Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. 9: 97-133 (1987)

Intraspecific Differentiation of *Rana tagoi* Elucidated by Electrophoretic Analyses of Enzymes and Blood Proteins

By

Midori NISHIOKA, Shigeru OHTA and Masayuki SUMIDA

Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Hiroshima 730, Japan

(With 22 Text-figures)

ABSTRACT

Intraspecific differentiation of *Rana tagoi* was examined by electrophoretic analyses of 14 enzymes extracted from the skeletal muscles and livers and two blood proteins. A total of 194 *Rana tagoi* collected from seven stations in the western Japan had 22 loci controlling the enzymes and blood proteins. Of the 22 loci, those of AAT-A, AK and CK showed a single phenotype produced by a single allele. The other loci showed 2~25 phenotypes produced by 2~10 alleles. At the 22 loci, there were 6.9 phenotypes produced by 4.3 alleles on the average.

The gene frequencies in the seven populations were examined at 19 of the 22 loci, except three loci of AAT-A, AK and CK, which consisted of a single allele. While these three loci were zero in Fst, five of the others were 0.015~0.068, four were 0.144~0.208, three were 0.312~0.377, three were 0.423~0.462, three were 0.516~0.621 and the remaining Hb locus was 1.000 in Fst. Of the seven populations, the three island populations, the Yaku, Hirado and Oki populations, were lower in average heterozygosity than the other four land populations, the Nabara, Kurama, Omogo and Ono populations. The proportions of polymorphic loci in the seven populations were 40.9~63.6%, 55.2% on the average. The mean numbers of alleles per locus in the seven populations were 1.6~2.5, 2.0 on the average.

The genetic distances among the seven populations were estimated on the basis of gene frequencies at the 22 loci. The largest distance (0.335) was found between the Yaku and Omogo populations, while the smallest (0.031) was found between the Nabara and Omogo populations. A dendrogram was drawn by the UPGMA clustering method. This dendrogram seems to indicate that the Yaku population (*Rana tagoi yakushimensis*) was differentiated earlier than the other six populations.

INTRODUCTION

Rana tagoi OKADA, TAGO's brown frog, is a Japanese endemic species named by OKADA in 1928. This species is widely distributed in mountain areas of Honshu, Shikoku and Kyushu and also in two islands, Yaku Isl. and Oki Isl. *Rana tagoi* always dwell along small mountain streams and usually do not migrate to any distance. Although they are very similar to each other in morphology and

ecology, there are slight local variations. As the population of Yaku Isl. somewhat differs in external characters from those of the mainlands of Japan, it was named *Rana tagoi yakushimensis* by NAKATANI and OKADA in 1966. While the population of Oki Isl. evidently differs from those of the mainlands and Yaku Isl. in size of mature frogs, no subspecific name has been given to this population. However, the latter is called by a popular name, TAGO's brown frog of Oki Isl.

In the present study, seven populations were biochemically compared with one another by electrophoresis. Three of these populations, the Yaku, Oki and Hirado populations, were collected from three small islands, Yaku, Oki and Hirado. Four other populations were obtained from three mainlands of Japan, the Ono population from Kyushu, the Omogo population from Shikoku and the Nabara and Kurama populations from Honshu. While the males are usually larger than the females in the Nabara and Omogo populations, in contrast to most frog species, the females are larger than the males in the other five populations. The males and females of the Kurama population are somewhat larger than those of the Nabara, Omogo, Ono and Hirado populations. It is considered interesting to compare these morphological differences with biochemical ones.

MATERIALS AND METHODS

The specimens of *Rana tagoi* OKADA used as materials in the present study were mature males and females collected in the summer of 1981 from seven stations of the western Japan: Nabara ravine, Hiroshima Prefecture, Omogo ravine, Ehime Prefecture, Mt. Kurama, Kyoto Prefecture, Ono, Oita Prefecture, Hirado Isl., Nagasaki Prefecture, Oki Isl., Shimane Prefecture and Yaku Isl., Kagoshima Prefecture. The numbers and body lengths of females and males collected from each of these seven localities are presented in Table 1.

TABLE 1

Specimens of *Rana tagoi* used in the present study

Localities	No. of frogs			Body length (mm)		
	Female	Male	Total	Female	Male	Total
Nabara, Hiroshima Pref.	16	11	27	33~50 (39.1±1.3)	31~48 (41.3±1.7)	31~50 (40.0±1.1)
Omogo, Ehime Pref.	10	23	33	31~52 (34.9±2.1)	32~50 (41.4±1.2)	31~52 (39.4±1.1)
Kurama, Kyoto Pref.	9	25	34	36~57 (51.4±2.1)	32~56 (46.6±1.5)	32~57 (47.9±1.3)
Ono, Oita Pref.	4	4	8	44~46 (45.0±0.4)	39~42 (40.5±0.6)	39~46 (42.8±1.0)
Hirado, Nagasaki Pref.	13	7	20	38~48 (42.2±0.8)	39~45 (41.6±0.8)	38~48 (42.0±0.6)
Oki, Shimane Pref.	7	22	29	51~57 (54.1±0.9)	39~50 (44.9±0.7)	39~57 (47.1±1.0)
Yaku, Kagoshima Pref.	22	21	43	31~52 (43.0±1.8)	30~51 (39.8±1.4)	30~52 (41.4±1.2)

The existence of reproductive isolating mechanisms between different populations of *Rana tagoi* was examined by crossing experiments using nine females of the Nabara population and four males of the Oki population and three males of the Yaku population. In addition to these males, four males of the Nabara population were used in the control matings.

The results of these experiments are shown in Table 2. While 73.8% and 81.0% of Nabara eggs cleaved normally by insemination with Nabara and Oki sperm, respectively, 55.8% of Nabara eggs did so by insemination with Yaku sperm. This seems to indicate that the Nabara and Yaku populations are slightly isolated by a low degree of gametic isolation. On the other hand, 78.3% and 72.4% of normally cleaved eggs in the control matings became normally feeding tadpoles and metamorphosed frogs, respectively. In the crossings between Nabara eggs and Oki sperm, 64.6% and 59.8% of normally cleaved eggs became normally feeding tadpoles and metamorphosed frogs, respectively. In the crossings between Nabara eggs and Yaku sperm, 65.8% and 53.2% of normally cleaved eggs became normally feeding tadpoles and metamorphosed frogs, respectively. These results seem to show that the Oki and Yaku populations are slightly isolated from the Nabara population by a low degree of hybrid inviability.

TABLE 2

Results of crossing experiments among one subspecies and two populations of *Rana tagoi*

Parents		No.of eggs	No. and % of normal cleavages		No. and % of normal tail-bud embryos		No. and % of normally hatched tadpoles		No. and % of normally feeding tadpoles		No. and % of metamorphosed frogs	
Female	Male											
Nabara	Nabara	275	203 (73.8%)	100	186 (67.6%)	91.6	170 (61.8%)	83.7	159 (57.8%)	78.3	147 (53.5%)	72.4
	Oki	258	209 (81.0%)	100	179 (69.4%)	85.6	154 (59.7%)	73.7	135 (52.3%)	64.6	125 (48.4%)	59.8
	Yaku*	283	158 (55.8%)	100	144 (50.9%)	91.1	130 (45.9%)	82.3	104 (36.7%)	65.8	84 (29.7%)	53.2

*, *R. t. yakushimensis*

Biochemical differences among the seven populations were examined by analyzing 14 enzymes extracted from skeletal muscles and livers and two blood proteins by the method of horizontal starch-gel electrophoresis. This method has been reported in detail by NISHIOKA, OHTANI and SUMIDA (1980). The buffer systems used in the electrophoresis are shown in Table 3, together with the abbreviations of the enzymes and proteins analyzed. The detection of each enzyme was performed by the agar overlay method of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification, and that of blood proteins was made with amido-black staining.

When each of multiple alleles exists in more than 1% frequency at a locus, this locus is considered to be polymorphic. As a standard to show the degree of genetic differentiation found at a definite locus among local populations, the fixation index (Fst) coined by WRIGHT (1978) was utilized. In order to show

TABLE 3
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	〃	〃
Mannose phosphate isomerase	MPI	〃	T-C pH 7.0
Peptidase	Pep	Liver	T-B-E pH 8.0
Phosphoglucomutase	PGM	Skeletal muscle	〃
Superoxide dismutase	SOD	〃	〃
Serum albumin	Ab	Blood serum	〃
Hemoglobin	Hb	Erythrocytes	T-B-E pH 8.6

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

quantitatively the genetic variations of local populations, the proportion of polymorphic loci and the average heterozygosity (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974) were utilized.

The genetic relationship among local populations was surmised by estimating the genetic identity (I) and the genetic distance (D) according to the method by NEI (1972, 1975). The systematic relationship of these local populations was surmised by the unweighted pair-group arithmetic average (UPGMA) clustering method (SOKAL and SNEATH, 1963; SNEATH and SOKAL, 1973; NEI, 1975) on the basis of the genetic distance (D).

OBSERVATION

I. Electrophoretic pattern and allelomorphs

Electrophoretic patterns of 12 enzymes extracted from skeletal muscles, two enzymes from livers, serum albumin and hemoglobin were analyzed in 81 mature females and 113 mature males obtained from the seven populations, Nabara, Omogo, Kurama, Ono, Hirado, Oki and Yaku. It was found that 22 loci participate in controlling these enzymes and blood proteins. The electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, ····· in the order of mobility from fast to slow, and the alleles were shown by *a, b, c,* ····· (Figs. 1~3).

Of the 22 loci, those of AAT-A, AK and CK showed a single phenotype produced by a single allele, *a*. At the three loci of IDH-A, LDH-A and Hb, two

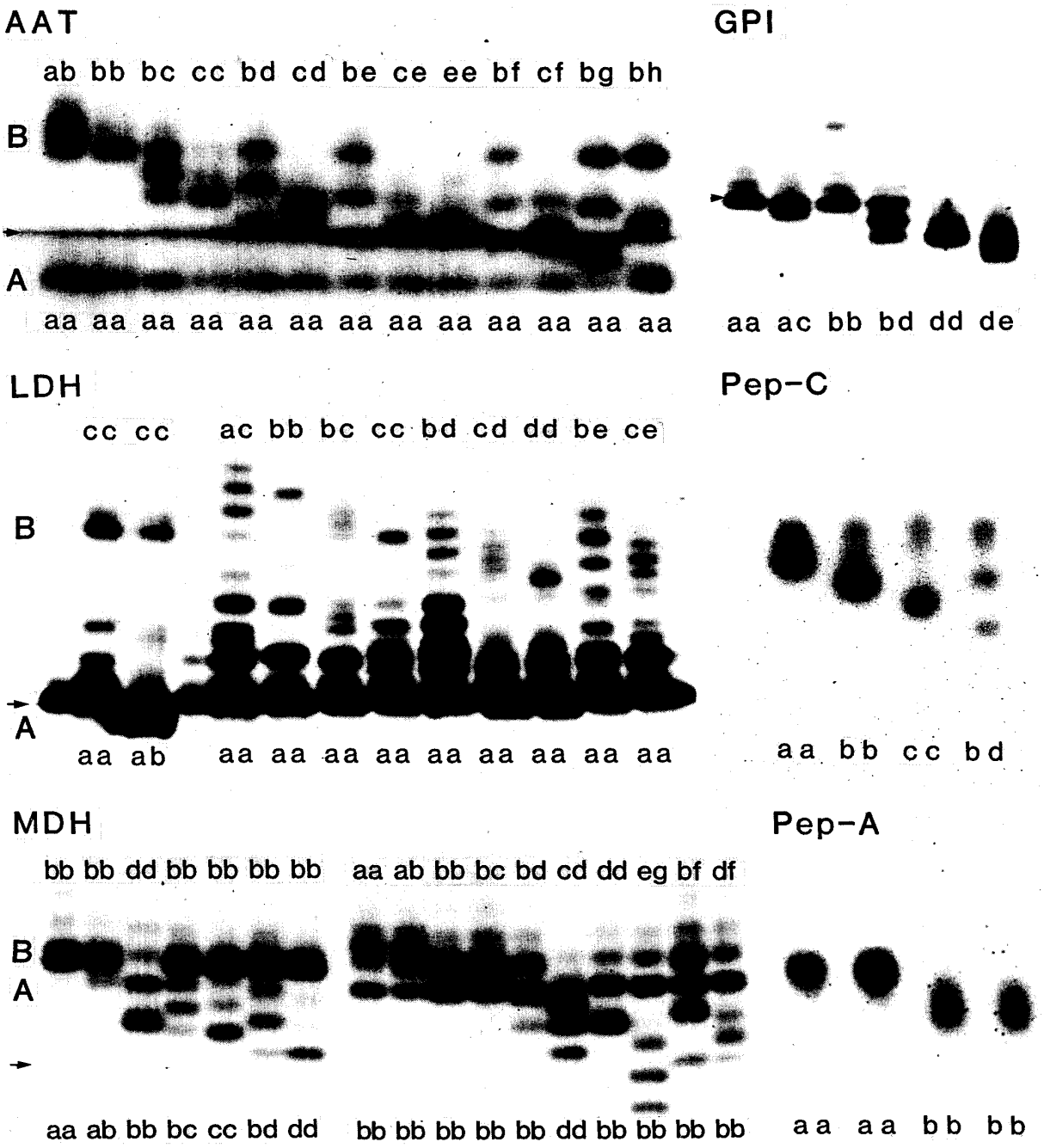

Fig. 1. Electrophoretic patterns of six enzymes, AAT, LDH, MDH, GPI, Pep-C and Pep-A, in one subspecies and six populations of *Rana tagoi*.

phenotypes produced by two alleles, *a* and *b*, were found, while three phenotypes were produced by two alleles, *a* and *b*, at the locus of Pep-A. There were four phenotypes produced by three alleles (*a*~*c*) at the locus of Fum, four to nine phenotypes produced by four alleles (*a*~*d*) at four loci of PGM, MDH-A, Pep-C and Ab, and five to nine phenotypes produced by five alleles (*a*~*e*) at four loci of *α*-GDH, GPI, SOD and LDH-B. There were eight and 10 phenotypes produced by six alleles (*a*~*f*) at the loci of IDH-B and Pep-D, respectively, 10 and 15

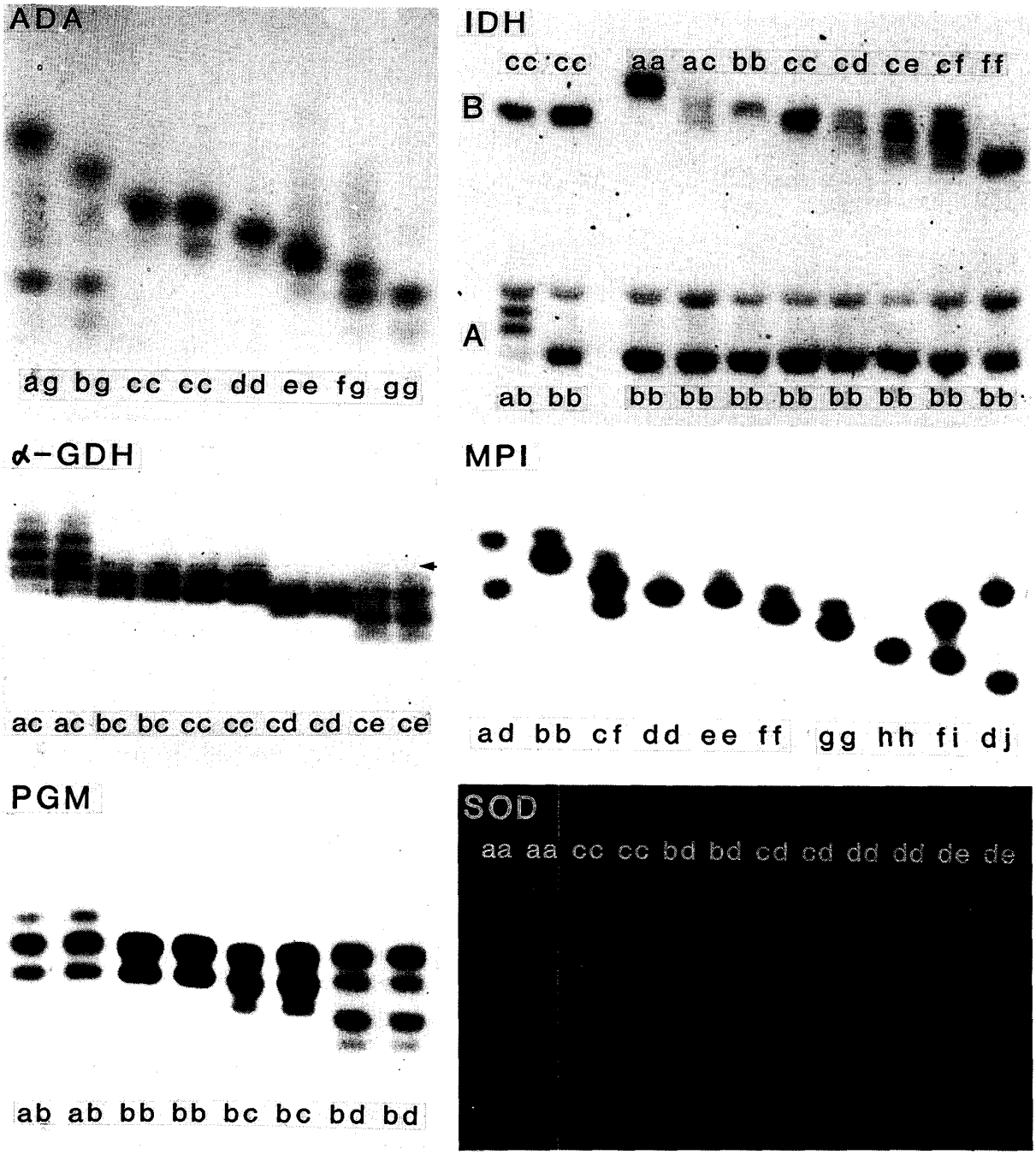

Fig. 2. Electrophoretic patterns of six enzymes, ADA, α-GDH, PGM, IDH, MPI and SOD, in one subspecies and six populations of *Rana tagoi*.

phenotypes produced by seven alleles (*a*~*g*) at the loci of MDH-B and ADA, respectively, and 13 phenotypes produced by eight alleles (*a*~*h*) at the locus of AAT-B. The most numerous phenotypes were found at the locus of MPI; there were 25 phenotypes produced by 10 alleles (*a*~*j*). At the foregoing 22 loci in total, there were 6.9 phenotypes produced by 4.3 alleles on the average (Table 4).

