in 1976.
11. *Rana lessonae* CAMERANO, from Luxembourg, collected by Mr. R. THORN in 1975.
12. *Rana lessonae* CAMERANO, from Poland, sent to Drs. R. Verhoeff-de FREMERY in 1978.
13. *Rana lessonae* CAMERANO, from Firenze, Italy, collected by Dr. B. LANZER (sent in 1977).
14. *Rana esculenta* LINNAEUS, from Wien, Austria, collected by Mr. SEAGER (sent

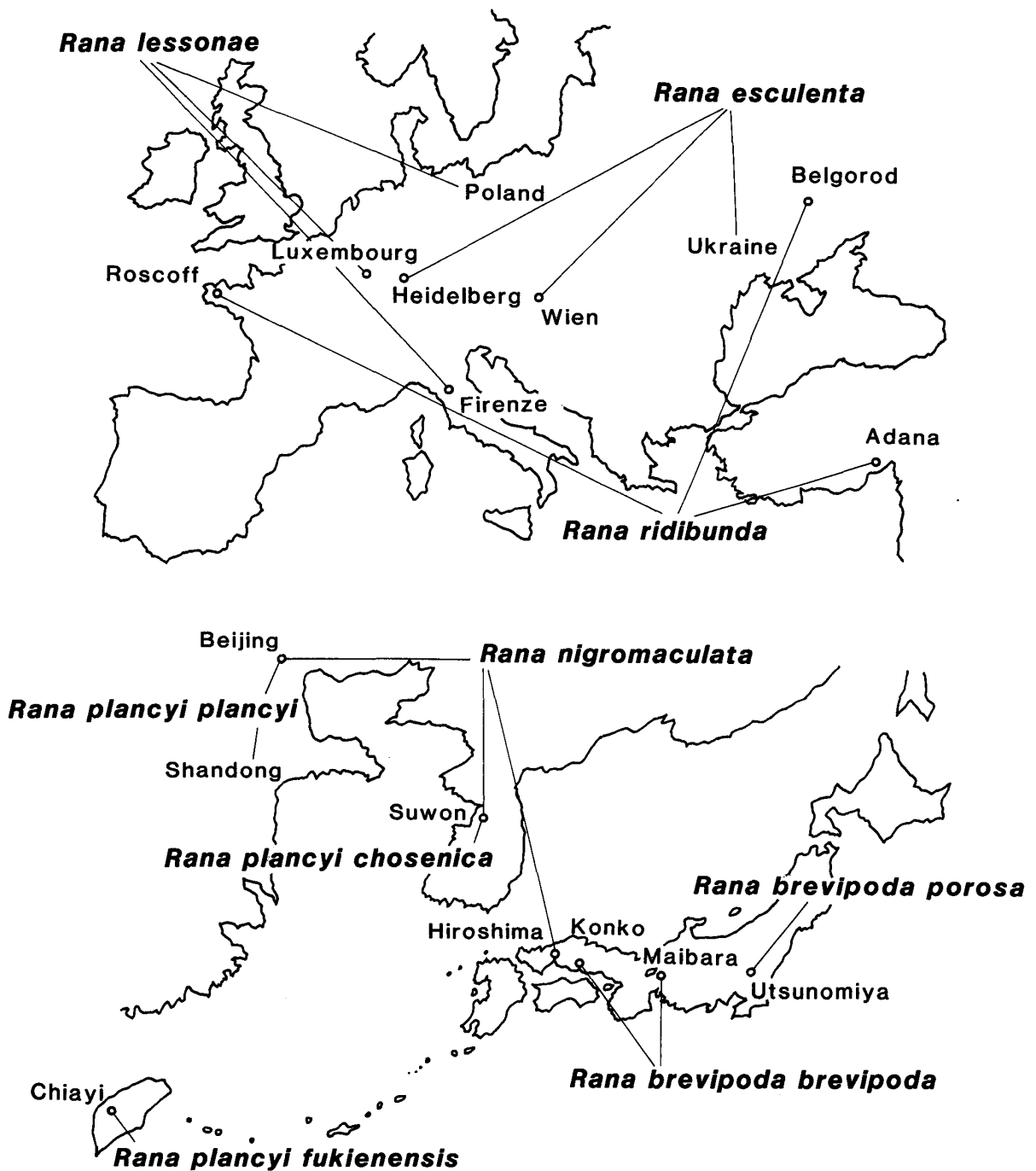


Fig. 1. Map showing localities of 19 populations of pond frogs used in this study.

to Dr. M. KURAMOTO in 1977).

15. *Rana esculenta* LINNAEUS, from Ukraine, collected by Dr. L. J. BORKIN (sent to Dr. M. KURAMOTO in 1976).
16. *Rana esculenta* LINNAEUS, from Heidelberg, Germany, collected by Dr. K. BACHMANN (sent in 1977).
17. *Rana ridibunda* PALLAS, from Belgorod, Ukraine, collected by Dr. L. J. BORKIN (sent to Dr. M. KURAMOTO in 1976).
18. *Rana ridibunda* PALLAS, from Roscoff, France, collected by Dr. J. VASSOROT in

1974.

19. *Rana ridibunda* PALLAS, from Adana, Turkey, collected by Dr. L. J. BORKIN (sent to Dr. M. KURAMOTO in 1975).

Eighteen kinds of enzymes extracted from skeletal muscles and livers and two kinds of blood proteins were analyzed by the method of horizontal starch-gel electrophoresis. 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 are shown in Table 2.

TABLE 2
Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	E.C.No.	Sample	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	3.5.4.4	„	„
Adenylate kinase	AK	2.7.4.3	„	„
Alcohol dehydrogenase	ADH	1.1.1.1	Liver	T-B-E pH 8.0
Aldolase	ALD	4.1.2.7	Skeletal muscle	T-C pH 7.0
Creatine kinase	CK	2.7.3.2	„	T-B-E pH 8.0
Fumarase	Fum	4.2.1.2	Liver	„
α -Glycerophosphate dehydrogenase	α -GDH	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	„	T-B-E pH 8.0
Isocitrate dehydrogenase	IDH	1.1.1.42	„	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	„	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	„	„
Malic enzyme	ME	1.1.1.40	„	T-C pH 7.0
Mannose phosphate isomerase	MPI	5.3.1.8	„	„
Peptidase	Pep	3.4.3.1	Liver	T-B-E pH 8.0
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	Skeletal muscle	T-C pH 7.0
Phosphoglucomutase	PGM	2.7.5.1	„	T-B-E pH 8.0
Superoxide dismutase	SOD	1.15.1.1	„	„
Serum albumin	Ab	—	Blood serum	„
Hemoglobin	Hb	—	Erythrocyte	T-B-E pH 8.6

T-C, Tris-citrate buffer

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

The method of electrophoresis has been previously reported in detail by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was performed by the agar overlay method of BREWER (1970) and HARRIS and HOPKINSON (1976), 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. 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, subspecies and populations were evaluated by calculating the

genetic distances (D) by the method of NEI (1975). A dendrogram for these species, subspecies 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 multiple alleles

The electrophoretic patterns of 18 enzymes extracted from skeletal muscles and livers and two kinds of blood proteins were analyzed in 350 pond frogs belonging to 19 populations of six species and three subspecies distributed in the Palearctic region. It was found that these enzymes and blood proteins were controlled by genes at 26 loci. The electrophoretic bands corresponding to multiple alleles at

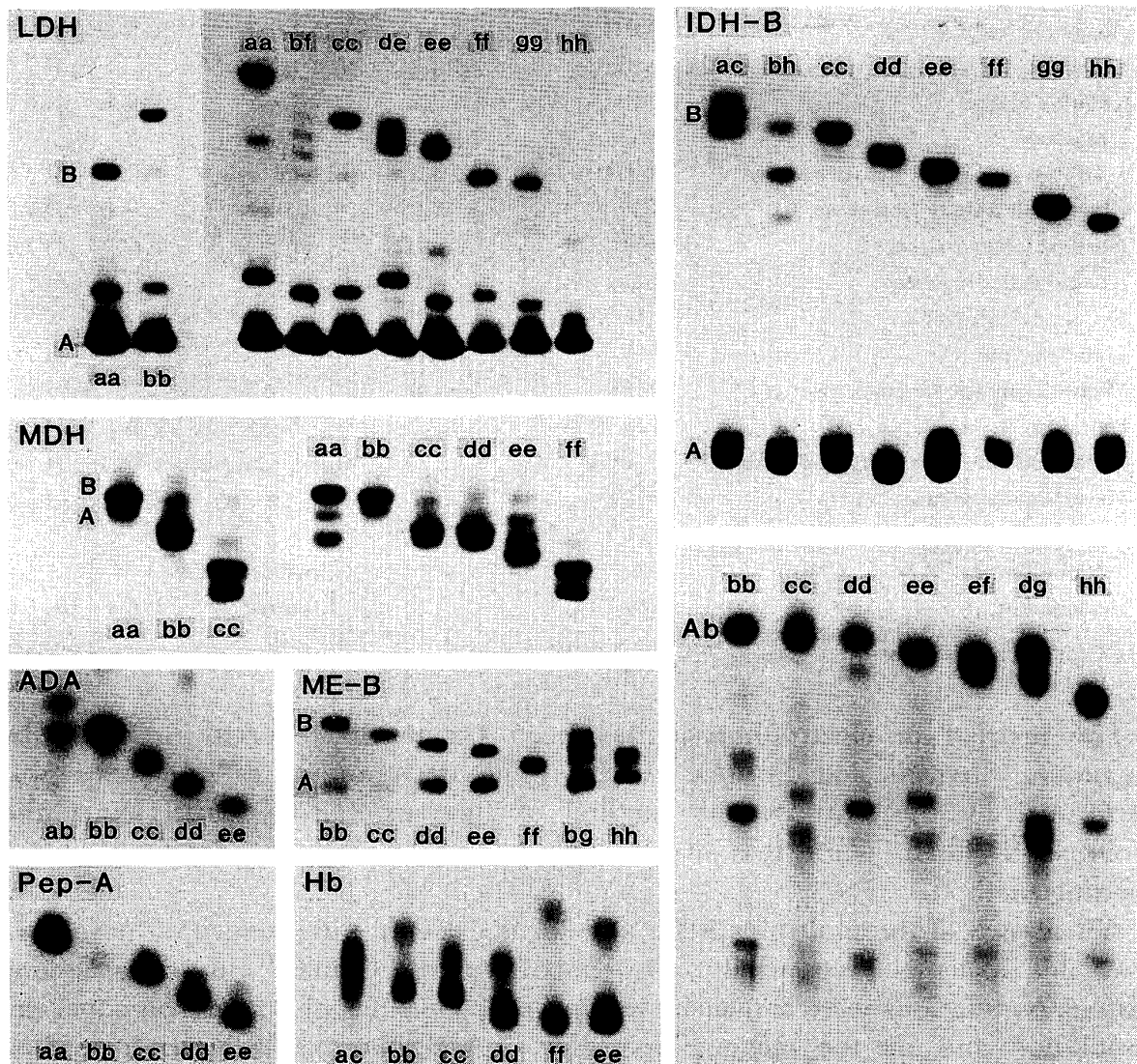


Fig. 2. Electrophoretic patterns of six enzymes, ADA, IDH-B, LDH, MDH, ME-B and Pep-A, and two blood proteins, Ab and Hb, in 19 populations of pond frogs in the Palearctic region.

each locus were named A, B, C, --- in the order of mobility from fast to slow, and the alleles were shown by *a, b, c, ---* (Fig. 2).

At the 26 loci, one to 25 phenotypes, 8.5 on the average, produced by one to 15 alleles, 5.3 on the average, were observed. Of the number of these phenotypes, the smallest was one produced by one allele at the IDH-A locus, followed by two produced by two alleles at the LDH-A locus. At each of seven loci, the AAT-A, AAT-B, AK, CK, MDH-A, PGM and SOD-A loci, four or five phenotypes produced by three alleles were observed. At each of three loci, the Fum, ME-A and SOD-B loci, there were five or six phenotypes produced by four alleles. At each of four loci, the ADA, GPI, Pep-A and 6-PGD loci, seven to 10 phenotypes produced by five alleles were observed. At the MDH-B and Hb loci, nine

TABLE 3
Number of alleles and phenotypes at each of 26 loci in 19 populations of pond frog species in the Palearctic region

