

# An Electrophoretic Study of Genetic Differentiation in 40 Populations of *Bufo japonicus* Distributed in Japan

By

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## ABSTRACT

Electrophoretic analyses of 14 kinds of enzymes and two kinds of blood proteins were made in 525 toads of 40 populations of *Bufo japonicus* distributed widely in Japan. These populations belong to five subspecies, *B. j. montanus*, *B. j. japonicus* (including *B. j. formosus* and *B. j. hokkaidoensis*), *B. j. torrenticola*, *B. j. yakushimensis* and *B. j. miyakonis*. The enzymes and blood proteins are controlled by genes at 22 loci.

The fixation indexes calculated according to WRIGHT (1978) showed that the Miyako population of *B. j. miyakonis* is remarkably differentiated from the other 39 populations belonging to four subspecies. The rates of average heterozygosity were 1.0~21.7%, 10.7% on the average. The proportions of polymorphic loci in the 40 populations were 5~65%, 36.0% on the average. The mean numbers of alleles per locus were 1.05~1.95, 1.46 on the average.

Examination of the geographic distribution of alleles at the 22 loci in the 40 populations of *B. japonicus* showed that there are distinct gradients from east to west in the alleles at the AAT-A, IDH-B, LDH-B, ME-B and MPI loci. Some of the alleles at the Ab and Hb loci are abundantly gathered in the populations distributed in some districts. At each of the 15 loci other than the foregoing seven, one of the alleles is overwhelmingly abundant in all the 40 populations or the 39 populations excluding the Miyako population which is exclusively or partly occupied by another allele.

The genetic relationships among the 40 populations of *Bufo japonicus* distributed in Japan were conjectured by estimating the genetic distances by the method of NEI (1975) and a dendrogram was drawn using the UPGMA clustering method. It was found that *B. japonicus* has evolved into the eastern and western groups, except that *B. j. miyakonis* seems to have come from the mainland of eastern Asia.

## INTRODUCTION

STEJNEGER (1907) has described three *Bufo* species as Japanese toads. They are *B. bufo formosus* BOULENGER, *B. smithi* (n. sp.) and *B. bufo japonicus* (SCHLEGEL). Thereafter, OKADA (1931) has reported that there are five subspecies, *B. vulgaris*

*formosus* (BOULENGER), *B. v. hokkaidoensis* OKADA, *B. v. japonicus* SCHLEGEL, *B. v. yakushimensis* OKADA and *B. bufo miyakonis* OKADA in Japan. In 1937, he added one more subspecies, *B. v. montanus* OKADA, to them. In contrast to these classifications, NAKAMURA and UENO (1963) combined the five subspecies of *Bufo vulgaris* into one subspecies, *B. bufo japonicus* SCHLEGEL, and changed *B. b. miyakonis* into *B. b. gargarizans* CANTOR. In 1966, OKADA changed the species name, *vulgaris* into *bufo*, and divided the Japanese *Bufo bufo* into six subspecies, *B. b. hokkaidoensis*, *B. b. formosus*, *B. b. japonicus*, *B. b. yakushimensis*, *B. b. miyakonis* and *B. b. montanus*. MATSUI (1975, 1976) has reported on a new species, *B. torrenticola*, distributed in the mountains of central Japan.

KAWAMURA, NISHIOKA and UEDA (1980) have made voluminous crossing experiments among Japanese, European and American toads in order to clarify the existence of reproductive isolation among them. The results of these experiments have shown that the Japanese toads are completely isolated from European toads by postmating isolating mechanisms. On the basis of this fact *B. b. japonicus* was elevated to the status of a species and named *B. japonicus*. On the other hand, *B. torrenticola* was also changed into a subspecies of *B. japonicus*, as it is not reproductively isolated from *B. japonicus*. As *B. b. japonicus* was changed into *B. j. japonicus*, all the subspecies of *B. bufo* distributed in Japan were changed into those of *B. japonicus* as a matter of course.

The present study elucidates biochemically the genetic differentiation of *Bufo japonicus* distributed widely in Japan by starch-gel protein electrophoresis. It is of interest to determine the genetic differences and to examine the phylogenetic relationships among the populations belonging to various subspecies of *Bufo japonicus*.

## MATERIALS AND METHODS

A total of 525 toads consisting of 154 females and 371 males was used in the present study. They were mostly collected in the four years of 1981~1984. They consisted of 27 (♀4, ♂23) toads of two populations belonging to *Bufo japonicus montanus* OKADA, 473 (♀138, ♂335) toads of 34 populations belonging to *Bufo japonicus japonicus* SCHLEGEL, 14 (♀6, ♂8) toads of two populations belonging to *Bufo japonicus torrenticola* MATSUI, 5 (♀2, ♂3) toads of one population belonging to *Bufo japonicus yakushimensis* OKADA, and 6 (♀4, ♂2) toads of one population belonging to *Bufo japonicus miyakonis* OKADA.

The prefecture, collecting station, date, number of toads, abbreviation of each subspecies and population name are as follows.

### A. *Bufo japonicus japonicus*

1. Hokkaido: Hakodate City. April 1982, 4 (♀2, ♂2), collected by Mr. M. SAKUYAMA. *jap.* Hakodate
2. Aomori Pref.: Namioka-cho, Minamitsugaru-gun. July~August 1981, 7 (♀1, ♂6). *jap.* Namioka

3. Aomori Pref.: Jonan, Hirosaki City. July~August 1981, 4(♀ 3, ♂ 1). *jap.* Hirosaki
4. Aomori Pref.: Mt. Iwaki, Hirosaki City. July 1981, 1(♂ 1). *jap.* Iwaki
5. Miyagi Pref.: Wakuya-machi, Toda-gun. July~August 1981, 9(♀ 3, ♂ 6). *jap.* Wakuya
6. Yamagata Pref.: Yamairakawa, Atsumi-cho, Nishitagawa-gun. May 1984, 11(♀ 4, ♂ 7), collected by Dr. T. OTSU. *jap.* Atsumi
7. Yamagata Pref.: Hayama, Murayama City. May 1984, 7(♀ 2, ♂ 5), collected by Dr. T. OTSU. *jap.* Hayama

#### B. *Bufo japonicus montanus*

8. Yamagata Pref.: Zao, Yamagata City. May 1984, 14(♀ 4, ♂ 10), collected by Dr. T. OTSU. *mon.* Zao  
Yamagata Pref.: Nishizao, Yamagata City. April 1984, 3(♂ 3), collected by Dr. T. OTSU. *mon.* Zao
9. Tochigi Pref.: Nikko City. June 1984, 10(♂ 10), collected by Dr. S. SHINOZAKI. *mon.* Nikko

#### C. *Bufo japonicus japonicus*

10. Ibaraki Pref.: Uenomiya, Kuji-gun. May 1984, 37(♀ 1, ♂ 36). *jap.* Kuji
11. Ibaraki Pref.: Watari, Mito City. April 1983, 42(♀ 10, ♂ 32). *jap.* Mito
12. Ibaraki Pref.: Minori-cho, Higashiibaraki-gun. March 1982, 3(♂ 3). *jap.* Minori
13. Chiba Pref.: Okura, Sawara City. April 1984, 42(♀ 10, ♂ 32). *jap.* Sawara
14. Chiba Pref.: Kashiwa City. March 1982, 8(♀ 1, ♂ 7). *jap.* Kashiwa
15. Gunma Pref.: Tochikubo, Nakanojo-cho, Agatsuma-gun. April 1983, 10(♀ 1, ♂ 9). *jap.* Azuma
16. Gunma Pref.: Niiharu-mura, Tone-gun. April 1983, 3(♂ 3). *jap.* Tone
17. Saitama Pref.: Otaki-mura, Chichibu-gun. May 1984, 42(♀ 10, ♂ 32). *jap.* Chichibu
18. Kanagawa Pref.: Sagami City. May 1984, 22(♀ 11, ♂ 11). *jap.* Sagami
19. Kanagawa Pref.: Irioya-cho, Zama City. March 1983, 26(♀ 7, ♂ 19). *jap.* Zama
20. Yamanashi Pref.: Shibireko, Ichikawadaimon-cho, Nishiyatsushiro-gun. April 1983, 47(♀ 17, ♂ 30). *jap.* Shibire
21. Yamanashi Pref.: Tabayama-mura, Kitatsuru-gun. April 1983, 20(♀ 2, ♂ 18). *jap.* Tabayama
22. Nagano Pref.: Shimosuwa-cho, Suwa-gun. April 1983, 13(♀ 4, ♂ 9), collected by Mr. R. SHIMOYAMA. *jap.* Suwa
23. Nagano Pref.: Chigataki-nakaku, Karuizawa-cho, Kitasaku-gun. April 1983, 21(♀ 11, ♂ 10). *jap.* Kitasaku
24. Nagano Pref.: Matsumoto City. May 1984, 2(♀ 1, ♂ 1). *jap.* Matsumoto
25. Nagano Pref.: Shiga-mura, Higashichikuma-gun. May 1984, 3(♀ 1, ♂ 2). *jap.* Shigamura

26. Shizuoka Pref.: Hamamatsu City. June 1981, 18(♀2, ♂16). *jap.* Hamamatsu  
 27. Gifu Pref.: Neo-mura, Motosu-gun. June~July 1983, 15(♀10, ♂5), collected by Dr. M. TADANO. *jap.* Neo

D. *Bufo japonicus torrenticola*

28. Gifu Pref.: Neo-mura, Motosu-gun. June 1983, 4(♀2, ♂2), collected by Dr. M. TADANO. *tor.* Neo  
 29. Nara Pref.: Odaigahara. April 1977, 10(♀4, ♂6), collected by Dr. M. MATSUI. *tor.* Odai

E. *Bufo japonicus japonicus*

30. Shiga Pref.: Mt. Hiei. March 1982, 3(♀3). *jap.* Hiei  
 31. Kyoto Pref.: Mt. Kurama. August 1981, 1(♂1). *jap.* Kurama  
 32. Kyoto Pref.: Arashiyama. March 1982, 9(♀3, ♂6). *jap.* Arashi  
 33. Hiroshima Pref.: Kanmurikogen, Yoshiwa-mura, Saeki-gun. March 1977, 19(♀5, ♂14). *jap.* Yoshiwa  
 34. Hiroshima Pref.: Sandankyo, Togochi-cho, Yamagata-gun. June 1983, 4(♀4). *jap.* Togochi  
 35. Hiroshima Pref.: Ujina, Hiroshima City. June 1984, 1(♀1). *jap.* Hiroshima  
 36. Ehime Pref.: Omogo-mura, Kamiukena-gun. 3(♀3). *jap.* Omogo  
 37. Oita Pref.: Asaji-cho, Ono-gun. 1(♀1). *jap.* Ono  
 38. Kagoshima Pref.: Taniyama, Kagoshima City. June 1979, 15(♀4, ♂11), collected by Dr. S. ISHIKUBO. *jap.* Kagoshima

F. *Bufo japonicus yakushimensis*

39. Kagoshima Pref.: Yaku Isl., Kumage-gun. August 1981, 5(♀2, ♂3), collected by Dr. M. KURAMOTO. *yak.* Yaku

G. *Bufo japonicus miyakonis*

40. Okinawa Pref.: Miyako Isl. April 1977 and February 1979, 6(♀4, ♂2), collected by Dr. M. KURAMOTO. *miy.* Miyako

Fourteen kinds of enzymes were extracted from the skeletal muscles or livers of each toad. These enzymes were aspartate aminotransferase (AAT), adenosine deaminase (ADA), adenylate kinase (AK), creatine kinase (CK),  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GDH), glucose phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), mannose phosphate isomerase (MPI), peptidase (Pep), phosphoglucomutase (PGM) and superoxide dismutase (SOD). The two kinds of blood proteins prepared in addition were serum albumin (Ab) and hemoglobin (Hb). The enzymes and blood proteins were analyzed 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

electrophoresis have been described by NISHIOKA, OHTA and SUMIDA (1987). The detection of each enzyme was performed by the agar overlay method of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. The detection of blood proteins was made by amido-black staining method.

When each of the multiple alleles existed at a frequency of more than 1% at a locus, this locus was considered to be polymorphic. The fixation index ( $F_{st}$ ) coined by WRIGHT (1978) was utilized as a standard to show the degree of genetic differentiation found at a definite locus among local populations. The proportion of polymorphic loci and average heterozygosity (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974) were utilized to denote quantitatively the genetic variations of local populations.

The genetic relationships among local populations were conjectured by estimating the genetic distances (D) according to NEI's method (1975). The systematic relationship among these local populations was established by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975) on the basis of genetic distances (D).

## OBSERVATION

### *I. Electrophoretic patterns and allelomorphs*

Electrophoretic patterns of 14 kinds of enzymes extracted from the skeletal muscles or livers and two kinds of blood proteins were analyzed in 525 toads of 40 populations belonging to five subspecies. The results showed that these enzymes and blood proteins were controlled by genes at 22 loci. The electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, ... in the order of mobility from fast to slow and the alleles were indicated by  $a, b, c, \dots$ .

Six of the 22 loci, AAT-A, AK, CK, IDH-A, SOD-A and SOD-B, showed two or three phenotypes produced by two alleles,  $a$  and  $b$ . At two loci, GPI and Pep-A, there were four phenotypes controlled by three alleles,  $a, b$  and  $c$ . At six loci, LDH-B, MDH-A, MDH-B, ME-A, PGM and Hb, four to six phenotypes controlled by four alleles,  $a, b, c$  and  $d$ , were observed. At four loci, AAT-B, ADA, IDH-B and LDH-A, there were five to eight phenotypes controlled by five alleles,  $a, b, c, d$  and  $e$ . At two loci,  $\alpha$ -GDH and Ab, eight or 10 phenotypes controlled by six alleles,  $a, b, c, d, e$  and  $f$ , were observed. At the locus of MPI, 15 phenotypes controlled by eight alleles,  $a, b, c, d, e, f, g$  and  $h$ , were observed. The locus of ME-B was the most polymorphic among the 22 analyzed loci. There were 23 phenotypes controlled by 10 alleles,  $a, b, c, d, e, f, g, h, i$  and  $j$ . At these 22 loci, there were 6.3 phenotypes produced by 4.2 alleles on the average (Table 1; Figs. 1 and 2).

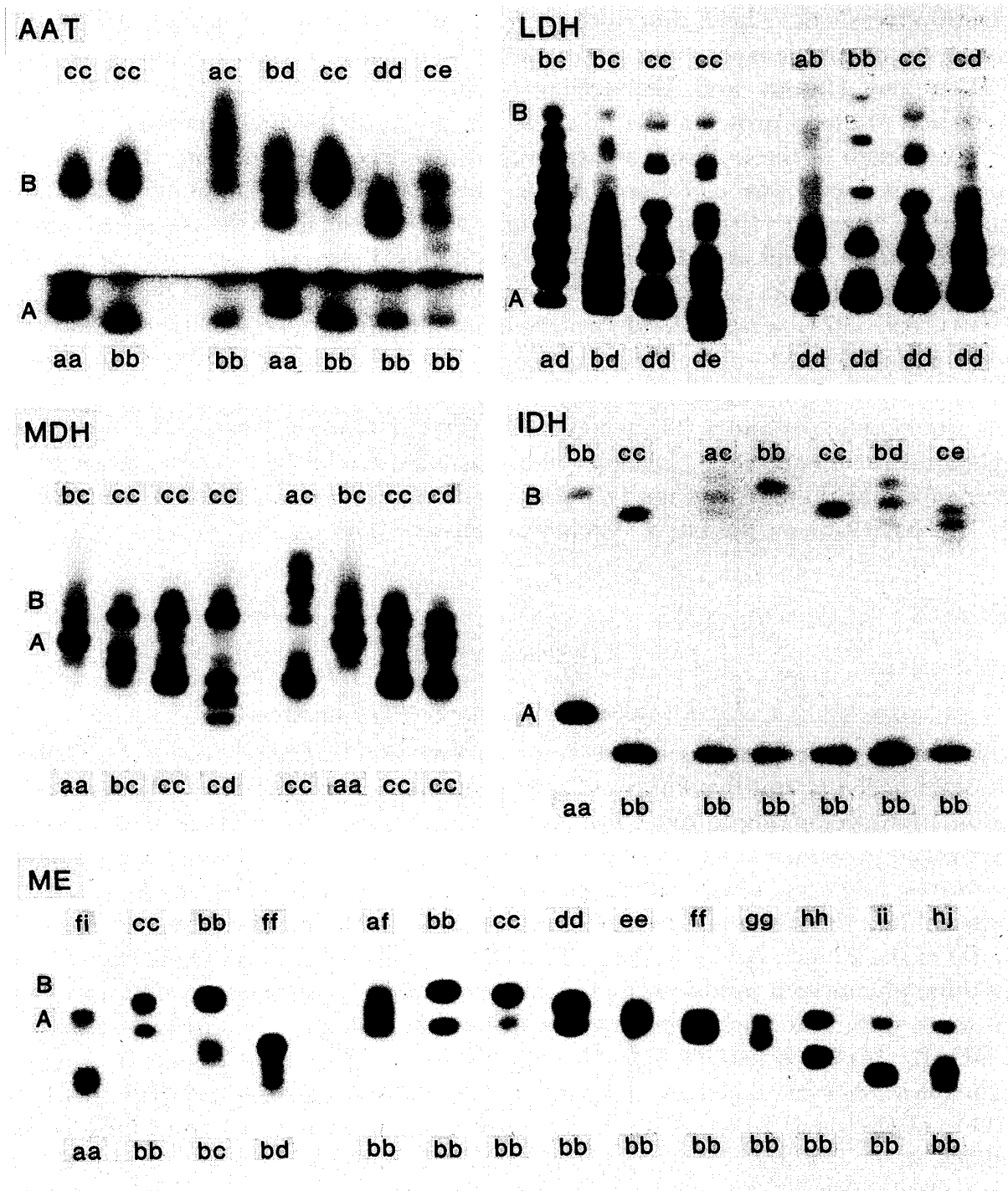


Fig. 1. Electrophoretic patterns of five enzymes, AAT, LDH, MDH, IDH and ME, in 40 populations of *Bufo japonicus*.