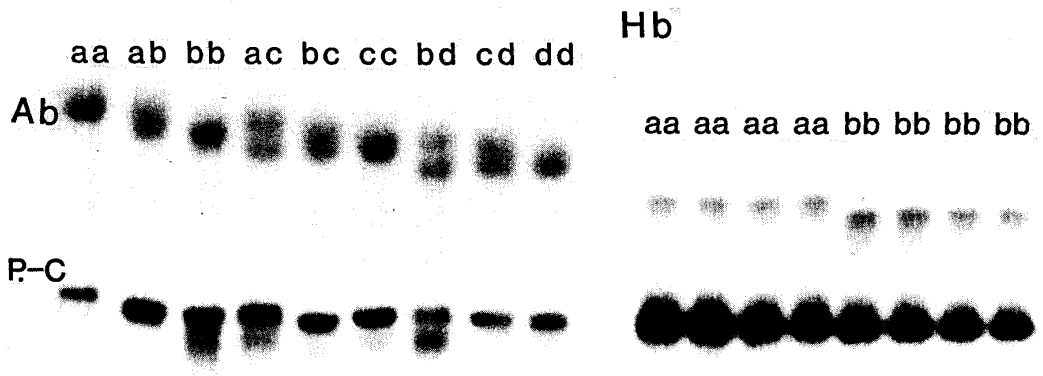

Fig. 3. Electrophoretic patterns of two proteins, Ab and Hb, in one subspecies and six populations of *Rana tagoi*.

TABLE 4
Number of phenotypes and alleles at 22 loci in one subspecies and six populations of *Rana tagoi*

Locus	No. of phenotypes	No. of alleles
AAT-A	1	1
AAT-B	13	8
ADA	15	7
AK	1	1
CK	1	1
Fum	4	3
α-GDH	5	5
GPI	6	5
IDH-A	2	2
IDH-B	8	6
LDH-A	2	2
LDH-B	9	5
MDH-A	7	4
MDH-B	10	7
MPI	25	10
Pep-A	3	2
Pep-C	8	4
Pep-D	10	6
PGM	4	4
SOD	7	5
Ab	9	4
Hb	2	2
Average	6.9	4.3

II. Gene frequency

Gene frequency in the seven populations was examined at 19 of the foregoing 22 loci (Table 5), except for three loci of AAT-A, AK and CK, which consisted of a single allele.

1. AAT-B locus

As stated above, 13 phenotypes produced by eight alleles, *a*, *b*, *c*, *d*, *e*, *f*, *g* and *h*, were found at the AAT-B locus in the seven populations. Of the 27 frogs of the Nabara population, 11 showed a homozygous band, BB, and three showed a homozygous band, CC, while four, one, six and two showed heterozygous bands, BC, BD, BF and CF, respectively. Of the 33 frogs of the Omogo population, 19 showed a homozygous band, BB, and one showed a homozygous band, CC, while six, one, three, one, one and one showed heterozygous bands, BC, BD, BE, BG, BH and CD, respectively. Of the 34 frogs of the Kurama population, 17 and two showed homozygous bands, BB and CC, respectively, while one and 14 showed heterozygous bands, AB and BC, respectively. Of the eight frogs of the Ono population, five and three showed a homozygous band, CC, and a heterozygous band, BC, respectively. Of the 20 frogs of the Hirado population, 11 and nine showed a homozygous band, CC, and a heterozygous band, BC, respectively. Of the 29 frogs of the Oki population, 20 and two showed homozygous bands, CC and EE, respectively, while seven showed a heterozygous band, CE. Of the 43 frogs of the Yaku population, 37 and one showed homozygous bands, CC and BB,

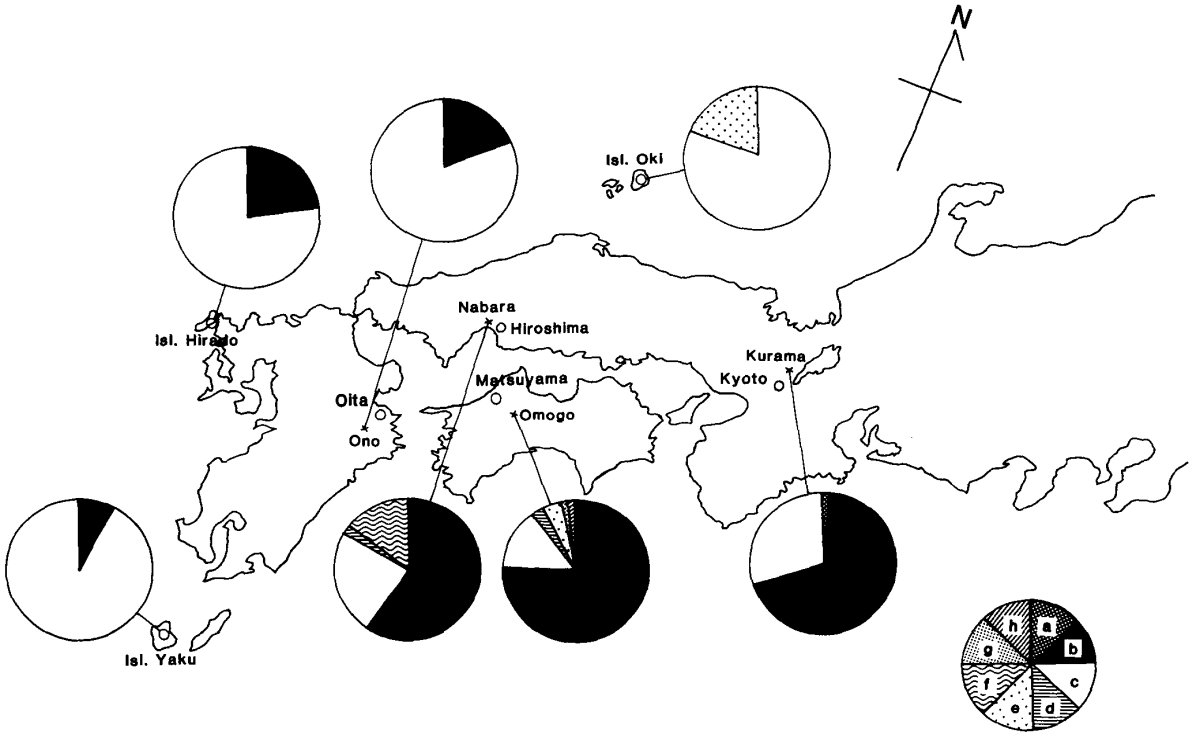

Fig. 4. Geographic distribution of AAT-B alleles among seven populations of *Rana tagoi* in western Japan.

respectively, while five showed a heterozygous band, BC. It was found that these numbers of homozygous and heterozygous frogs in each population almost agreed with the expected values calculated according to HARDY-WEINBERG law.

The gene frequency in each of the seven populations indicated that the six populations other than the Oki consisted of two alleles, *b* and *c*. In the Nabara, Omogo and Kurama populations, the frequency of allele *b* was high, being 0.611, 0.758 and 0.721, respectively, while allele *c* was low, being 0.222, 0.136 and 0.265, respectively. In the other three populations, Ono, Hirado and Yaku, the frequency of allele *c* was high, being 0.813, 0.775 and 0.919, respectively, while allele *b* was low, being 0.188, 0.225 and 0.081, respectively. In the Oki population, allele *c* was high in frequency, being 0.810. Allele *e* was found in place of allele *b* and was 0.190 in frequency. In the Nabara population, the frequencies of alleles *d* and *f* were 0.019 and 0.148, respectively, in addition to alleles *b* and *c*. In the Omogo population, there were four alleles, *d*, *e*, *g* and *h*, whose frequencies were 0.030, 0.045, 0.015 and 0.015, respectively, in addition to alleles *b* and *c*. In the Kurama population, a characteristic allele *a* was found in a frequency of 0.015, in addition to alleles *b* and *c* (Table 5; Fig. 4).

2. ADA locus

At the ADA locus, 15 phenotypes produced by seven alleles, *a*, *b*, *c*, *d*, *e*, *f* and *g*, were observed in the seven populations. Of the 27 frogs of the Nabara population, 12 showed a homozygous band, GG, while two, one, eight and four showed heterozygous bands, AG, CE, CG and EG, respectively. Of the 33 frogs of the Omogo population, 21 showed a homozygous band, GG, while five and seven showed heterozygous bands, CG and FG, respectively. Of the 34 frogs of the Kurama population, eight and three showed homozygous bands, CC and GG, while eight, five, one and nine showed heterozygous bands, BC, BG, CF and CG, respectively. Of the eight frogs of the Ono population, four showed a homozygous band, DD, while two, one and one showed heterozygous bands, DE, CD and DG, respectively. Of the 20 frogs of the Hirado population, 16 showed a homozygous band, DD, and four showed a heterozygous band, DG. Of the 29 frogs of the Oki population, 23 showed a homozygous band, DD, while four and two showed heterozygous bands, CD and DE, respectively. Of the 43 frogs of the Yaku population, 29 and one showed homozygous bands, DD and CC, respectively, while nine, one and three showed heterozygous bands, CD, CF and DF, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

The gene frequency in each population indicated that allele *g* was the highest, being 0.704 and 0.818, in the Nabara and Omogo populations, while it was 0.294, 0.063 and 0.100 in the Kurama, Ono and Hirado populations, respectively. In the remaining Oki and Yaku populations, allele *g* was zero in frequency. The frequency of allele *d* was the highest in the Ono, Hirado, Oki and Yaku populations, being 0.750, 0.900, 0.897 and 0.814, respectively. In the remaining three populations, Nabara, Omogo and Kurama, there was no allele *d*. In the

TABLE 5
Gene frequencies at 22 loci in one subspecies

Locality		Nabara	Omogo	Kurama	Ono	Hirado	Oki	Yaku*
Sample size		27	33	34	8	20	29	43
Locus	Allele							
AAT-A	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-B	*a*			0.015				
	b	0.611	0.758	0.721	0.188	0.225		0.081
	c	0.222	0.136	0.265	0.813	0.775	0.810	0.919
	d	0.019	0.030					
	e		0.045				0.190	
	f	0.148						
	g		0.015					
	h		0.015					
ADA	*a*	0.037						
	b			0.191				
	c	0.167	0.076	0.500	0.063		0.069	0.140
	d				0.750	0.900	0.897	0.814
	e	0.093			0.125		0.034	
	f		0.106	0.015				0.047
	g	0.704	0.818	0.294	0.063	0.100		
AK	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fum	*a*	0.037	0.030		0.063			0.047
	b	0.963	0.970	1.000	0.938	0.725	1.000	0.953
	c					0.275		
α-GDH	*a*				0.063			
	b	0.019						
	c	0.981	0.955	0.956	0.938	1.000	1.000	0.919
	d			0.044				0.081
	e		0.045					
GPI	*a*							0.884
	b	0.130	0.273	0.853	0.714	1.000	1.000	
	c							0.116
	d	0.852	0.712	0.147	0.286			
	e	0.019	0.015					
IDH-A	*a*							0.047
	b	1.000	1.000	1.000	1.000	1.000	1.000	0.953
IDH-B	*a*	0.111						
	b				0.125			
	c	0.889	0.818	1.000	0.813	1.000	0.569	1.000
	d		0.030		0.063			
	e		0.076					
	f		0.076				0.431	
LDH-A	*a*	1.000	1.000	1.000	1.000	1.000	0.983	1.000
	b						0.017	
LDH-B	*a*		0.045					0.012
	b	0.296	0.106					
	c	0.704	0.803	1.000	0.563	0.200	1.000	0.988
	d		0.015		0.438	0.800		
	e		0.030					

*, *R. t. yakushimensis*

and six populations of *Rana tagoi*

Locality		Nabara	Omogo	Kurama	Ono	Hirado	Oki	Yaku*
Sample size		27	33	34	8	20	29	43
Locus	Allele							
MDH-A	*a*			0.515				
	b	0.944	1.000	0.485	1.000	0.500	0.897	1.000
	c	0.037					0.103	
	d	0.019				0.500		
MDH-B	*a*			0.029				0.047
	b	0.352		0.632	0.750	0.850	1.000	0.826
	c					0.100		
	d	0.556	1.000	0.338	0.250	0.050		0.128
	e	0.019						
	f	0.056						
	g	0.019						
MPI	*a*	0.019					0.017	
	b				0.125		0.448	
	c	0.019		0.029				
	d	0.093	0.197	0.324		0.175		
	e						0.534	
	f	0.056		0.176				0.333
	g	0.574	0.500	0.294	0.750	0.800		
	h	0.148		0.176		0.025		0.667
	i	0.056	0.288		0.063			
	j	0.037	0.015		0.063			
Pep-A	*a*	1.000	0.922	1.000	1.000	1.000	1.000	1.000
	b		0.078					
Pep-C	*a*	0.056	0.061	0.353		0.025		0.023
	b	0.741	0.909	0.500	0.813	0.975	1.000	0.674
	c	0.167	0.015	0.147	0.188			0.279
	d	0.037	0.015					0.023
Pep-D	*a*						0.052	
	b				0.063			0.023
	c	0.130	0.047	0.029	0.375	0.075	0.448	0.058
	d	0.815	0.953	0.971	0.438	0.925	0.448	0.907
	e	0.056			0.125		0.052	
	f							0.012
PGM	*a*							0.058
	b	1.000	0.985	1.000	1.000	0.975	1.000	0.942
	c					0.025		
	d		0.015					
SOD	*a*						0.034	0.512
	b			0.015				
	c	0.019	0.379		0.688	1.000	0.966	0.488
	d	0.981	0.606	0.985	0.313			
	e		0.015					
Ab	*a*			0.044			0.897	1.000
	b	0.212	0.076	0.162			0.069	
	c	0.712	0.621	0.794	0.429	0.875	0.034	
	d	0.077	0.303		0.571	0.125		
Hb	*a*							1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	

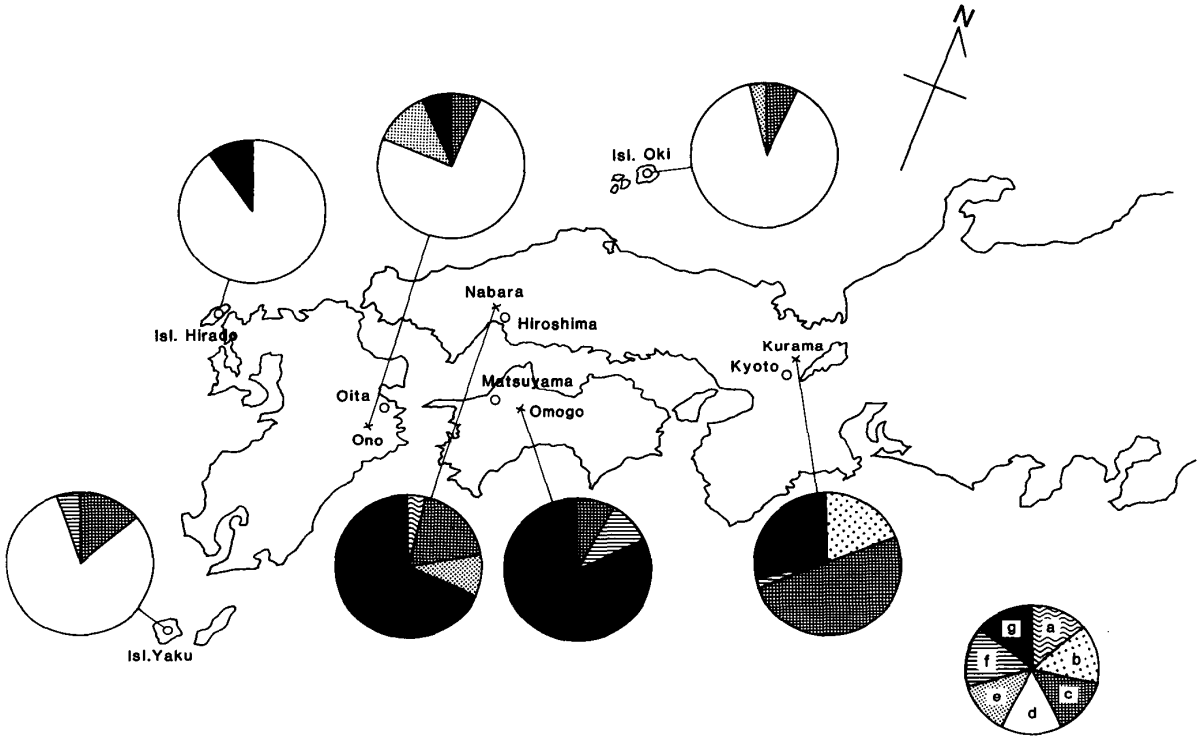

Fig. 5. Geographic distribution of ADA alleles among seven populations of *Rana tagoi* in western Japan.