Locus	Alleles		Phenotypes	
	No.	Kind	No.	Kind
AAT-A	3	<i>a~c</i>	5	AA, BB, CC, AB, BC
AAT-B	3	<i>a~c</i>	4	BB, CC, AB, BC
ADA	5	<i>a~e</i>	10	AA, BB, CC, DD, EE, AB, AC, BC, BD, CD
ADH-B	7	<i>a~g</i>	11	AA, BB, CC, DD, EE, FF, GG, AD, BG, DE, DF
AK	3	<i>a~c</i>	4	AA, BB, AB, BC
ALD	7	<i>a~g</i>	11	BB, CC, DD, EE, FF, GG, AG, BG, CD, DG, EF
CK	3	<i>a~c</i>	4	AA, BB, CC, BC
Fum	4	<i>a~d</i>	5	BB, CC, DD, AB, BC
α -GDH	7	<i>a~g</i>	9	AA, BB, DD, EE, AD, BD, CE, DF, DG
GPI	5	<i>a~e</i>	7	CC, DD, EE, AD, BC, BD, CE
IDH-A	1	<i>a</i>	1	AA
IDH-B	9	<i>a~i</i>	15	CC, DD, EE, FF, GG, HH, AC, BH, CF, DF, EF, EH, EI, FH, FI
LDH-A	2	<i>a, b</i>	2	AA, BB
LDH-B	8	<i>a~h</i>	13	AA, BB, CC, EE, FF, GG, HH, AC, BE, BF, DE, EF, GH
MDH-A	3	<i>a~c</i>	4	AA, BB, CC, BC
MDH-B	6	<i>a~f</i>	9	AA, BB, CC, DD, EE, FF, CE, CF, DF
ME-A	4	<i>a~d</i>	6	AA, CC, DD, AB, AC, CD
ME-B	10	<i>a~j</i>	19	BB, CC, DD, EE, FF, HH, II, AB, BC, BD, BG, BI, BJ, CD, DE, DF, DG, DJ, FH
MPI	15	<i>a~o</i>	25	AA, DD, FF, GG, HH, II, JJ, MM, NN, BC, BF, CF, DG, DJ, EG, EJ, FH, GJ, GK, GL, GM, HI, JN, MN, NO
Pep-A	5	<i>a~e</i>	10	AA, BB, CC, DD, EE, AB, AC, AE, BE, CD
6-PGD	5	<i>a~e</i>	8	AA, BB, CC, DD, EE, AC, CD, BE
PGM	3	<i>a~c</i>	5	AA, BB, CC, AB, BC
SOD-A	3	<i>a~c</i>	4	AA, BB, CC, AC
SOD-B	4	<i>a~d</i>	6	AA, BB, CC, DD, AC, CD
Ab	8	<i>a~h</i>	15	BB, CC, DD, EE, FF, GG, HH, AB, BC, CE, DF, DG, EF, FH, GH
Hb	6	<i>a~f</i>	9	AA, BB, CC, DD, EE, FF, AC, BE, DF
Average	5.3		8.5	

phenotypes produced by six alleles, at the ADH-B, ALD and α -GDH loci, nine or 11 phenotypes produced by seven alleles, and at the LDH-B and Ab loci, 13 or 15 phenotypes produced by eight alleles were observed. At the IDH-B locus, 15 phenotypes produced by nine alleles, and at the ME-B locus, 19 phenotypes produced by 10 alleles were observed. The largest number of phenotypes at the 26 loci was 25 produced by 15 alleles, which was found at the MPI locus (Table 3).

II. Gene frequency

1. AAT-A locus

The analyses of the electrophoretic patterns at the AAT-A locus in the 350 pond frogs belonging to the 19 populations showed that there were five pheno-

TABLE 4
Gene frequencies at 26 loci in 19 populations of

Species	Population	Sample size	AAT-A			AAT-B			ADA					ADH-B												
			a	b	c	a	b	c	a	b	c	d	e	a	b	c	d	e	f	g						
<i>R. nigro.</i>	Hiroshima	38	1.000			0.105	0.895				0.974	0.026					0.461	0.539								
"	Suwon	38	1.000			1.000					0.306	0.639	0.056				1.000									
"	Beijing	21	1.000			1.000					0.310	0.476	0.167	0.048			1.000									
<i>R. bre. b.</i>	Konko	38	1.000			1.000					1.000						1.000									
"	Maibara	46	1.000			1.000					0.033	0.967				0.032	0.968									
<i>R. bre. p.</i>	Utsunomiya	56	1.000			1.000					0.750	0.250					0.844	0.156								
<i>R. pla. c.</i>	Suwon	33	1.000			1.000					1.000					0.650	0.350									
<i>R. pla. p.</i>	Beijing	24	1.000			1.000					1.000					0.761	0.239									
"	Shandong	12	1.000			1.000					1.000					0.083	0.917									
<i>R. pla. f.</i>	Chiayi	7	0.286	0.714		1.000					0.917	0.083				0.167	0.833									
<i>R. less.</i>	Luxembourg	10	1.000			1.000					1.000					1.000										
"	Poland	9	1.000			1.000					1.000					1.000										
"	Firenze	6	1.000			1.000					1.000					1.000										
<i>R. escu.</i>	Wien	1	0.500	0.500		0.500	0.500				1.000					0.500									0.500	
"	Ukraine	1	0.500	0.500		0.500	0.500				1.000					0.500									0.500	
"	Heidelberg	2	0.500	0.500		0.500	0.500				1.000					0.500									0.500	
<i>R. ridi.</i>	Belgorod	2	1.000			1.000					1.000					1.000									1.000	
"	Roscoff	3	0.167	0.833		0.833	0.167				1.000					1.000									1.000	
"	Adana	3	1.000			1.000					1.000					1.000									1.000	

TABLE 4
Gene frequencies at 26 loci in 19 populations of

Species	Population	Sample size	GPI					IDH-A	IDH-B									LDH-A									
			a	b	c	d	e	a	a	b	c	d	e	f	g	h	i	a	b								
<i>R. nigro.</i>	Hiroshima	38			1.000			1.000						0.789	0.211												1.000
"	Suwon	38			0.917		0.083	1.000							0.224	0.513		0.211	0.053							1.000	
"	Beijing	21			0.929		0.071	1.000			0.071							0.929								1.000	
<i>R. bre. b.</i>	Konko	38			1.000			1.000									1.000									1.000	
"	Maibara	46			0.011		0.989	1.000									1.000									1.000	
<i>R. bre. p.</i>	Utsunomiya	56			1.000			1.000									1.000									1.000	
<i>R. pla. c.</i>	Suwon	33			1.000			1.000									1.000									1.000	
<i>R. pla. p.</i>	Beijing	24			1.000			1.000									1.000									1.000	
"	Shandong	12			1.000			1.000									1.000									1.000	
<i>R. pla. f.</i>	Chiayi	7			1.000			1.000									1.000									1.000	
<i>R. less.</i>	Luxembourg	10					1.000	1.000									1.000									1.000	
"	Poland	9					1.000	1.000			0.278						1.000									1.000	
"	Firenze	6	0.167				0.833	1.000								0.083										1.000	
<i>R. escu.</i>	Wien	1		0.500		0.500		1.000								1.000										1.000	
"	Ukraine	1		0.500		0.500		1.000								1.000										1.000	
"	Heidelberg	2		0.500		0.500		1.000								1.000										1.000	
<i>R. ridi.</i>	Belgorod	2		0.250		0.750		1.000								1.000										1.000	
"	Roscoff	3				1.000		1.000								1.000										1.000	
"	Adana	3				1.000		1.000								1.000										1.000	

types, AA, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*.

Of the 19 populations examined, 13 including the three of *R. nigromaculata*, the three of *R. brevipoda*, the two of *R. p. plancyi*, the one of *R. p. chosenica*, the Firenze of *R. lessonae* and the three of *R. ridibunda* almost all had allele *b*, while the Roscoff of *R. ridibunda* had allele *a* in a frequency of 0.167 in addition to allele *b*. The Luxembourg and Poland populations of *R. lessonae* had only allele *a*. The three populations of *R. esculenta* had alleles *a* and *b* each in a frequency of 0.500. It was found that *R. esculenta* had the alleles of *R. lessonae* and *R. ridibunda* in the same frequency. The Chiayi population of *R. plancyi fukiensis* had alleles *c* and *b* in frequencies of 0.714 and 0.286, respectively (Table 4-I; Fig. 3).

2. AAT-B locus

The electrophoretic patterns at the AAT-B locus in the 350 pond frogs belonging to the 19 populations showed four phenotypes, BB, CC, AB and BC,

pond frog species in the Palearctic region (I)

AK			ALD							CK			Fum				α-GDH							
<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	
0.987	0.013						1.000			1.000			1.000								1.000			
1.000							1.000			1.000			0.028	0.944	0.028						0.895		0.026	0.079
1.000							0.738	0.262		1.000			0.024	0.950	0.024						1.000			
1.000			0.152						0.848	1.000			1.000				1.000				1.000			
1.000			1.000							1.000			1.000				1.000				0.989		0.011	
0.156	0.844		1.000							1.000			1.000				1.000				1.000			
1.000						1.000				1.000			0.065	0.935			1.000				1.000			
1.000						1.000				1.000			0.913	0.087			1.000				1.000			
1.000						1.000				1.000			0.792	0.208			0.792				1.000		0.208	
1.000						1.000				1.000			1.000				1.000				1.000			
1.000						1.000				0.150	0.850					1.000					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					0.083		0.917	
1.000						0.500	0.500			1.000						1.000					0.500		0.500	
1.000						0.500		0.500		1.000						1.000					0.500		0.500	
1.000						0.500		0.500		1.000						1.000					0.500		0.500	
1.000						1.000				1.000						1.000					1.000		1.000	
1.000						1.000				1.000						1.000					1.000		1.000	
1.000			0.333						0.667	1.000						1.000					1.000		1.000	
1.000						0.667			0.333	1.000						1.000					1.000		1.000	

pond frog species in the Palearctic region (II)

LDH-B								MDH-A			MDH-B						ME-A							
<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>				
0.013	1.000							1.000			1.000										1.000			
	0.987							1.000						0.987	0.013						1.000			
	1.000							0.929	0.071					0.881	0.119		0.024				0.976			
						1.000		1.000						1.000							1.000			
						0.044	0.956	1.000						1.000							0.044	0.956		
						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					1.000			
1.000								1.000						0.500		0.286	0.214				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			
						0.500	0.500	1.000						1.000							1.000			
						0.500	0.500	1.000						1.000							1.000			
						0.500		1.000						1.000							1.000			
						0.750		1.000						1.000							1.000			
						0.500		1.000						1.000							1.000			
						1.000		1.000						1.000							0.667	0.333		

pond frog species in the Palearctic region (III)

							Pep-A					6-PGD					PGM			SOD-A			
<i>i</i>	<i>j</i>	<i>k</i>	<i>l</i>	<i>m</i>	<i>n</i>	<i>o</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	
				0.197					1.000			0.053		0.947					1.000				1.000
				0.056					0.972	0.028		0.250		0.750					0.972	0.028			1.000
			0.024	0.071					0.976	0.024		0.024		0.976					0.810	0.190			1.000
	0.211						0.674		0.326					1.000					0.940	0.060			1.000
		0.109					1.000							0.911	0.089				1.000				1.000
									1.000					0.391	0.609				0.984	0.016			1.000
					1.000				1.000					1.000			0.424		0.576				1.000
	0.198				0.813				1.000			0.854		0.146					1.000				1.000
	0.333				0.667				1.000			0.750		0.250					1.000				1.000
				0.357	0.500	0.143			1.000					1.000					1.000				1.000
0.333										1.000		1.000							1.000				1.000
										1.000		1.000							1.000				1.000
										1.000		1.000							1.000				1.000
							0.500			0.500		0.500			0.500				0.500	0.500	0.500		0.500
								0.500		0.500		0.500			0.500				1.000				0.500
									0.500	0.500		0.500			0.500				0.500	0.500	0.500		0.500
										1.000					1.000				0.500	0.500	1.000		1.000
							0.167		0.833						1.000				0.167	0.833	1.000		1.000
							0.167		0.833						1.000				1.000		1.000		1.000

frogs belonging to the 19 populations showed that there were 10 phenotypes, AA, BB, CC, DD, EE, AB, AC, BC, BD and CD, produced by five alleles, *a~e*.

Of the 19 populations examined, all the nine of the three species distributed in Europe had only allele *e*. While all the two of *R. p. plancyi* and the Suwon of *R. plancyi chosonica* had allele *d*. The Chiayi population of *R. plancyi fukienensis* and the three of *R. brevipoda* had allele *c* in frequencies of 0.750~1.000. Of these populations, the two of *R. plancyi fukienensis* and *R. brevipoda porosa* had allele *d* in frequencies of 0.083 and 0.250, respectively, in addition to allele *c*. The Maibara population of *R. b. brevipoda* had allele *b* in a frequency of 0.033 in addition to allele *c*. The Konko population of *R. b. brevipoda* had only allele *c*. The Hiroshima population of *R. nigromaculata* had alleles *b* and *c* in frequencies of 0.974 and 0.026, respectively, while the Suwon population of this species had alleles *c*, *b* and *d* in frequencies of 0.639, 0.306 and 0.056, respectively. The Beijing population of the same species had alleles *b*, *a*, *c* and *d* in frequencies of 0.476, 0.310, 0.167 and 0.048, respectively (Table 4-I; Fig. 3).

4. ADH-B locus

The electrophoretic patterns at the ADH-B locus in the 350 pond frogs belonging to the 19 populations showed that there were 11 phenotypes, AA, BB, CC, DD, EE, FF, GG, AD, BG, DE and DF, produced by seven alleles, *a~g*.