## II. Gene frequency

### 1. AAT-A locus

The electrophoretic patterns of AAT-A obtained from 521 toads of the 40 populations belonging to *Bufo japonicus* showed three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*.

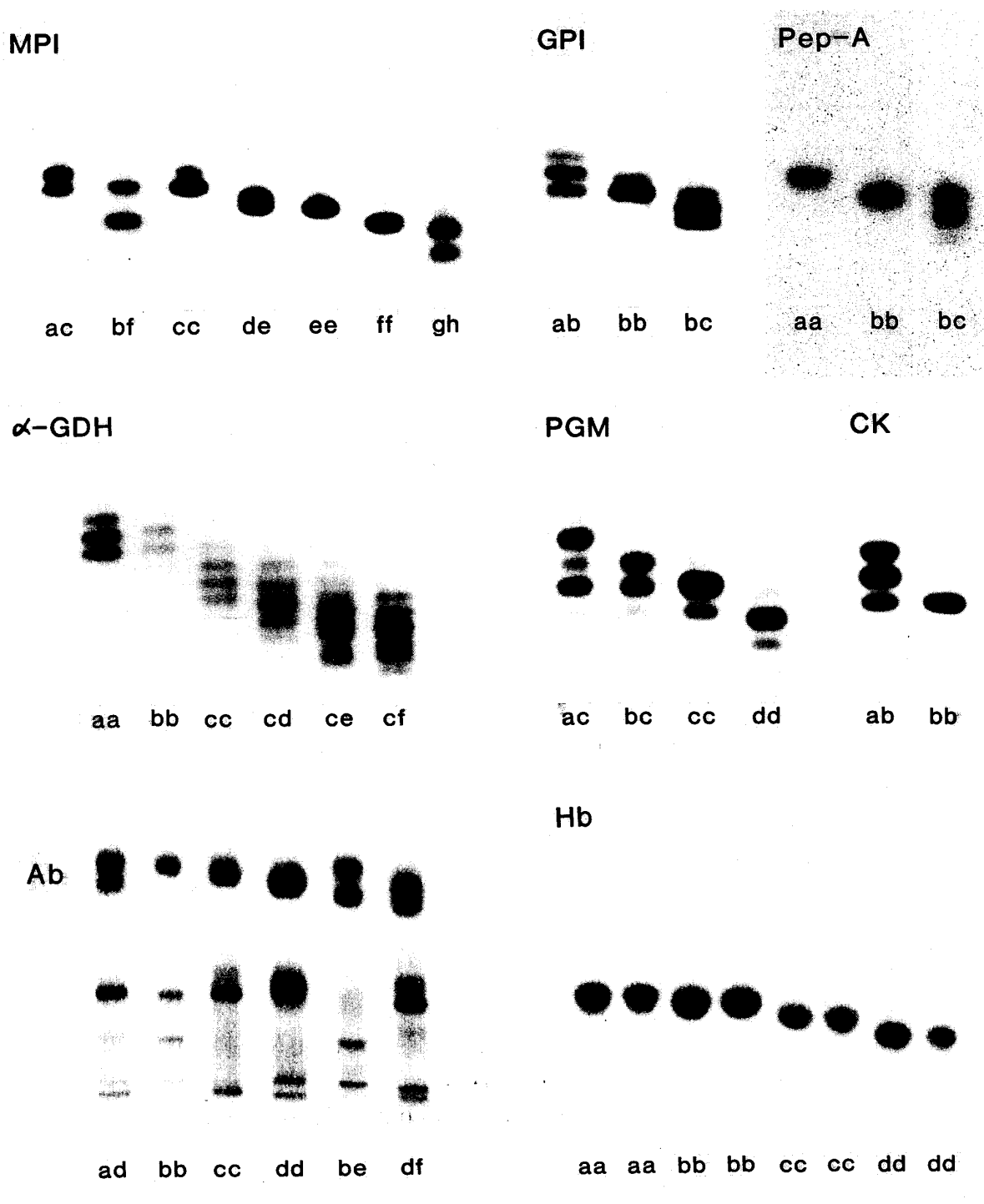


Fig. 2. Electrophoretic patterns of six enzymes, MPI, GPI, Pep-A,  $\alpha$ -GDH, PGM and CK, and two blood proteins, Ab and Hb, in 40 populations of *Bufo japonicus*.

While all the 12 toads of three populations of Namioka, Hirosaki and Iwaki of *jap*, showed a homozygous AA band, all the 72 toads of the 12 populations, including nine populations of Hakodate, Hiei, Kurama, Yoshiwa, Togochi, Hiroshima, Omogo, Ono and Kagoshima of *jap*, the Odai population of *tor*, the Yaku population of *yak*, and the Miyako population of *miy* showed a homozygous

TABLE 1  
Number of phenotypes and alleles at 22 loci in 40  
populations of *Bufo japonicus*

Locus	No. of phenotypes	No. of alleles
AAT-A	3	2
AAT-B	8	5
ADA	7	5
AK	2	2
CK	2	2
$\alpha$ -GDH	8	6
GPI	4	3
IDH-A	3	2
IDH-B	8	5
LDH-A	5	5
LDH-B	6	4
MDH-A	4	4
MDH-B	6	4
ME-A	5	4
ME-B	23	10
MPI	15	8
Pep-A	4	3
PGM	5	4
SOD-A	2	2
SOD-B	2	2
Ab	10	6
Hb	6	4
Average	6.3	4.2

#### BB band.

In three populations of Wakuya, Atsumi and Tone of *jap*, four of the nine toads, 10 of the 11 toads and one of the three toads, respectively, showed an AA band, while the remaining five, one and two toads, respectively, showed an AB band. In six populations including the Minori, Azuma, Matsumoto, Shigamura and Arashi populations of *jap* and the Neo population of *tor*, two of the three toads, seven of the 10 toads, one of the two toads, two of the three toads, six of the nine toads and three of the four toads, respectively, showed a BB band, while the remaining one, three, one, one, three and one, respectively, showed an AB band. In three populations including the Zao and Nikko populations of *mon* and the Kuji population of *jap*, eight of the 17 toads, four of the 10 toads and 16 of the 37 toads, respectively, showed an AA band, four, one and five, respectively, showed a BB band, and the remaining five, five and 16, respectively, showed an AB band. In four populations of Hayama, Mito, Kashiwa and Chichibu of *jap*, one of the seven toads, 14 of the 42 toads, two of the eight toads and nine of the 42 toads, respectively, showed an AA band, two, eight, three and nine, respectively, showed



TABLE 2  
Gene frequencies at seven loci, AAT-A, AAT-B, ADA, AK, CK,  $\alpha$ -GDH and GPI, in 40 populations of *Bufo japonicus*

Species	Locality	Sample size	AAT-A		AAT-B					ADA					AK		CK					$\alpha$ -GDH					GPI		
			a	b	a	b	c	d	e	a	b	c	d	e	a	b	a	b	a	b	c	d	e	f	a	b	c		
<i>B. j. jap.</i>	Hakodate	4	1.000		1.000					1.000						1.000		1.000		1.000				1.000			0.125	0.875	
"	Namioka	7	1.000		0.071	0.929				1.000						1.000		1.000		1.000				1.000			0.143	0.857	
"	Hirosaki	4	1.000		1.000				0.125	0.875						1.000		1.000		1.000				1.000			1.000		
"	Iwaki	1	1.000		1.000				1.000							1.000		1.000		1.000				1.000			1.000		
"	Wakuya	9	0.722	0.278	0.056	0.944			1.000							1.000		1.000		1.000				1.000			1.000		
"	Atsumi	11	0.955	0.045	0.091	0.909			0.955	0.045						1.000		1.000		1.000				1.000			1.000		
"	Hayama	7	0.429	0.571	0.786	0.214			1.000							1.000		1.000		1.000				1.000			0.857	0.143	
<i>B. j. mon.</i>	Zao	17	0.618	0.382	0.971	0.029			0.118	0.853			0.029			1.000		1.000		1.000				1.000			0.971	0.029	
"	Nikko	10	0.650	0.350	0.100	0.650	0.200	0.050	0.100	0.900						1.000		1.000		1.000				1.000			1.000		
<i>B. j. jap.</i>	Kuji	37	0.649	0.351	0.041	0.932			0.027	0.108	0.878		0.014			0.986	0.014	1.000		1.000				1.000			0.986	0.014	
"	Mito	42	0.571	0.429	0.071	0.917			0.012	0.036	0.952		0.012			1.000		1.000		1.000				1.000			0.976	0.024	
"	Minori	3	0.167	0.833	0.167	0.833			0.833		0.167					1.000		1.000		1.000				1.000			0.976	0.024	
"	Sawara	42	0.226	0.774	0.083	0.893	0.024			0.131	0.869					1.000		1.000		1.000				1.000			0.976	0.024	
"	Kashiwa	8	0.438	0.563	0.063	0.875	0.063			0.375	0.500	0.125				1.000		1.000		1.000				1.000			0.976	0.024	
"	Azuma	10	0.150	0.850	0.100	0.850	0.050			0.250	0.750					1.000		1.000		1.000				1.000			0.976	0.024	
"	Tone	3	0.667	0.333	0.167	0.667	0.167			0.333	0.667					1.000		1.000		1.000				1.000			0.976	0.024	
"	Chichibu	42	0.500	0.500	0.143	0.702	0.155			1.000						1.000		1.000		1.000				1.000			0.964	0.012	0.012
"	Sagami	22	0.250	0.750	0.023	0.818	0.159			0.977			0.023			1.000		1.000		1.000				1.000			0.976	0.024	
"	Zama	26	0.135	0.865	0.077	0.923			0.942		0.058					1.000		1.000		1.000				1.000			0.976	0.024	
"	Shibire	47	0.191	0.809	0.128	0.670	0.202			0.942			0.058			1.000		1.000		1.000				1.000			0.976	0.024	
"	Tabayama	20	0.225	0.775	0.075	0.750	0.175			1.000						1.000		1.000		1.000				1.000			0.976	0.024	
"	Suwa	13	0.269	0.731	0.154	0.769	0.077			1.000						1.000		1.000		1.000				1.000			0.976	0.024	
"	Kitasaku	21	0.381	0.619	0.119	0.857	0.024			0.190	0.810					1.000		1.000		1.000				1.000			0.976	0.024	
"	Matsumoto	2	0.250	0.750	0.250	0.750			1.000							1.000		1.000		1.000				1.000			0.976	0.024	
"	Shigamuro	3	0.167	0.833	0.056	0.861	0.083			1.000						1.000		1.000		1.000				1.000			0.976	0.024	
"	Hamamatsu	18	0.344	0.656	0.056	0.861	0.083			0.139	0.861					1.000		1.000		1.000				1.000			0.976	0.024	
"	Neo	15	0.385	0.615	0.056	0.861	0.083			0.033	0.967					1.000		1.000		1.000				1.000			0.976	0.024	
<i>B. j. tor.</i>	Neo	4	0.125	0.875	1.000				1.000							1.000		1.000		1.000				1.000			0.875	0.125	
"	Odai	10	1.000		1.000				0.050	0.950						1.000		1.000		1.000				1.000			0.950	0.050	
<i>B. j. jap.</i>	Hici	3	1.000		1.000				1.000							1.000		1.000		1.000				1.000			0.167	0.833	
"	Kurama	1	1.000		1.000				1.000							1.000		1.000		1.000				1.000			0.167	0.833	
"	Arashi	9	0.167	0.833	0.944	0.056			1.000							0.944	0.056	0.111	0.889	1.000				1.000			0.056	0.944	
"	Yoshiwa	19	1.000		0.026	0.632	0.342			1.000						1.000		1.000		1.000				1.000			0.079	0.921	
"	Togochi	4	1.000		0.750	0.250			1.000							1.000		1.000		1.000				1.000			0.974	0.026	
"	Hiroshima	1	1.000		1.000				1.000							1.000		1.000		1.000				1.000			0.875	0.125	
"	Omogo	3	1.000		1.000				1.000							1.000		1.000		1.000				1.000			1.000		
"	Ono	1	1.000		0.833	0.167			1.000							1.000		1.000		1.000				1.000			0.167	0.833	
"	Kagoshima	15	1.000		0.500	0.500			1.000							1.000		1.000		1.000				1.000			0.167	0.833	
<i>B. j. yak.</i>	Yaku	5	1.000		1.000				0.067	0.933						1.000		1.000		1.000				1.000			1.000		
<i>B. j. miy.</i>	Miyako	6	1.000		1.000				1.000				1.000			1.000		1.000		1.000				1.000			0.500	0.500	
																												0.167	0.833

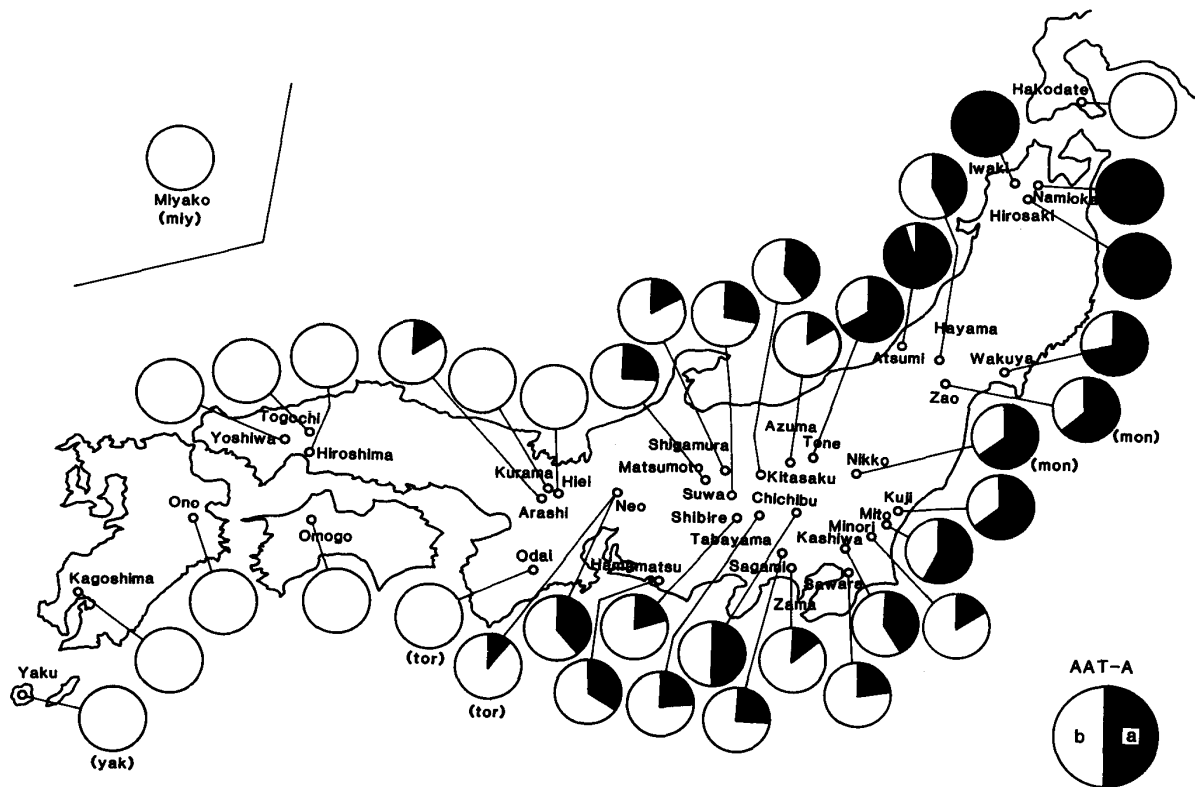


Fig. 3. Geographic distribution of AAT-A alleles among 40 populations of *Bufo japonicus*.

a BB band, and the remaining four, 20, three and 24, respectively, showed an AB band. In the remaining nine populations of Sawara, Sagami, Zama, Shibire, Tabayama, Suwa, Kitasaku, Neo and Hamamatsu of *jap*, two of the 42 toads, two of the 22 toads, one of the 26 toads, one of the 47 toads, one of the 20 toads, two of the 13 toads, four of the 21 toads, two of the 13 toads and four of the 16 toads, respectively, showed an AA band, 25, 13, 20, 30, 12, eight, nine, five and nine, respectively, showed a BB band, and the remaining 15, seven, five, 16, seven, three, eight, six and three, respectively, showed an AB band.