Kurama population, allele *c* was 0.500 in frequency, while this allele was 0.167, 0.140, 0.076, 0.069 and 0.063 in the Nabara, Yaku, Omogo, Oki and Ono populations, respectively. Allele *a* existed in the Nabara population alone in a frequency of only 0.037. Allele *b* also existed in the Kurama population alone in a frequency of 0.191. Allele *e* was found in the Nabara, Ono and Oki populations in the frequencies of 0.093, 0.125 and 0.034, respectively. In contrast, allele *f* in the Omogo, Kurama and Yaku populations was 0.106, 0.015 and 0.047 in frequency, respectively (Table 5; Fig. 5).

3. Fum locus

At the Fum locus, four phenotypes produced by three alleles, *a*, *b* and *c*, were found in the seven populations. In all these seven populations, the frogs showing a homozygous band, BB, were overwhelmingly numerous. In the Kurama and Oki populations, 34 and 29 frogs, respectively, showed a homozygous band, BB. In the Nabara, Omogo, Ono and Yaku populations, 25 (92.6%), 31 (93.9%), 7 (87.5%) and 39 (90.7%) frogs showed a homozygous band, BB, respectively. In the Hirado population, 11 (55.0%) frogs showed a homozygous band, BB, and two showed a homozygous band, CC, and seven showed a heterozygous band, BC. Two, two, one and four frogs of the Nabara, Omogo, Ono and Yaku populations, respectively, showed a heterozygous band, AB. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

It was found that all the seven populations were almost occupied by allele *b*,

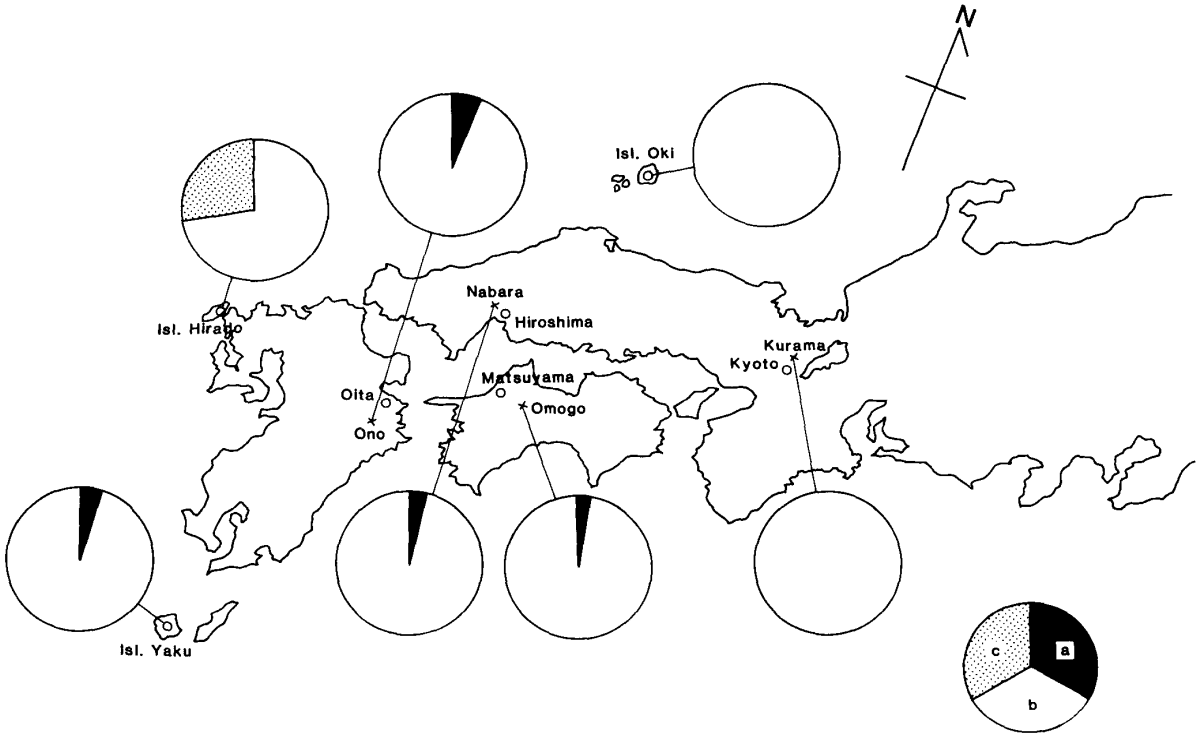

Fig. 6. Geographic distribution of Fum alleles among seven populations of *Rana tagoi* in western Japan.

being 0.725~1.000 in gene frequency. Allele *a* in the Nabara, Omogo, Ono and Yaku populations was 0.030~0.063 in frequencies. Allele *c* found in the Hirado population alone was 0.275 in a frequency (Table 5; Fig. 6).

4. α-GDH locus

At the α-GDH locus, five phenotypes produced by five alleles, *a, b, c, d* and *e,* were found. The frogs which were CC in phenotype were overwhelmingly numerous in all the seven populations: 26 (96.3%) frogs of the Nabara, 30 (90.9%) of the Omogo, 31 (91.2%) of the Kurama, seven (87.5%) of the Ono, 20 (100%) of the Hirado, 29 (100%) of the Oki and 36 (83.7%) of the Yaku population showed a homozygous band, CC. One frog of the Ono population and one frog of the Nabara population showed heterozygous bands, AC and BC, respectively. Three of the Kurama population and seven of the Yaku population showed a heterozygous band, CD, while three of the Omogo population showed a heterozygous band, CE. These numbers of frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

In gene frequency, allele *c* was 0.919~1.000 in all the seven populations. Besides, allele *b* was 0.019 in the Nabara population and allele *e* was 0.045 in the Omogo population. Allele *d* was 0.044 and 0.081 in the Kurama and Yaku populations, respectively. Allele *a* was 0.063 in the Ono population (Table 5; Fig. 7).

5. GPI locus

At the GPI locus, six phenotypes produced by five alleles, *a, b, c, d* and *e,* were

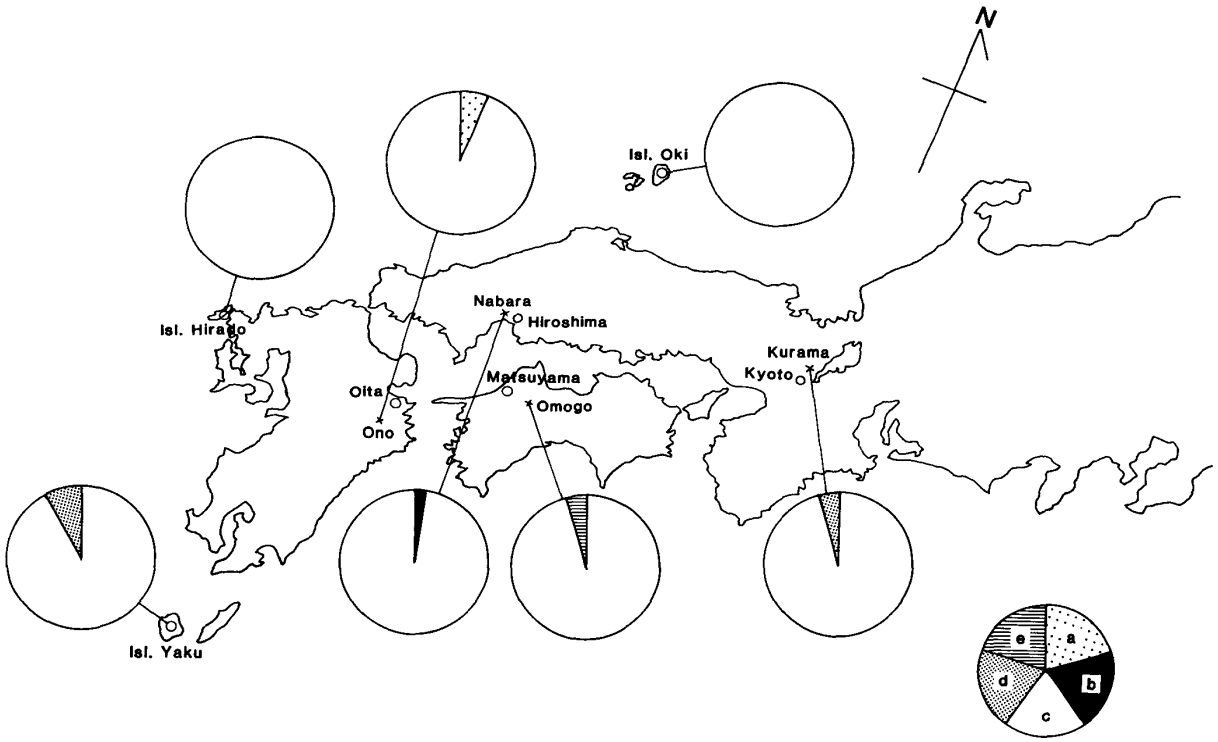

Fig. 7. Geographic distribution of α–GDH alleles among seven populations of *Rana tagoi* in western Japan.

found in the seven populations. In the Nabara and Omogo populations, 20 of the 27 frogs and 17 of the 33 frogs, respectively, showed a homozygous band, DD, and one and three frogs, respectively, showed a homozygous band, BB. In contrast, 26 of the 34 frogs of the Kurama, three of the seven frogs of the Ono, all of the 20 frogs of the Hirado and all of the 29 frogs of the Oki population showed a homozygous band, BB. While two frogs of the Kurama population showed a homozygous band, DD, there were no frogs showing DD in the other three populations. In the Yaku population, 33 of the 43 frogs showed a homozygous band, AA, and 10 showed a heterozygous band, AC. Five frogs of the Nabara, 12 frogs of the Omogo, six frogs of the Kurama and four frogs of the Ono population showed a heterozygous band, BD, while one frog of the Nabara and one frog of the Omogo population showed a heterozygous band, DE. These numbers of frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

The Yaku population distinctly differed from the other six populations in having alleles *a* and *c*. Alleles *a* and *c* were 0.884 and 0.116 in frequency, respectively. In the six populations other than the Yaku population, the locus of GPI was almost occupied by allele *b* or *d*, or by these two alleles. In the Nabara and Omogo populations, allele *d* was 0.852 and 0.712, and allele *b* was 0.130 and 0.273 in frequency, respectively. In the Kurama, Ono, Hirado and Oki populations, allele *b* was high in frequency, being 0.853, 0.714, 1.000 and 1.000, respectively. In the Kurama and Ono populations, allele *d* was 0.147 and 0.286, respectively, while no allele *d* was found in the Hirado and Oki populations. In the Nabara

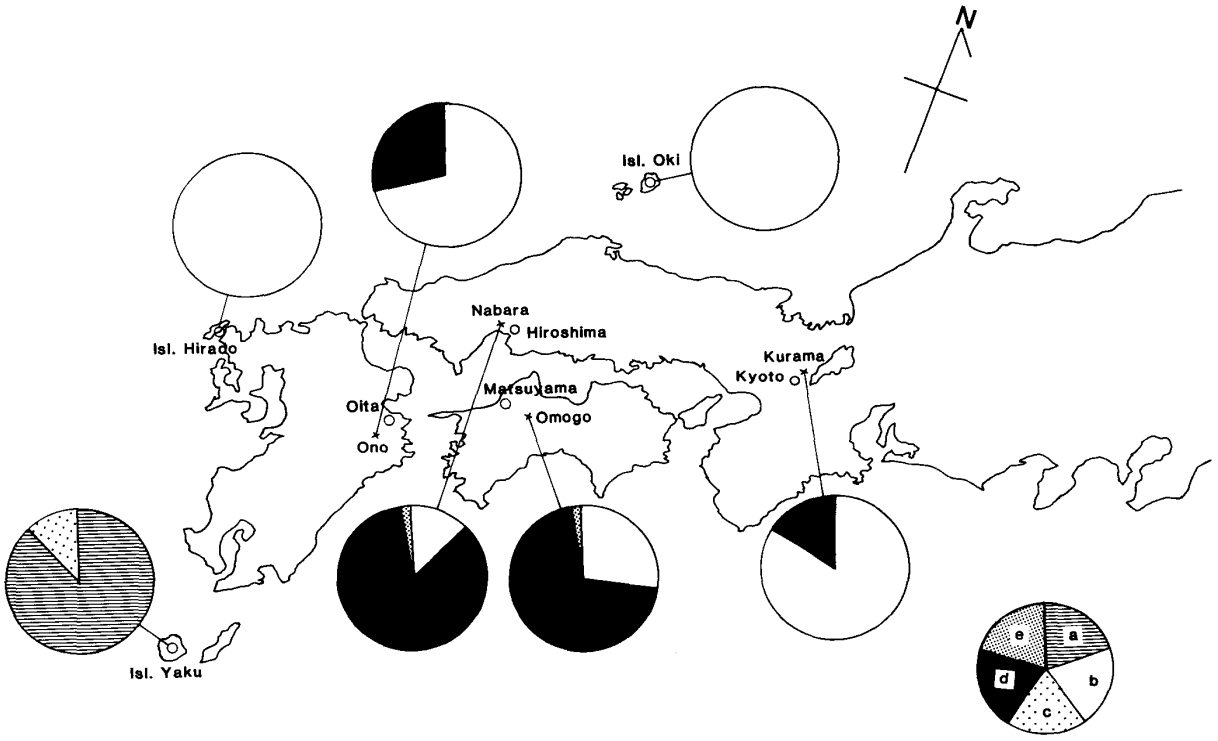

Fig. 8. Geographic distribution of GPI alleles among seven populations of *Rana tagoi* in western Japan.

and Omogo populations, allele *e* was slightly found, being 0.019 and 0.015 in frequency, respectively (Table 5; Fig. 8).

6. IDH-A locus

At the IDH-A locus, two phenotypes produced by two alleles, *a* and *b*, were observed. In the Yaku population, 39 of 43 frogs showed a homozygous band, BB, while the other four showed a heterozygous band, AB. In the six populations other than the Yaku population, all the frogs showed a homozygous band, BB.

In the Yaku population, alleles *b* and *a* were 0.953 and 0.047 in frequency, respectively. The other six populations were occupied by allele *b* alone (Table 5; Fig. 9).