Of the 19 populations, seven including the Suwon and Beijing of *R. nigromaculata*, the three of *R. brevipoda*, the Shandong of *R. p. plancyi* and the Chiayi of *R. p. fukienensis* had allele *d* in frequencies of 0.833~1.000. Of these populations, the Maibara of *R. b. brevipoda* and the two of *R. plancyi* had allele *a* in frequencies of 0.032~0.167 in addition to allele *d*, and the Utsunomiya population of *R. brevipoda porosa* had allele *f* in a frequency of 0.156. The other three populations had only allele *d*. The Suwon population of *R. plancyi chosonica* and the Beijing population of *R. p. plancyi* had allele *a* in frequencies of 0.650 and 0.761, respectively, and had

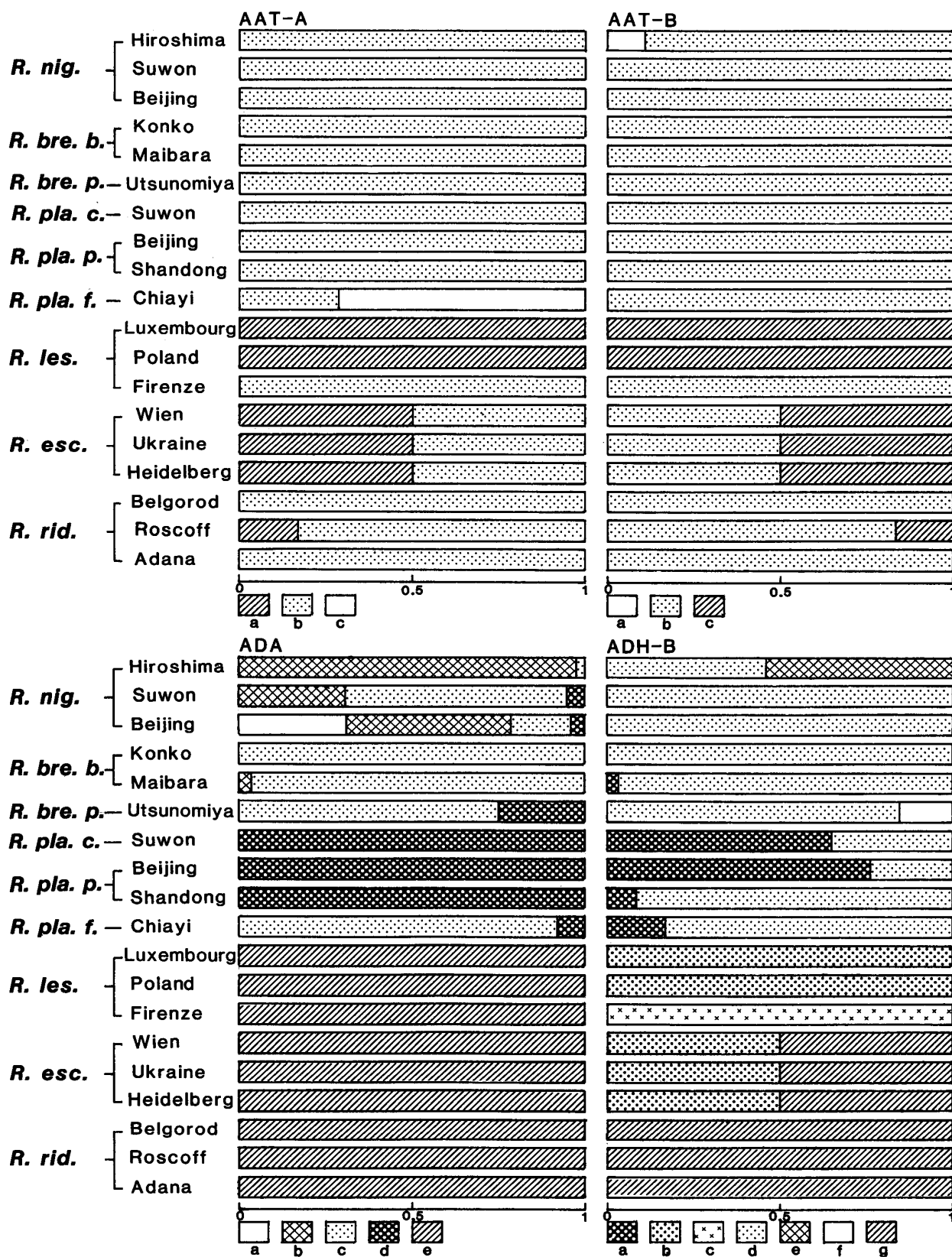


Fig. 3. Gene frequencies at four loci, AAT-A, AAT-B, ADA and ADH-B, in 19 populations of pond frogs in the Palearctic region.

allele *d* in frequencies of 0.350 and 0.239, respectively. The Hiroshima population of *R. nigromaculata* had alleles *e* and *d* in frequencies of 0.539 and 0.461, respectively.

The three populations of *R. ridibunda* had only allele *g* and the Luxembourg and Poland populations of *R. lessonae* had only allele *b*. The three populations of *R. esculenta* had allele *b* derived from *R. lessonae* and allele *g* derived from *R. ridibunda* in frequencies of 0.500 and 0.500, respectively. The Firenze population of *R. lessonae* had only allele *c* (Table 4-I; Fig. 3).

5. AK locus

The analyses of the electrophoretic patterns at the AK locus in the 350 pond frogs belonging to the 19 populations showed that there were four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*, *b* and *c*.

All the frogs of the 19 populations examined had allele *b*, except that the Hiroshima population of *R. nigromaculata* had allele *c* in a frequency of 0.013 in addition to allele *b*, and the Utsunomiya population of *R. brevipoda porosa* had allele *a* in a frequency of 0.156 (Table 4-I; Fig. 4).

6. ALD locus

The analyses of the electrophoretic patterns at the ALD locus in the 350 pond frogs belonging to the 19 populations showed that there were 11 phenotypes, BB, CC, DD, EE, FF, GG, AG, BG, CD, DG and EF, produced by seven alleles, *a*~*g*.

Of the 19 populations examined, six including the three of *R. nigromaculata* and three of the four of *R. plancyi* other than *R. p. fukienensis* had only allele *e*, except that the Beijing of *R. nigromaculata* had allele *f* in a frequency of 0.262 in addition to allele *e*. The Chiayi population of *R. plancyi fukienensis* and the Belgorod population of *R. ridibunda* had only allele *g*. While the Maibara and Utsunomiya populations of *R. brevipoda* had only allele *b*, the Konko population of the same species had alleles *g* and *b* in frequencies of 0.848 and 0.152, respectively. The three populations of *R. lessonae* had only allele *d*. The Roscoff population of *R. ridibunda* had alleles *g* and *a* in frequencies of 0.667 and 0.333, respectively. While the three populations of *R. esculenta* had allele *d* derived from *R. lessonae* in a frequency of 0.500, the Wien had allele *c* derived from *R. ridibunda* in a frequency of 0.500, and the Ukraine and Heidelberg had allele *g* derived from *R. ridibunda* in a frequency of 0.500 (Table 4-I; Fig. 4).

7. CK locus

The analyses of the electrophoretic patterns at the CK locus in the 350 pond frogs of the 19 populations showed that there were four phenotypes, AA, BB, CC and BC, produced by three alleles, *a*, *b* and *c*.

Of the 19 populations examined, seven including the three of *R. nigromaculata*, the three of *R. brevipoda* and the Chiayi of *R. plancyi fukienensis* had only allele *b*. The three populations of *R. plancyi chosenuca* and *R. p. plancyi* had only allele *a*.

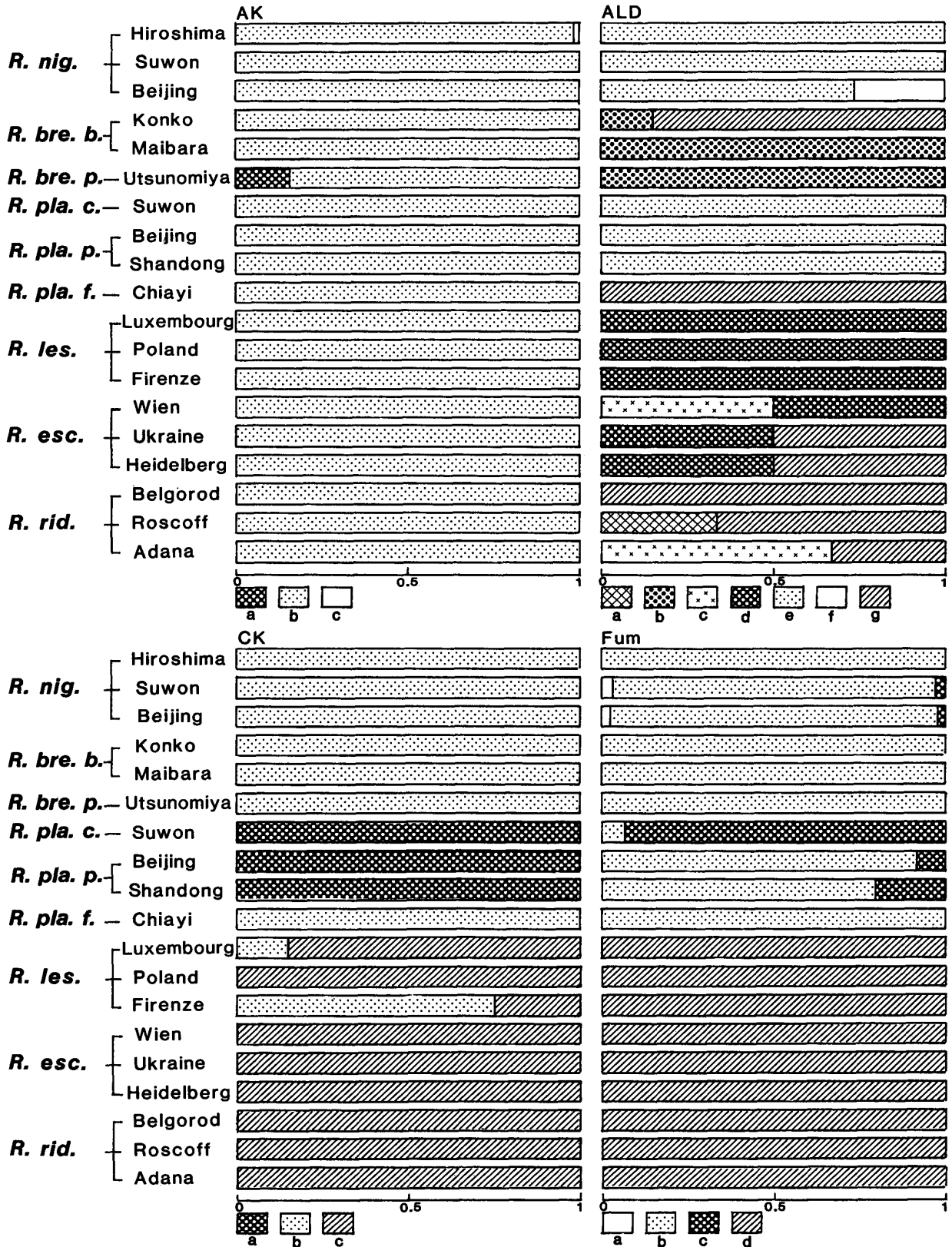


Fig. 4. Gene frequencies at four loci, AK, ALD, CK and Fum, in 19 populations of pond frogs in the Palearctic region.

Seven populations including the three of *R. ridibunda*, the three of *R. esculenta* and the Poland population of *R. lessonae* had only allele *c*. The Luxembourg population of *R. lessonae* had alleles *c* and *b* in frequencies of 0.850 and 0.150, respectively, while the Firenze population of the same species had alleles *b* and *c* in frequencies of 0.750 and 0.250, respectively (Table 4-I; Fig. 4).

8. Fum locus

The analyses of the electrophoretic patterns at the Fum locus in the 350 pond frogs of the 19 populations showed that there were five phenotypes, BB, CC, DD, AB and BC, produced by four alleles, *a*~*d*.

Of the 19 populations of the six species examined, all the nine populations of the three species distributed in Europe had only allele *d*, and the nine populations of the three species distributed in the East except the Suwon population of *R. plancyi chosonica* had allele *b* in frequencies of 0.792~1.000. Of these populations, the Suwon population of *R. nigromaculata* had alleles *a* and *c* each in a frequency of 0.028, while the Beijing population of the same species had alleles *a* and *c* each in a frequency of 0.024. The two populations of *R. p. plancyi* had allele *c* in frequencies of 0.087 and 0.208. The remaining five populations had only allele *b*. The Suwon population of *R. plancyi chosonica* had alleles *c* and *b* in frequencies of 0.935 and 0.065, respectively (Table 4-I; Fig. 4).

9. α -GDH locus

The analyses of the electrophoretic patterns at the α -GDH locus in the 350 pond frogs of the 19 populations showed that there were nine phenotypes, AA, BB, DD, EE, AD, BD, CE, DF and DG, produced by seven alleles, *a*~*g*.

Of the 19 populations of the six species examined, the three populations of *R. nigromaculata* were all occupied by allele *d*, except that the Suwon population had alleles *f* and *g* in frequencies of 0.026 and 0.079, respectively, in addition to allele *d*. The three populations of *R. brevipoda* and the Chiayi population of *R. plancyi fukienensis* had only allele *b*, except that the Maibara population of *R. brevipoda* had allele *d* in a frequency of 0.011 in addition to allele *b*. Three of the four populations of *R. plancyi* other than *R. p. fukienensis* had only allele *a*, except that the Shandong population had allele *d* in a frequency of 0.208 in addition to allele *a*. The populations of *R. lessonae* and *R. ridibunda* had only allele *e*, except that the Firenze population had allele *c* in a frequency of 0.083 in addition to allele *e*. The three populations of *R. esculenta* had alleles *c* and *e* each in a frequency of 0.500 (Table 4-I; Fig. 5).

10. GPI locus

The analyses of the electrophoretic patterns at the GPI locus in the 350 pond frogs of the 19 populations showed that there were seven phenotypes, CC, DD, EE, AD, BC, BD and CE, produced by five alleles, *a*~*e*.