It was found that the distribution of alleles *a* and *b* in the 40 populations of *Bufo japonicus* indicated a distinct gradient in frequency from east to west. In the eastern populations, allele *a* was abundantly found. All the toads of the three populations of *jap* in Aomori Prefecture had allele *a*. Allele *a* in the Wakuya and Atsumi populations of *jap* was 0.722 and 0.955 in frequency, respectively. While allele *a* in the Zao and Nikko populations of *mon* and the Kuji, Mito and Tone populations of *jap* was 0.571~0.667 in frequency, alleles *a* and *b* in the Chichibu population of *jap* were both 0.500 in frequency. In contrast, allele *b* in nine populations of Hakodate, Hiei, Kurama, Yoshiwa, Togochi, Hiroshima, Omogo, Ono and Kagoshima of *jap*, the Odai population of *tor*, the Yaku population of *yak* and the Miyako population of *miy* was 1.000 in frequency. In the Neo population of *tor* and 11 populations of Minori, Sawara, Azuma, Sagami, Zama, Shibire, Tabayama, Suwa, Matsumoto, Shigamura and Arashi of *jap*, allele *b* was 0.731~0.875 in frequency, while in five populations of Hayama, Kashiwa, Kitasaku,

Hamamatsu and Neo of *jap*, allele *b* was 0.571~0.659 in frequency (Table 2; Fig. 3).

## 2. AAT-B locus

The electrophoretic patterns of AAT-B obtained from 523 toads of the 40 populations of *Bufo japonicus* showed that there were eight phenotypes, BB, CC, DD, AC, BC, BD, CD and CE, produced by five alleles, *a*, *b*, *c*, *d* and *e*.

All the 39 toads of 10 populations including the Hakodate, Hirosaki, Iwaki, Hiei, Kurama and Hiroshima populations of *jap*, the Neo and Odai populations of *tor*, the Yaku population of *yak* and the Miyako population of *miy* showed a CC band. In six populations of Atsumi, Shigamura, Arashi, Omogo and Kagoshima of *jap*, and the Zao population of *mon*, 10 of the 11 toads, two of the three toads, eight of the nine toads, two of the three toads, six of the 15 toads and 16 of the 17 toads, respectively, showed a CC band and the other one, one, one, one, nine and one, respectively, showed a CD band. Of the four toads of the Togoichi population of *jap*, three showed a CC band, and the other showed a DD band. The one toad of the Ono population of *jap* showed a CD band. In the Hayama and Neo populations of *jap*, five of the seven toads and six of the 13 toads, respectively, showed a CC band, one and one, respectively, showed a DD band, and the remaining one and six, respectively, showed a CD band. In five populations of Namioka, Wakuya, Minori, Zama and Matsumoto of *jap*, six of the seven toads, eight of the nine toads, two of the three toads, 22 of the 26 toads and one of the two toads, respectively, showed a CC band, and the remaining one, one, one, four and one, respectively, showed a BC band. In four populations of Kashiwa, Azuma, Tabayama and Kitasaku of *jap*, six of the eight toads, seven of the 10 toads, 10 of the 20 toads and 15 of the 21 toads, respectively, showed a CC band, one, two, three and five, respectively, showed a BC band, and the remaining one, one, seven and one, respectively, showed a CD band. In three populations of Kuji, Mito and Tone of *jap*, 32 of the 37 toads, 35 of the 42 toads and one of the three toads, respectively, showed a CC band, three, six and one, respectively, showed a BC band, and the remaining two, one and one, respectively, showed a CE band. In the Sagami and Hamamatsu populations of *jap*, 15 of the 22 toads and 14 of the 18 toads, respectively, showed a CC band, one and one, respectively, showed a DD band, one and two, respectively, showed a BC band, and the remaining five and one, respectively, showed a CD band. Of the 13 toads of the Suwa population of *jap*, one and eight showed BB and CC bands, respectively, and the other two and two showed BC and CD bands, respectively. Of the 19 toads of the Yoshiwa population of *jap*, nine and four showed CC and DD bands, respectively, and the other one and five showed AC and CD bands, respectively. Of the 42 toads of the Sawara population of *jap*, one and 35 showed BB and CC bands, respectively, and the other four, one and one toad showed BC, BD and CD bands, respectively. Of the 10 toads of the Nikko population of *mon*, five and one showed CC and DD bands, respectively, while the other one, one, one and one showed BC, BD, CD and CE bands, respectively. Of the 42 toads of the

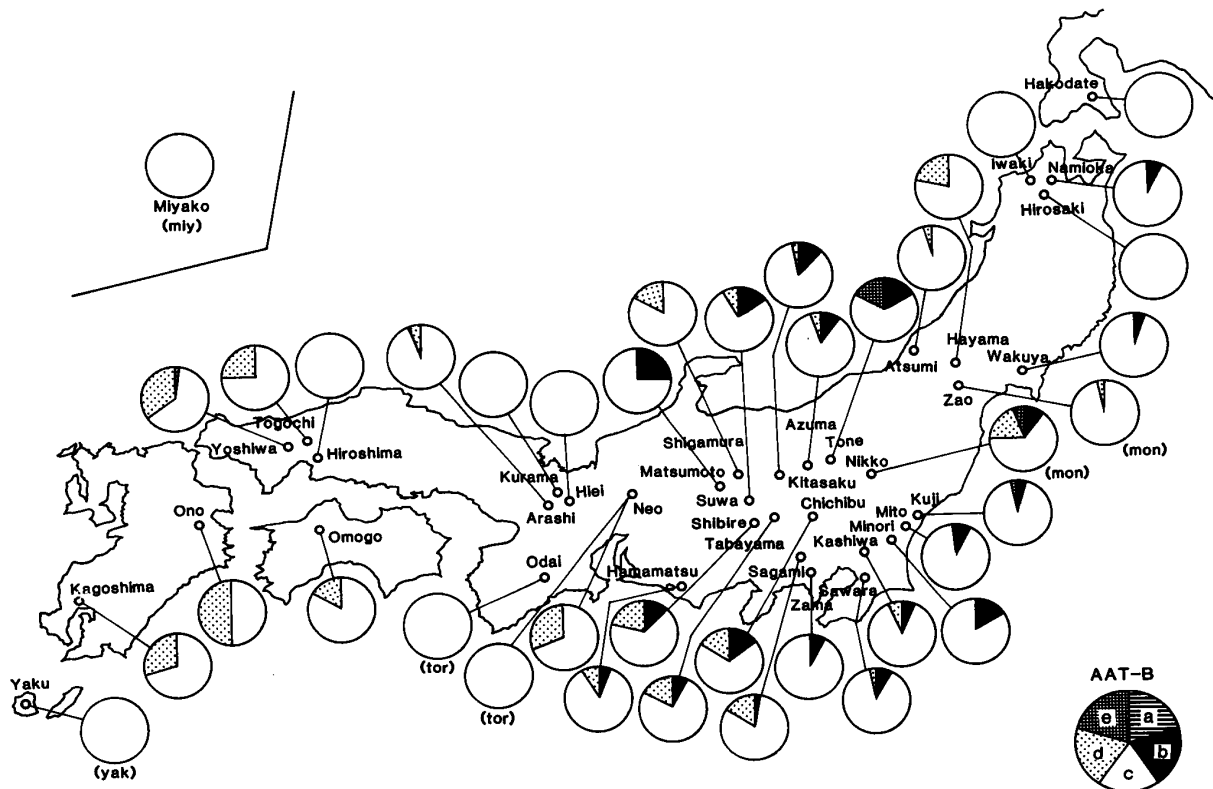


Fig. 4. Geographic distribution of AAT-B alleles among 40 populations of *Bufo japonicus*.

Chichibu population of *jap*, one, 21 and two showed BB, CC and DD bands, respectively, and the other nine, one and eight showed BC, BD and CD bands, respectively. Of the 47 toads of the Shibire population of *jap*, one, 22 and four showed BB, CC and DD bands, respectively, and the other nine, one and 10 showed BC, BD and CD bands, respectively.

In all the 40 populations of *Bufo japonicus*, allele *c* was the highest in frequency, being 0.500~1.000. Allele *d* was found in 20 populations of *jap* and the two populations of *mon*, being 0.024~0.500 in frequency. Allele *b* was found in 18 populations of *jap* and the Nikko population of *mon*, being 0.023~0.250 in frequency. Allele *e* was 0.012~0.167 in frequency and was found in the Kuji, Mito and Tone populations of *jap* and the Nikko population of *mon*. Allele *a* was only found in the Yoshiwa population of *jap*, being 0.026 in frequency (Table 2; Fig. 4).

### 3. ADA locus

The electrophoretic patterns of ADA obtained from 525 toads of the 40 populations of *Bufo japonicus* showed that there were seven phenotypes, AA, BB, EE, AB, AC, BD and BE, produced by five alleles, *a*, *b*, *c*, *d* and *e*.

In nine populations including the Hirosaki, Atsumi, Tone, Shibire, Neo, Togochi and Kagoshima populations of *jap*, the Nikko population of *mon* and the Odai population of *tor*, three of the four toads, nine of the 11 toads, one of the three toads, 46 of the 47 toads, 14 of the 15 toads, three of the four toads, 13 of the 15 toads, eight of the 10 toads and nine of the 10 toads, respectively, showed a BB

band, and the other one, two, two, one, one, one, two, two and one, respectively, showed an AB band. In the Minori, Sagami and Zama populations of *jap*, two of the three toads, 21 of the 22 toads and 23 of the 26 toads, respectively, showed a BB band and the other one, one and three, respectively, showed a BD band. In four populations of Sawara, Azuma, Kitasaku and Hamamatsu of *jap*, two of the 42 toads, one of the 10 toads, one of the 21 toads and one of the 18 toads, respectively, showed an AA band, 33, six, 14 and 14, respectively, showed a BB band, and the remaining seven, three, six and three showed an AB band. Of the 42 toads of the Mito population of *jap*, 38 showed a BB band, three others showed an AB band and the remainder showed a BD band. Of the 17 toads of the Zao population of *mon*, 12 showed a BB band, four others showed an AB band and the remainder showed a BE band. Of the 37 toads of the Kuji population of *jap*, one showed an AA band, 29 showed a BB band, six showed an AB band and the remainder showed a BD band. Of the eight toads of the Kashiwa population of *jap*, one showed an AA band, three others showed a BB band and the remaining two and two showed AB and AC bands, respectively. All the six toads of the Miyako population of *miy* showed an EE band. The remaining 154 toads of 19 populations including 17 populations of *jap*, the Neo population of *tor* and the Yaku population of *yak*, all showed a BB band.

In the 39 populations of *Bufo japonicus* other than the Miyako population which had only allele *e*, allele *b* was the highest in frequency, being 0.500~1.000. In 14 populations of *jap*, the two populations of *mon* and the Odai population of *tor*, allele

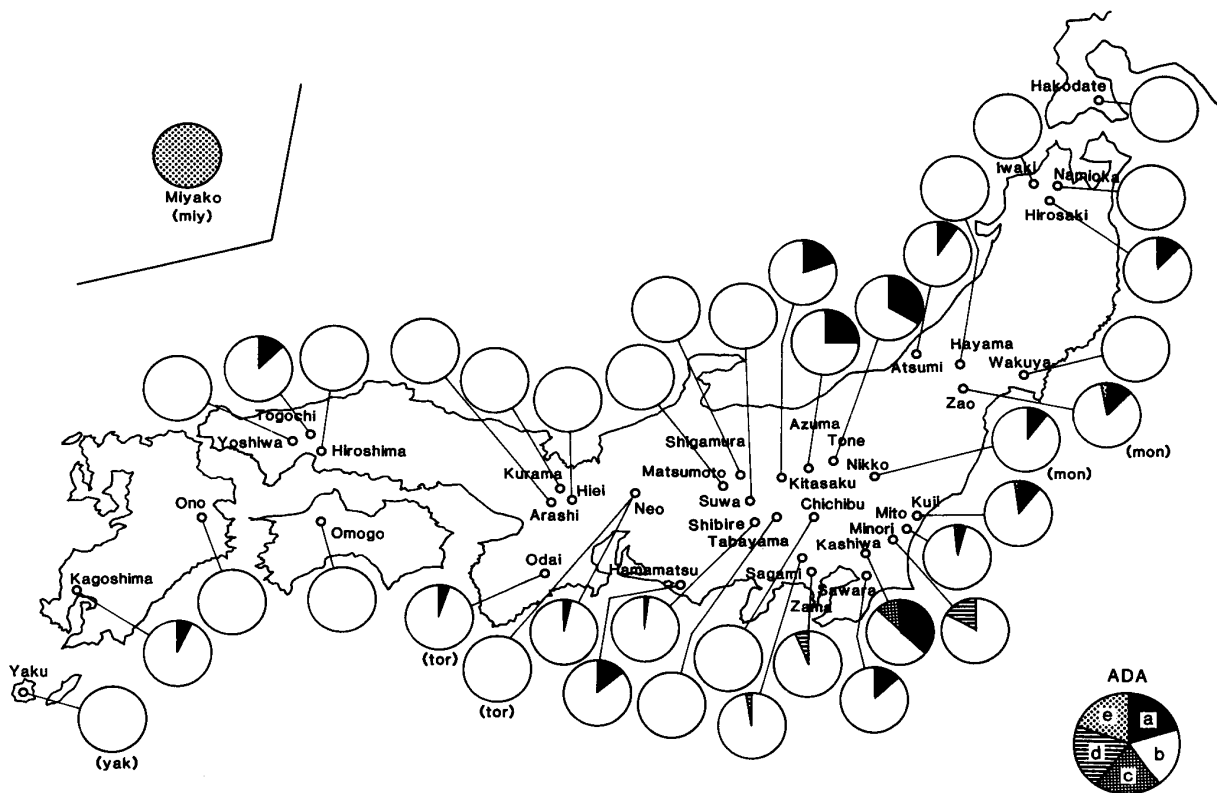


Fig. 5. Geographic distribution of ADA alleles among 40 populations of *Bufo japonicus*.

*a* was 0.011~0.333 in frequency. In the Kuji, Mito, Minori, Sagami and Zama populations of *jap*, allele *d* was 0.012~0.167 in frequency. Allele *c* was 0.125 in frequency in the Kashiwa population of *jap*. Allele *e* was 0.029 in frequency in the Zao population of *mon*, while the Miyako population of *miy* had only allele *e* (Table 2; Fig. 5).

#### 4. AK locus

The electrophoretic patterns of AK obtained from 525 toads of the 40 populations of *Bufo japonicus* showed that there were two phenotypes, AA and AB, produced by two alleles, *a* and *b*.

In eight populations of Wakuya, Kuji, Sagami, Zama, Shibire, Neo, Hamamatsu and Arashi of *jap*, eight of the nine toads, 36 of the 37 toads, 19 of the 22 toads, 25 of the 26 toads, 42 of the 47 toads, 13 of the 15 toads, 12 of the 18 toads and eight of the nine toads, respectively, showed an AA band, and the other one, one, three, one, five, two, six and one, respectively, showed an AB band. All the remaining 290 toads of 26 populations of *jap*, 27 toads of the two populations of *mon*, five toads of the Yaku population of *yak*, 14 toads of the two populations of *tor* and six toads of the Miyako population of *miy* showed an AA band.