7. IDH-B locus

At the IDH-B locus, eight phenotypes produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*, were found in the seven populations, although 155 (79.9%) of 194 frogs in total showed a homozygous band, CC. In addition to the homozygous band, CC, one and four frogs of the Nabara population showed a homozygous band, AA, and a heterozygous band, AC, respectively. In the Omogo population, two, five and five frogs showed heterozygous bands, CD, CE and CF, respectively. In the Ono population, one showed a homozygous band, BB, and one a heterozygous band, CD. In the Oki population, five and 15 frogs showed a homozygous band, FF, and a heterozygous band, CF, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

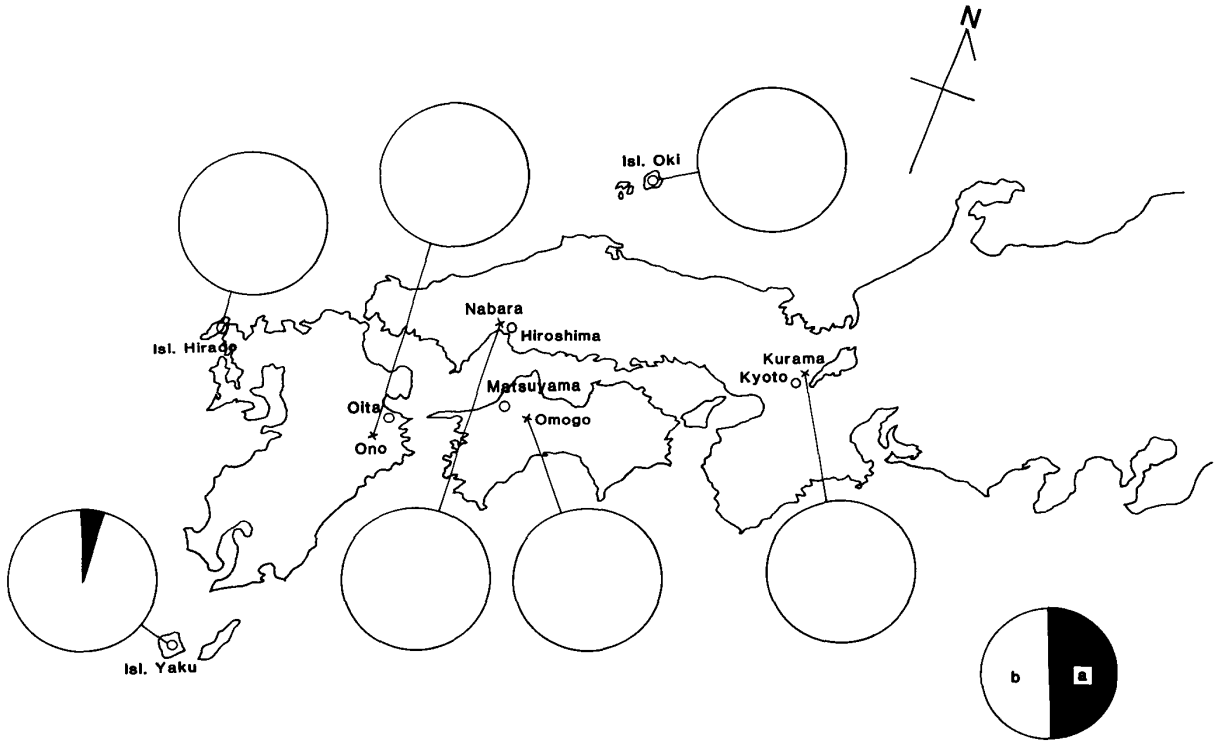

Fig. 9. Geographic distribution of IDH-A alleles among seven populations of *Rana tagoi* in western Japan.

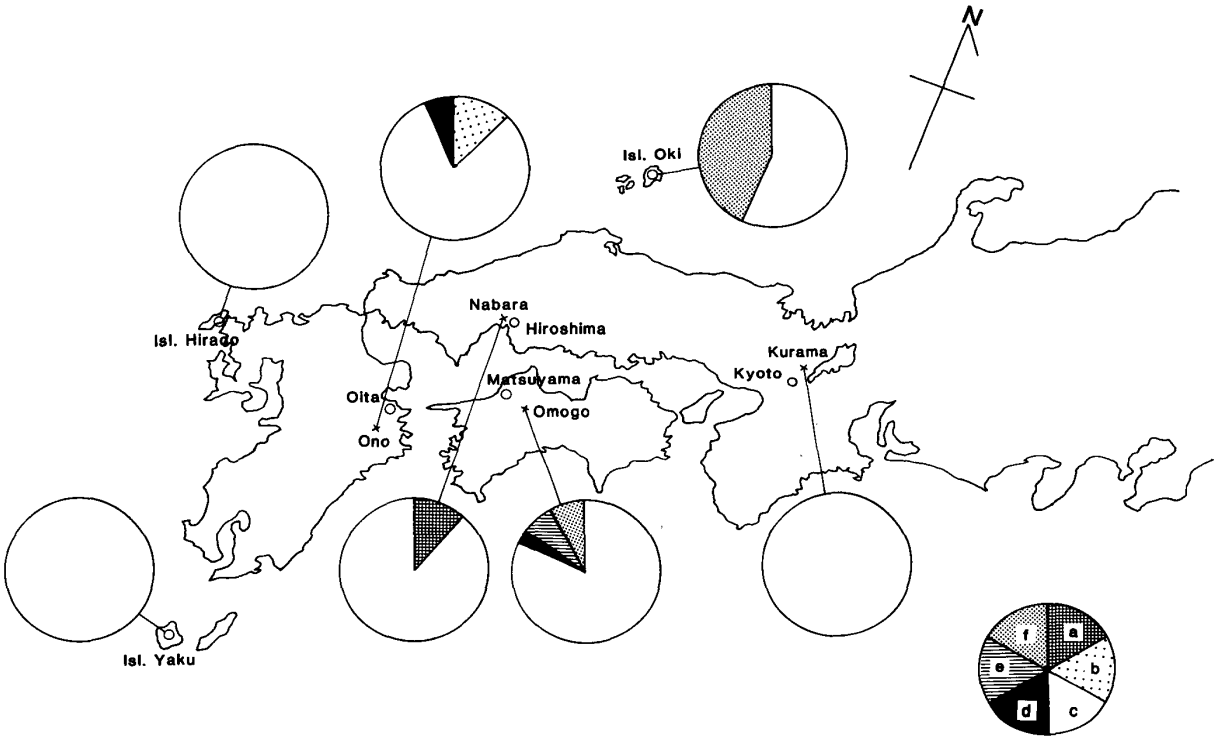

Fig. 10. Geographic distribution of IDH-B alleles among seven populations of *Rana tagoi* in western Japan.

In the Nabara population, alleles *a* and *c* were 0.111 and 0.889 in frequency, respectively. In the Omogo population, alleles *c*, *d*, *e* and *f* were 0.818, 0.030, 0.076 and 0.076, respectively. In the Ono population, alleles *b*, *c* and *d* were 0.125, 0.813 and 0.063, respectively. In the Oki population, alleles *c* and *f* were 0.569 and 0.431, respectively. In the other three populations, no alleles other than *c* were found (Table 5; Fig. 10).

8. LDH-A locus

Of 194 frogs in the seven populations, 193 showed a homozygous band, AA, while one frog in the Oki population showed a heterozygous band, AB. The existence of this single heterozygous frog showed that alleles *a* and *b* were 0.983 and 0.017 in frequency, respectively, in the Oki population (Table 5; Fig. 11).

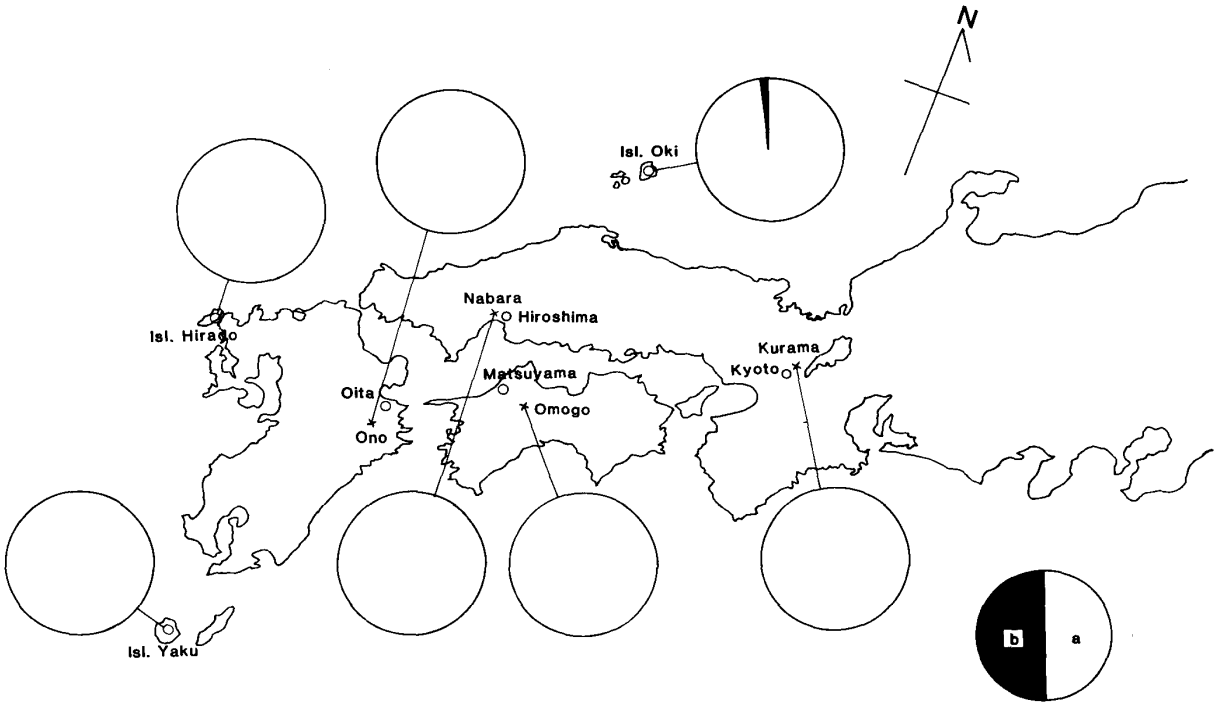

Fig. 11. Geographic distribution of LDH-A alleles among seven populations of *Rana tagoi* in western Japan.

9. LDH-B locus

At the LDH-B locus, nine phenotypes produced by five alleles, *a*, *b*, *c*, *d* and *e*, were observed. While a homozygous band, CC, was found in six populations other than the Hirado, the Kurama and Oki populations showed a homozygous band, CC, alone. In the Yaku population, 42 of 43 frogs showed a homozygous band, CC, and the remainder showed a heterozygous band, AC. In the Omogo population, 22 of 33 frogs showed a homozygous band, CC, while three, five, one, one and one showed heterozygous bands, AC, BC, BD, BE and CE, respectively. In the Nabara population, 12 of 27 frogs showed a homozygous band, CC, while one and 14 showed a homozygous band, BB, and a heterozygous band, BC, respectively. In the Ono population, three, two and three of eight frogs showed

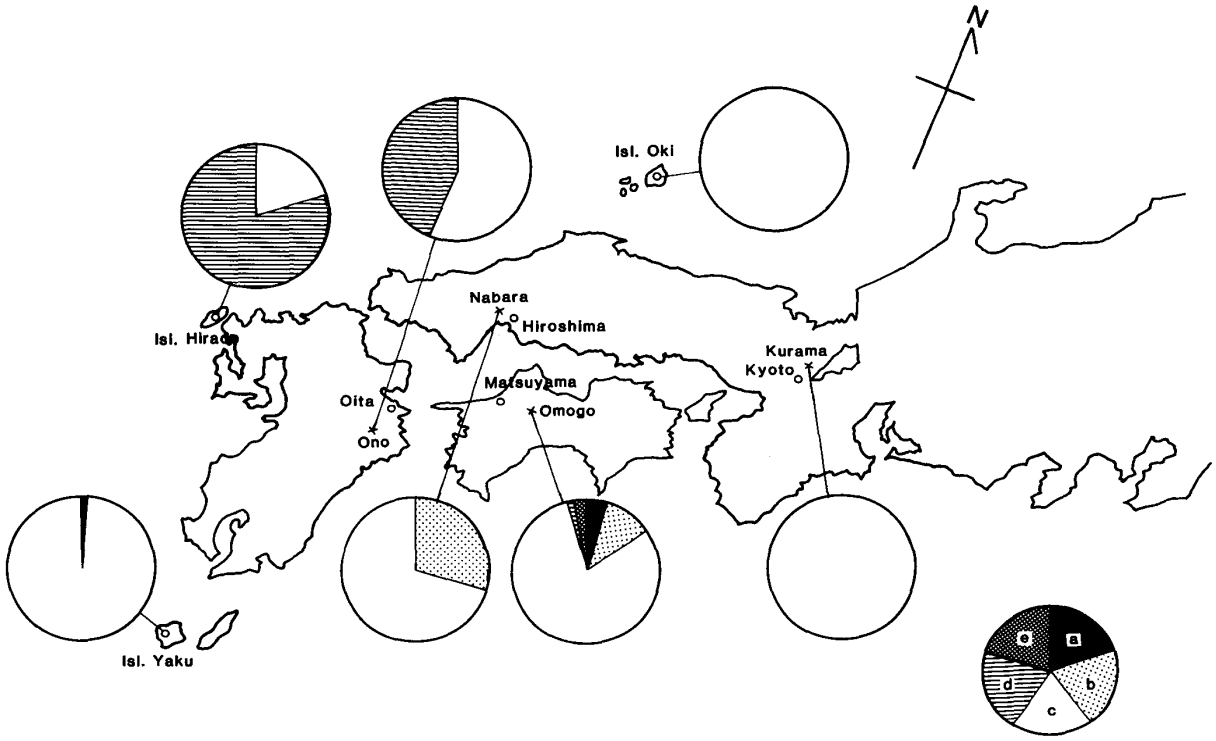

Fig. 12. Geographic distribution of LDH-B alleles among seven populations of *Rana tagoi* in western Japan.

homozygous bands, CC and DD, and a heterozygous band, CD, respectively. In the Hirado population, 12 and eight of the 20 frogs showed a homozygous band, DD, and a heterozygous band, CD, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law, although there was a slight disagreement between them.

Allele *c* was found in all the seven populations. This allele was 0.704~1.000 in frequency in five populations other than the Ono and Hirado populations. In the Ono population, alleles *c* and *d* were 0.563 and 0.438 in frequency, respectively. In the Hirado population, alleles *c* and *d* were 0.200 and 0.800, respectively. In the Omogo population, allele *e* was found in a low frequency, being 0.030. Allele *a* was 0.045 and 0.012 in the Omogo and Yaku populations, respectively. Allele *b* was 0.296 and 0.106 in the Nabara and Omogo populations, respectively. Allele *d* was 0.015 in the Omogo population (Table 5; Fig. 12).

10. MDH-A locus

At the MDH-A locus, there were seven phenotypes produced by four alleles, *a, b, c* and *d*. Frogs having a homozygous band, BB, were found in all the seven populations. All the frogs of the Omogo, Ono and Yaku populations showed a homozygous band, BB. Of the 27 frogs of the Nabara population, 24 showed a homozygous band, BB, and two and one showed heterozygous bands, BC and BD, respectively. In the Oki population, 25 of the 29 frogs showed a homozygous band, BB, two showed a homozygous band, CC, and two showed a heterozygous

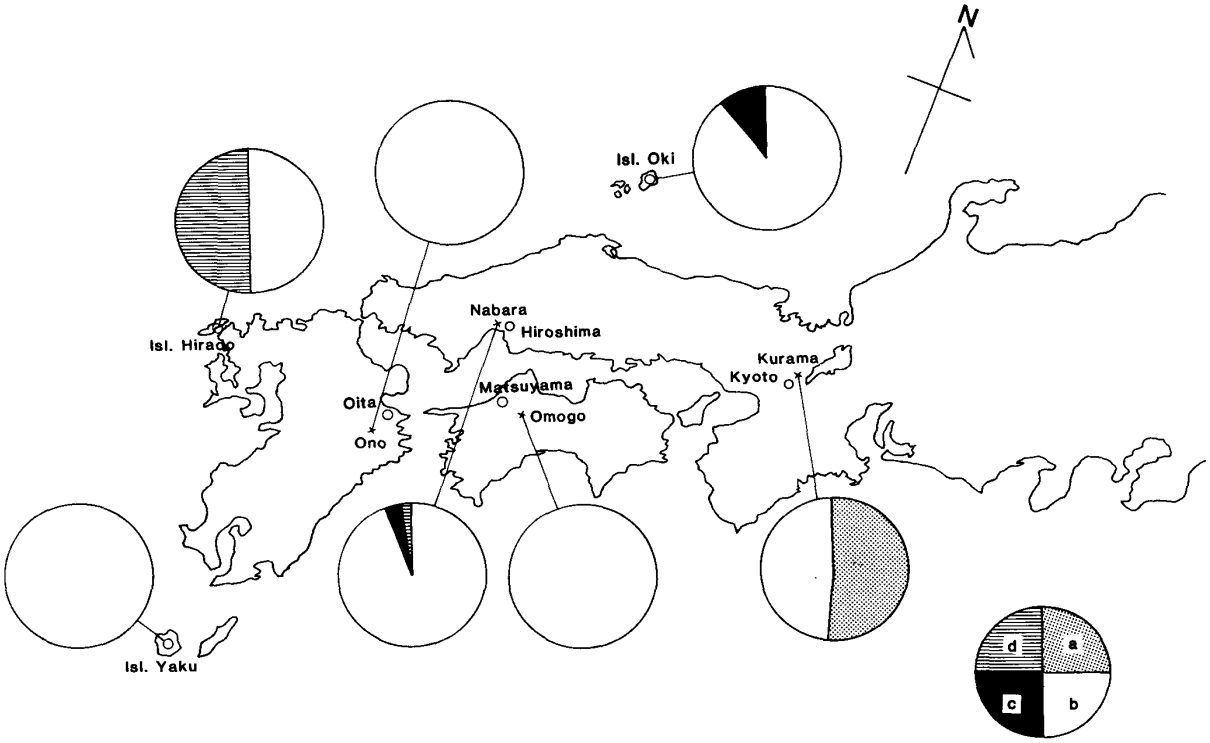

Fig. 13. Geographic distribution of MDH-A alleles among seven populations of *Rana tagoi* in western Japan.

band, BC. In contrast to those five populations, eight, nine and 17 of the 34 frogs showed homozygous bands, BB and AA, and a heterozygous band, AB, respectively, in the Kurama population. In the Hirado population, four, four and 12 of the 20 frogs showed homozygous bands, BB and DD, and a heterozygous band, BD, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

The frequency of allele *b* was usually high in the seven populations. The MDH-A locus of the Omogo, Ono and Yaku populations were occupied only by allele *b*, which was 0.944, 0.485, 0.500 and 0.897 in frequency in the Nabara, Kurama, Hirado and Oki populations, respectively. Allele *a* was found only in the Kurama population, being 0.515 in frequency. Allele *c* was 0.037 and 0.103 in the Nabara and Oki populations, respectively, while allele *d* was 0.019 and 0.500 in the Nabara and Hirado populations, respectively (Table 5; Fig. 13).