Of the 19 populations, 10 including the three of *R. nigromaculata*, the three of *R. brevipoda* and the four of *R. plancyi*, had allele *c* in frequencies of 0.917~1.000. In

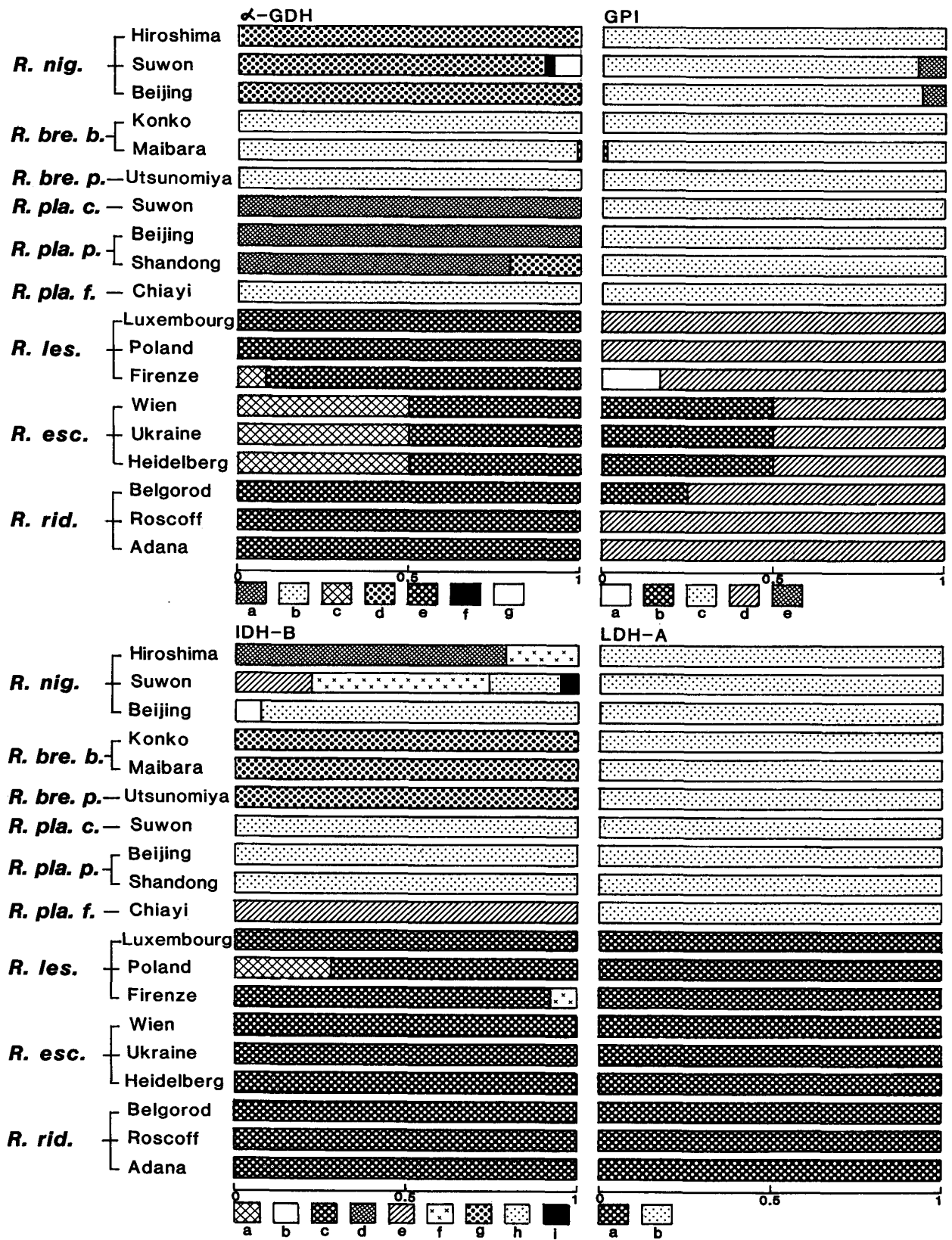


Fig. 5. Gene frequencies at four loci, α -GDH, GPI, IDH-B and LDH-A, in 19 populations of pond frogs in the Palearctic region.

addition to allele *c*, the Suwon and Beijing populations of *R. nigromaculata* had allele *e* in frequencies of 0.083 and 0.071, respectively, and the Maibara population of *R. brevipoda* had allele *b* in a frequency of 0.011, while the remaining seven populations had only allele *c*.

The three populations of *R. lessonae* and the three populations of *R. ridibunda* had allele *d* in frequencies of 0.750~1.000. In addition to allele *d*, the Firenze population of *R. lessonae* had allele *a* in a frequency of 0.167, and the Belgorod population of *R. ridibunda* had allele *b* in a frequency of 0.250, while the remaining four populations had only allele *d*. The three populations of *R. esculenta* had alleles *b* and *d*, each of which was 0.500 in frequency (Table 4-II; Fig. 5).

11. IDH-A locus

All the 350 pond frogs of the 19 populations had AA band produced by allele *a* at the IDH-A locus when their electrophoretic patterns were analyzed (Table 4-II).

12. IDH-B locus

The analyses of the electrophoretic patterns at the IDH-B locus in the 350 pond frogs of the 19 populations showed that there were 15 phenotypes, CC, DD, EE, FF, GG, HH, AC, BH, CF, DF, EF, EH, EI, FH and FI, produced by nine alleles, *a*~*i*.

Of the 19 populations of the six species, the nine of the three species distributed in Europe had allele *c* in frequencies of 0.722~1.000. In addition to allele *c*, the Poland population of *R. lessonae* had allele *a* in a frequency of 0.278 and the Firenze population of the same species had allele *f* in a frequency of 0.083. The three populations of *R. brevipoda* had only allele *g* and the Chiayi population of *R. plancyi fukienensis* had only allele *e*. The remaining three populations of *R. plancyi* had only allele *h*. *R. nigromaculata* was abundant in the kind of alleles; the Hiroshima population had alleles *d* and *f* in frequencies of 0.789 and 0.211, respectively, the Beijing population had alleles *h* and *b* in frequencies of 0.929 and 0.071, respectively, and the Suwon population had alleles *f*, *e*, *h* and *i* in frequencies of 0.513, 0.224, 0.211 and 0.053, respectively (Table 4-II; Fig. 5).

13. LDH-A locus

The analyses of the electrophoretic patterns at the LDH-A locus in the 350 pond frogs of the 19 populations showed that there were two phenotypes, AA and BB, produced by two alleles, *a* and *b*.

It was found that all the pond frogs of the nine populations of the three species distributed in Europe had only allele *a*, while all the pond frogs of the 10 populations of the three species distributed in the East had only allele *b* (Table 4-II; Fig. 5).

14. LDH-B locus

The analyses of the electrophoretic patterns at the LDH-B locus in the 350

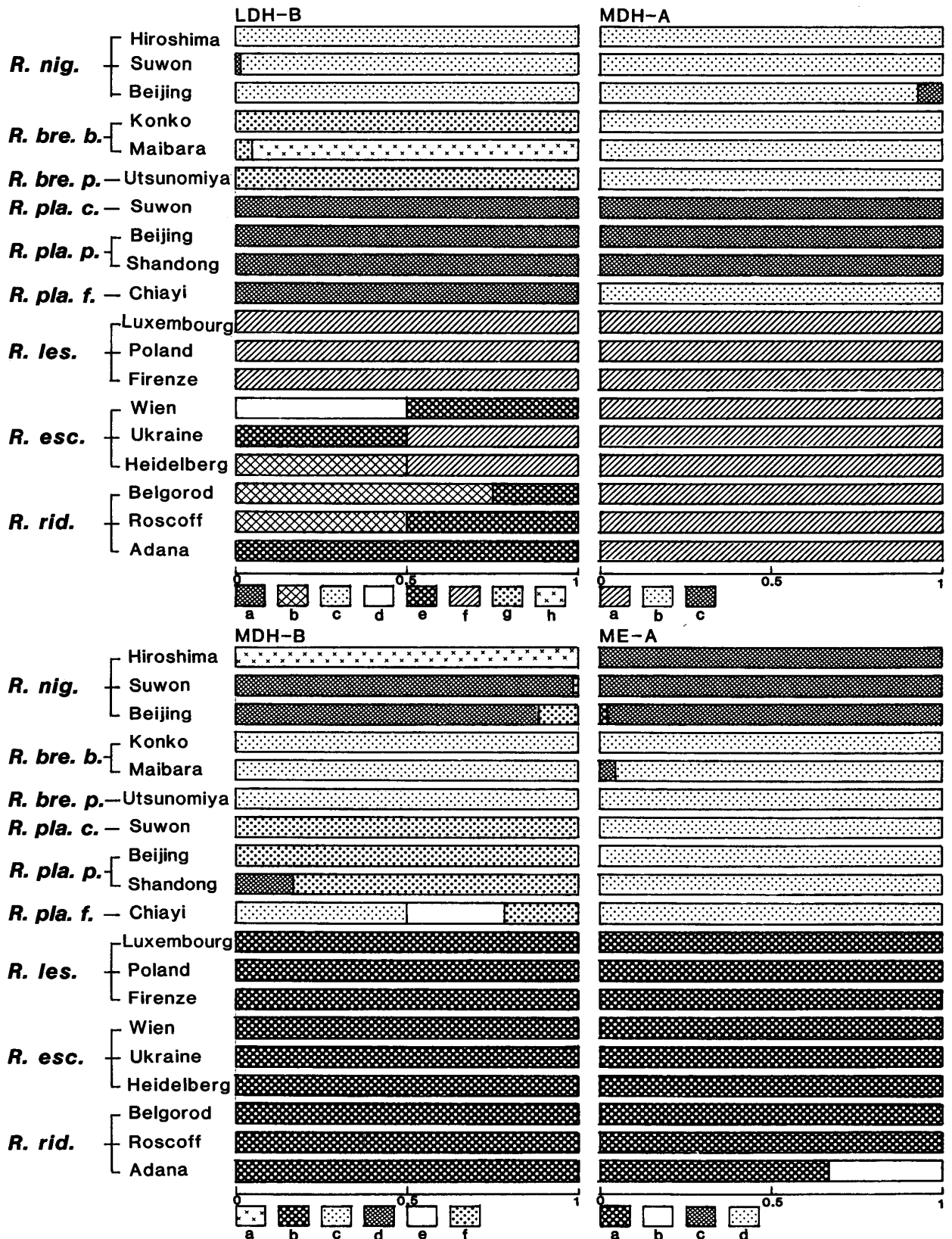


Fig. 6. Gene frequencies at four loci, LDH-B, MDH-A, MDH-B and ME-A, in 19 populations of pond frogs in the Palearctic region.

pond frogs of the 19 populations showed that there were 13 phenotypes, AA, BB, CC, EE, FF, GG, HH, AC, BE, BF, DE, EF and GH, produced by eight alleles, *a-h*.

The three populations of *R. nigromaculata* had only allele *c*, except that the Suwon had allele *a* in a frequency of 0.013 in addition to allele *c*. The Maibara population of *R. b. brevipoda* had alleles *h* and *g* in frequencies of 0.956 and 0.044, respectively, while the Konko population of the same subspecies and the Utsunomiya population of *R. brevipoda porosa* had only allele *g*. The four populations of *R. plancyi* had only allele *a*, and the three populations of *R. lessonae* had only allele *f*. The Belgorod population of *R. ridibunda* had alleles *b* and *e* in frequencies of 0.750 and 0.250, respectively, and the Roscoff population had alleles *b* and *e* each in a frequency of 0.500, while the Adana population had only allele *e*. The Wien population of *R. esculenta* had alleles *d* and *e* each in a frequency of 0.500, the Ukraine population had alleles *e* and *f* each in a frequency of 0.500, and the Heidelberg population had alleles *b* and *f* each in a frequency of 0.500 (Table 4-II; Fig. 6).

15. MDH-A locus

The analyses of the electrophoretic patterns at the MDH-A locus in the 350 pond frogs of the 19 populations showed that there were four phenotypes, AA, BB, CC and BC, produced by three alleles, *a*, *b* and *c*.

Of the 19 populations, the three populations of *R. nigromaculata* had only allele *b*, except that the Beijing had allele *c* in a frequency of 0.071 in addition to allele *b*. The three populations of *R. brevipoda* and the Chiayi population of *R. plancyi fukienensis* had only allele *b*. The two populations of *R. p. plancyi* and the population of *R. plancyi chosenica* had only allele *c*. The nine populations of the three species distributed in Europe had only allele *a* (Table 4-II; Fig. 6).

16. MDH-B locus

The analyses of the electrophoretic patterns at the MDH-B locus in the 350 pond frogs of the 19 populations showed that there were nine phenotypes, AA, BB, CC, DD, EE, FF, CE, CF and DF, produced by six alleles, *a-f*.

Of the three populations of *R. nigromaculata*, the Hiroshima had only allele *a*, while the Suwon and Beijing had allele *d* in frequencies of 0.987 and 0.881, respectively, and had allele *f* in frequencies of 0.013 and 0.119, respectively. The three populations of *R. brevipoda* had only allele *c*. The Chiayi population of *R. plancyi fukienensis* had alleles *c*, *e* and *f* in frequencies of 0.500, 0.286 and 0.214, respectively, while the remaining three populations of *R. plancyi* had only allele *f*, except that the Shandong population had allele *d* in a frequency of 0.167. All the nine populations of the three species distributed in Europe had only allele *b* (Table 4-II; Fig. 6).

17. ME-A locus

The analyses of the electrophoretic patterns at the ME-A locus in the 350 pond

frogs of the 19 populations showed that there were six phenotypes, AA, CC, DD, AB, AC and CD, produced by four alleles, $a\sim d$.

The three populations of *R. nigromaculata* had only allele c , except that the Beijing population had allele a in a frequency of 0.024 in addition to allele c . The three populations of *R. brevipoda* and the four populations of *R. plancyi* had only allele d , except that the Maibara population of *R. brevipoda* had allele c in a frequency of 0.044 in addition to allele d . All the eight of the nine populations of the three species distributed in Europe, other than the Adana population of *R. ridibunda*, had only allele a . The Adana population had alleles a and b in frequencies of 0.667 and 0.333, respectively (Table 4-II; Fig. 6).