In eight populations of *jap*, allele *a* was 0.833~0.986 in frequency, while allele *b* was 0.014~0.167. The toads of the remaining 32 populations had only allele *a* (Table 2; Fig. 6).

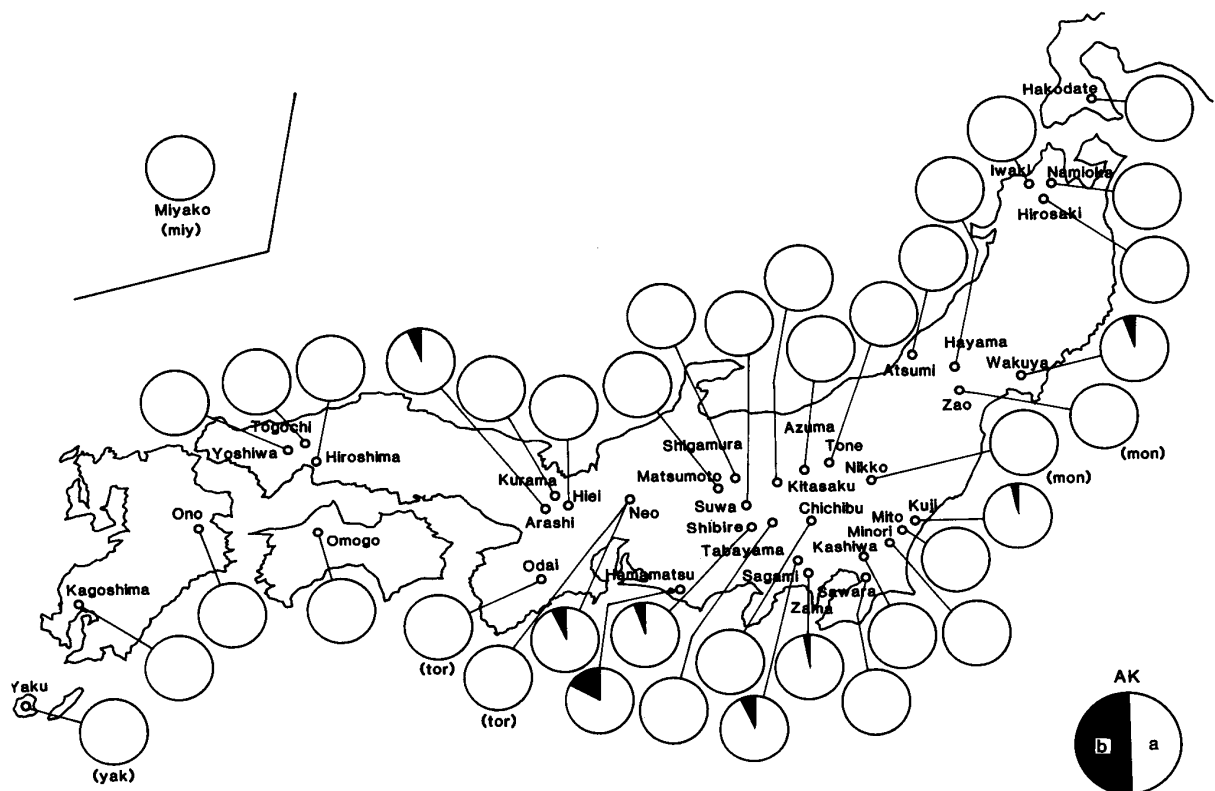


Fig. 6. Geographic distribution of AK alleles among 40 populations of *Bufo japonicus*.

### 5. CK locus

The electrophoretic patterns of CK obtained from 525 toads of the 40 populations of *Bufo japonicus* showed that there were two phenotypes, BB and AB, produced by two alleles, *a* and *b*.

All the 513 toads of the 38 populations other than the Hiei and Arashi populations of *jap* showed a homozygous BB band. In the Hiei and Arashi populations, two of the three toads and seven of the nine toads, respectively, showed a BB band, while the remaining one and two, respectively, showed an AB band.

In gene frequency, allele *b* in the Hiei and Arashi populations of *jap* was 0.833 and 0.889, respectively, while allele *a* was 0.167 and 0.111, respectively. All the other 38 populations had only allele *b* (Table 2).

### 6. $\alpha$ -GDH locus

The electrophoretic patterns of  $\alpha$ -GDH obtained from 525 toads of the 40 populations of *Bufo japonicus* showed that there were eight phenotypes, AA, BB, CC, AC, BC, CD, CE and CF, produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*.

In six populations including the Namioka, Atsumi, Kuji, Mito and Neo populations of *jap* and the Zao population of *mon*, six of the seven toads, 10 of the 11 toads, 36 of the 37 toads, 40 of the 42 toads, 14 of the 15 toads and 16 of the 17 toads, respectively, showed a CC band, and the other one, one, one, two, one and one, respectively, showed an AC band. In the Yoshiwa and Togochi populations of *jap*, 18 of the 19 toads and three of the four toads, respectively, showed a CC band and the other one and one, respectively, showed a CD band. Of the 18 toads of the Hamamatsu population of *jap*, one and 16 showed AA and CC bands, respectively, and the remainder showed an AC band. Of the 20 toads of the Tabayama population of *jap*, 15 showed a CC band and the other two and three showed AC and CF bands, respectively. Of the 47 toads of the Shibire population of *jap*, four and 25 showed AA and CC bands, respectively, while the other 16 and two showed AC and CF bands, respectively. Of the 42 toads of the Chichibu population of *jap*, 39 showed a CC band and the other one, one and one showed AC, CD and CE bands, respectively. Of the six toads of the Miyako population of *miy*, two and two showed BB and CC bands, respectively, and the other two showed a BC band. The remaining 240 toads of 27 populations including 23 populations of *jap*, the Nikko population of *mon*, the two populations of *tor* and the Yaku population of *yak* all showed a CC band.

In the Miyako population of *miy*, each of alleles *b* and *c* was 0.500 in frequency. In the other 39 populations of *Bufo japonicus*, allele *c* was the highest in frequency, being 0.723~1.000. The frequency of allele *a* was 0.012~0.255 in nine populations of *jap* and the Zao population of *mon*. Allele *d* was 0.012~0.125 in frequency in three populations of *jap*. Allele *e* was 0.012 in frequency and found only in the Chichibu population of *jap*. Allele *f* was 0.021 and 0.075 in frequency in the Shibire and Tabayama populations of *jap*, respectively (Table 2; Fig. 7).

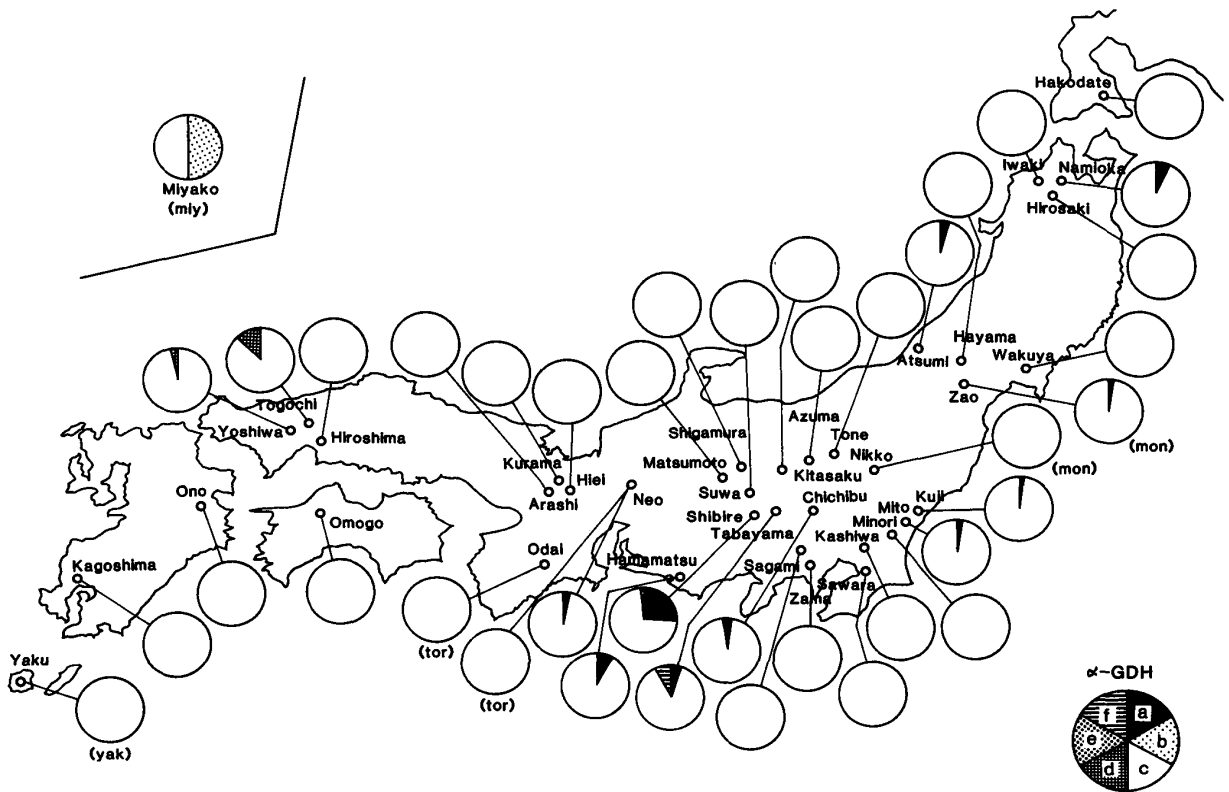


Fig. 7. Geographic distribution of  $\alpha$ -GDH alleles among 40 populations of *Bufo japonicus*.

## 7. GPI locus

The electrophoretic patterns of GPI obtained from 525 toads of the 40 populations of *Bufo japonicus* showed that there were four phenotypes, BB, CC, AB and BC, produced by three alleles, *a*, *b* and *c*.

In 19 populations including the 18 populations of Hakodate, Namioka, Minori, Sawara, Kashiwa, Chichibu, Zama, Shibire, Tabayama, Suwa, Kitasaku, Matsumoto, Neo, Hamamatsu, Hiei, Arashi, Yoshiwa and Omogo of *jap* and the Miyako population of *miy*, three of the four toads, five of the seven toads, one of the three toads, 37 of the 42 toads, five of the eight toads, 38 of the 42 toads, 19 of the 26 toads, 46 of the 47 toads, 19 of the 20 toads, 10 of the 13 toads, 16 of the 21 toads, one of the two toads, 14 of the 15 toads, 15 of the 18 toads, two of the three toads, eight of the nine toads, 16 of the 19 toads, two of the three toads and four of the six toads, respectively, showed a BB band, and the other one, two, two, five, three, four, seven, one, one, three, five, one, one, three, one, one, three, one and two toads, respectively, showed an AB band. Of the 42 toads of the Mito population of *jap*, 31 and three showed BB and CC bands, respectively, and the other one and seven showed AB and BC bands, respectively. In six populations including the Hayama, Kuji and Sagami populations of *jap*, the Neo and Odai populations of *tor*, and the Zao population of *mon*, five of the seven toads, 36 of the 37 toads, 21 of the 22 toads, three of the four toads, nine of the 10 toads and 15 of the 17 toads, respectively, showed a BB band, and the other two, one, one, one, one and two



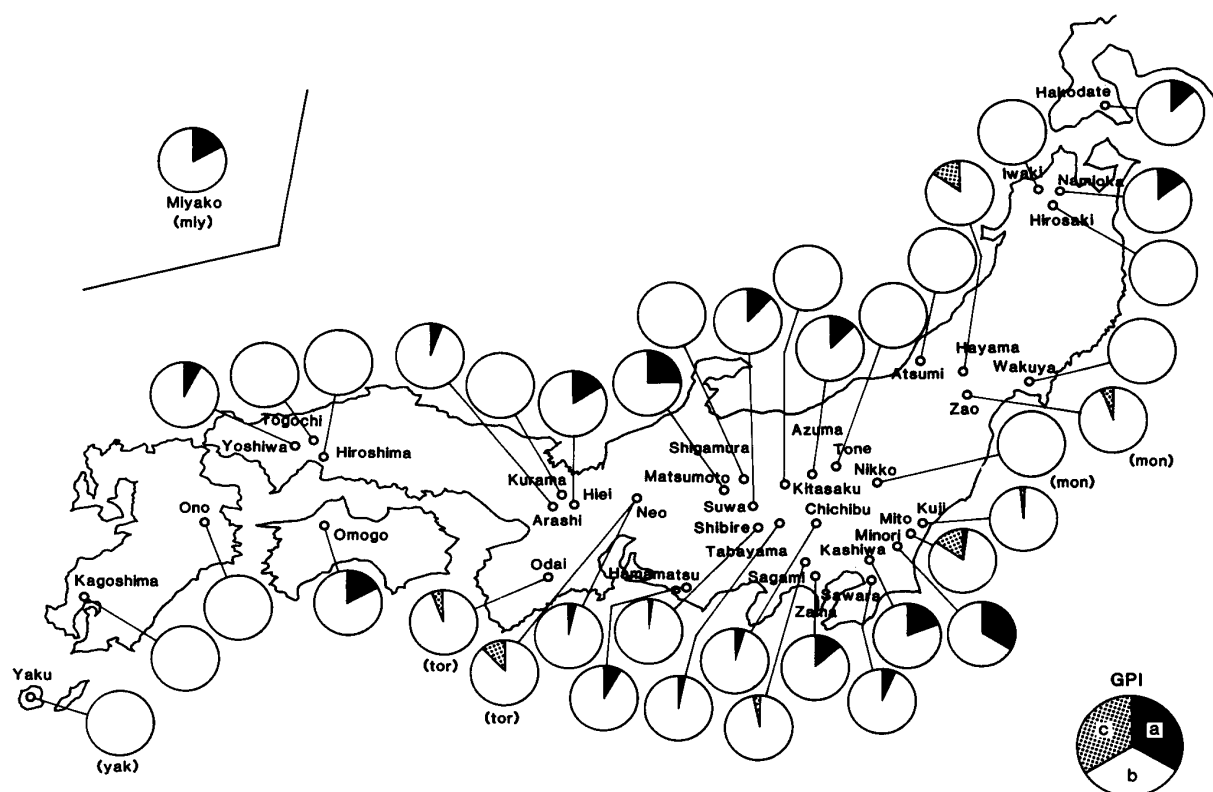


Fig. 8. Geographic distribution of GPI alleles among 40 populations of *Bufo japonicus*.

toads, respectively, showed a BC band. All the remaining 78 toads of 14 populations, including 12 populations of *jap*, the Nikko population of *mon* and the Yaku population of *yak* showed a BB band.

In the 40 populations of *Bufo japonicus*, allele *b* was the highest in frequency, being 0.667~1.000. Allele *a* was 0.011~0.333 in frequency in 19 populations of *jap* and the Miyako population of *miy*. Allele *c* was 0.014~0.155 in frequency in seven populations including four populations of *jap*, the Zao population of *mon* and the two populations of *tor* (Table 2; Fig. 8).

#### 8. IDH-A locus

The electrophoretic patterns of IDH-A obtained from 525 toads of the 40 populations of *Bufo japonicus* showed three phenotypes, AA, BB and AB, produced by two alleles, *a* and *b*.

All the 469 toads of 37 populations other than three populations including the Mito and Kashiwa populations of *jap* and the Miyako population of *miy*, showed a BB band. In the Mito and Kashiwa populations, 40 of the 42 toads and seven of the eight toads, respectively, showed a BB band, while the other two and one, respectively, showed an AB band. All the six toads of the Miyako population of *miy* showed an AA band.