11. MDH-B locus

At the MDH-B locus of the seven populations, 10 phenotypes were produced by seven alleles, *a, b, c, d, e, f* and *g*. Nine frogs of the Nabara, 33 frogs of the Omogo, three frogs of the Kurama and one frog of the Ono population showed a homozygous band, DD. Three frogs of the Nabara, 13 frogs of the Kurama, five frogs of the Ono, 15 frogs of the Hirado, 29 frogs of the Oki and 29 frogs of the Yaku population showed a homozygous band, BB. One frog of the Kurama and one frog of the Yaku population showed a homozygous band, AA. Eleven frogs of

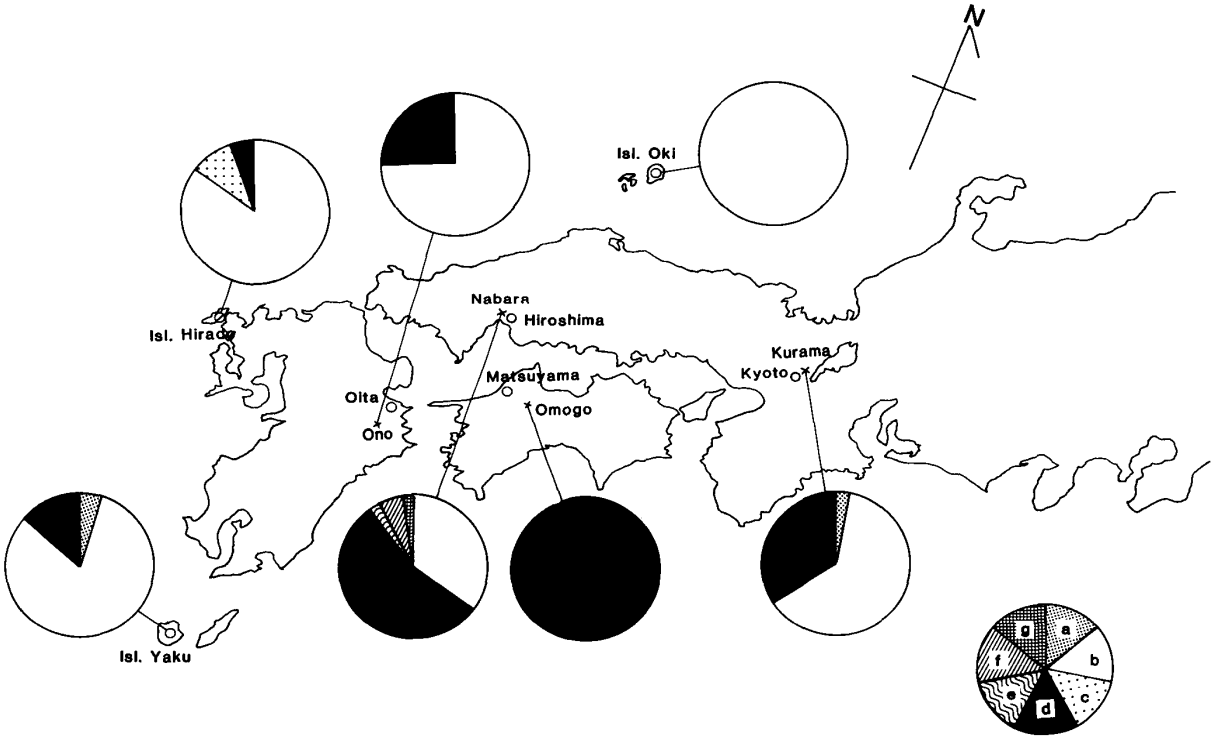

Fig. 14. Geographic distribution of MDH-B alleles among seven populations of *Rana tagoi* in western Japan.

the Nabara, 17 frogs of the Kurama, two frogs of the Ono, one frog of the Hirado and 11 frogs of the Yaku population showed a heterozygous band, BD. In addition to these phenotypes, two, one and one frogs of the Nabara population showed heterozygous bands, BF, DF and EG, respectively, three and one frogs of the Hirado population showed heterozygous bands, BC and CD, respectively, and two frogs of the Yaku population showed a heterozygous band, AB. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law, although there was a slight disagreement between them.

Allele *d* in six populations, the Omogo, Nabara, Kurama, Ono, Yaku and Hirado populations, was 1.000, 0.556, 0.338, 0.250, 0.128 and 0.050 in frequency, respectively, while the Oki population was occupied by allele *b* alone. Allele *b* was also fairly high in frequency in five other populations, being 0.352, 0.632, 0.750, 0.850 and 0.826 in the Nabara, Kurama, Ono, Hirado and Yaku populations, respectively. In the Nabara population, specific alleles *e*, *f* and *g* were found to be 0.019, 0.056 and 0.019 in frequency, respectively. Allele *a* was 0.029 and 0.047 in the Kurama and Yaku populations, respectively. In the Hirado population, allele *c* was 0.100 in frequency (Table 5; Fig. 14).

12. MPI locus

The MPI locus is most polymorphic and had the most numerous alleles among the 22 loci. At this locus, there were 25 phenotypes produced by 10 alleles, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i* and *j*. In the 27 frogs in the Nabara population, the most numerous

kinds of phenotypes were found; nine and one frogs showed homozygous bands, GG and HH, respectively, and three, two and five frogs showed heterozygous bands, DG, FG and GH, respectively. The remaining seven frogs showed heterozygous bands, AD, CG, DJ, FI, GI, GJ or HI. Of the 33 frogs of the Omogo population, one, nine and three showed homozygous bands, DD, GG and II, respectively, and six, five, eight and one showed heterozygous bands, DG, DI, GI and GJ, respectively. Of the 34 frogs of the Kurama population, four, one, two and one showed homozygous bands, DD, FF, GG and HH, respectively, and two, three, eight, three, three, two and five showed heterozygous bands, CF, DF, DG, DH, FG, FH and GH, respectively. Of the eight frogs of the Ono population, four, two, one and one showed a homozygous band, GG, and heterozygous bands, BG, GI and GJ, respectively. Of the 20 frogs of the Hirado population, two and 14 showed homozygous bands, DD and GG, respectively, and three and one showed heterozygous bands, DG and GH, respectively. Of the 29 frogs of the Oki population, eight and 11 showed homozygous bands, BB and EE, respectively, and one and nine showed heterozygous bands, AB and BE, respectively. Of the 42 frogs of the Yaku population, 10 and 24 showed homozygous bands, FF and HH, respectively, and only eight showed a heterozygous band, FH. When these numbers of homozygous and heterozygous frogs were compared with the expected values calculated according to HARDY-WEINBERG law, somewhat remarkable disagreement was found in the three island populations, especially in the Yaku and Oki populations.

Allele *g* was the highest in frequency in the seven populations. This allele was

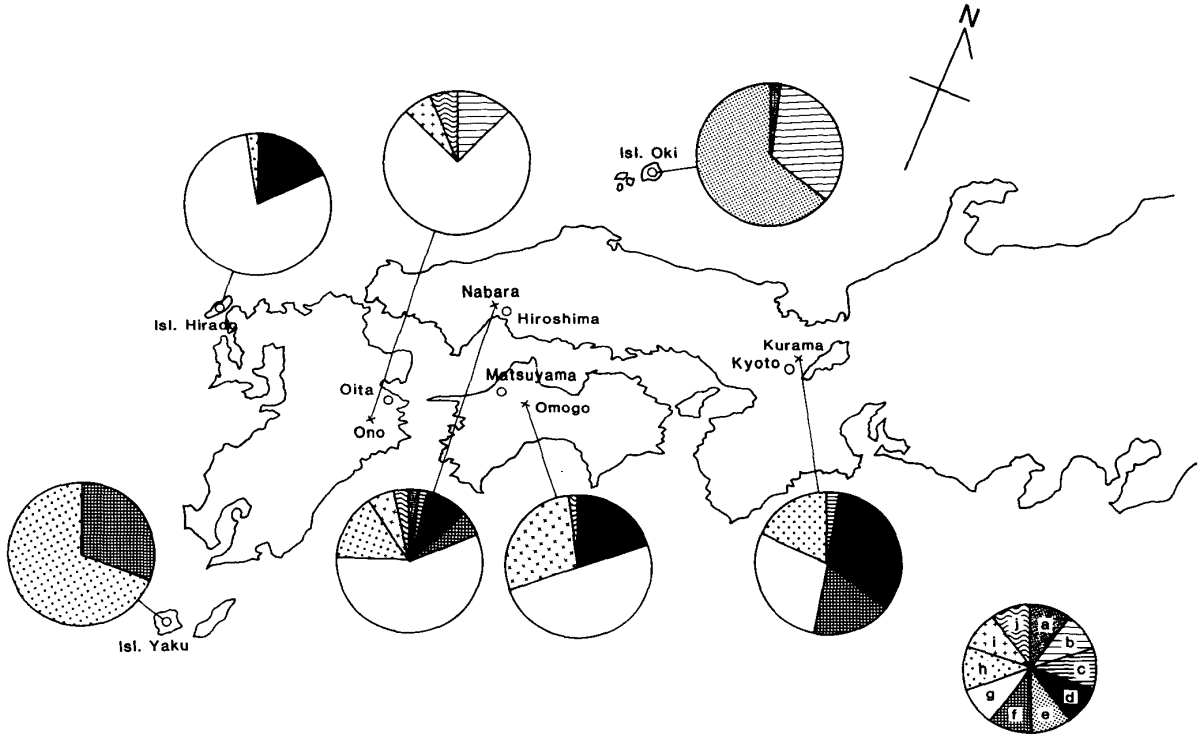

Fig. 15. Geographic distribution of MPI alleles among seven populations of *Rana tagoi* in western Japan.

0.800 and 0.750 in the Hirado and Ono populations, respectively, 0.574 and 0.500 in the Nabara and Omogo populations, respectively, 0.294 in the Kurama population, and was absent in the Oki and Yaku populations. In addition to allele *g*, allele *h* was 0.148, and six alleles, *a*, *c*, *d*, *f*, *i* and *j*, were 0.019~0.093 in frequency in the Nabara population, and alleles *i*, *d* and *j* were 0.288, 0.197 and 0.015 in frequency, respectively, in the Omogo population. Also in addition to allele *g*, alleles *d*, *f*, *h* and *c* were 0.324, 0.176, 0.176 and 0.029, respectively, in the Kurama population, alleles *b*, *i* and *j* were 0.063~0.125 in frequency in the Ono population, and alleles *d* and *h* were 0.175 and 0.025, respectively, in the Hirado population. In the Oki population, alleles *a*, *b* and *e* were 0.017, 0.448 and 0.534 in frequency, respectively. In the Yaku population, alleles *f* and *h* were 0.333 and 0.667 in frequency, respectively (Table 5; Fig. 15).

13. Pep-A locus

Three phenotypes produced by two alleles, *a* and *b*, were found at the Pep-A locus in the seven populations. Of the 32 frogs of the Omogo population, one showed a homozygous band, BB, while three showed a heterozygous band, AB. The remaining frogs all showed a homozygous band, AA. In the six populations other than the Omogo population, all the frogs showed a homozygous band, AA.

In the Omogo population, allele *a* was 0.922 and allele *b* was 0.078 in frequency. The other six populations were occupied by allele *a* alone (Table 5).

14. Pep-C locus

At the Pep-C locus, eight phenotypes were produced by four alleles, *a*, *b*, *c* and *d*, in the seven populations. A homozygous band, BB, was found in all the seven populations. Of the 27 frogs of the Nabara population, 15 and two showed homozygous bands, BB and CC, respectively, while three, five and two showed heterozygous bands, AB, BC and BD, respectively. Of the 33 frogs of the Omogo population, 27 showed a homozygous band, BB, and four, one and one showed heterozygous bands, AB, BC and BD, respectively. Of the 34 frogs of the Kurama population, 10, six and two showed homozygous bands, BB, AA and CC, respectively, while 10, two and four showed heterozygous bands, AB, AC and BC, respectively. Of the eight frogs of the Ono population, five showed a homozygous band, BB, while the other three showed a heterozygous band, BC. Of the 43 frogs of Yaku population, 22 and three showed homozygous bands, BB and CC, respectively, while two, 14 and two showed heterozygous bands, AC, BC and CD, respectively. All the 29 frogs of the Oki population showed a homozygous band, BB. Of the 20 frogs of the Hirado population, 19 showed a homozygous band, BB, while the remainder showed a heterozygous band, AB. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

Allele *b* was the highest in frequency, being 0.500~1.000, in all the seven populations, followed by allele *c*. In the Nabara, Kurama, Ono and Yaku populations, it was 0.167, 0.147, 0.188 and 0.279 in frequency, respectively, while

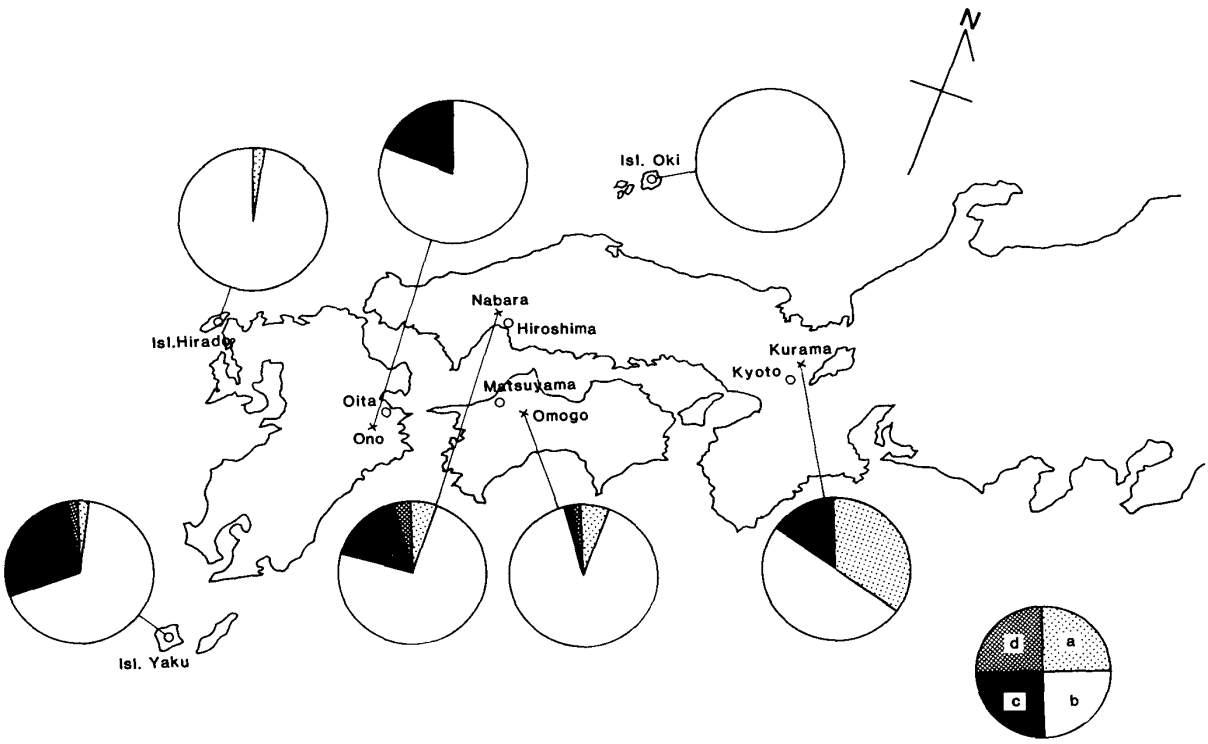

Fig. 16. Geographic distribution of Pep–C alleles among seven populations of *Rana tagoi* in western Japan.

it was only 0.015 in the Omogo population and was not present in the Hirado and Oki populations. Allele *a* was 0.353 in frequency in the Kurama population, while it was 0.023~0.061 in the Nabara, Omogo, Hirado and Yaku populations and was not present in the Ono and Oki populations. Allele *d* was 0.015~0.037 in frequency in the Nabara, Omogo and Yaku populations and was not present in the other four populations (Table 5; Fig. 16).