18. ME-B locus

The analyses of the electrophoretic patterns at the ME-B locus in the 350 pond frogs of the 19 populations showed that there were 19 phenotypes, BB, CC, DD, EE, FF, HH, II, AB, BC, BD, BG, BI, BJ, CD, DE, DF, DG, DJ and FH, produced by 10 alleles, $a\sim j$.

Of the three populations of *R. nigromaculata*, the Hiroshima had alleles d , e and g in frequencies of 0.816, 0.158 and 0.026, respectively. The Beijing population had alleles b and d in frequencies of 0.905 and 0.095, respectively, and the Suwon population had allele b in a frequency of 0.639 and, moreover, had alleles d , g , i and j in frequencies of 0.056~0.167. The two populations of *R. brevipoda* other than the Maibara and the three populations of *R. plancyi* other than the Beijing had only allele b . The Maibara population had alleles b , c , d and a in frequencies of 0.567, 0.367, 0.056 and 0.011, respectively. The Beijing population had alleles b , d and c in frequencies of 0.625, 0.271 and 0.104, respectively. The Poland and Firenze populations of *R. lessonae* and the Wien population of *R. esculenta* had only allele d , while the Luxembourg population of *R. lessonae* had alleles d and e in frequencies of 0.450 and 0.550, respectively. The Ukraine and Heidelberg populations of *R. esculenta* had alleles d and f each in a frequency of 0.500. The Belgorod and Roscoff populations of *R. ridibunda* had only allele f , while the Adana population of the same species had alleles f and h each in a frequency of 0.500 (Table 4-III; Fig. 7).

19. MPI locus

The analyses of the electrophoretic patterns at the MPI locus in the 350 pond frogs of the 19 populations showed that there were 25 phenotypes, AA, DD, FF, GG, HH, II, JJ, MM, NN, BC, BF, CF, DG, DJ, EG, EJ, FH, GJ, GK, GL, GM, HI, JN, MN and NO, produced by 15 alleles, $a\sim o$.

The three populations of *R. nigromaculata* had allele g in high frequencies, being 0.803~0.917. In addition to allele g , the Hiroshima population had allele m in a frequency of 0.197, the Suwon population had alleles e and m in frequencies of 0.028 and 0.056, respectively, and the Beijing population had alleles e , l and m in frequencies of 0.024, 0.024 and 0.071, respectively. The three populations of *R. brevipoda* had allele g in high frequencies, being 0.433~0.875. In addition to allele

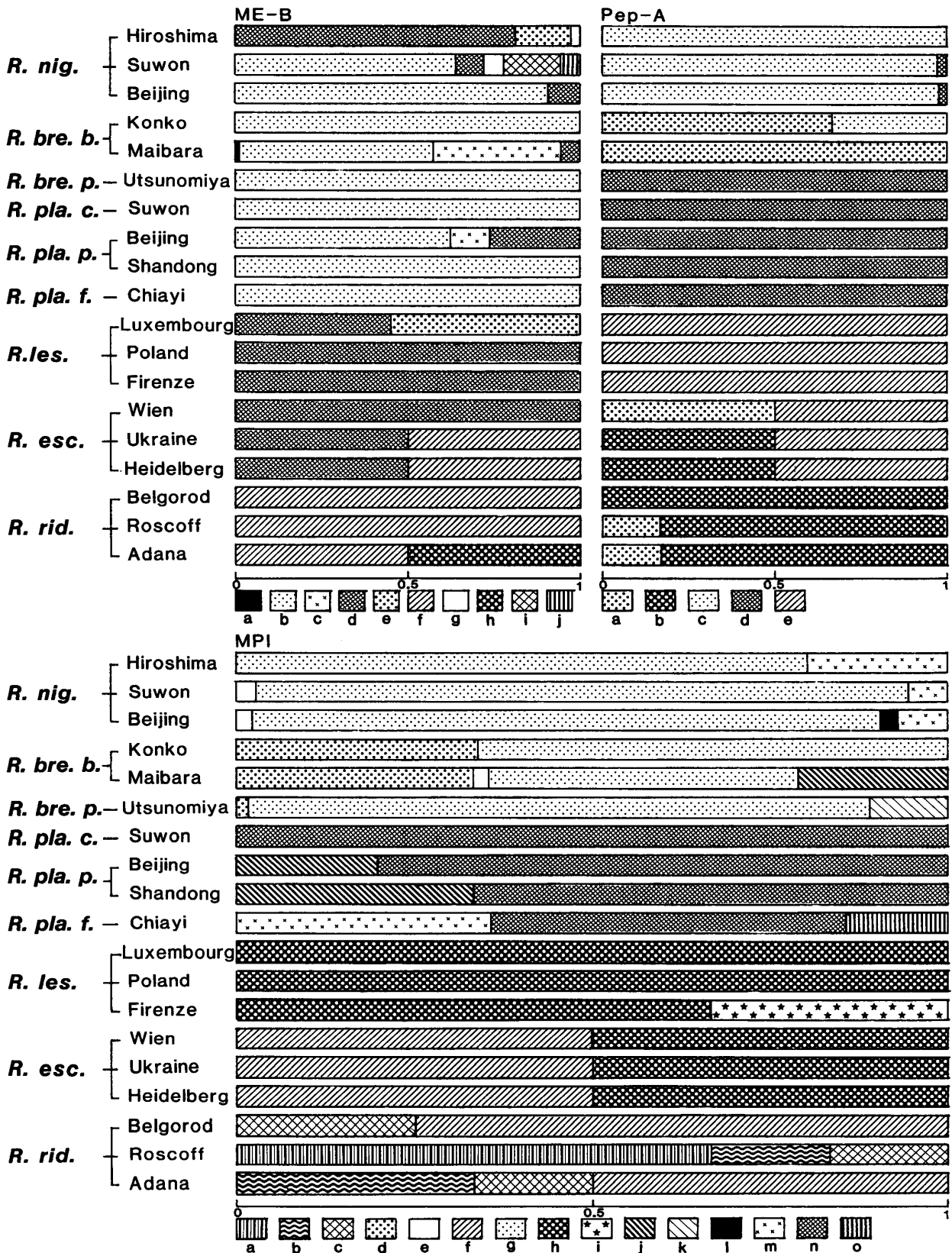


Fig. 7. Gene frequencies at three loci, ME-B, Pep-A and MPI, in 19 populations of pond frogs in the Palearctic region.

g, the Konko population had allele *d* in a frequency of 0.340, the Maibara population had alleles *d*, *j* and *e* in frequencies of 0.333, 0.211 and 0.022, respectively, and the Utsunomiya population had alleles *k* and *d* in frequencies of 0.109 and 0.016, respectively. The four populations of *R. plancyi* had allele *n* in high frequencies, being 0.500~1.000. In addition to allele *n*, the two populations of *R. p. plancyi* had allele *j* in frequencies of 0.198 and 0.333, and the Chiayi population of *R. p. fukienensis* had alleles *m* and *o* in frequencies of 0.357 and 0.143, respectively. The Suwon population of *R. p. chosenica* had only allele *n*. In Europe, the three populations of *R. lessonae* had allele *h* in high frequencies. The Luxembourg and Poland populations had only allele *h*, while the Firenze population had allele *i* in a frequency of 0.333 in addition to allele *h*. The three populations of *R. esculenta* had alleles *f* and *h* each in a frequency of 0.500. The Belgorod population of *R. ridibunda* had alleles *f* and *c* in frequencies of 0.750 and 0.250, respectively. The Roscoff population had alleles *a*, *b* and *c* in frequencies of 0.667, 0.167 and 0.167, respectively. The Adana population had alleles *f*, *b* and *c* in frequencies of 0.500, 0.333 and 0.167, respectively (Table 4-III; Fig. 7).

20. Pep-A locus

The analyses of the electrophoretic patterns at the Pep-A locus in the 350 pond frogs of the 19 populations showed that there were 10 phenotypes, AA, BB, CC, DD, EE, AB, AC, AE, BE and CD, produced by five alleles, *a*~*e*.

The three populations of *R. nigromaculata* had allele *c* in high frequencies, being 0.972~1.000. While the Hiroshima population had only allele *c*, the Suwon and Beijing populations had allele *d* in frequencies of 0.028 and 0.024, respectively, in addition to allele *c*. In *R. brevipoda*, the Konko population had alleles *a* and *c* in frequencies of 0.674 and 0.326, respectively, the Maibara population had only allele *a*, and the Utsunomiya population had only allele *d*. The four populations of *R. plancyi* had only allele *d*. In Europe, the three populations of *R. lessonae* had only allele *e*, and the three populations of *R. ridibunda* had allele *b* in high frequencies, being 0.833~1.000. The Roscoff and Adana populations had allele *a* in a frequency of 0.167 in addition to allele *b*, while the Belgorod population had only allele *b*. The three populations of *R. esculenta* had allele *e* derived from *R. lessonae* in a frequency of 0.500, and had allele *a* or *b* derived from *R. ridibunda* in a frequency of 0.500. All the three populations of *R. esculenta* showed a hybrid band, AE or BE, between *R. lessonae* and *R. ridibunda* in phenotype (Table 4-III; Fig. 7).

21. 6-PGD locus

The analyses of the electrophoretic patterns at the 6-PGD locus in the 350 pond frogs of the 19 populations showed that there were eight phenotypes, AA, BB, CC, DD, EE, AC, CD and BE, produced by five alleles, *a*~*e*.

The three populations of *R. nigromaculata* had allele *c* in high frequencies, being 0.750~0.976, and had allele *a* in frequencies of 0.024~0.250. The two populations of *R. b. brevipoda* had also allele *c* in high frequencies. The Konko popula-

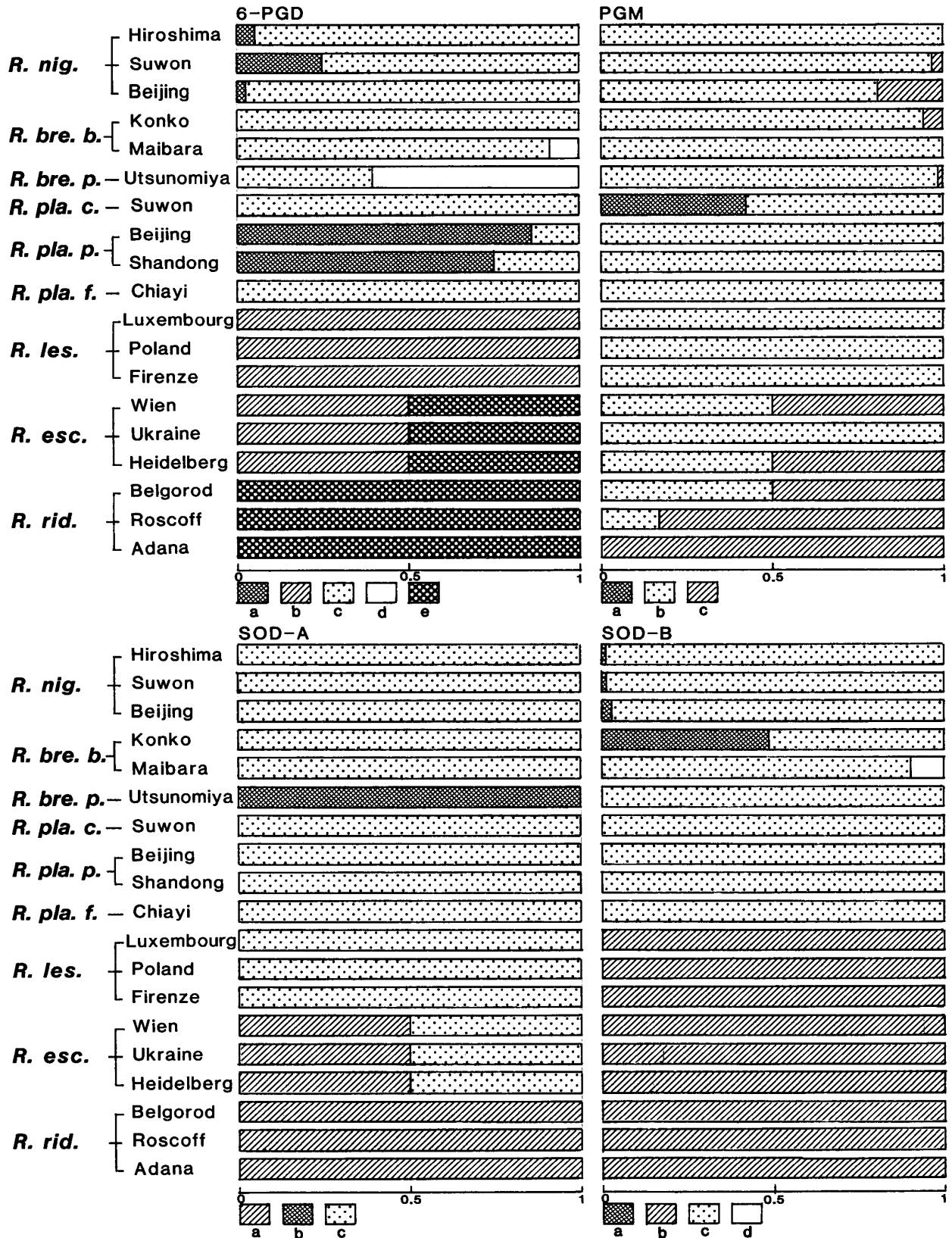


Fig. 8. Gene frequencies at four loci, 6-PGD, PGM, SOD-A and SOD-B, in 19 populations of pond frogs in the Palearctic region.

tion had only allele *c*, while the Maibara population had allele *d* in a frequency of 0.089 in addition to allele *c*. *R. brevipoda porosa* had alleles *d* and *c* in frequencies of 0.609 and 0.391, respectively. The two populations of *R. p. plancyi* had allele *a* in frequencies of 0.854 and 0.750, and allele *c* in frequencies of 0.146 and 0.250. The two populations of *R. p. chosenica* and *R. p. fukiensis* had only allele *c*.