In the Miyako population of *miy*, there was only allele *a*. In the Mito and Kashiwa populations of *jap*, allele *a* was 0.024 and 0.063 in frequency, respectively, while allele *b* was 0.976 and 0.938, respectively. There was only allele *b* in the



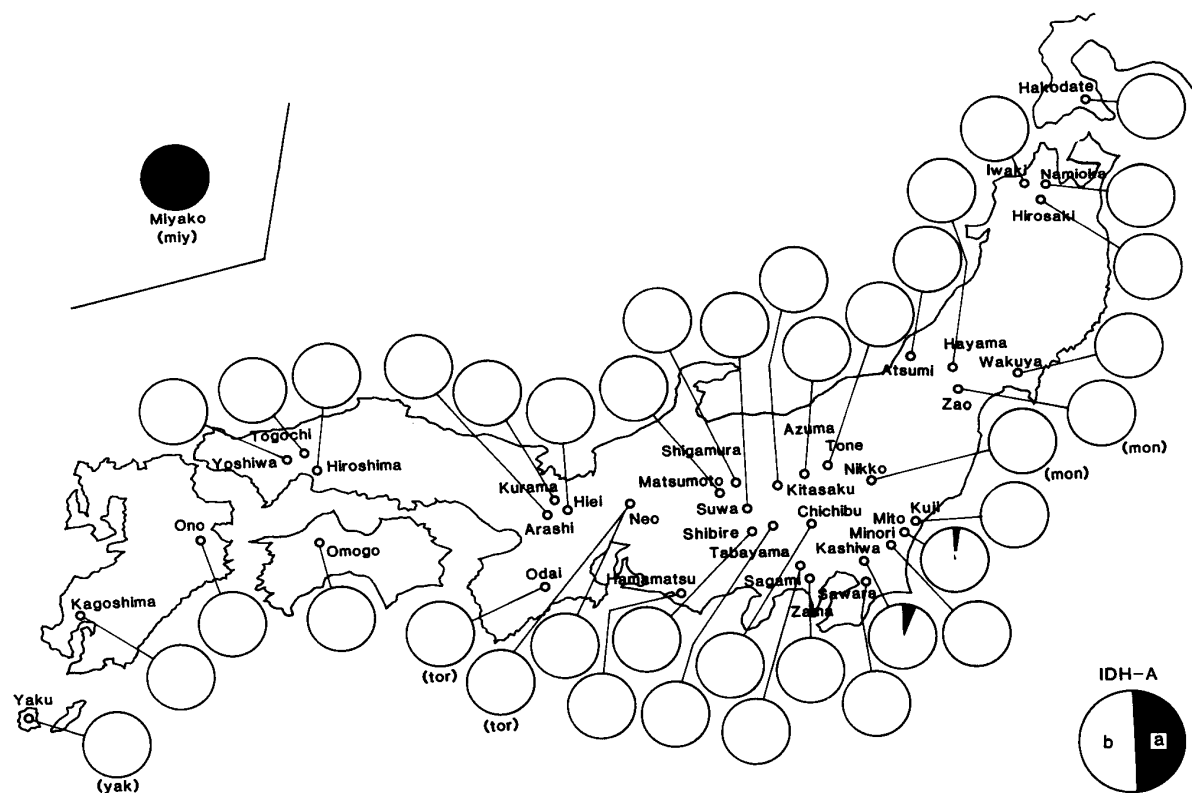


Fig. 9. Geographic distribution of IDH-A alleles among 40 populations of *Bufo japonicus*.

other 37 populations (Table 3; Fig. 9).

### 9. IDH-B locus

The electrophoretic patterns of IDH-B obtained from 523 toads of the 40 populations of *Bufo japonicus* showed eight phenotypes, BB, CC, DD, AC, BC, BD, CD and CE, produced by five alleles, *a*, *b*, *c*, *d* and *e*. The western districts remarkably differed from the eastern districts in kind and distribution of alleles at the IDH-B locus.

All the 207 toads of 16 eastern populations of Hakodate, Namioka, Hirosaki, Iwaki, Atsumi, Minori, Kashiwa, Tone, Chichibu, Shibire, Tabayama, Suwa, Matsumoto, Shigamura, Neo and Hamamatsu of *jap* and the Miyako population of *miy* showed a BB band, while all the 30 toads of six western populations including the Togoichi, Hiroshima, Ono and Kagoshima populations of *jap*, the Yaku population of *yak* and the Neo population of *tor* showed a CC band. In five populations of Wakuya, Hayama, Sagami and Kitasaku of *jap* and the Nikko population of *mon*, six of the nine toads, four of the six toads, 19 of the 22 toads, 20 of the 21 toads and nine of the 10 toads, respectively, showed a BB band, while the other three, two, three, one and one, respectively, showed a BC band. Of the 10 toads of the Azuma population of *jap*, eight showed a BB band and the other two showed a BD band. In three populations of Kuji, Mito and Zama of *jap* and the Zao population of *mon*, 30 of the 37 toads, 26 of the 41 toads, 20 of the 26 toads and 12 of the 17 toads, respectively, showed a BB band, one, one, one and one,

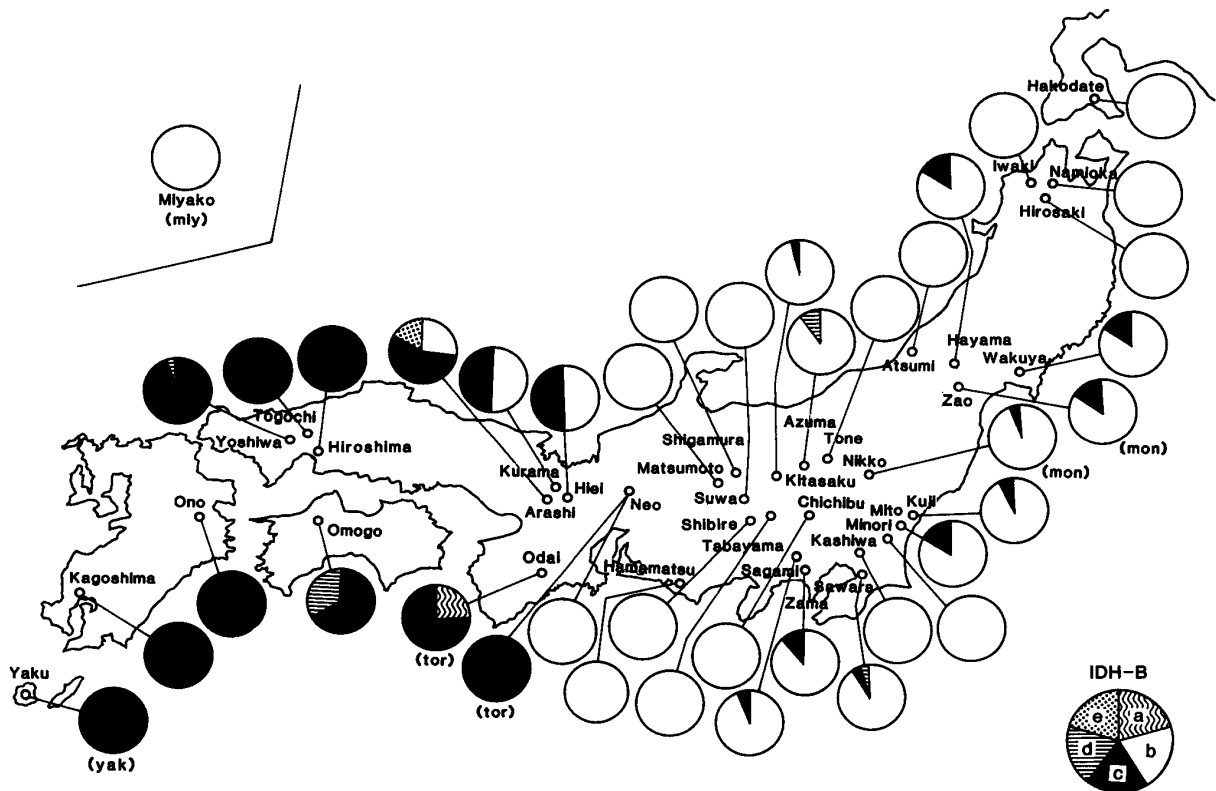


Fig. 10. Geographic distribution of IDH-B alleles among 40 populations of *Bufo japonicus*.

respectively, showed a CC band, and the remaining six, 14, five and four, respectively, showed a BC band. In two populations of Hiei and Kurama of *jap*, all the four toads showed a BC band. Of the 42 toads of the Sawara population of *jap*, 36 showed a BB band and the other three and three showed BC and BD bands, respectively. Of the 10 toads of the Odai population of *tor*, five showed a CC band, while the other five showed an AC band. Of the three toads of the Omogo population of *jap*, two showed a CC band, while the remainder showed a DD band. Of the 19 toads of the Yoshiwa population of *jap*, 18 and one showed CC and CD bands, respectively. Of the nine toads of the Arashi population of *jap*, one and two showed BB and CC bands, respectively, while the other three and three showed BC and CE bands, respectively.

In each of the 27 populations of *jap* and *mon* distributed in eastern Japan, allele *b* was the highest in frequency, being 0.805~1.000, while in each of the nine populations of *jap*, *tor* and *yak* distributed in western Japan, allele *c* was the highest in frequency, being 0.667~1.000. In the three populations of Hiei, Kurama and Arashi of *jap*, distributed in the Kinki district, alleles *b* and *c* were 0.278~0.500 and 0.500~0.556 in frequency, respectively. The Miyako population of *my* had only allele *b*. Allele *c* was 0.024~0.195 in frequency in 10 populations of *jap* and *mon* distributed in eastern Japan, while allele *d* was 0.026~0.333 in frequency in four populations of Sawara, Azuma, Yoshiwa and Omogo of *jap*. Allele *a* was found in the Odai population of *tor* in frequency of 0.250, while allele *e* was found in the Arashi population of *jap* in frequency of 0.167 (Table 3; Fig. 10).

10. LDH-A locus

The electrophoretic patterns of LDH-A obtained from 525 toads of the 40 populations of *Bufo japonicus* showed five phenotypes, CC, DD, AD, BD and DE, produced by five alleles, *a*, *b*, *c*, *d* and *e*.

All the 452 toads of 35 populations other than the Mito, Suwa, Hiei and Arashi populations of *jap* and the Miyako population of *miy* showed a DD band. Of the 42 toads of the Mito population of *jap*, one showed a BD band, while the other 41 showed a DD band. Of the 13 toads of the Suwa population of *jap*, 12 and one showed DD and AD bands, respectively. In the Hiei and Arashi populations of *jap*, two of the three toads and eight of the nine toads, respectively, showed a DD band, and the other one and one, respectively, showed a DE band. All the six toads of the Miyako population of *miy* showed a CC band.

While the Miyako population of *miy* had only allele *c*, in the other 39 populations, allele *d* was overwhelmingly high in frequency, being 0.833~1.000. Allele *a* was 0.038 in frequency in the Suwa population of *jap*, and allele *b* was 0.012 in the Mito population of *jap*. Allele *e* was 0.167 and 0.056 in frequency in the Hiei and Arashi populations of *jap*, respectively (Table 3; Fig. 11).

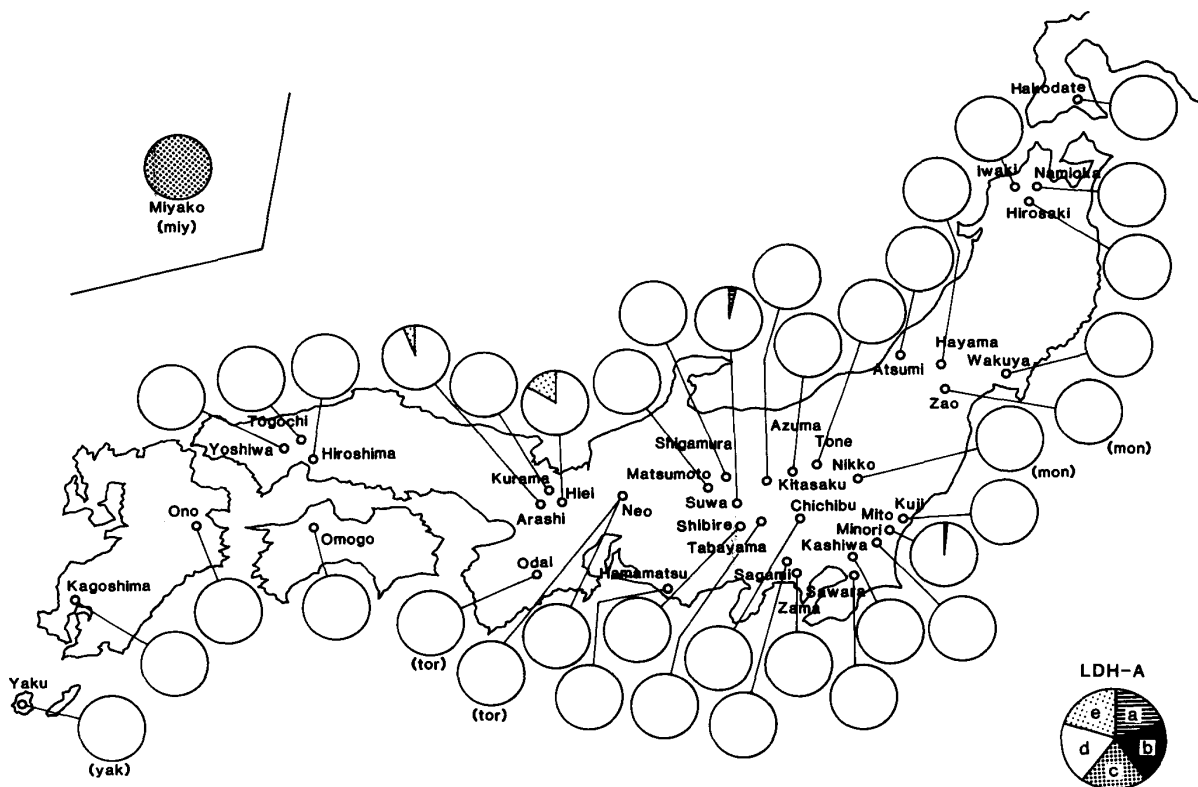


Fig. 11. Geographic distribution of LDH-A alleles among 40 populations of *Bufo japonicus*.

## 11. LDH-B locus

The electrophoretic patterns of LDH-B obtained from 524 toads of the 40 populations of *Bufo japonicus* showed six phenotypes, BB, CC, AB, AC, BC and CD, produced by four alleles, *a*, *b*, *c* and *d*.