15. Pep–D locus

Ten phenotypes produced by six alleles, *a, b, c, d, e* and *f*, were found at the Pep–D locus in the seven populations. A homozygous band, DD, and a heterozygous band, CD, were found in all the seven populations. A homozygous band, DD, and a heterozygous band, CD, were found in 29 and three of the 32 frogs of the Omogo population, in 32 and two of the 34 frogs of the Kurama population and in 17 and three of the 20 frogs of the Hirado population, respectively. In the Nabara population, 17 of the 27 frogs showed a homozygous band, DD, while seven and three showed heterozygous bands, CD and DE, respectively. In the Yaku population, 36 of the 43 frogs showed a homozygous band, DD, while one, one, four and one showed heterozygous bands, BC, BD, CD and DF, respectively. In the Oki population, six and seven of the 29 frogs showed homozygous bands, CC and DD, respectively, while one, two, 10 and three showed heterozygous bands, AC, AD, CD and CE, respectively. In the Ono population, one and one of the eight frogs showed homozygous bands, CC and DD, respectively, while one, three, one and one showed heterozygous bands,

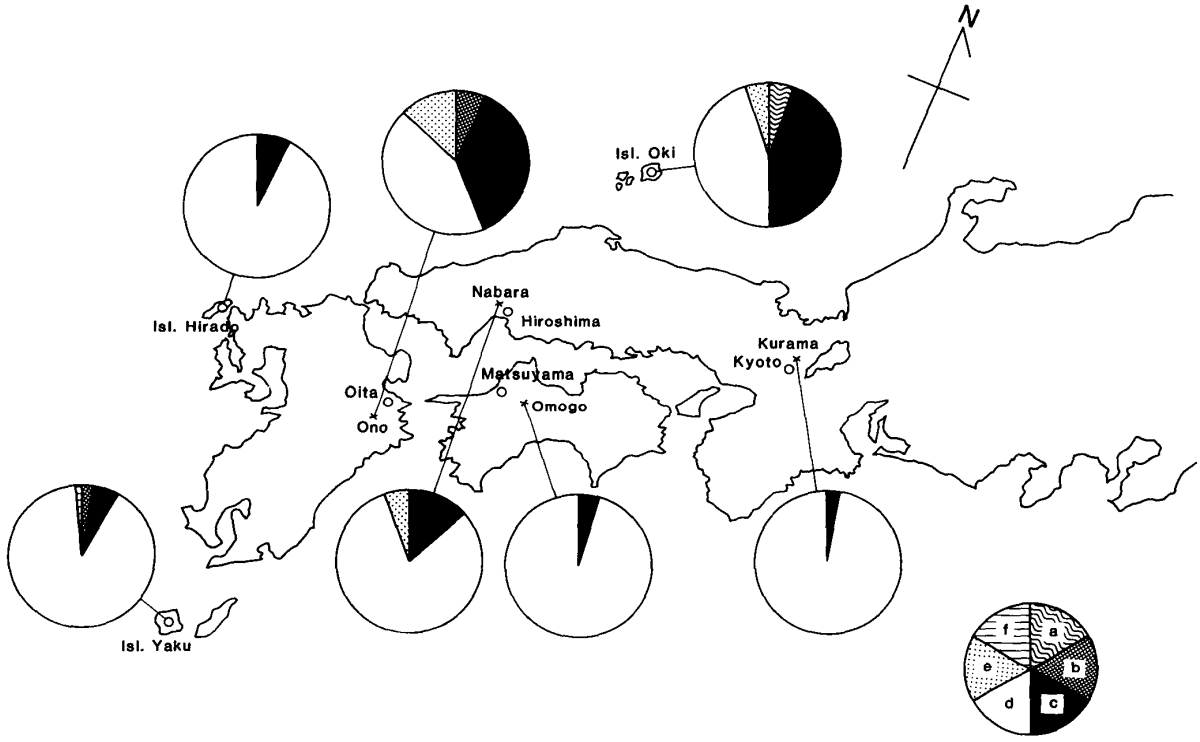

Fig. 17. Geographic distribution of Pep-D alleles among seven populations of *Rana tagoi* in western Japan.

BD, CD, CE and DE, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

Alleles *d* and *c* were found in all seven populations. In the Ono population, alleles *d* and *c* were 0.438 and 0.375 in frequency, respectively. In the Oki population, each of these alleles was 0.448. In the remaining five populations, allele *d* was overwhelmingly high in frequency, being 0.815~0.971, while allele *c* was low in frequency, being 0.029~0.130. Allele *a* was found in the Oki population alone and it was 0.052 in frequency, while allele *f* was found in the Yaku population alone and it was 0.012. Allele *b* was found in the Ono and Yaku populations, and it was 0.063 and 0.023 in frequency. Allele *e* was found in the Nabara, Ono and Oki populations, and it was 0.052~0.125 in frequency (Table 5; Fig. 17).

16. PGM locus

Four phenotypes produced by four alleles, *a, b, c* and *d,* were found at the PGM locus in the seven populations. It was remarkable that the variations of phenotypes were very scarce. Of the 194 frogs of the seven populations, 187 showed a homozygous band, BB, and the remaining seven showed three kinds of heterozygous bands. Five frogs of the Yaku, one of the Hirado and one of the Omogo population showed heterozygous bands, AB, BC and BD, respectively. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

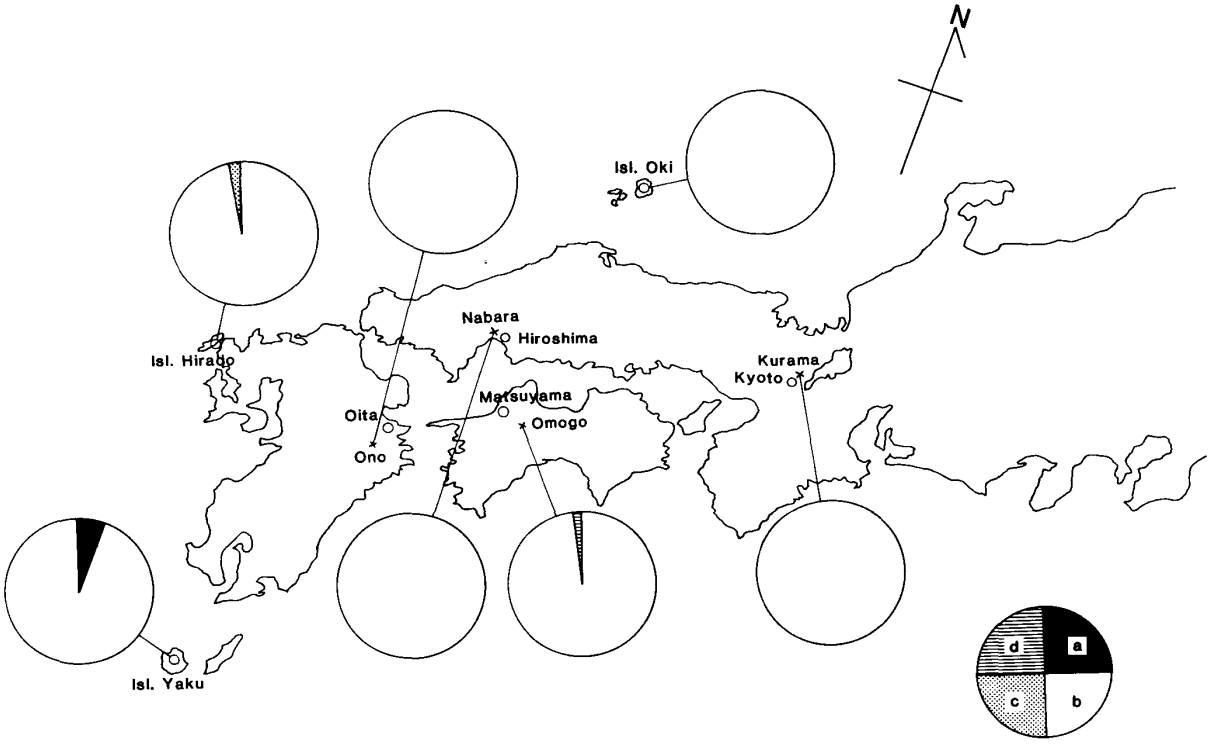

Fig. 18. Geographic distribution of PGM alleles among seven populations of *Rana tagoi* in western Japan.

Allele *b* was overwhelmingly high in each of the seven populations, being 0.942~1.000 in frequency. In addition, allele *d* was 0.015 in the Omogo population, allele *c* was 0.025 in the Hirado population and allele *a* was 0.058 in frequency in the Yaku population (Table 5; Fig. 18).

17. SOD locus

Seven phenotypes produced by five alleles, *a, b, c, d* and *e,* were found at the SOD locus in the seven populations. In the Nabara population, 26 of the 27 frogs showed a homozygous band, DD, and the remainder showed a heterozygous band, CD. In the Kurama population, 33 of the 34 frogs showed a homozygous band, DD, and the remainder showed a heterozygous band, BD. In the Omogo population, 11 and four showed homozygous bands, DD and CC, respectively, while the remaining 17 and one showed heterozygous bands, CD and DE, respectively. In the Hirado population, all the 20 frogs showed a homozygous band, CC. Of the eight frogs of the Ono population, four and one showed homozygous bands, CC and DD, respectively, and three showed a heterozygous band, CD. In the Oki population, 27 of the 29 frogs showed a homozygous band, CC, and two showed a heterozygous band, AC. In the Yaku population, 13 and 12 of the 43 frogs showed homozygous bands, AA and CC, respectively, and 18 showed a heterozygous band, AC. These numbers of homozygous and heterozygous frogs almost agreed with the expected values calculated according to HARDY-WEINBERG law.

Allele *d* was remarkably high in frequency in the Nabara, Kurama and Omogo

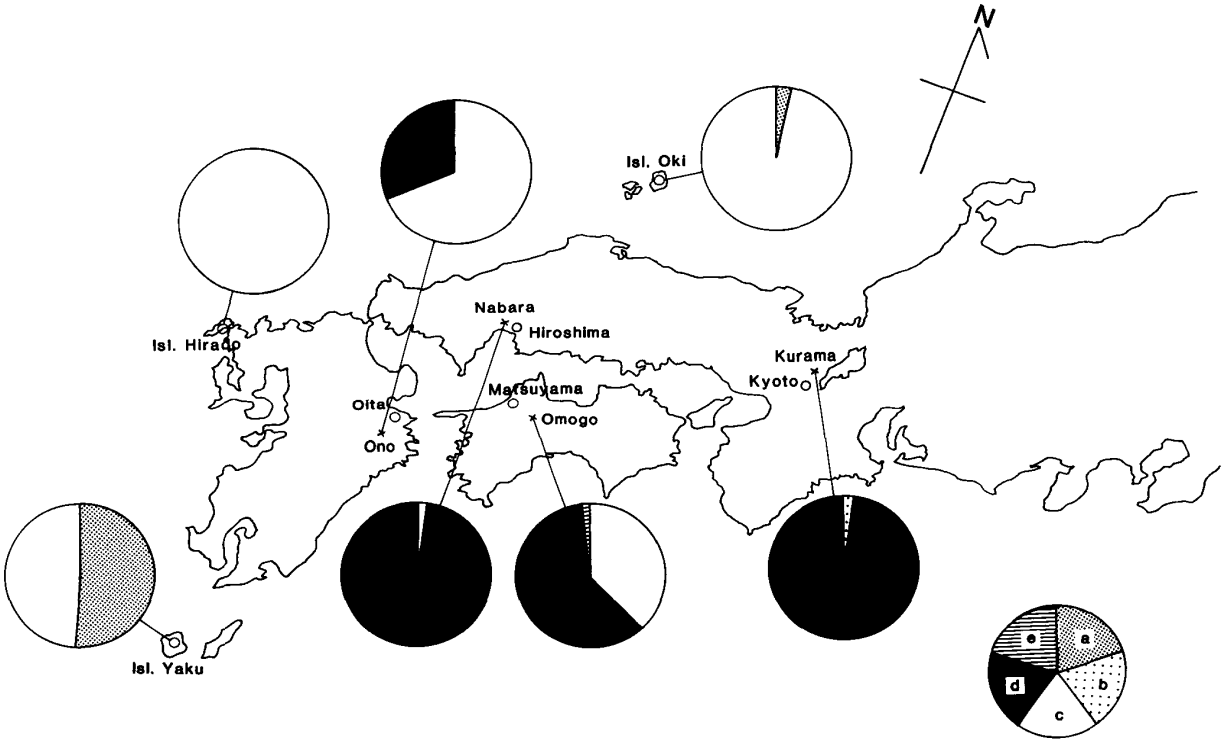

Fig. 19. Geographic distribution of SOD alleles among seven populations of *Rana tagoi* in western Japan.

populations, being 0.981, 0.985 and 0.606, respectively. It was fairly high in the Ono population, being 0.313 in frequency. On the other hand, allele *c* was distinctly high in frequency in the Hirado, Oki and Ono populations, being 1.000, 0.966 and 0.688, respectively. It was fairly high in frequency in the Yaku and Omogo populations, being 0.488 and 0.379, respectively. Allele *c* was 0.019 in frequency in the Nabara population and was not present in the Kurama population. Allele *a* was 0.512 and 0.034 in frequency in the Yaku and Oki populations, respectively. Alleles *b* and *e* were slightly found in the Kurama and Omogo populations, both being 0.015 in frequency (Table 5; Fig. 19).

18. Ab locus

Nine phenotypes produced by four alleles, *a, b, c* and *d,* were found at the Ab locus in the seven populations. Homozygous bands, CC, AA and DD, were abundantly shown in the Nabara, Kurama, Omogo and Hirado populations, in the Oki and Yaku populations and in the Ono population, respectively. In the Nabara population, two and 16 of the 26 frogs showed homozygous bands, BB and CC, respectively, and four, three and one showed heterozygous bands, BC, BD and CD, respectively. In the Omogo population, one, 15 and three of the 33 frogs showed homozygous bands, BB, CC and DD, respectively, and three and 11 showed heterozygous bands, BD and CD, respectively. In the Kurama population, 23 and one of the 34 frogs showed homozygous bands, CC and BB, and two, one and seven showed heterozygous bands, AB, AC and BC, respectively. In the

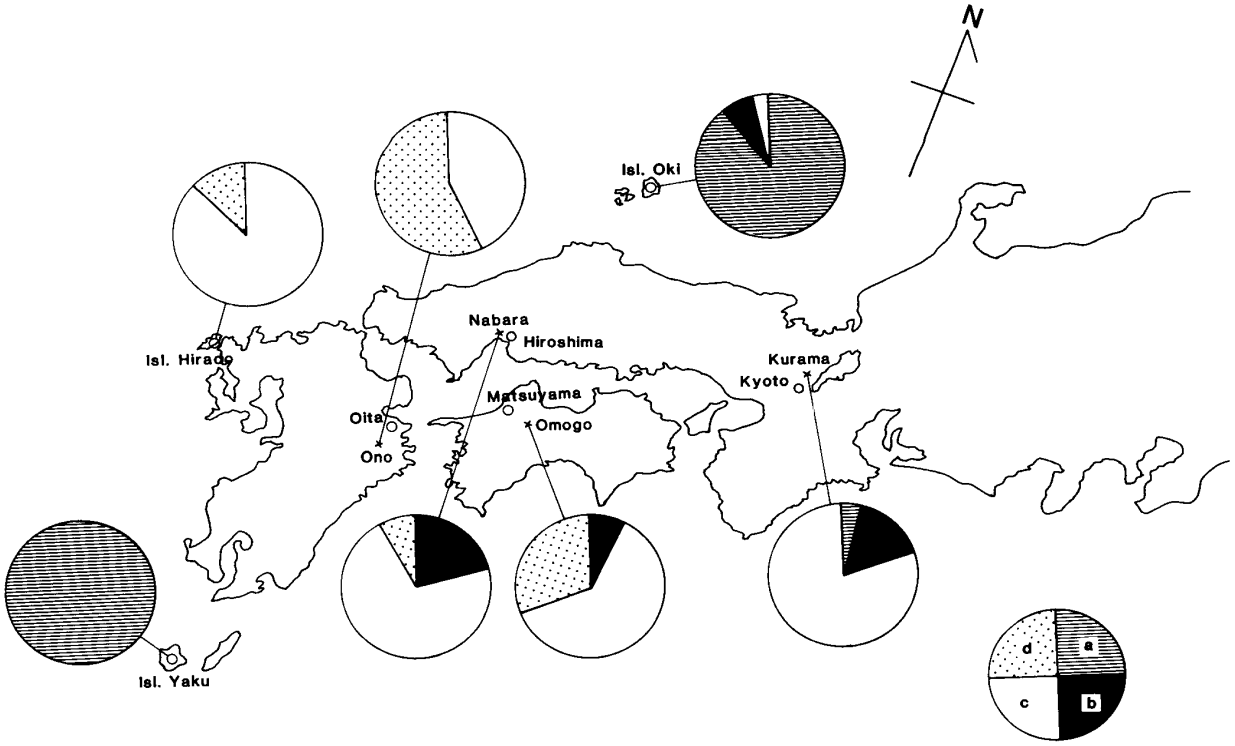

Fig. 20. Geographic distribution of Ab alleles among seven populations of *Rana tagoi* in western Japan.