In Europe, the three populations of *R. lessonae* had only allele *b*, while the three populations of *R. ridibunda* had only allele *e*. The three populations of *R. esculenta* had allele *b* derived from *R. lessonae* in a frequency of 0.500 and allele *e* derived from *R. ridibunda* in a frequency of 0.500. All the three populations of *R. esculenta* show the hybrid band, BE, in phenotype (Table 4-III; Fig. 8).

22. PGM locus

The analyses of the electrophoretic patterns at the PGM locus in the 350 pond frogs of the 19 populations showed that there were five phenotypes, AA, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*.

Twelve populations including the three of *R. nigromaculata*, the three populations of *R. brevipoda*, the three populations of *R. plancyi* other than *R. plancyi chosenica* and the three populations of *R. lessonae* had allele *b* in very high frequencies, being 0.810~1.000. Of these populations, the Suwon and Beijing of *R. nigromaculata*, the Konko of *R. b. brevipoda* and the Utsunomiya of *R. b. porosa* had allele *c* in frequencies of 0.016~0.190 in addition to allele *b*. The remaining eight populations had only allele *b*. The Suwon population of *R. p. chosenica* had alleles *a* and *b* in frequencies of 0.424 and 0.576, respectively. The one species of the Ukraine population of *R. esculenta* had allele *b*, while the Adana population of *R. ridibunda* had only allele *c*. The Roscoff population of *R. ridibunda* had alleles *b* and *c* in frequencies of 0.167 and 0.833, respectively. The Wien and Heidelberg populations of *R. esculenta* and the Belgorod population of *R. ridibunda* had alleles *b* and *c* each in a frequency of 0.500 (Table 4-III; Fig. 8).

23. SOD-A locus

The analyses of the electrophoretic patterns at the SOD-A locus in the 350 pond frogs of the 19 populations showed that there were four phenotypes, AA, BB, CC and AC, produced by three alleles, *a*, *b* and *c*.

Of the 19 populations, 12 including the three populations of *R. nigromaculata*, the two populations of *R. b. brevipoda*, the four populations of *R. plancyi* and the three populations of *R. lessonae* had only allele *c*, the Utsunomiya population of *R. brevipoda porosa* had only allele *b*, and the three populations of *R. ridibunda* had only allele *a*. The three populations of *R. esculenta* had allele *a* derived from *R. ridibunda* in a frequency of 0.500 and allele *c* derived from *R. lessonae* in a frequency of 0.500. The phenotype of *R. esculenta* showed the hybrid band, AC, derived from the two species (Table 4-III; Fig. 8).

24. SOD-B locus

The analyses of the electrophoretic patterns at the SOD-B locus in the 350 pond

frogs of the 19 populations showed that there were six phenotypes, AA, BB, CC, DD, AC and CD, produced by four alleles, *a*~*d*.

Of the 19 populations, the nine distributed in the East except the Konko population of *R. b. brevipoda* had allele *c* in very high frequencies, being 0.900~1.000. Of these populations, the three of *R. nigromaculata* had allele *a* in frequencies of 0.013~0.024 in addition to allele *c*. The Maibara population of *R. b. brevipoda* had allele *d* in a frequency of 0.100 in addition to allele *c*. The Konko population of the same subspecies had alleles *c* and *a* in frequencies of 0.513 and 0.487, respectively. All the nine populations of the three species distributed in Europe had only allele *b* (Table 4-IV; Fig. 8).

25. Ab locus

The analyses of the electrophoretic patterns at the Ab locus in the 350 pond frogs of the 19 populations showed that there were 15 phenotypes, BB, CC, DD, EE, FF, GG, HH, AB, BC, CE, DF, DG, EF, FH and GH, produced by eight alleles, *a*~*h*.

While the Hiroshima population of *R. nigromaculata* had only allele *b*, the Suwon population had allele *a* in a frequency of 0.083 in addition to allele *b*, and the Beijing population had alleles *c* and *b* in frequencies of 0.846 and 0.154, respectively. The two populations of *R. b. brevipoda* had allele *e* in high frequencies, being 0.786 and 0.978; the Konko population had allele *f* in a frequency of 0.214 and the

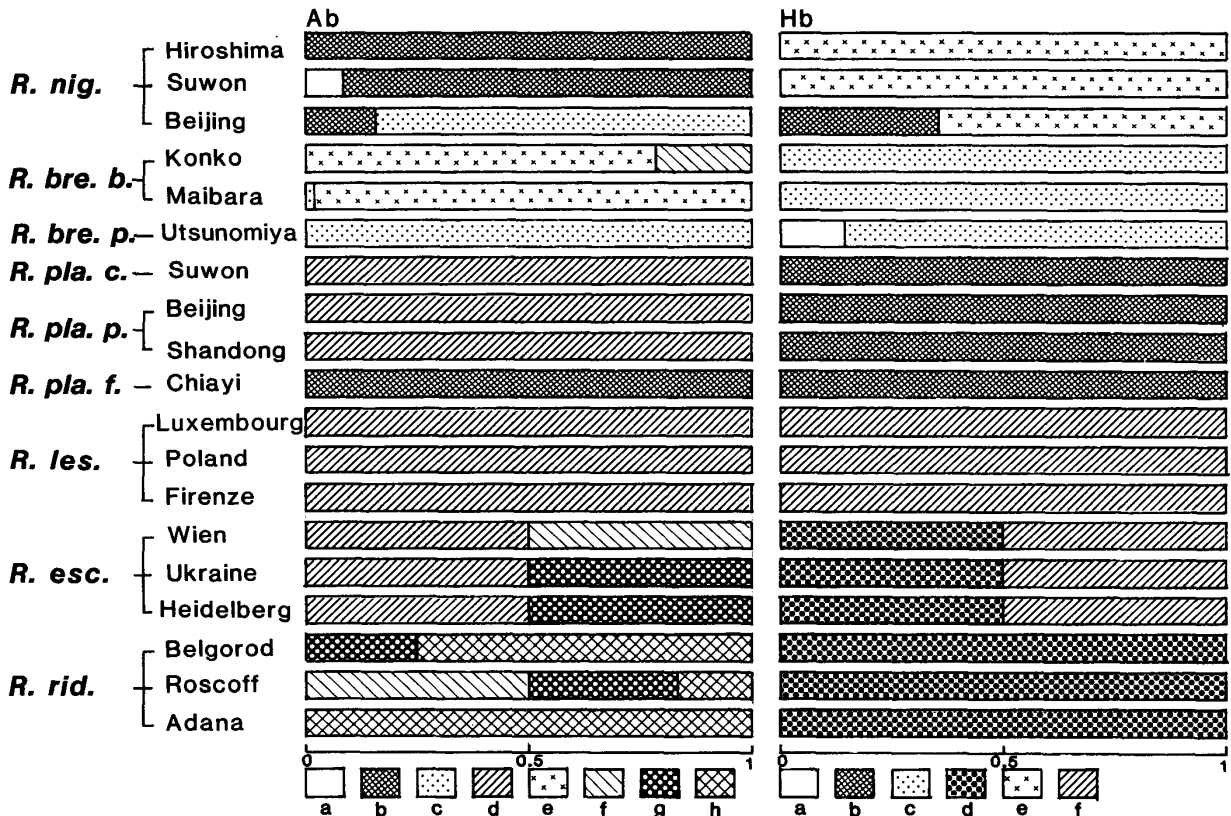


Fig. 9. Gene frequencies at two loci, Ab and Hb, in 19 populations of pond frogs in the Palearctic region.

Maibara population had allele *c* in a frequency of 0.022 in addition to allele *e*. The Utsunomiya population of *R. brevipoda porosa* had only allele *b*. The two populations of *R. p. plancyi* and the Suwon population of *R. plancyi chosenica* had only allele *d*, while the Chiayi population of *R. plancyi fukienensis* had only allele *b*.

In Europe, the three populations of *R. lessonae* had only allele *d*. While the Adana population of *R. ridibunda* had only allele *h*, the Belgorod population had alleles *h* and *g* in frequencies of 0.750 and 0.250, respectively. The Roscoff population had alleles *f*, *g* and *h* in frequencies of 0.500, 0.333 and 0.167, respectively. All the three populations of *R. esculenta* had allele *d* derived from *R. lessonae* in a frequency of 0.500. The Wien population had allele *f* derived from *R. ridibunda* in a frequency of 0.500, and the Ukraine and Heidelberg populations had allele *g* derived from *R. ridibunda* in a frequency of 0.500. All the three populations of *R. esculenta* showed the hybrid band, DF or DG, between *R. lessonae* and *R. ridibunda* in phenotype (Table 4-IV; Fig. 9).

26. Hb locus

The analyses of the electrophoretic patterns at the Hb locus in the 350 pond frogs of the 19 populations showed that there were nine phenotypes, AA, BB, CC, DD, EE, FF, AC, BE and DF, produced by six alleles, *a~f*.

The three populations of *R. nigromaculata* had allele *e* in frequencies of 0.643 and 1.000. The Beijing population had allele *b* in a frequency of 0.357 in addition to allele *e*, while the Hiroshima and Suwon populations had only allele *e*. The two populations of *R. b. brevipoda* had only allele *c*, while the Utsunomiya population of *R. brevipoda porosa* had alleles *c* and *a* in frequencies of 0.855 and 0.145, respectively. All the four populations of *R. plancyi* had only allele *b*. In Europe, the three populations of *R. lessonae* had only allele *f*, and the three populations of *R. ridibunda* had only allele *d*. All the three populations of *R. esculenta* had allele *f* derived from *R. lessonae* in a frequency of 0.500 and allele *d* derived from *R. ridibunda* in a frequency of 0.500. All of them showed a hybrid band, DF, in phenotype (Table 4-IV; Fig. 9).

III. Genetic variation

1. Proportion of heterozygous loci

The degree of differentiation at numerous loci of each individual is shown by the mean proportion of heterozygous loci. Then, in each of the 19 populations belonging to the six species and three subspecies distributed in the Palearctic region, the mean proportion of heterozygous loci per individual was estimated at 26 loci. The results showed that the mean proportions of heterozygous loci in the 19 populations were 2.2~57.7%, 14.8% on the average, in the actual values, 1.5~28.9%, 10.6% on the average, in the expected values. When the three populations of *R. esculenta* were excluded, as it has been clarified that *R. esculenta* is a kind of hybrids derived from *R. lessonae* and *R. ridibunda*, the mean proportions of heterozygous loci in the 16 populations belonging to the remaining five species and

TABLE 5
Genetic variabilities at 26 loci in 19 populations of pond frog species 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
<i>R. nigro.</i>	Hiroshima	38	6.0 (7.1)	34.6	1.38
“	Suwon	38	11.5 (11.5)	53.9	1.88
“	Beijing	21	11.9 (12.8)	61.5	1.81
<i>R. bre. b.</i>	Konko	38	8.0 (8.1)	23.1	1.23
“	Maibara	46	7.3 (7.4)	42.3	1.58
<i>R. bre. p.</i>	Utsunomiya	56	6.2 (7.2)	26.9	1.31
<i>R. pla. c.</i>	Suwon	33	3.3 (4.1)	11.5	1.12
<i>R. pla. p.</i>	Beijing	24	6.3 (6.1)	19.2	1.23
“	Shandong	12	7.4 (7.3)	23.1	1.23
<i>R. pla. f.</i>	Chiayi	7	11.6 (7.9)	19.2	1.27
<i>R. lesso.</i>	Luxembourg	10	3.2 (2.9)	7.7	1.08
“	Poland	9	2.2 (1.5)	3.9	1.04
“	Firenze	6	5.9 (5.4)	19.2	1.19
<i>R. escu.</i>	Wien	1	53.8 (26.9)	53.9	1.54
“	Ukraine	1	53.8 (26.9)	53.9	1.54
“	Heidelberg	2	57.7 (28.9)	57.7	1.58
<i>R. ridi.</i>	Belgorod	2	7.7 (7.7)	19.2	1.19
“	Roscoff	3	11.5 (12.2)	30.8	1.38
“	Adana	3	6.6 (8.8)	19.2	1.23
Average		18.4	14.8 (10.6)	30.6	1.36

Parentheses show an expected value.

three subspecies were 2.2~11.9, 7.3% on the average, in the actual values.