In five populations of Hirosaki, Hayama, Shibire, Suwa and Arashi of *jap*, two of the four toads, three of the seven toads, 42 of the 47 toads, 11 of the 13 toads and eight of the nine toads, respectively, showed a CC band, while the other two, four, five, two and one, respectively, showed a BC band. All the four toads of the Hakodate population of *jap* showed a BC band. Of the three toads of the Tone population of *jap*, two showed a BB band, and the other showed a BC band. Of the nine toads of the Wakuya population of *jap*, two showed a BB band, and the other seven showed a CC band. Of the 15 toads of the Neo population of *jap*, 14 showed a CC band, and the other showed an AC band. In 14 populations including 12 populations of Namioka, Atsumi, Kuji, Mito, Sawara, Kashiwa, Azuma, Chichibu, Sagami, Zama, Tabayama and Kitasaku of *jap* and the Zao and Nikko populations of *mon*, one of the seven toads, one of the 11 toads, nine of the 37 toads, 10 of the 42 toads, nine of the 42 toads, two of the eight toads, two of the 10 toads, three of the 42 toads, five of the 22 toads, one of the 26 toads, one of the 20 toads, four of the 21 toads, three of the 16 toads and one of the 10 toads, respectively, showed a BB band, four, seven, 13, nine, 15, two, four, 31, nine, 16, 15, nine, six and six others, respectively, showed a CC band, and the remaining

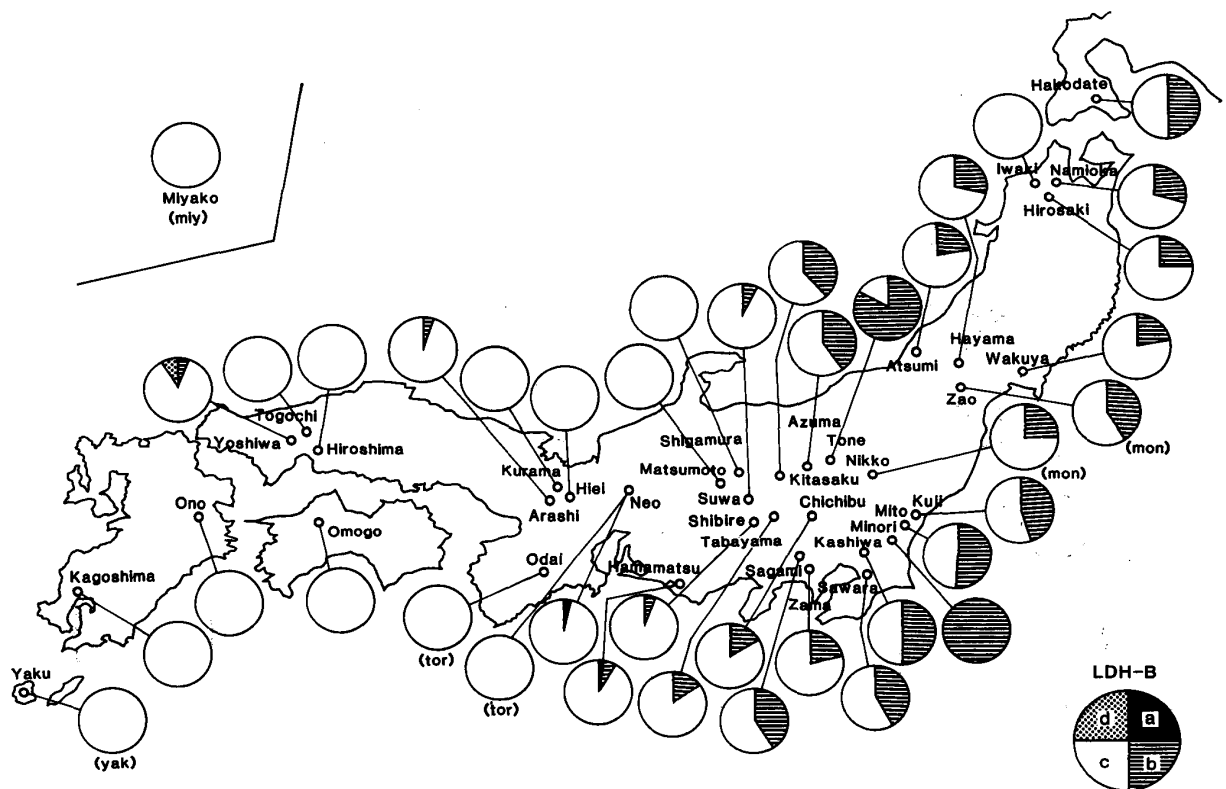


Fig. 12. Geographic distribution of LDH-B alleles among 40 populations of *Bufo japonicus*.

two, three, 15, 23, 18, four, four, eight, eight, nine, four, eight, seven and three, respectively, showed a BC band. In the Hamamatsu population of *jap*, 16 of the 18 toads showed a CC band, and the remaining one and one showed AB and BC bands, respectively. Of the 19 toads of the Yoshiwa population of *jap*, 14 showed a CC band and the remaining two and three showed BC and CD bands, respectively. All the three toads of the Minori population of *jap* showed a BB band. A total of 59 toads of the 14 populations including the remaining 10 populations of *jap*, the Yaku population of *yak*, the two populations of *tor* and the Miyako population of *miy* showed a CC band.

There was only allele *c* in the 10 populations of *jap*, the one population of *yak*, the two populations of *tor* and the one population of *miy*, while only allele *b* was found in the Minori population of *jap*. In the Neo population of *jap*, alleles *c* and *a* were 0.967 and 0.033 in frequency, respectively. In each of the Hakodate and Kashiwa populations of *jap*, alleles *b* and *c* were 0.500 in frequency, respectively. In the Tone population of *jap*, alleles *b* and *c* were 0.833 and 0.167 in frequency, respectively. In the other 21 populations of *jap* and *mon*, allele *b* was 0.053~0.512 in frequency, while allele *c* was 0.488~0.947. However, in the Hamamatsu and Yoshiwa populations of *jap*, there were alleles *a* and *d* in frequency of 0.028 and 0.079, respectively, in addition to alleles *b* and *c* (Table 3; Fig. 12).

### 12. MDH-A locus

The electrophoretic patterns of MDH-A obtained from 525 toads of the 40

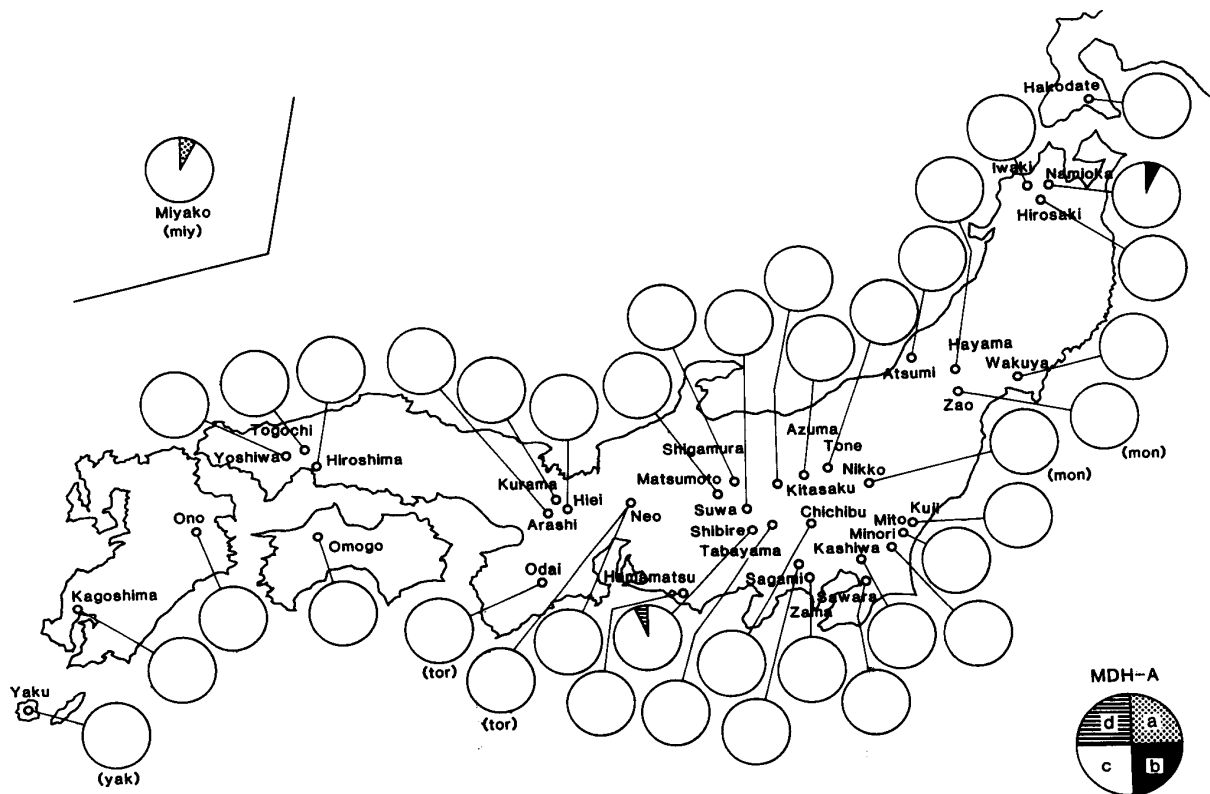


Fig. 13. Geographic distribution of MDH-A alleles among 40 populations of *Bufo japonicus*.

populations of *Bufo japonicus* showed four phenotypes, CC, AC, BC and CD, produced by four alleles, *a*, *b*, *c* and *d*.

A total of 465 toads belonging to 37 populations other than the Namioka and Shibire populations of *jap* and the Miyako population of *miy* showed a CC band. Of the seven toads of the Namioka population, one showed a BC band, while the other six showed a CC band. Of the 47 toads of the Shibire population of *jap*, six showed a CD band, while 41 showed a CC band. Of the six toads of the Miyako population of *miy*, one showed an AC band, while the other five showed a CC band.

In 37 of the 40 populations, there was only allele *c*, while in the other three populations, allele *c* was overwhelmingly high in frequency, being 0.917~0.936. Allele *a* was 0.083 in frequency in the Miyako population of *miy*, allele *b* was 0.071 in the Namioka population of *jap* and allele *d* was 0.064 in the Shibire population of *jap* (Table 3; Fig. 13).

### 13. MDH-B locus

The electrophoretic patterns of MDH-B obtained from 525 toads of the 40 populations of *Bufo japonicus* showed six phenotypes, AA, CC, DD, AC, BC and CD, produced by four alleles, *a*, *b*, *c* and *d*.

A total of 409 toads of 34 populations other than six populations including the Sawara, Azuma, Sagami and Zama populations of *jap*, the Odai population of *tor*

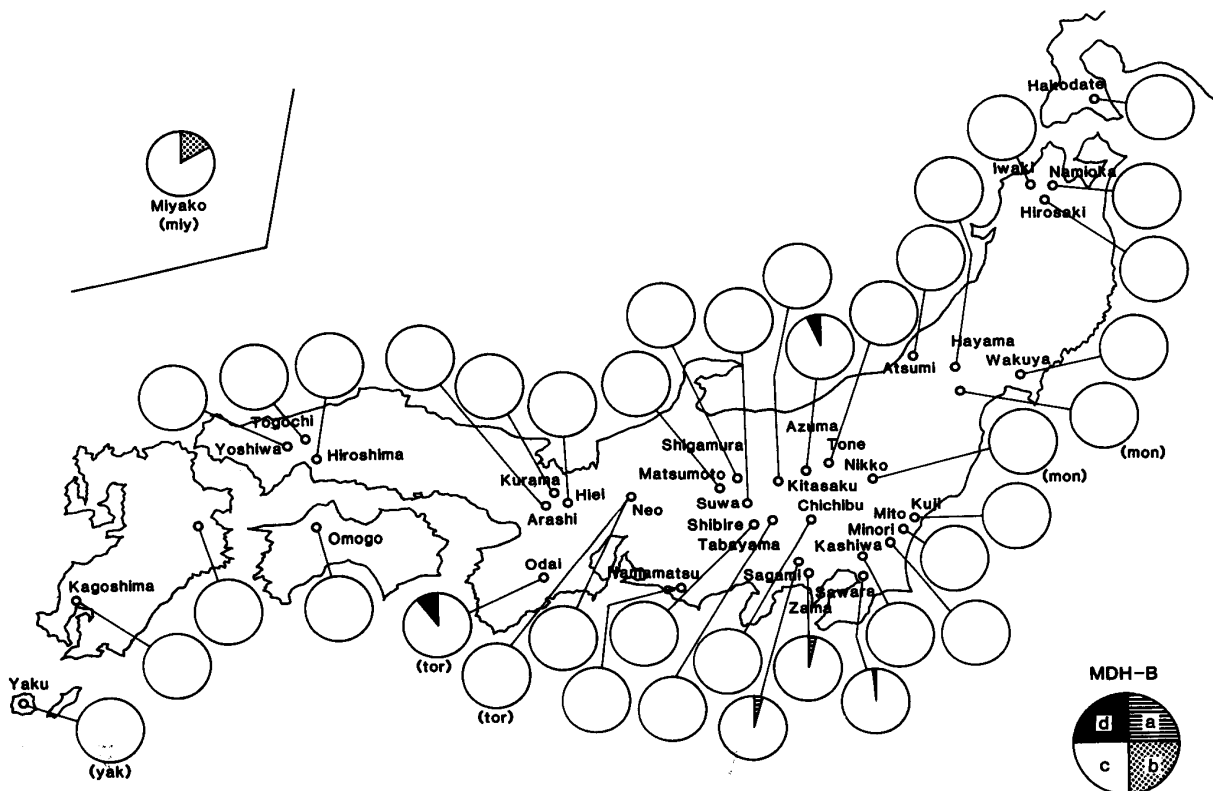


Fig. 14. Geographic distribution of MDH-B alleles among 40 populations of *Bufo japonicus*.



and the Miyako population of *miy*, showed a CC band. In the exceptional six populations, a CC band was also very abundantly found. In the Sawara and Azuma populations of *jap*, 41 of the 42 toads and nine of the 10 toads, respectively, showed a CC band, while the other one and one, respectively, showed a CD band. Of the 22 toads of the Sagami population of *jap*, 20 showed a CC band and the other two showed an AC band. Of the 26 toads of the Zama population of *jap*, 25 showed a CC band, and the other showed an AA band. Of the 10 toads of the Odai population of *tor*, nine showed a CC band, and the other showed a DD band. Of the six toads of the Miyako population of *miy*, four showed a CC band, and the other two showed a BC band.

Of the 40 populations, 34 had only allele *c*. In the other six populations, allele *c* was overwhelmingly high in frequency, being 0.833~0.988. Allele *a* was found in the Sagami and Zama populations of *jap* in frequencies of 0.045 and 0.038, respectively. Allele *b* was 0.167 in frequency in the Miyako population of *miy*. Allele *d* was 0.012~0.100 in frequency in the Sawara and Azuma populations of *jap* and the Odai population of *tor* (Table 3; Fig. 14).

#### 14. ME-A locus

The electrophoretic patterns of ME-A obtained from 525 toads of the 40 populations of *Bufo japonicus* showed five phenotypes, AA, BB, AB, BC and BD, produced by four alleles, *a*, *b*, *c* and *d*.

A total of 348 toads of 30 populations other than 10 populations including the nine populations of Iwaki, Wakuya, Atsumi, Mito, Sawara, Chichibu, Azuma, Matsumoto and Ono of *jap* and the Zao population of *mon* showed a BB band. In four populations including the Atsumi, Mito and Sawara populations of *jap* and the Zao population of *mon*, 10 of the 11 toads, 40 of the 42 toads, 41 of the 42 toads and 16 of the 17 toads, respectively, showed a BB band, and the other one, two, one and one, respectively, showed a BD band. In the Azuma and Matsumoto populations of *jap*, nine of the 10 toads and one of the two toads, respectively, showed a BB band, and the remaining one and one, respectively, showed an AB band. In the Wakuya and Chichibu populations of *jap*, five of the nine toads and 33 of the 42 toads, respectively, showed a BB band, one and one others, respectively, showed an AA band, and the remaining three and eight, respectively, showed an AB band. In each of the Iwaki and Ono populations of *jap*, one of the two toads showed an AB band and the other showed a BC band.

In the Iwaki and Ono populations each of which contained only one toad, there were alleles *a* and *b*, and alleles *b* and *c*, respectively. Each of these alleles was 0.500 in frequency. In the other 38 populations, allele *b* was very high in frequency. In four populations of Wakuya, Azuma, Chichibu and Matsumoto of *jap*, allele *b* was 0.722~0.950 in frequency, while allele *a* was 0.050~0.278. In four populations including the Atsumi, Mito and Sawara populations of *jap* and the Zao population of *mon*, allele *b* was 0.955~0.988 in frequency, and allele *d* was 0.012~0.045. The remaining 30 populations had only allele *b* (Table 4; Fig. 15).