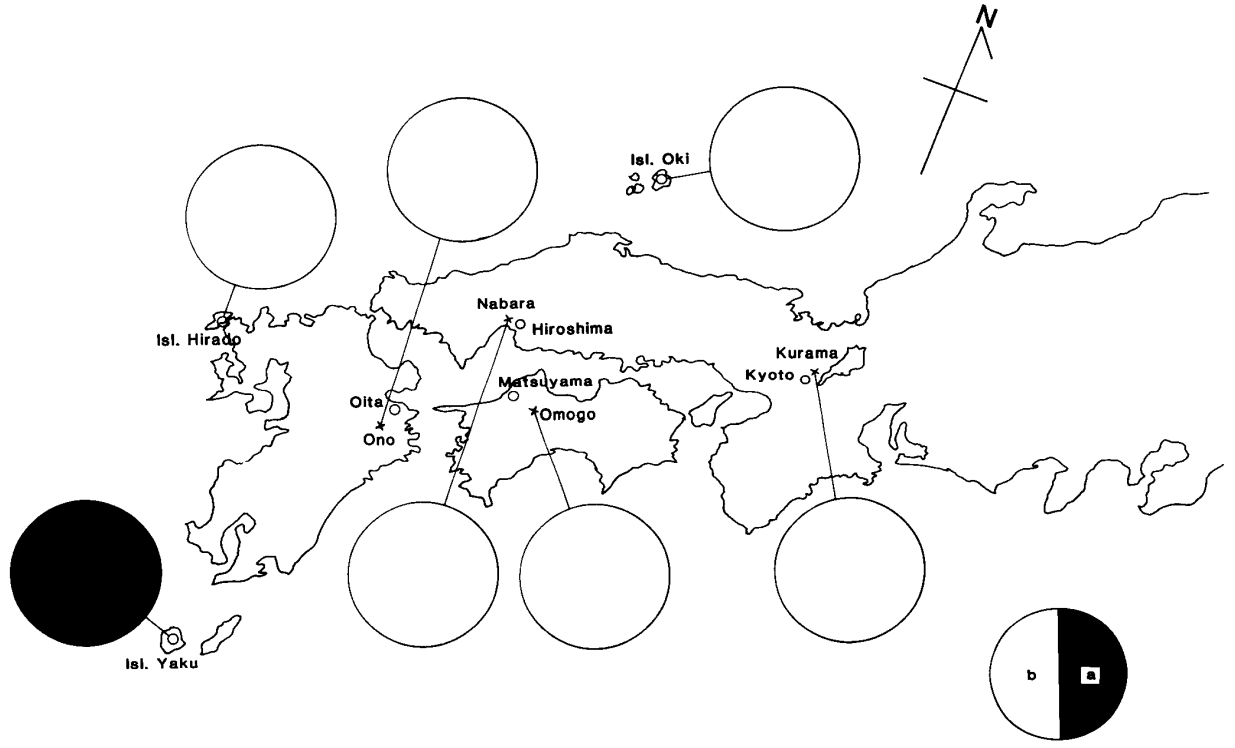

Fig. 21. Geographic distribution of Hb alleles among seven populations of *Rana tagoi* in western Japan.

Hirado population, 16 and one of the 20 frogs showed homozygous bands, CC and DD, respectively, and the remaining three showed a heterozygous band, CD. In the Ono population, two and three of the seven frogs showed homozygous bands, CC and DD, and the other two showed a heterozygous band, CD. In the Oki population, 23 of the 29 showed a homozygous band, AA, and four and two showed heterozygous bands, AB and AC, respectively. In the Yaku population, all the 43 frogs showed a homozygous band, AA. These numbers of homozygous and heterozygous frogs nearly agreed with the expected values calculated according to HARDY-WEINBERG law.

Allele *c* was high in frequency in the Nabara, Omogo, Kurama and Hirado populations, being 0.712, 0.621, 0.794 and 0.875, respectively. This allele was 0.429 in frequency in the Ono population and 0.034 in the Oki population. Allele *d* was 0.571, 0.303, 0.125 and 0.077 in frequency in the Ono, Omogo, Hirado and Nabara populations, respectively. Allele *a* was 0.897 and 1.000 in frequency in the Oki and Yaku populations, respectively, while it was only 0.044 in frequency in the Kurama population. Allele *b* was 0.212, 0.162, 0.076 and 0.069 in frequency in the Nabara, Kurama, Omogo and Oki populations, respectively (Table 5; Fig. 20).

19. Hb locus

Two phenotypes produced by two alleles, *a* and *b*, were found at the Hb locus in the seven populations. In the Yaku population alone, all the frogs showed a homozygous band, AA, while in the other six populations, all the frogs showed a homozygous band, BB. Thus, allele *a* was 1.000 in frequency in the Yaku population, and allele *b* was 1.000 in the other six populations (Table 5; Fig. 21).

20. Fixation index (Fst)

The fixation index (Fst) was calculated according to WRIGHT (1978) at 22 loci in

TABLE 6
Fixation index at 22 loci in one subspecies and six populations of *Rana tagoi*

Locus	Fixation index (Fst)	Locus	Fixation index (Fst)
AAT-A	0	LDH-B	0.432
AAT-B	0.351	MDH-A	0.377
ADA	0.462	MDH-B	0.423
AK	0	MPI	0.312
CK	0	Pep-A	0.068
Fum	0.144	Pep-C	0.152
α-GDH	0.036	Pep-D	0.208
GPI	0.621	PGM	0.033
IDH-A	0.041	SOD	0.610
IDH-B	0.202	Ab	0.516
LDH-A	0.015	Hb	1.000

the 194 frogs belonging to the seven populations (Table 6). When the gene frequencies at a definite locus are the same in the seven local populations, the fixation index is zero, while this is 1.000 when there is no common allele in one or more populations. The results of examination showed that three loci of AAT-A, AK and CK which consisted of a single allele were zero in fixation index and were most stable. Five loci of LDH-A, PGM, α-GDH, IDH-A and Pep-A were 0.015~0.068 in fixation index and indicated a slight genetic differentiation. Four loci of Fum, Pep-C, IDH-B and Pep-D were 0.144~0.208, three loci of MPI, AAT-B and MDH-A were 0.312~0.377, three loci of MDH-B, LDH-B and ADA were 0.423~0.462, and three loci of Ab, SOD and GPI were 0.516~0.621 in fixation index. These values of the 13 loci in fixation index show various degrees of genetic differentiation. The Hb locus was 1.000 in fixation index (Table 6), as this locus is completely differentiated in the Yaku population. This seems to show that the Yaku population remarkably differs from the other six populations in this respect.

III. Genetic variation and genetic distance

1. Genetic variations among the seven populations

In order to show the genetic variations in the seven populations of *Rana tagoi*, three genetic parameters, mean number of alleles per locus, mean proportion of heterozygous loci per individual and mean proportion of polymorphic loci per population were estimated (Tables 5 and 7).

The mean number of multiple alleles at each locus was examined in the seven populations. The results showed that the Nabara and Omogo populations were the most numerous, being 2.5 and 2.4 in mean number, respectively. Each of the Kurama, Ono and Yaku populations was 1.9 in mean number, while each of the Oki and Hirado populations was 1.6. The mean numbers of alleles in the seven

TABLE 7

Estimates of genetic variabilities at 22 loci in one subspecies and six populations of *Rana tagoi*

Population	Sample size	Mean number of alleles per locus	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)
Nabara	27	2.50	20.1 (20.5)	63.6
Omogo	33	2.41	18.1 (18.2)	63.6
Kurama	34	1.91	18.2 (18.8)	50.0
Ono	8	1.91	21.4 (21.9)	59.1
Hirado	20	1.59	13.0 (12.7)	50.0
Oki	29	1.64	10.2 (11.6)	40.9
Yaku*	43	1.86	12.0 (13.7)	59.1
Mean (Total)	27.7 (194)	1.97	16.1 (16.8)	55.2

*, *R. t. yakushimensis* Parentheses show an expected value.

populations were 2.0 on the average (Table 7).

When the proportion of heterozygous loci was examined at the 22 loci in the seven populations, the highest was found in the Ono population, being 21.4%. Those of the Nabara, Kurama and Omogo populations were 20.1%, 18.2% and 18.1%, respectively. The other three populations, Hirado, Yaku and Oki, were 13.0%, 12.0% and 10.2%, in proportion, respectively. The proportions of heterozygous loci in the total seven populations were 16.1% on the average (Table 7).

The proportion of polymorphic loci containing multiple alleles at the rate of more than 1% was estimated in each of the seven populations. The results showed that the polymorphic loci occupied 63.6% in each of the Nabara and Omogo populations. They occupied 59.1% in each of the Ono and Yaku populations, 50.0% in each of the Kurama and Hirado populations, and 40.9% in the Oki population. They occupied 55.2% on the average among the seven populations (Table 7).

2. Genetic distance and dendrogram

The genetic distance (D) and genetic identity (I) among the seven populations were estimated on the basis of gene frequencies at the 22 loci (NEI, 1972, 1975). The results showed that the genetic distances between the mainland and island populations were comparatively large. Those between the Yaku (Isl.) population and four mainland populations, the Nabara, Omogo, Kurama and Ono populations, were 0.314, 0.335, 0.301 and 0.225, respectively. Those between the Oki (Isl.) population and three mainland populations, the Nabara, Omogo and Kurama populations, were 0.283, 0.278 and 0.237, respectively. Those between the Hirado (Isl.) population and three mainland populations, the Nabara, Omogo and Kurama populations, were 0.226, 0.223 and 0.192, respectively. The genetic distances between the Yaku (Isl.) population and the two other island populations, the Hirado and Oki populations, were also large, being 0.285 and 0.182, respectively, while those between the Oki (Isl.) population and two other

TABLE 8
Genetic identity (I) and genetic distance (D) among one subspecies and six populations of *Rana tagoi*

Population	Nabara	Omogo	Kurama	Ono	Hirado	Oki	Yaku*
Nabara	—	0.969	0.928	0.874	0.798	0.754	0.730
Omogo	0.031	—	0.901	0.868	0.800	0.757	0.715
Kurama	0.075	0.104	—	0.859	0.825	0.789	0.740
Ono	0.135	0.142	0.152	—	0.939	0.904	0.798
Hirado	0.226	0.223	0.192	0.063	—	0.860	0.752
Oki	0.283	0.278	0.237	0.101	0.151	—	0.833
Yaku*	0.314	0.335	0.301	0.225	0.285	0.182	—

Genetic identity (I) is given above the diagonal and genetic distance (D) is given below.
*, *R. t. yakushimensis*

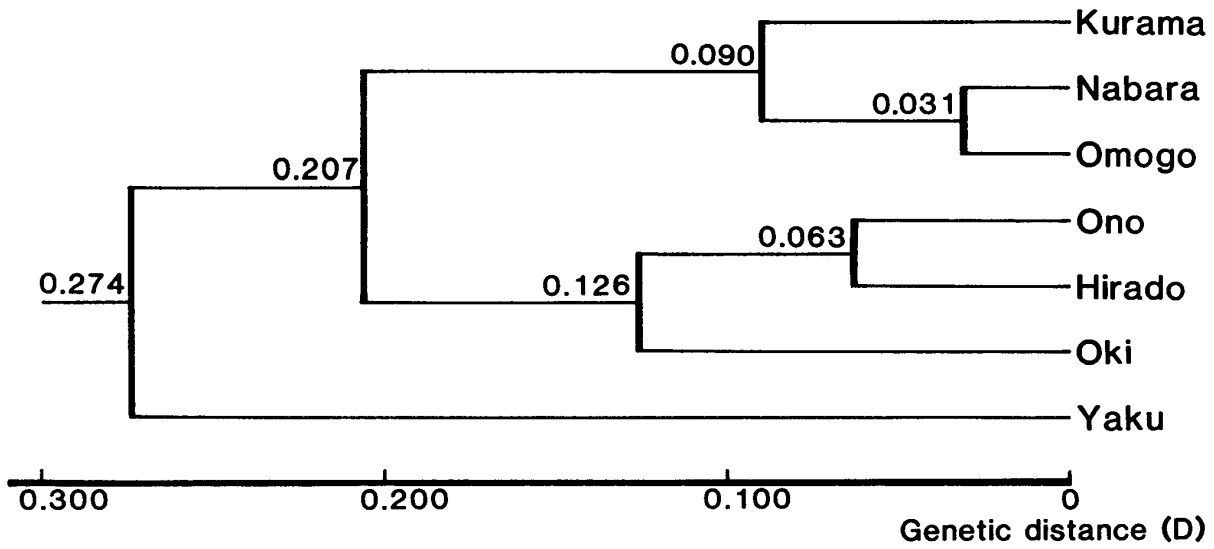

Fig. 22. Dendrogram for one subspecies and six populations of *Rana tagoi*.

populations, the Ono and Hirado (Isl.) populations, and between the Hirado (Isl.) and Ono populations were small, being 0.101, 0.151 and 0.063, respectively. The genetic distances among the four mainland populations were small, being 0.031~0.152. The size of genetic distance values seems to be closely related to that of geographical distances on the whole (Table 8).

As the genetic distances among populations of a species show the degree of genetic differentiation of this species, a dendrogram of the species can be drawn by using the genetic distances. Although several methods are known in order to establish a dendrogram, the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975) was used in the seven populations of *Rana tagoi*. The dendrogram established by this method seems to indicate that the Yaku population was first differentiated in Yaku Island. The remaining frogs were thereafter divided into two groups. From one of these groups, the Oki population was first differentiated in Oki Island, and then the Hirado and Ono populations were produced. From the other group, the Kurama population was first produced and then the Nabara and Omogo populations were differentiated (Fig. 22).

DISCUSSION

1. Genetic variability

Of the three estimates of genetic variabilities, the proportion of heterozygous loci per individual seems to show an evident difference between the four land populations, Nabara, Omogo, Kurama and Ono populations, and the three island populations, Hirado, Oki and Yaku populations. While the land populations were 18.1~21.4% in average heterozygosity, the island populations were 10.2 ~13.0%. This seems to be principally due to a difference in the size of the

TABLE 9
Genetic variability

Species	Sample size	Number of populations	Mean number of alleles per locus	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)	Authors
Rana tagoi	194	7	1.97	16.1	55.2	NISHIOKA et al. (1987)
Rana ridibunda	203	7	1.46	7.3	38.0	NEVO (1976)
Buergeria japonica	118	3	1.55	8.7	40.0	NISHIOKA et al. (1987)
Buergeria buergeri	40	1	2.00	11.4	56.0	〃
Rhacophorus arboreus	42	2	1.56	12.6	48.0	〃
Rhacophorus schlegelii	37	2	2.06	20.7	64.0	〃
Rhacophorus viridis	14	2	1.28	8.0	24.0	〃
Bufo cognatus	150	5	—	12.1	52.0	ROGERS (1973)
Bufo speciosus	116	6	—	10.7	42.0	〃
Bufo americanus	620	25	1.45	11.6	25.7	GUTTMAN (1975)
Bufo arenarum	407	15	—	16.3	39.6	MATTHEWS (1975)
Bufo viridis	507	11	1.65	13.4	42.3	DESSAUER, NEVO & CHUANG (1975)
〃	294	7	1.69	14.1	47.0	NEVO (1976)
Hyla arborea savignyi	218	27	1.62	7.2	39.4	NEVO & YANG (1979)
〃	211	7	1.68	7.4	43	NEVO (1976)
Hyla chrysoscelis	238	9	1.43	6.8	32.4	RALIN & SELANDER (1979)
Hyla versicolor (4n)	132	4	1.98	32.2	47.9	〃
Pelobates syriacus	56	2	1.09	2.3	9	NEVO (1976)
Scaphiopus holbrooki	48	2	—	3.9	23.8	SATTLER (1980)
Scaphiopus couchi	21	1	—	2.6	19.0	〃
Scaphiopus bombifrons	106	5	—	8.2	52.4	〃
Scaphiopus multiplicatus	117	4	—	8.3	38.1	〃
Scaphiopus hammondi	4	1	—	1.5	9.5	〃
Taricha granulosa	116	4	1.49	9.2	39.8	HEDGECOCK (1976)
Taricha torosa	99	5	1.22	4.6	20.5	〃
Taricha rivularis	532	12	1.24	6.8	20.9	HEDGECOCK (1978)
Triturus vulgaris	105	3	1.44	8.7	34.9	KALEZIĆ & HEDGECOCK (1979)
Triturus cristatus	110	3	1.28	5.2	27.8	〃
Triturus alpestris	131	2	1.62	13.5	49.2	〃
Eurycea lucifuga	66	6	1.14	3.9	13.9	MERKLE & GUTTMAN (1977)
Plethodon yonahlossee	166	7	1.14	2.3	8.2	GUTTMAN, KARLIN & LABANICK (1978)
Aneides flavipunctatus	405	22	1.44	10.3	—	LARSON (1980)

natural population. Of the seven populations, the Nabara and Omogo populations were 2.5 and 2.4 in mean number of alleles per locus, respectively, and 63.6% in proportion of polymorphic loci, while the Hirado and Oki populations were 1.6 in mean number of alleles per locus and 50.0% and 40.9% in proportion of polymorphic loci, respectively. These differences seem to reflect the facts that the Nabara and Omogo populations are especially large in the size of natural population, while the Hirado and Oki populations are very small.