Of the 19 populations, the highest in the mean proportion of heterozygous loci were the three populations of *R. esculenta* derived from *R. lessonae* and *R. ridibunda*. They were 53.8~57.7%, in the actual values, which were very larger than the expected values of 26.9~28.9%. The next were the Beijing and Suwon populations of *R. nigromaculata*, the Chiayi population of *R. plancyi fukiensis*, and the Roscoff population of *R. ridibunda*. These were 11.9~11.5%, 11.6% on the average, and they were in the expected values 7.9~12.8%, 11.1% on the average. The third was the two populations of *R. b. brevipoda*, the Shandong population of *R. p. plancyi* and the Belgorod population of *R. ridibunda*, which were 8.0~7.3%, 7.6% on the average. The expected values of these populations were 8.1~7.3%, 7.6% on the average. The fourth were the Adana population of *R. ridibunda*, the Beijing population of *R. p. plancyi*, the Utsunomiya population of *R. brevipoda porosa*, the Hiroshima population of *R. nigromaculata* and the Firenze population of *R. lessonae*. Their actual values were 6.6~5.9%, 6.2% on the average, and their expected values were 8.8~5.4%, 6.9% on the average. The lowest group were the Suwon population of *R. plancyi chosenica*, and the Luxembourg and Poland populations of *R. lessonae*. These three populations were 3.3~2.2%, 2.9% on the average. The

expected values were 4.1~1.5%, 2.8% on the average (Table 5).

2. Proportion of polymorphic loci

The mean proportion of polymorphic loci at which each of the alleles was contained at the rate of more than 1% was estimated in each of the 19 populations belonging to the six species and three subspecies of pond frogs distributed in the Palearctic region. It was found that the mean proportions of polymorphic loci in the 19 populations were 3.9~61.5%, 30.6% on the average. The highest was 61.5% in the Beijing population of *R. nigromaculata*. The second were 57.7% and 53.9% in the three populations of *R. esculenta*. The third was 42.3% in the Maibara population of *R. b. brevipoda*, and the fourth was 34.6% in the Hiroshima population of *R. nigromaculata*. This was followed by 30.8% in the Roscoff population of *R. ridibunda*, 26.9% in the Utsunomiya population of *R. b. porosa*, 23.1% in the Konko population of *R. b. brevipoda* and the Shandong population of *R. p. plancyi*, 19.2% in the Beijing population of *R. p. plancyi*, the Chiayi population of *R. plancyi fukienensis*, the Firenze population of *R. lessonae*, and the Belgorod and Adana populations of *R. ridibunda*, 11.5% in the Suwon population of *R. plancyi chosenica*, and 7.7% in the Luxembourg population of *R. lessonae*. The lowest was 3.9% in the Poland population of *R. lessonae* (Table 5).

3. Mean number of alleles per locus

The mean number of alleles at each of the 26 loci in the 19 populations of the six species and three subspecies of pond frogs was counted. It was 1.04~1.88, 1.36 on the average. The largest was 1.88 in the Suwon population of *R. nigromaculata*. The next was 1.81 in the Beijing population of the same species. The third was 1.58 in the Maibara population of *R. b. brevipoda* and the Heidelberg population of *R. esculenta*. The fourth was 1.54 in the Wien and Ukraine populations of *R. esculenta*, which was followed in order by 1.38 in the Hiroshima population of *R. nigromaculata* and the Roscoff population of *R. ridibunda*, 1.31 in the Utsunomiya population of *R. brevipoda porosa*, and 1.27 in the Chiayi population of *R. plancyi fukienensis*. This was followed by 1.23 in the Konko population of *R. b. brevipoda*, and the Beijing and Shandong populations of *R. p. plancyi* and the Adana population of *R. ridibunda*. It decreased from 1.19 in the Firenze population of *R. lessonae* and the Belgorod population of *R. ridibunda* to 1.12 in the Suwon population of *R. plancyi chosenica*, and 1.08 in the Luxembourg population of *R. lessonae*. The smallest was 1.04 in the Poland population of *R. lessonae* (Table 5).

IV. Genetic distance

The genetic distances among the 19 populations belonging to the six species and three subspecies of pond frogs distributed in the Palearctic region were estimated on the basis of the gene frequencies by the method of NEI (1975).

1. Differences in the Eastern pond frogs

a. Differences between populations or subspecies

The genetic distances among the Hiroshima, Suwon and Beijing populations of *R. nigromaculata* were 0.073~0.177, 0.124 on the average. Those among the Konko and Maibara populations of *R. b. brevipoda* and the Utsunomiya population of *R. brevipoda porosa* were 0.096~0.212, 0.166 on the average. Those among the Suwon population of *R. plancyi chosenica*, the Beijing and Shandong populations of *R. p. plancyi*, and the Chiayi population of *R. plancyi fukienensis* were 0.029~0.480, 0.260 on the average. Those among the Suwon population of *R. plancyi chosenica*

TABLE 6
Genetic distance (D) among 19 populations of pond frog species in the Palearctic region

Species	Population	No.	1	2	3	4	5	6	7	8	9	10
<i>R. nigro.</i>	Hiroshima	1	—									
"	Suwon	2	0.121	—								
"	Beijing	3	0.177	0.073	—							
<i>R. bre. b.</i>	Konko	4	0.567	0.431	0.437	—						
"	Maibara	5	0.575	0.463	0.477	0.096	—					
<i>R. bre. p.</i>	Utsunomiya	6	0.684	0.583	0.489	0.190	0.212	—				
<i>R. pla. c.</i>	Suwon	7	0.838	0.724	0.606	0.755	0.772	0.765	—			
<i>R. pla. p.</i>	Beijing	8	0.765	0.676	0.603	0.753	0.737	0.714	0.075	—		
"	Shandong	9	0.735	0.576	0.500	0.659	0.664	0.638	0.075	0.029	—	
<i>R. pla. f.</i>	Chiayi	10	0.571	0.439	0.485	0.293	0.357	0.381	0.480	0.471	0.425	—
<i>R. less.</i>	Luxembourg	11	1.683	1.757	1.782	1.793	1.776	2.115	1.702	1.578	1.596	1.779
"	Poland	12	1.644	1.789	1.813	1.837	1.812	2.173	1.709	1.556	1.603	1.823
"	Firenze	13	1.183	1.245	1.263	1.288	1.274	1.474	1.326	1.215	1.246	1.391
<i>R. escu.</i>	Wien	14	1.506	1.633	1.617	1.568	1.545	1.857	1.625	1.507	1.559	1.767
"	Ukraine	15	1.487	1.533	1.555	1.477	1.551	1.721	1.560	1.433	1.454	1.525
"	Heidelberg	16	1.583	1.630	1.615	1.559	1.655	1.843	1.611	1.523	1.546	1.625
<i>R. ridi.</i>	Belgorod	17	1.704	1.653	1.640	1.499	1.675	1.712	1.741	1.682	1.676	1.611
"	Roscoff	18	1.839	1.783	1.742	1.613	1.768	1.851	1.847	1.818	1.811	1.789
"	Adana	19	1.819	1.758	1.707	1.676	1.747	1.824	1.905	1.794	1.788	1.885

Species	Population	No.	11	12	13	14	15	16	17	18	19
<i>R. less.</i>	Luxembourg	11	—								
"	Poland	12	0.016	—							
"	Firenze	13	0.166	0.163	—						
<i>R. escu.</i>	Wien	14	0.207	0.191	0.245	—					
"	Ukraine	15	0.165	0.164	0.216	0.082	—				
"	Heidelberg	16	0.179	0.178	0.232	0.083	0.027	—			
<i>R. ridi.</i>	Belgorod	17	0.631	0.648	0.537	0.289	0.192	0.165	—		
"	Roscoff	18	0.587	0.603	0.544	0.259	0.213	0.180	0.055	—	
"	Adana	19	0.671	0.688	0.576	0.262	0.267	0.254	0.081	0.086	—

and the Beijing and Shandong populations of *R. p. plancyi* were 0.029~0.075, 0.060 on the average. Those among the Chiayi population of *R. plancyi fukienensis* and the other three populations of *R. plancyi* were 0.425~0.480, 0.459 on the average (Table 6).

b. Differences between species

The genetic distances among *R. nigromaculata*, *R. brevipoda* and *R. plancyi* were 0.293~0.838, 0.597 on the average.

The genetic distances between the three populations, the Hiroshima, Suwon and Beijing, of *R. nigromaculata* and the three populations, the Konko, Maibara and Utsunomiya, of *R. brevipoda* were 0.431~0.684, 0.523 on the average. The genetic distances between the three populations of *R. nigromaculata* and the three populations, the Beijing, Shandong and Suwon, of *R. plancyi* were 0.500~0.838, 0.669 on the average, while those between the three populations of *R. nigromaculata* and the Chiayi population of *R. plancyi fukienensis* were 0.439~0.571, 0.498 on the average. The genetic distances between the three populations of *R. brevipoda* and the three populations, the Beijing, Shandong and Suwon, of *R. plancyi* were 0.638~0.772, 0.717 on the average, while those between the three populations of *R. brevipoda* and the Chiayi population of *R. plancyi fukienensis* were 0.293~0.381, 0.344 on the average (Table 6).

2. Differences in the European pond frogs

a. Differences between populations

The genetic distances among the Luxembourg, Poland and Firenze populations of *R. lessonae* were 0.016~0.166, 0.115 on the average, while those between the Firenze population and the other two populations were 0.163 and 0.166, 0.165 on the average. These were fairly larger than that between the Luxembourg and Poland populations which was 0.016. The genetic distances among the three populations of *R. esculenta* were 0.027~0.083, 0.064 on the average, and those among the three populations of *R. ridibunda* were 0.055~0.086, 0.074 on the average (Table 6).

b. Differences between species

The genetic distances between the three populations of *R. esculenta* and the three populations of *R. lessonae* were 0.164~0.245, 0.197 on the average, while those between the former and the three populations of *R. ridibunda* were 0.165~0.289, 0.231 on the average. The genetic distances between the three populations of *R. lessonae* and the three populations of *R. ridibunda* were 0.537~0.688, 0.609 on the average (Table 6).

The genetic distances between the three populations of *R. esculenta* and the Firenze population of *R. lessonae* were 0.216~0.245, 0.231 on the average, while those between the former and the Luxembourg and Poland populations of *R. lessonae* were 0.164~0.207, 0.181 on the average. On the other hand, the genetic distances between the three populations of *R. ridibunda* and the Firenze population

of *R. lessonae* were 0.537~0.576, 0.552 on the average, while those between the former and the Luxembourg and Poland populations of *R. lessonae* were 0.587~0.688, 0.638 on the average (Table 6).

3. Differences between the Eastern and European pond frogs

The genetic distances between the three species including 10 populations distributed in the East and the three species including nine populations distributed in Europe were 1.183~2.173, 1.647 on the average.

a. Differences between *R. lessonae* and the Eastern pond frogs

The genetic distances between the three populations of *R. lessonae* and the three population of *R. nigromaculata* were 1.183~1.813, 1.573 on the average. Those between the former and the three populations of *R. brevipoda* were 1.274~2.173, 1.727 on the average, while those between the former and the four populations of *R. plancyi* were 1.215~1.823, 1.544 on the average. The genetic distances between the three populations of *R. lessonae* and the 10 populations of the three Eastern species were 1.183~2.173, 1.608 on the average (Table 6).

The genetic distances between the Firenze population of *R. lessonae* and the populations of *R. nigromaculata*, *R. brevipoda* and *R. plancyi* were 1.230, 1.345 and 1.295, respectively. Those between the Luxembourg and Poland populations of *R. lessonae* and the populations of *R. nigromaculata*, *R. brevipoda* and *R. plancyi* were 1.745, 1.918 and 1.668, respectively. The genetic distances between the Firenze population of *R. lessonae* and 10 populations of the three Eastern species were 1.183~1.474, 1.291 on the average, while those between the Luxembourg and Poland populations of *R. lessonae* and the 10 populations of the three Eastern species were 1.556~2.173, 1.766 on the average (Table 6).

b. Differences between *R. esculenta* and the Eastern pond frogs

The genetic distances between the three populations of *R. esculenta* and the three populations of *R. nigromaculata* were 1.487~1.633, 1.573 on the average, those between the former and the three populations of *R. brevipoda* were 1.477~1.857, 1.642 on the average, and those between the former and the four populations of *R. plancyi* were 1.433~1.767, 1.561 on the average. The genetic distances between the three populations of *R. esculenta* and the 10 populations of the three Eastern species were 1.433~1.857, 1.589 on the average (Table 6).

c. Differences between *R. ridibunda* and the Eastern pond frogs

The genetic distances between the three populations of *R. ridibunda* and the three populations of *R. nigromaculata* were 1.640~1.839, 1.738 on the average, those between the former and the three populations of *R. brevipoda* were 1.499~1.851, 1.707 on the average, and those between the former and the four populations of *R. plancyi* were 1.611~1.905, 1.779 on the average. The genetic distances between the three populations of *R. ridibunda* and the 10 populations of the three Eastern species were 1.499~1.905, 1.745 on the average (Table 6).

V. Dendrogram

A dendrogram for the pond frogs belonging to the 19 populations of the six species and three subspecies distributed in the Palearctic region was drawn on the basis of genetic distances by the unweighted pair-group arithmetic average (UP-GMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975). This dendrogram showed that the pond frogs were first divided into the Eastern and European groups. From the Eastern group, *R. p. plancyi* and *R. p. chosenica* were derived in China and Korea, respectively. The remainder of the Eastern group was divided into two subgroups. From one subgroup, *R. plancyi fukiensis* was first deviated in Taiwan, and then *R. brevipoda* was differentiated in Japan. *R. nigromaculata* was differentiated from the other group in the East and distributed in China, Korea and Japan (Fig. 10).

The European group was divided into two subgroups, the *R. ridibunda* and *R. lessonae* including *R. esculenta* which was produced by crossing with *R. ridibunda* (Fig. 10).