TABLE 4  
Gene frequencies at three loci, ME-A, ME-B and MPI, in 40 populations of *Bytho japonicus*

Species	Locality	Sample size	ME-A				ME-B				MPI						
			a	b	c	d	a	b	c	d	e	f	g	h			
<i>B. j. japh.</i>	Hakodate	4	1.000				0.250	0.625						0.875		0.125	
"	Namioka	7	1.000				1.000							0.857		0.143	
"	Hirosaki	4	1.000				1.000							0.875		0.125	
"	Iwaki	1	0.500	0.500			0.500							1.000			
"	Wakuya	9	0.278	0.722			0.667		0.167	0.167				0.889			
"	Atsumi	11	0.955		0.045		0.800		0.050	0.150				0.273	0.045	0.682	
"	Hayama	7	1.000				0.857		0.143					0.214	0.143	0.643	
<i>B. j. mon.</i>	Zao	17	0.971		0.029		0.029	0.824		0.029	0.118			0.118		0.882	
"	Nikko	10	1.000				0.600			0.400				0.950		0.050	
<i>B. j. japh.</i>	Kuji	37	1.000				0.108	0.622		0.068	0.189	0.014		0.149		0.797	0.041
"	Mito	42	0.976		0.024		0.714		0.083	0.155	0.048		0.155		0.560	0.286	
"	Minori	3	1.000				0.667			0.333			0.167		0.667	0.167	
"	Sawara	42	0.988		0.012		0.029	0.750		0.176	0.015		0.179		0.583	0.238	
"	Kashiwa	8	1.000				0.688			0.313			0.100		0.938	0.063	
"	Azuma	10	0.050	0.950			0.150	0.600		0.250			0.100		0.650	0.250	
"	Tone	3	1.000				1.000						0.167		0.833		
"	Chichibu	42	0.119	0.881			0.071	0.667		0.262			0.060		0.917	0.012	0.012
"	Sagami	22	1.000				0.023	0.591		0.386			0.364		0.500	0.136	
"	Zama	26	1.000				0.558			0.442			0.250		0.385	0.365	
"	Shibire	47	1.000				0.074	0.713		0.213			0.096		0.840	0.064	
"	Tabayama	20	1.000				0.625			0.375			0.026		0.974		
"	Suwa	13	1.000				0.192	0.538		0.269			0.038		0.962		
"	Kitasaku	21	1.000				0.024	0.595		0.381			0.053		0.868	0.079	
"	Matsumoto	2	0.250	0.750			0.750			0.250			0.750		0.250	0.333	
"	Shigamura	3	1.000				0.667			0.333			0.667		0.333		
"	Hamamatsu	18	1.000				0.083	0.667		0.250			0.028		0.972		
"	Neo	15	1.000				0.033	0.733		0.233			0.233		0.767		
<i>B. j. tor.</i>	Neo	4	1.000				1.000						0.625		0.375		
"	Odai	10	1.000				0.100	0.900					0.100	0.050	0.850		
<i>B. j. japh.</i>	Hiei	3	1.000				0.167	0.167		0.167			0.667		0.333		
"	Kurama	1	1.000				0.500	0.500					0.500		0.500		
"	Arashi	9	1.000				0.444	0.222	0.222				0.722		0.278		
"	Yoshiwa	19	1.000				0.526	0.316	0.105	0.053		0.026	0.553		0.421		
"	Togochi	4	1.000				0.250	0.125	0.500	0.125			0.625		0.375		
"	Hiroshima	1	1.000				0.500						0.500		0.500		
"	Omogo	3	1.000				0.500	0.167					1.000		0.000		
"	Ono	1	0.500	0.500			1.000						1.000		0.000		
"	Kagoshima	15	1.000				0.967	0.033		0.233			0.233		0.733	0.033	
<i>B. j. yak.</i>	Yaku	5	1.000				0.900	0.100					1.000		0.083	0.083	0.833
<i>B. j. miy.</i>	Miyako	6	1.000				1.000						0.083	0.083	0.833		

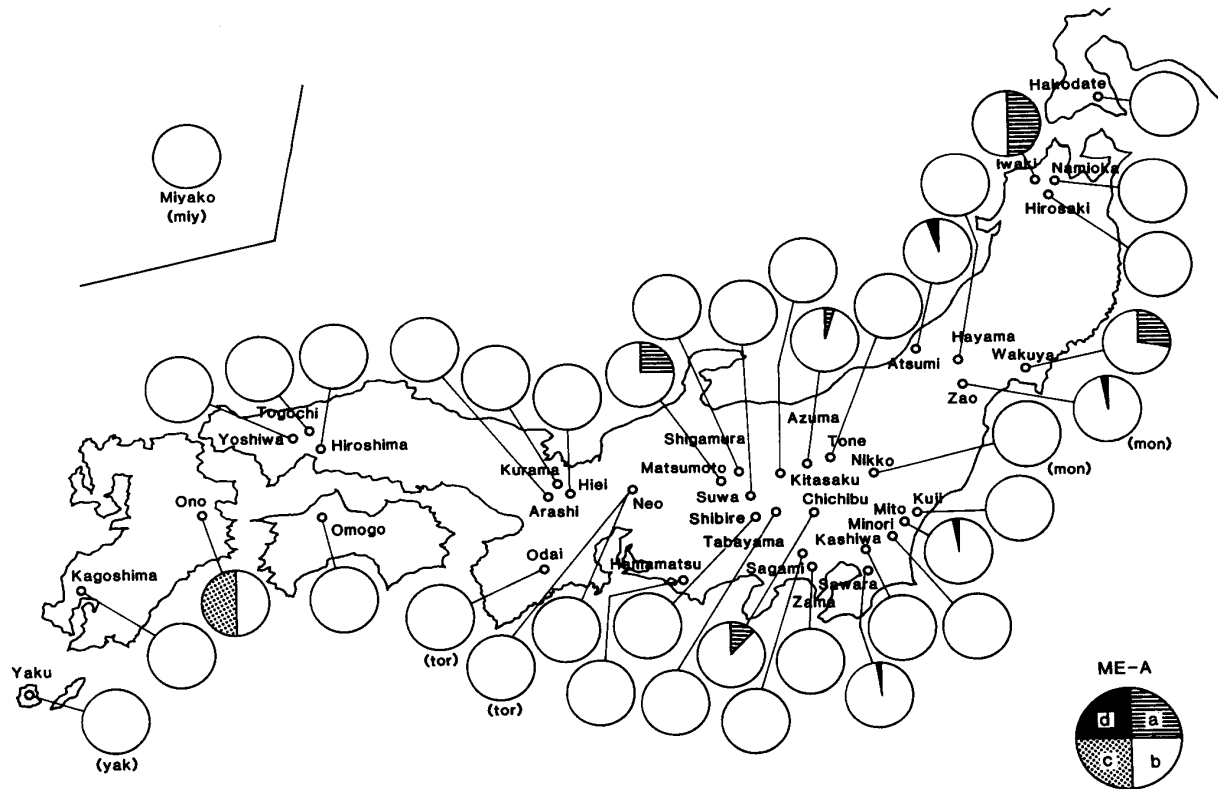


Fig. 15. Geographic distribution of ME-A alleles among 40 populations of *Bufo japonicus*.

### 15. ME-B locus

The electrophoretic patterns of ME-B obtained from 516 toads of the 40 populations of *Bufo japonicus* were extremely polymorphic, showing 23 phenotypes, BB, CC, DD, EE, FF, GG, HH, II, AF, BD, BE, BF, BI, DE, DF, EF, EI, FG, FH, FI, FJ, HI and HJ, produced by 10 alleles, *a*~*j*.

In eastern Japan, all the 14 toads of three populations of Namioka, Hirosaki and Tone of *jap* showed an FF band. The one toad of the Iwaki population of *jap* showed an FI band. In the Hayama, Minori, Matsumoto and Shigamura populations of *jap*, five of the seven toads, one of the three toads, one of the two toads and one of the three toads, respectively, showed an FF band, and the remaining two, two, one and two, respectively, showed an FI band. In four populations including the Kashiwa, Zama and Tabayama populations of *jap* and the Nikko population of *mon*, four of the eight toads, seven of the 26 toads, eight of the 20 toads and three of the 10 toads, respectively, showed an FF band, one, four, three and one others, respectively, showed an II band, and the remaining three, 15, nine and six showed an FI band. Of the four toads of the Hakodate population of *jap*, two, one and one showed FF, EF and EI bands, respectively. In the Wakuya and Atsumi populations of *jap*, three of the nine toads and six of the 10 toads, respectively, showed an FF band, three and one others, respectively, showed an FH band, and the remaining three and three, respectively, showed an FI band. In the Kitasaku, Neo and Hamamatsu populations of *jap*, eight of the

21 toads, nine of the 15 toads and nine of the 18 toads, respectively, showed an FF band, four, two and three others, respectively, showed an II band, one, one and three others, respectively, showed an EF band, and the remaining eight, three and three, respectively, showed an FI band. Of the 22 toads of the Sagami population of *jap*, six showed an FF band, one showed an II band, one showed an EI band and the remaining 14 showed an FI band. In the Azuma and Suwa populations of *jap*, three of the 10 toads and three of the 13 toads, respectively, showed an FF band, two and three, respectively, showed an EF band, one and two, respectively, showed an EI band and the remaining four and five, respectively, showed an FI band. Of the 17 toads of the Zao population of *mon*, 12 showed an FF band and the other one, one and three showed EI, FH and FI bands, respectively. Of the 42 toads of the Chichibu population of *jap*, 19 and three showed FF and II bands, respectively, while the other four, two and 14 showed EF, EI and FI bands, respectively. Of the 34 toads of the Sawara population of *jap*, 19 showed an FF band. The remaining two, two, 10 and one toads showed BI, EF, FI and FJ bands, respectively. Of the 47 toads of the Shibire population of *jap*, one, 26 and one showed EE, FF and II bands, respectively, while the other one, four and 14 showed EF, EI and FI bands, respectively. The Mito and Kuji populations of *jap* were the most polymorphic. Of the 42 toads of the Mito population, 25, one and two showed FF, HH and II bands, respectively, while the other one, seven, two, two and two showed FH, FI, FJ, HI and HJ bands, respectively. Of the 37 toads of the Kuji population, two, 17, one and two showed EE, FF, HH and II bands, respectively, while the other one, three, three, seven and one showed EF, EI, FH, FI and FJ bands, respectively.

Of the three populations of *jap* in the central districts of Japan, one toad of the Kurama population showed a DE band and three toads of the Hiei population showed BD, BE and BI bands. Of the nine toads of the Arashi population, two showed BB and EE bands, and the other three, one, two and one showed BD, BE, BI and DE bands, respectively.

In western Japan, one toad of the Ono population of *jap* showed a BB band. One toad of the Hiroshima population of *jap* showed a BD band. Of the 15 toads of the Kagoshima population of *jap*, 14 showed a BB band and the remainder showed a BD band. Of the five toads of the Yaku population of *yak*, four showed a BB band and the remainder showed a BD band. Of the four toads of the Togoichi population of *jap*, one, two and one showed BB, EE and DF bands, respectively. The three toads of the Omogo population of *jap* showed EE, AF and BE bands. The 19 toads of the Yoshiwa population of *jap* were extremely polymorphic. Eight, four and one of them showed homozygous BB, DD and EE bands, respectively, while the other two, one, one, one and one showed BD, BE, BF, DE and DF bands, respectively. In the Neo population of *tor*, all the four toads showed a GG band, while of the 10 toads of the Odai population of *tor*, eight showed a GG band and the other two showed an FG band. All the six toads of the Miyako population of *miy* showed a CC band.

There was a distinct difference in gene frequency between the toads distributed

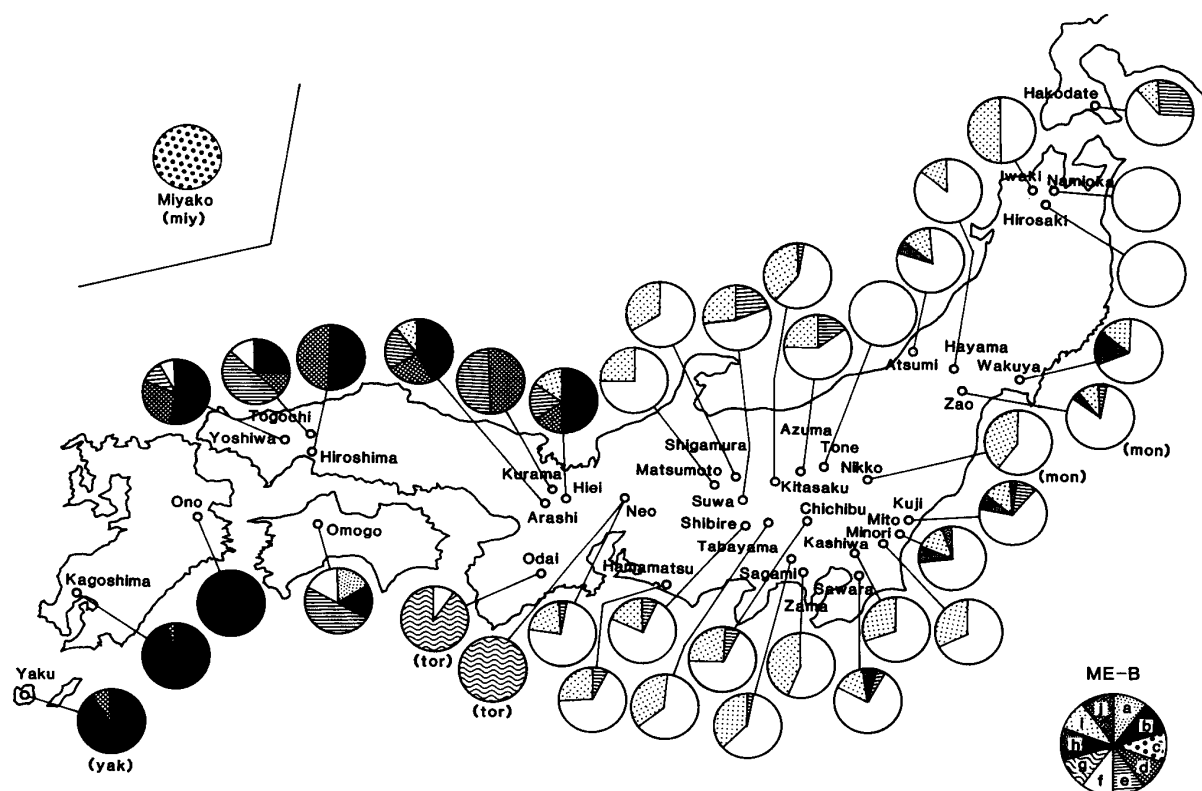


Fig. 16. Geographic distribution of ME-B alleles among 40 populations of *Bufo japonicus*.

in eastern and western Japan. In each of the 27 populations of *jap* and *mon* distributed in eastern Japan, allele *f* was abundant, being 0.500~1.000 in frequency. Allele *i* was 0.118~0.500 in frequency in 24 of the 27 populations, allele *e* was 0.023~0.250 in 12 populations, allele *h* was 0.029~0.167 in five populations and allele *j* was 0.014~0.048 in three populations. Allele *b* was 0.029 in frequency in the Sawara population.

In the Odai and Neo populations of *tor*, allele *g* was 1.000 and 0.900 in frequency, respectively, and allele *c* was 1.000 in the Miyako population of *miy*. In three populations including the Ono and Kagoshima populations of *jap* and the Yaku population of *yak*, allele *b* was 0.900~1.000 in frequency, and was 0.167~0.500 in six of the other 10 populations distributed in western Japan. Allele *d* was 0.033~0.500 in eight populations and allele *e* was 0.105~0.500 in frequency in six populations. In three populations of Yoshiwa, Togocho and Omogo of *jap*, and the Odai population of *tor*, allele *f* was low in frequency, being 0.053~0.167. In the two populations of Hiei and Arashi of *jap*, allele *i* was 0.167 and 0.111 in frequency, respectively. Allele *a* was 0.167 in frequency in the Omogo population (Table 4; Fig. 16).

#### 16. MPI locus

The electrophoretic patterns of MPI obtained from 522 toads of the 40 populations of *Bufo japonicus* showed 15 phenotypes, CC, EE, FF, GG, AC, AE, BF, CD, CE, CG, DE, DF, EG, EH and GH, produced by eight alleles, *a*~*h*.

In four populations of eastern Japan of Hakodate, Hirosaki, Kashiwa and Matsumoto of *jap*, three of the four toads, three of the four toads, seven of the eight toads and one of the two toads, respectively, showed an EE band, while the other one, one, one and one, respectively, showed an EG band. The toad of the Iwaki population of *jap* showed only an EE band. Of the seven toads of the Namioka population of *jap*, six showed an EE band and the remainder showed a GG band. In the Tone, Tabayama, Suwa and Hamamatsu populations of *jap* and the Zao population of *mon*, two of the three toads, 18 of the 19 toads, 12 of the 13 toads, 17 of the 18 toads and 13 of the 17 toads, respectively, showed an EE band, and the other one, one, one, one and four, respectively, showed a CE band. Of the nine toads of the Wakuya population of *jap*, eight showed an EE band, and the remainder showed a CC band. In the Shigamura population of *jap* and the Nikko population of *mon*, one of the three toads and nine of the 10 toads, respectively, showed an EE band, and the other two and one, respectively, showed an EH band. Of the 15 toads of the Neo population of *jap*, one and nine showed CC and EE bands, respectively, and the remaining five showed a CE band. Of the 11 toads of the Atsumi population of *jap*, five showed an EE band, another showed a CD band and the remaining five showed a CE band. The three toads of the Minori population of *jap* showed EE, CE and EG bands. Of the seven toads of the Hayama population of *jap*, one showed a CC band, three showed an EE band, one showed a CE band, and the remaining two showed a DE band. Of the 42 toads of the Chichibu population of *jap*, 35 showed an EE band, and the remaining five, one and one showed CE, EG and EH bands, respectively. Of the 47 toads of the Shibire population of *jap*, one showed a CC band, 33 showed an EE band, seven showed a CE band, and the remaining six showed an EH band. Of the 19 toads of the Kitasaku population of *jap*, 15 showed an EE band and the other one, one and two showed CE, CG and EG bands, respectively. In the Mito and Azuma populations of *jap*, 13 of the 42 toads and five of the 10 toads, respectively, showed an EE band, three and one, respectively, showed a GG band, eight and one, respectively, showed a CE band, five and one, respectively, showed a CG band, and the remaining 13 and two, respectively, showed an EG band. Of the 22 toads of the Sagami population of *jap*, three and five showed CC and EE bands, respectively, and the other eight, two and four showed CE, CG and EG bands, respectively. Of the 37 toads of the Kuji population of *jap*, one and 24 showed CC and EE bands, respectively, and the other nine, two and one showed CE, EG and GH, respectively. Of the 42 toads of the Sawara population of *jap*, one, 12 and two showed CC, EE and GG bands, respectively, and the other 11, two and 14 showed CE, CG and EG bands, respectively. Of the 26 toads of the Zama population of *jap*, two, four and four showed CC, EE and GG bands, respectively, and the other five, four and seven showed CE, CG and EG bands, respectively.