The genetic variability in allopatric populations has been studied in various amphibian species by many authors. DESSAUER, NEVO and CHUANG (1975) have reported that *Bufo viridis* distributed in Israel is of the highest genetic variation in any vertebrates. The genetic variations found in some anurans and urodeles are shown in Table 9. It is remarkable that the three parameters showing the genetic variation in *Rana tagoi* are extraordinarily high. They are 1.97, 16.1% and 55.2% in mean number of alleles per locus, mean proportion of heterozygous loci per individual and mean proportion of polymorphic loci per population, respectively, while those in *Bufo viridis* are 1.65, 13.4% and 42.3%, respectively (DESSAUER, NEVO and CHUANG, 1975), or 1.69, 14.1% and 47.0%, respectively (NEVO, 1976). In addition to these two species, there are some species which are remarkably high in the degree of genetic variability. In another paper of the present volume, NISHIOKA, SUMIDA, OHTA and SUZUKI (1987) have reported that the three parameters showing genetic variabilities are 2.00, 11.4% and 56.0% in *Buergeria buergeri,* 1.55, 8.7% and 40.0% in *Buergeria japonica,* 2.06, 20.7% and 64.0% in *Rhacophorus schlegelii,* and 1.56, 12.6% and 48.0% in *Rh. arboreus. Buergeria buergeri, Rhacophorus arboreus* and *Rh. schlegelii* are rhacophorids endemic in Japan. They are all distributed in mountain districts like *Rana tagoi. Bufo cognatus* (ROGERS,1973), *Hyla versicolor* (RALIN and SELANDER, 1979), *Scaphiopus bombifrons* (SATTLER, 1980) and *Triturus alpestris* (KALEZIĆ and HEDGECOCK, 1979) are also high in the degree of genetic variability, being more than 47% in mean proportion of polymorphic loci per population (Table 9).

The high degree of genetic variability observed in the seven populations of *Rana tagoi* seems to be attributable to the selection for heterozygosity which is operating as an adaptive strategy in the ecologically variable environment where they live, as suggested in *Bufo viridis* by DESSAUER, NEVO and CHUANG (1975). All the foregoing species seem to be inactive in behavior and considerably difficult in moving to any distance. Thus, it seems necessary for them to adapt themselves to the ecologically variable environment.

2. Intraspecific differentiation

The genetic distances for the seven populations of *Rana tagoi* are 0.031~0.335, as shown in Table 8. Of these populations, the Yaku population is described as a subspecies, *Rana tagoi yakushimensis,* by NAKATANI and OKADA (1966). The genetic distances between the Yaku and the other six populations are 0.182~0.335. Those between two island populations, the Oki and Hirado populations, and three mainland populations, the Nabara, Omogo and Kurama populations, are 0.192

~0.283. In contrast, the genetic distances among the four mainland populations, between the Ono and two island populations, the Hirado and Oki populations, and between two island populations, the Hirado and Oki populations, are 0.031~0.152.

The genetic identity and genetic distance have been estimated in various species and species groups by many investigators. NISHIOKA, SUMIDA, OHTA and SUZUKI (1987) have estimated the genetic identity (I) and genetic distance (D) among species and populations of *Buergeria* and *Rhacophorus*. The genetic distances among four island populations, the Amami-I, Amami-II, Tokara and Okinawa populations, of *Buergeria japonica* are 0.003~0.270, while those between *Buergeria buergeri* and the four populations of *B. japonica* are 2.045~2.243. The genetic distance between two subspecies, *Rhacophorus v. viridis* of Okinawa Island and *Rh. v. owstoni* of Ishigaki Island is 0.819. On the other hand, the genetic distances between *Rh. arboreus* and *Rh. schlegelii* are 0.301~0.387. As these two species closely resemble each other in appearance, the former was placed at first as a variety of the latter (OKADA and KAWANO, 1924).

CASE (1978) has estimated NEI's genetic distances of *Rana boylei* and *R. muscosa*. Those of six populations of *R. boylei* and five populations of *R. muscosa* are 0.01~0.02 and 0.02~0.05, respectively, while the genetic distances between the two species are 0.68~0.77. The genetic distances among 17 populations of *Hyla regilla* are 0.01~0.49 (CASE, HANELINE and SMITH, 1975). The coefficients of genetic identity among eight populations of *Hyla arborea savignyi* are 0.869~0.998 (NEVO and YANG, 1979), while those and the genetic distances among nine populations of *H. chrysoscelis* are 0.871~0.999 and 0.001~0.138, respectively (RALIN and SELANDER, 1979). The coefficients of genetic identity among 15 Argentine populations of *Bufo arenarum* are 0.76~0.95 (MATTHEWS, 1975). NEI's genetic distances and ROGER's coefficients of genetic similarity among four populations of *Bufo punctatus* are 0.052~0.207 and 0.808~0.935, respectively (FEDER, 1979). The genetic distances among five populations of a New Zealand frog species, *Leiopelma hochstetteri*, are 0~0.11 (DAUGHERTY, BELL, ADAMS and MAXSON, 1981).

In urodeles, HEDGECOCK and AYALA (1974) have estimated genetic variation in three species, *Taricha granulosa, T. rivularis* and *T. torosa*, and one subspecies *T. t. sierrae*. The mean genetic similarity and genetic distances between consubspecific populations are 0.972 ± 0.009 and 0.029 ± 0.010, respectively. The genetic distance between southern and northern populations of *T. t. torosa* is 0.109. HEDGECOCK (1976) has reported that the genetic distances among three populations of *Taricha t. torosa* and between two populations of *T. t. sierrae* are 0.018~0.140 and 0.071, respectively, while those between these two subspecies are 0.162~0.309. The genetic distances among four populations of *Taricha granulosa* are 0.006~0.176. HEDGECOCK (1978) has also reported that the genetic distances among 12 populations of *Taricha rivularis* are 0.001~0.023. According to LARSON and HIGHTON (1978), *Plethodon dorsalis* is divided into two groups of populations which electrophoretically diverge from each other to the degree of different species. The genetic distances of 18 populations of one of these groups are 0.03~0.46, while

those of eight populations of the other group are 0.01~0.20. DUNCAN and HIGHTON (1979) have reported that the genetic distances among 10 populations of *Plethodon ouachitae* and among three populations of *P. caddoensis*, both endemic to the Ouachita Mountains of Arkansas and Oklahoma, are 0~0.28 and 0.04~0.11, respectively. WAKE, MAXSON and WURST (1978) have described that the genetic distances among five populations of *Hydromantes shastae* occurring in California are 0.003~0.275. TILLEY and SCHWERDTFEGER (1981) have also described that the genetic distances among 10 populations of *Desmognathus fuscus* found in eastern North America are 0.002~0.290. KALEZIĆ and HEDGECOCK (1979) have calculated genetic identity and genetic distance from allele frequencies to estimate the amount of genetic divergence within and between certain Yugoslavian populations of *Triturus vulgaris, T. alpestris, T. cristatus dobrogicus* and *T. c. karelinii*. Mean values of genetic distance between consubspecific, subspecific and specific taxa are 0.031, 0.347 and 0.906, respectively. RAGGHIANTI and WAKE (1986) have recently reported that NEI's genetic distances among 11 populations of *Triturus italicus* are 0.001~0.190.

It was found that the species having large genetic distances among populations are divided into two or three groups which must be diverged in old ages. As evidently recognized in dendrograms, the populations of *Hyla regilla, Bufo arenarum, Plethodon ouachitae, P. dorsalis, Desmognathus fuscus* and *Hydromantes shastae* are divided into three groups, while those of *Buergeria japonica* and *Aneides flavipunctatus* are divided into two groups. Similarly, the six populations of *Rana tagoi* are divided into two groups, one including the Nabara, Omogo and Kurama populations and the other including the Ono, Hirado and Oki populations. On the other hand, the genetic distance values between two subspecies of amphibians have been estimated in a few species. Those between *Rhacophorus v. viridis* and *Rh. v. owstoni* (NISHIOKA, SUMIDA, OHTA and SUZUKI, 1987), between *Taricha t. torosa* and *T. t. sierrae* (HEDGECOCK, 1976) and between *Triturus cristatus dobrogicus* and *T. c. karelinii* (KALEZIĆ and HEDGECOCK, 1979) are 0.819, 0.104~0.309 and 0.347, respectively. The genetic distances between the six populations of *Rana t. tagoi* and the one population of *R. t. yakushimensis* are 0.182~0.335. As *R. t. yakushimensis* is electrophoretically separated from the other six populations, in addition to the morphological characters as well as the presence of slight reproductive isolating mechanisms found between the Yaku and Nabara populations, its status as a subspecies of *Rana tagoi* seems to be proper.

ACKNOWLEDGMENTS

The authors are especially indebted to Emeritus Professor Toshijiro KAWAMURA for his encouragement and guidance during the course of this work and for his critical review of the manuscript.

LITERATURE

BREWER, G. J. 1970. An Introduction to Isozyme Techniques. Academic Press, New York and London, 186 pp.

CASE, S. M. 1978. Electrophoretic variation in two species of ranid frogs, *Rana boylei* and *R. muscosa*. Copeia, 1978: 311–320.

CASE, S. M., P. G. HANELINE and M. F. SMITH 1975. Protein variation in several species of *Hyla*. Syst. Zool., **24**: 281–295.

DAUGHERTY, C. H., B. D. BELL, M. ADAMS and L. R. MAXSON 1981. An electrophoretic study of genetic variation in the New Zealand frog genus *Leiopelma*. New Zealand J. of Zoology, **8**: 543–550.

DESSAUER, H. C., E. NEVO and K.-C. CHUANG 1975. High genetic variability in an ecologically variable vertebrate, *Bufo viridis*. Biochem. Genet., **13**: 651–661.

DUNCAN, R. and R. HIGHTON 1979. Genetic relationships of the eastern large *Plethodon* of the Ouachita Mountains. Copeia, 1979: 95–110.

FEDER, J. H. 1979. Natural hybridization and genetic divergence between the toads *Bufo boreas* and *Bufo punctatus*. Evolution, **33**: 1089–1097.

GUTTMAN, S. I. 1975. Genetic variation in the genus *Bufo*. II. Isozymes in northern allopatric populations of the American toad, *Bufo americanus*. Isozymes IV. Genetics and Evolution, edited by C. L. MARKERT, pp. 679–697. Academic Press, New York and London.

GUTTMAN, S. I., A. A. KARLIN and G. M. LABANICK 1978. A biochemical and morphological analysis of the relationship between *Plethodon longicrus* and *Plethodon yonahlossee* (Amphibia, Urodela, Plethodontidae). J. Herpetology, **12**: 445–454.

HARRIS, H. and D. A. HOPKINSON 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publ. Co., Amsterdam.

HEDGECOCK, D. 1976. Genetic variation in two widespread species of salamanders, *Taricha granulosa* and *Taricha torosa*. Biochem. Genet., **14**: 561–576.

———— 1978. Population subdivision and genetic divergence in the red-bellied newt, *Taricha rivularis*. Evolution, **32**: 271–286.

HEDGECOCK, D. and F. J. AYALA 1974. Evolutionary divergence in the genus *Taricha* (Salamandridae). Copeia, 1974: 738–747.

KALEZIĆ, M. L. and D. HEDGECOCK 1979. Genetic variation and differentiation of three common European newts (*Triturus*) in Yugoslavia. British J. Herpetology, **6**: 49–57.

LARSON, A. 1980. Paedomorphosis in relation to rates of morphological and molecular evolution in the salamander *Aneides flavipunctatus* (Amphibia, Plethodontidae). Evolution, **34**: 1–17.

LARSON, A. and R. HIGHTON 1978. Geographic protein variation and divergence in the salamanders of the *Plethodon welleri* group (Amphibia, Plethodontidae). Syst. Zool., **27**: 431–448.

LEWONTIN, R. C. 1974. The Genetic Basis of Evolutionary Change. Columbia University Press, New York.

LEWONTIN, R. C. and J. L. HUBBY 1966. A molecular approach to the study of genic heterozygosity in natural populations, II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics, **54**: 595–609.

MATTHEWS, T. C. 1975. Biochemical polymorphism in populations of the Argentine toad, *Bufo arenarum*. Copeia, 1975: 454–465.

MERKLE, D. A. and S. I. GUTTMAN 1977. Geographic variation in the cave salamander *Eurycea lucifuga*. Herpetologica, **33**: 313–321.

NAKATANI, T. and Y. OKADA 1966. *Rana tagoi yakushimensis* n. Subsp. from Yakushima, Kagoshima Prefecture, Japan. Acta Herpetologica Japonica, **1**: 64–66.

NEI, M. 1972. Genetic distance between populations. Amer. Natur., **106**: 283–292.

———— 1975. Molecular Population Genetics and Evolution. North-Holland Publ. Co., Amsterdam. 288 pp.

NEVO, E. 1976. Adaptive strategies of genetic systems in constant and varying environments. Population

Genetics and Ecology, edited by A. A. KARLIN and E. NEVO, pp. 141–158. Academic Press, New York.

NEVO, E. and S. Y. YANG 1979. Genetic diversity and climatic determinants of tree frogs in Israel. Oecologia(Berl.), **41**: 47–63.

NISHIOKA, M., H. OHTANI and M. SUMIDA 1980. Detection of chromosomes bearing the loci for seven kinds of proteins in Japanese pond frogs. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **4**: 127–184.

NISHIOKA, M., M. SUMIDA, S. OHTA and H. SUZUKI 1987. Speciation of three allied genera, *Buergeria, Rhacophorus* and *Polypedates,* elucidated by the method of electrophoretic analyses. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **9**: 53–96.

OKADA, Y. 1928. Notes on Japanese frogs. Annot. Zool. Japonenses, **11**: 269–277.

OKADA, Y. and U. KAWANO 1924. Two new varieties of Japanese green frogs and their ecological distribution. (In Japanese) Zool. Mag. (Tokyo), **36**: 104–109, 140–153.

RAGGHIANTI, M. and D. B. WAKE 1986. Genic variation and its evolutionary implications in the Italian newt, *Triturus italicus*. Herpetologica, **42**: 206–214.

RALIN, D. B. and R. K. SELANDER 1979. Evolutionary genetics of diploid-tetraploid species of treefrogs of the genus *Hyla*. Evolution, **33**: 595–608.

ROGERS, J. S. 1973. Protein polymorphism, genic heterozygosity and divergence in the toads, *Bufo cognatus* and *B. speciosus*. Copeia, 1973: 322–330.

SATTLER, P. W. 1980. Genetic relationships among selected species of North American *Scaphiopus*. Copeia, 1980: 605–610.

SNEATH, P. H. A. and R. R. SOKAL 1973. Numerical Taxonomy. W. H. FREEMAN and Co., San Francisco.

SOKAL, R. R. and P. H. A. SNEATH 1963. Principles of Numerical Taxonomy. W. H. FREEMAN and Co., San Francisco.

TILLEY, S. G. and P. M. SCHWERDTFEGER 1981. Electrophoretic variation in Appalachian populations of the *Desmognathus fuscus* complex (Amphibia: Plethodontidae). Copeia, 1981: 109–119.

WAKE, D. B., L. R. MAXSON and G. Z. WURST 1978. Genetic differentiation, albumin evolution, and their biogeographic implications in plethodontid salamanders of California and southern Europe. Evolution, **32**: 529–539.

WRIGHT, S. 1978. Evolution and The Genetics of Populations. Vol. 4. Variability within and among natural populations. pp.79–103. Univ. of Chicago Press, Chicago and London.