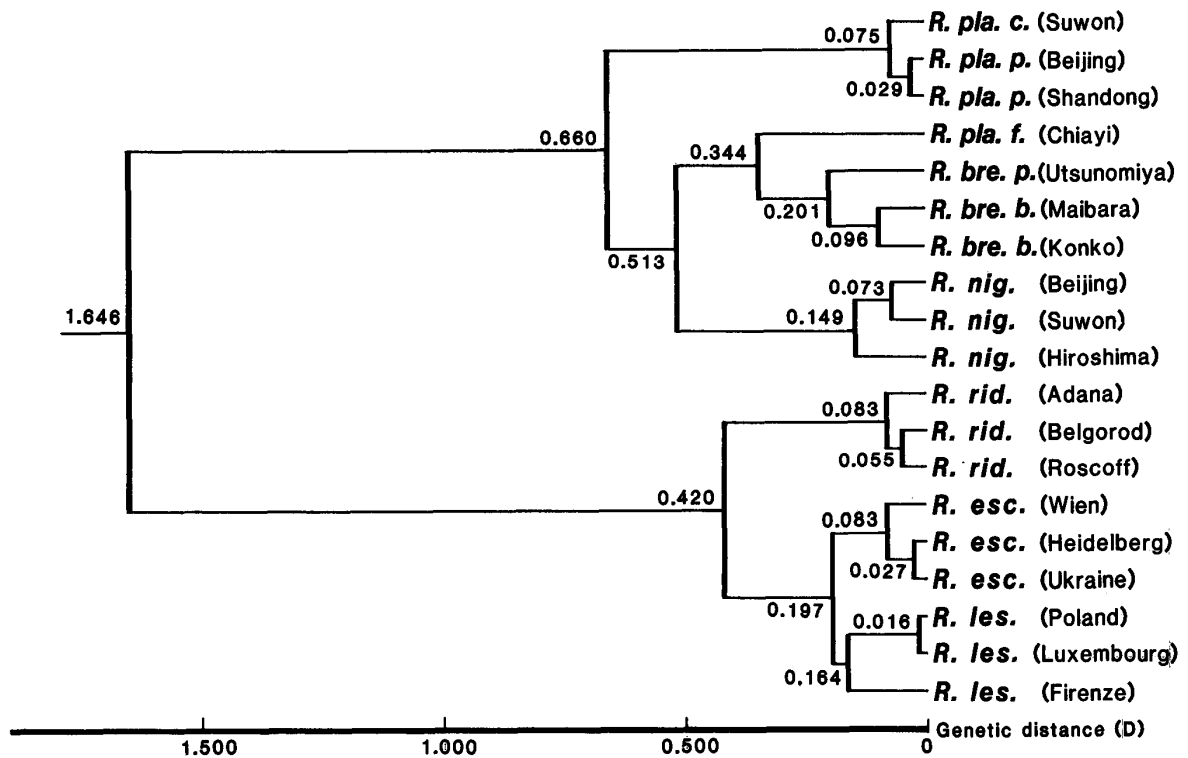


Fig. 10. Dendrogram for 19 populations of pond frogs in the Palearctic region based on genetic distances.

DISCUSSION

The dendrogram drawn for the European and Far Eastern pond (water or green) frogs in the present study shows that the two groups have been divided at a very old age. In the Far East, there are three species and three subspecies, *Rana*

nigromaculata, *R. brevipoda brevipoda*, *R. brevipoda porosa*, *R. plancyi plancyi*, *R. plancyi chosenica* and *R. plancyi fukienensis*. Of these frogs, *R. b. brevipoda* and *R. b. porosa* distributed in the Mainland of Japan, while *R. nigromaculata* is widely distributed in Japan, Korea and the eastern part of China. The dendrogram seems to show that *R. brevipoda* was derived from *R. plancyi*.

After the ancestor of the pond frogs was divided into the European and Far Eastern groups, the latter was divided into two groups in the beginning. One of these groups contained *R. plancyi* which was distributed in the eastern part of China including Beijing, Fukien and Korea. The specimens collected from China and Korea are named *R. p. plancyi* and *R. p. chosenica*, respectively, although these two subspecies are similar to each other in external characters and coloration. The other group was divided into three subgroups, *R. plancyi fukienensis*, *R. brevipoda* and *R. nigromaculata*. TING (1939) reported that reciprocal crosses between *R. nigromaculata* and *R. plancyi* collected from Beijing produced healthy hybrids. He suggested that *R. plancyi* probably arose from *R. nigromaculata* by mutation, since the latter species is more widely distributed. Later, TING (1948) described that a male of the two-year-old hybrids which he obtained from the above crossings produced active backcrosses by mating with a female *R. nigromaculata*.

R. plancyi fukienensis of the three subspecies was first described as a new species, *R. fukienensis*, by POPE (1929) and it was suggested by POPE and BORING (1940) that this species may be a hybrid between *R. nigromaculata* and *R. plancyi*. According to TING (1948), *R. fukienensis* may have been derived from *R. plancyi* of the north by gradual mutation according as this spread southward. The subspecific status of *R. plancyi* at the southern end of the range was suggested by BORING (1938), who gave the name, *R. plancyi fukienensis*. TING (1951) accepted BORING's (1938) suggestion that the Fukien pond frog should be *R. plancyi fukienensis*. TING, TSAI and LIU (1965) have insisted a close relationship of *R. nigromaculata* to *R. plancyi fukienensis* from the result of reciprocal crosses between them. The assumption that *R. plancyi* distributed in Taiwan which is an island situated near Fukien is *R. plancyi fukienensis* has been recognized by LIN and HUANG (1979), KAWAMURA and NISHIOKA (1973, 1975, 1977, 1979), KURAMOTO (1983), and NISHIOKA, OKUMOTO and RYUZAKI (1987) who made studies on karyotypes and speciation of pond frogs distributed in the Palearctic region.

Of the three subgroups including *R. plancyi fukienensis*, *R. brevipoda* and *R. nigromaculata*, *R. p. fukienensis* differs from the other two subspecies of *R. plancyi* in genetic distance. While the genetic distances among *R. p. chosenica* and the two populations of *R. p. plancyi* are 0.029~0.075, those between these three populations and the Chiayi population of *R. p. fukienensis* are 0.425~0.480 (Table 6). It is quite clear that *R. p. fukienensis* differs genetically from the other two subspecies of *R. plancyi*. On the other hand, the genetic distances between *R. plancyi fukienensis* and the three populations of *R. brevipoda* were 0.293~0.381, while those between the three populations of *R. p. plancyi* and *R. p. chosenica* and the three populations of *R. brevipoda* were 0.638~0.772. This seems to show that *R. p. fukienensis* has many genes which invaded from *R. brevipoda* by natural hybridization and introgression.

Similar invasion seems to have occurred from *R. nigromaculata* into *R. p. fukienensis*. The genetic distances between *R. p. fukienensis* and the Hiroshima, Suwon and Beijing populations of *R. nigromaculata* were 0.571, 0.439 and 0.485, respectively, while those between the three populations of *R. plancyi* including subspecific *plancyi* and *chosonica* and the three populations of *R. nigromaculata* were 0.500~0.838. Thus, *R. p. fukienensis* seems to have many genes which invaded from *R. nigromaculata* by natural hybridization and introgression. It seems very interesting that the genetic distances between *R. plancyi fukienensis* and the three populations of *R. brevipoda* distributed in the Mainland of Japan are remarkably smaller than those between *R. plancyi fukienensis* and the three populations of *R. nigromaculata* distributed widely in the Far East.

The results of analyses of the electrophoretic patterns in seven specimens of *R. p. fukienensis* at the 26 loci showed that the alleles of five loci differ from those of the same loci in 36 *R. p. plancyi* and 33 *R. p. chosonica*, while they were the same as those of the same loci in 84 *R. b. brevipoda* and 56 *R. b. porosa*. The allele *c* at the ADA locus was found in *R. p. fukienensis* at the rate of 0.92 and in the Konko, Maibara and Utsunomiya populations of *R. brevipoda* at the rates of 1.00, 0.97 and 0.75, respectively, while allele *d* was found at the rate of 1.00 each in the Suwon, Beijing and Shandong populations of *R. plancyi*. The allele *b* at the CK locus was found at the rate of 1.00 in *R. p. fukienensis* and in the Konko, Maibara and Utsunomiya populations of *R. brevipoda*, while allele *a* was found at the rate of 1.00 in each of the Suwon, Beijing and Shandong populations of *R. plancyi*. The allele *b* at the α -GDH locus was found at the rate of 1.00 in *R. p. fukienensis* and it was found in the Konko, Maibara and Utsunomiya populations of *R. brevipoda* at the rates of 1.00, 0.99 and 1.00, respectively, while the allele *a* was found in the Suwon, Beijing and Shandong populations of *R. plancyi* at the rates of 1.00, 1.00 and 0.79, respectively. The allele *b* at the MDH-A locus was found at the rate of 1.00 in *R. p. fukienensis* and in the Konko, Maibara and Utsunomiya populations of *R. brevipoda*, while allele *c* was found at the rate of 1.00 each in the Suwon, Beijing and Shandong populations of *R. plancyi*. The allele *c* at the MDH-B locus was found at the rate of 0.50 in *R. p. fukienensis* and at the rate of 1.00 each in the Konko, Maibara and Utsunomiya populations of *R. brevipoda*, while allele *f* was found in the Suwon, Beijing and Shandong populations of *R. plancyi* at the rates of 1.00, 1.00 and 0.83, respectively.

On the other hand, the alleles of three loci in *R. p. fukienensis* differ from those of the same loci in *R. p. chosonica* and *R. p. plancyi*, while they were the same as those of the same loci in *R. nigromaculata*. The allele *b* at the CK locus was found in *R. p. fukienensis* at the rate of 1.00 and in the Hiroshima, Suwon and Beijing populations of *R. nigromaculata* each at the rate of 1.00, while allele *a* was found in the Suwon, Beijing and Shandong populations of *R. plancyi* each at the rate of 1.00. The allele *b* at the MDH-A locus was found in *R. p. fukienensis* at the rate of 1.00, and in the Hiroshima, Suwon and Beijing populations of *R. nigromaculata* at the rates of 1.00, 1.00 and 0.93, respectively, while allele *c* was found in the Suwon, Beijing and Shandong populations of *R. plancyi* each at the rate of 1.00. The allele

b at the Ab locus was found in *R. p. fukienensis* at the rate of 1.00, and in the Hiroshima, Suwon and Beijing populations of *R. nigromaculata* at the rates of 1.00, 0.92 and 0.15, respectively, while allele *d* was found in the Suwon, Beijing and Shandong populations of *R. plancyi* each at the rate of 1.00.

The fact that all three populations of *R. brevipoda* distributed only in the Mainland of Japan have the same alleles as those at the five of the 26 loci in only *R. plancyi fukienensis* of the four populations of *R. plancyi* seems to show that *R. brevipoda* is closely related to *R. p. fukienensis*. It is probable that the ancestor of *R. brevipoda* was distributed in the southeastern part of China including Fukien and Taiwan.

The Hiroshima, Suwon and Beijing populations of *R. nigromaculata* have the same alleles as those at the three of the 26 loci in only *R. plancyi fukienensis*. This seems to show that *R. nigromaculata* is somewhat closely related to *R. p. fukienensis* as found between *R. nigromaculata* and *R. brevipoda* in the Mainland of Japan, and also to show that *R. nigromaculata* was distributed near *R. p. fukienensis* in olden times.

The dendrogram drawn for the European pond frogs shows that *R. lessonae* and *R. ridibunda* were differentiated early. BERGER (1966, 1967, 1968) first insisted that *R. esculenta* is a hybrid between *R. lessonae* and *R. ridibunda*. After BERGER, the hybrid origin of *R. esculenta* has been approved by many investigators, such as GÜNTHER (1967, 1968, 1973), BLANKENHORN, HEUSSER and VOGEL (1971), BLANKENHORN (1973), BLANKENHORN, HEUSSER and NOTTER (1973), and KAWAMURA and NISHIOKA (1986) from the results of field observations and crossing experiments, and by TUNNER (1970, 1972, 1973), ENGELMANN (1972, 1973, 1974), UZZELL and BERGER (1975), TUNNER and DOBROWSKY (1976), VOGEL and CHEN (1976a, b, 1977) and EBENDAL (1977) from the results of analyses of serum proteins and several enzymes in pond frogs collected from various areas of Europe.

In the present study, the hybrid origin of *R. esculenta* was also clarified by analyzing various enzymes extracted from the skeletal muscles and blood proteins. As there were no differences in the alleles at 11 of the 26 loci examined between *R. lessonae* and *R. ridibunda*, it was impossible to know the derivation of the alleles at these loci in *R. esculenta*. In contrast, as the alleles of *R. lessonae* differed from those of *R. ridibunda* at the other 15 loci, including AAT-A, AAT-B, ADH-B, ALD, α -GDH, GPI, LDH-B, ME-B, MPI, Pep-A, 6-PGD, PGM, SOD-A, Ab and Hb, it was possible to presume the origin of *R. esculenta*. At each locus, there were two different alleles which came from *R. lessonae* and *R. ridibunda* and each allele was found at the rate of 0.500. Although the origin of the allele *d* found at the rate of 0.500 at the LDH-B locus in the Wien population of *R. esculenta* was obscure, probably owing to scarcity of specimens, it was quite clear that *R. esculenta* was a hybrid between *R. lessonae* and *R. ridibunda*, as it has been insisted by BERGER and the other investigators listed above.

The genetic distances estimated from gene frequencies showed that the European pond frogs extremely differ from the Far Eastern, being 1.183~2.173. On the other hand, the genetic distances among the three Far Eastern species seemed to be near to those among the three European species. The former were

0.293~0.838, while the latter were 0.164~0.688. This seems to show that the differentiation of each species occurred at nearly the same ages in both Europe and the Far East.

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