In western Japan, all the nine toads of the Omogo and Ono populations of *jap* and the Yaku population of *yak* showed a CC band. The two toads of the Kurama and Hiroshima populations of *jap* showed a CE band. Of the three toads of the Hiei population of *jap*, one showed a CC band and the other two

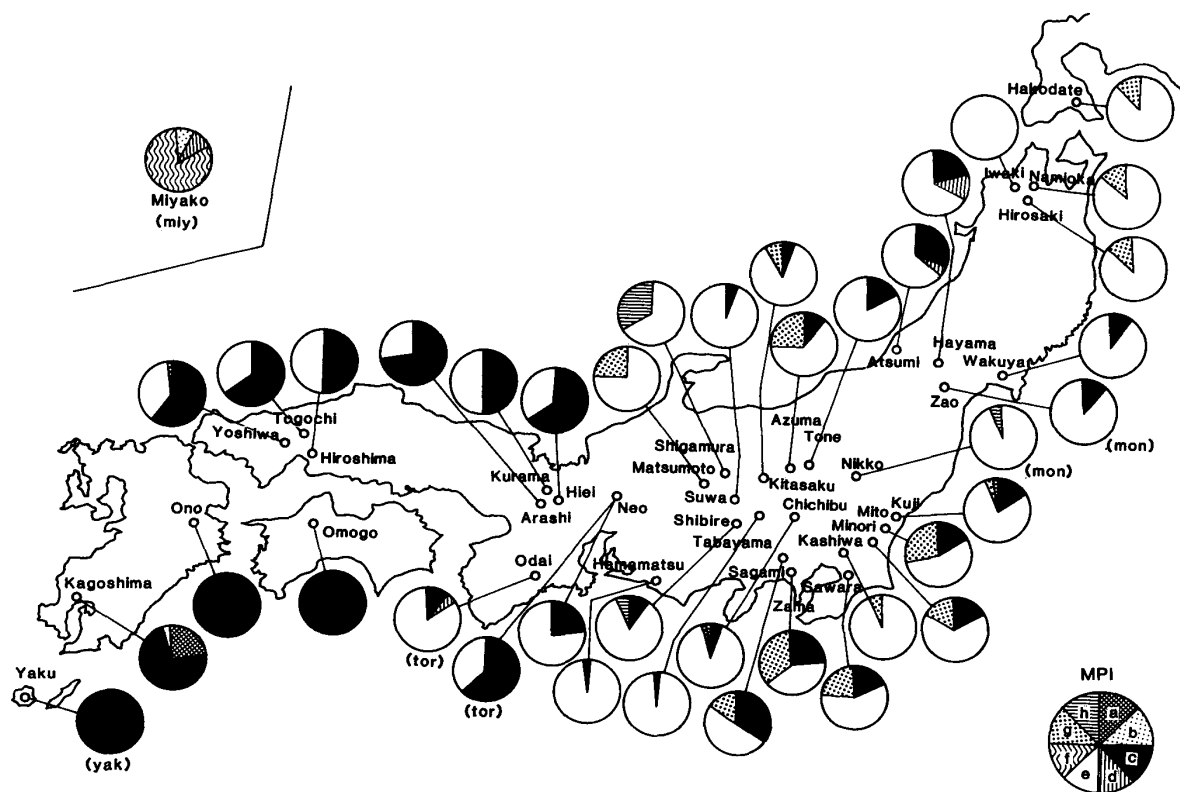


Fig. 17. Geographic distribution of MPI alleles among 40 populations of *Bufo japonicus*.

showed a CE band. In the Arashi and Togochi populations of *jap*, five of the nine toads and two of the four toads, respectively, showed a CC band, one and one, respectively, showed an EE band, and the remaining three and one, respectively, showed a CE band. Of the 15 toads of the Kagoshima population of *jap*, eight showed a CC band, and the other six and one showed AC and CE bands, respectively. Of the 19 toads of the Yoshiwa population of *jap*, seven and four showed CC and EE bands, respectively, and the remaining one and seven showed AE and CE bands, respectively.

In the Neo population of *tor*, two, one and one of the four toads showed CC, EE and CE bands, respectively, while in the Odai population of the same subspecies, seven, two and one of the 10 toads showed EE, CE and DE bands, respectively. Of the six toads of the Miyako population of *miy*, four, one and one showed FF, BF and DF bands, respectively.

There was a distinct gradient in the direction from east to west in gene frequency. In the 27 population of *jap* and *mon* distributed in eastern Japan, allele *e* was the highest in frequency, being 0.385~1.000. In addition, allele *c* was 0.026~0.364 in frequency in 19 of these populations, allele *g* was 0.012~0.365 in 14 populations, allele *h* was 0.012~0.333 in five populations, and allele *d* was 0.045 and 0.143 in two populations. On the other hand, allele *c* was the highest in frequency, being 0.500~1.000 in the 10 populations of *jap* and *yak* distributed in western Japan. In addition, allele *e* was 0.033~0.500 in frequency in seven of these populations and allele *a* was 0.026 and 0.233 in two populations. In the





Neo population of *tor*, alleles *c* and *e* were 0.625 and 0.375 in frequency, respectively. On the other hand, in the Odai population of the same subspecies, alleles *c*, *d* and *e* were 0.100, 0.050 and 0.850 in frequency, respectively. In the Miyako population of *miy*, alleles *b*, *f* and *d* were 0.083, 0.833 and 0.083 in frequency, respectively (Table 4; Fig. 17).

### 17. Pep-A locus

The electrophoretic patterns of Pep-A obtained from 525 toads of the 40 populations of *Bufo japonicus* showed four phenotypes, AA, BB, AB and BC, produced by three alleles, *a*, *b* and *c*.

All the 284 toads of 26 populations of *jap* other than the Hayama, Kuji, Mito, Azuma, Chichibu, Shibire, Neo and Hamamatsu populations, the Nikko population of *mon*, the Yaku population of *yak* and the Neo and Odai populations of *tor*, showed a BB band. In the Hayama and Chichibu populations of *jap* and the Zao population of *mon*, one of the seven toads, one of the 42 toads and one of the 17 toads, respectively, showed a BC band, while all the remaining toads of these three populations showed a BB band. In the other six populations of *jap*, a BB band was overwhelmingly abundant. In the Kuji, Mito, Azuma, Shibire, Neo and Hamamatsu populations of *jap*, 35 of the 37 toads, 41 of the 42 toads, nine of the 10 toads, 46 of the 47 toads, 14 of the 15 toads and 16 of the 18 toads, respectively, showed a BB band, while the other two, one, one, one, one and two, respectively, showed an AB band. Of the six toads of the Miyako population of *miy*, four showed an AA band, and the other two showed an AB band.

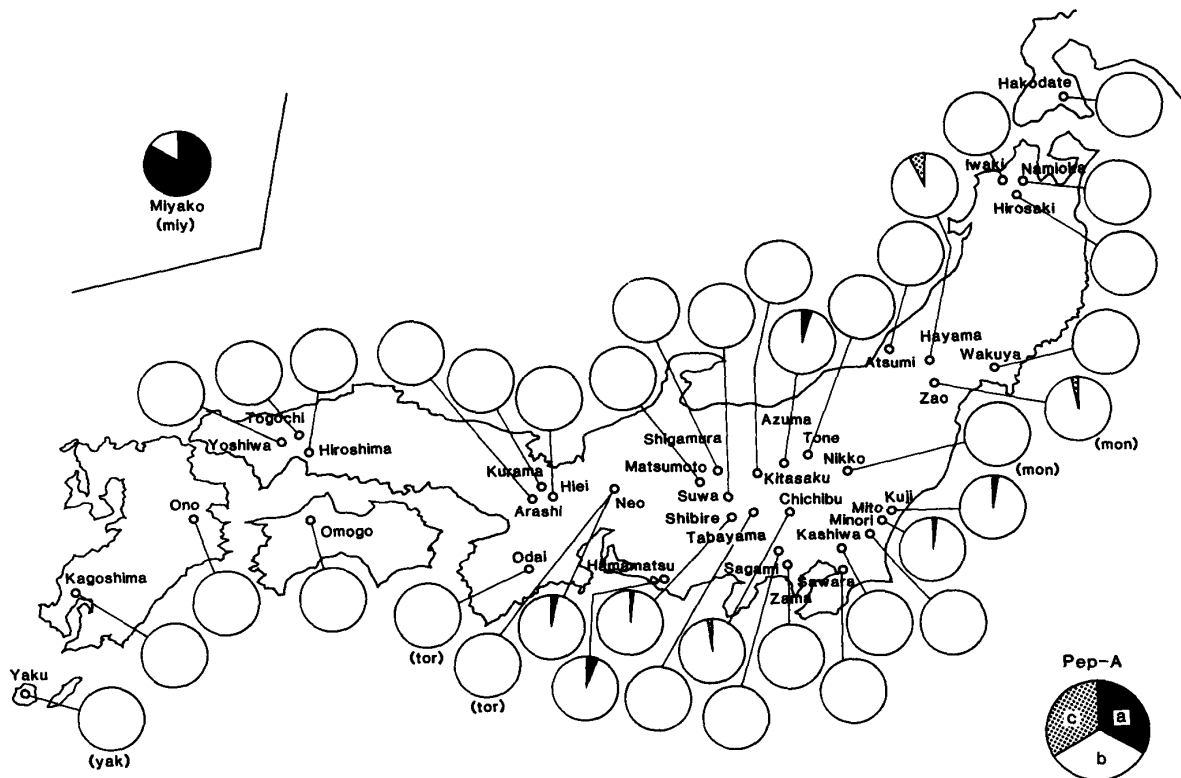


Fig. 18. Geographic distribution of Pep-A alleles among 40 populations of *Bufo japonicus*.



18 of the 21 toads, respectively, showed a CC band, and the other one, two, one and three toads showed a BC band. Of the 42 toads of the Sawara population of *jap*, 40 showed a CC band, another showed a BC band and the remainder showed a CD band. Of the 15 toads of the Neo population of *jap*, 12 showed a CC band, two showed an AC band, and the remainder showed a CD band. Of the six toads of the Miyako population of *miy*, three showed a CC band, another showed a DD band, and the remaining two showed a CD band.

Allele *c* was the highest in frequency in all the populations. While allele *c* was 0.667~0.977 in frequency in the 13 populations of *jap* and the population of *miy*, all the other 26 populations including *jap*, *mon*, *tor* and *yak* had only allele *c*. Allele *a* was found in the Neo population of *jap* in frequency of 0.067. Allele *b* was 0.012~0.071 in frequency in five populations of Sawara, Kashiwa, Tabayama, Suwa and Kitasaku of *jap*. Allele *d* was 0.012~0.333 in frequency in 10 populations including the Namioka, Hirosaki, Wakuya, Sawara, Chichibu, Sagami, Neo, Hiei and Arashi populations of *jap* and the Miyako population of *miy* (Table 5; Fig. 19).

#### 19. SOD-A locus

The electrophoretic patterns of SOD-A obtained from 525 toads of the 40 populations of *Bufo japonicus* showed two phenotypes, AA and AB, produced by two alleles, *a* and *b*.

All the 524 toads of the 40 populations other than one toad of the Neo population of *tor* showed an AA band. This exceptional toad of the Neo population of *tor* showed an AB band. Alleles *a* and *b* were 0.875 and 0.125 in frequency, respectively, in this population. The other 39 populations had only allele *a* (Table 5).

#### 20. SOD-B locus

The electrophoretic patterns of SOD-B obtained from 525 toads of the 40 populations of *Bufo japonicus* showed two phenotypes, BB and AB, produced by two alleles, *a* and *b*.

All the 521 toads of the 40 populations other than one toad of the Kuji population of *jap* and three toads of the Yoshiwa population of *jap* showed a BB band. The remaining four toads showed an AB band. Allele *b* was 0.986 and 0.921 in frequency in the Kuji and Yoshiwa populations, respectively, while allele *a* was 0.014 and 0.079, respectively, in these two populations. All the toads of the other 38 populations had only allele *b* (Table 5).

#### 21. Ab locus

The electrophoretic patterns of Ab obtained from 419 toads of 27 populations of *Bufo japonicus* showed 10 phenotypes, AA, BB, CC, DD, AB, AC, AD, BD, BE and DF, produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*.

In eight populations of eastern Japan including seven populations of Atsumi, Hayama, Kuji, Mito, Tone, Shigamura and Hamamatsu of *jap* and the Nikko

population of *mon*, six of the 10 toads, four of the seven toads, 35 of the 37 toads, 34 of the 36 toads, two of the three toads, one of the three toads, seven of the 12 toads and seven of the eight toads, respectively, showed a DD band, and the other four, three, two, two, one, two, five and one toads, respectively, showed a BD band. The two toads of the Matsumoto population of *jap* showed a DD band. Of the 20 toads of the Tabayama population of *jap*, eight and 12 showed BB and BD bands, respectively. In eight populations of Sawara, Azuma, Chichibu, Sagami, Zama, Suwa, Kitasaku and Neo of *jap*, three of the 42 toads, one of the eight toads, 18 of the 42 toads, six of the 17 toads, 12 of the 24 toads, three of the 12 toads, two of the 16 toads and three of the 14 toads, respectively, showed a BB band, 23, three, four, two, three, six, four and three, respectively, showed a DD band, and the remaining 16, four, 20, nine, nine, three, 10 and eight toads, respectively, showed a BD band. Of the 17 toads of the Zao population of *mon*, eight showed a DD band, seven showed a BD band, and the remaining two showed a DF band. Of the 44 toads of the Shibire population of *jap*, 25 showed a BB band, four showed a DD band, and the other three, one and 11 showed AB, AD and BD bands, respectively.

In the population of western Japan, the one toad of the Togochi population of *jap* showed a DD band. Of the seven toads of the Yoshiwa population of *jap*, five showed a DD band, and the other one and one showed AC and AD bands, respectively. Of the 15 toads of the Kagoshima population of *jap*, seven showed an AA band, another showed a CC band, and the remaining seven showed an AC band. Of the three toads of the Yaku population of *yak*, one showed an AA band, and the other two showed an AC band. In the two populations of *tor*, all the four toads of the Neo population showed a DD band, while three and three of the nine toads of the Odai population showed BB and DD bands, respectively, and the remaining two and one showed BD and BE bands, respectively. All the six toads of the Miyako population of *miy* showed only an AA band.

In seven populations of Atsumi, Hayama, Kuji, Mito, Sawara, Azuma and Tone of *jap*, and the two populations of *mon* of eastern Japan, allele *d* was 0.625~0.973 in frequency and allele *b* was 0.027~0.375. In five populations of Suwa, Kitasaku, Shigamura, Hamamatsu and Neo of *jap*, allele *d* was 0.500~0.792 in frequency, and allele *b* was 0.208~0.500. The Matsumoto population of *jap* had only allele *d*. In five populations of Chichibu, Sagami, Zama, Shibire and Tabayama of *jap*, allele *b* was 0.618~0.727 in frequency, and allele *d* was 0.227~0.382.

The Togochi population of *jap* in western Japan had only allele *d*. In the Yoshiwa population of *jap*, alleles *d*, *a* and *c* were 0.786, 0.143 and 0.071 in frequency, respectively. The Neo population of *tor* had only allele *d*, while in the Odai population alleles, *b*, *d* and *e* were 0.500, 0.444 and 0.056 in frequency, respectively. In the Kagoshima population of *jap* and the Yaku population of *yak*, allele *a* was high in frequency, being 0.700 and 0.667, respectively, while allele *c* was 0.300 and 0.333, respectively. In the districts other than western Japan, allele *a* was found in the Shibire population of *jap* in a very low frequency, being

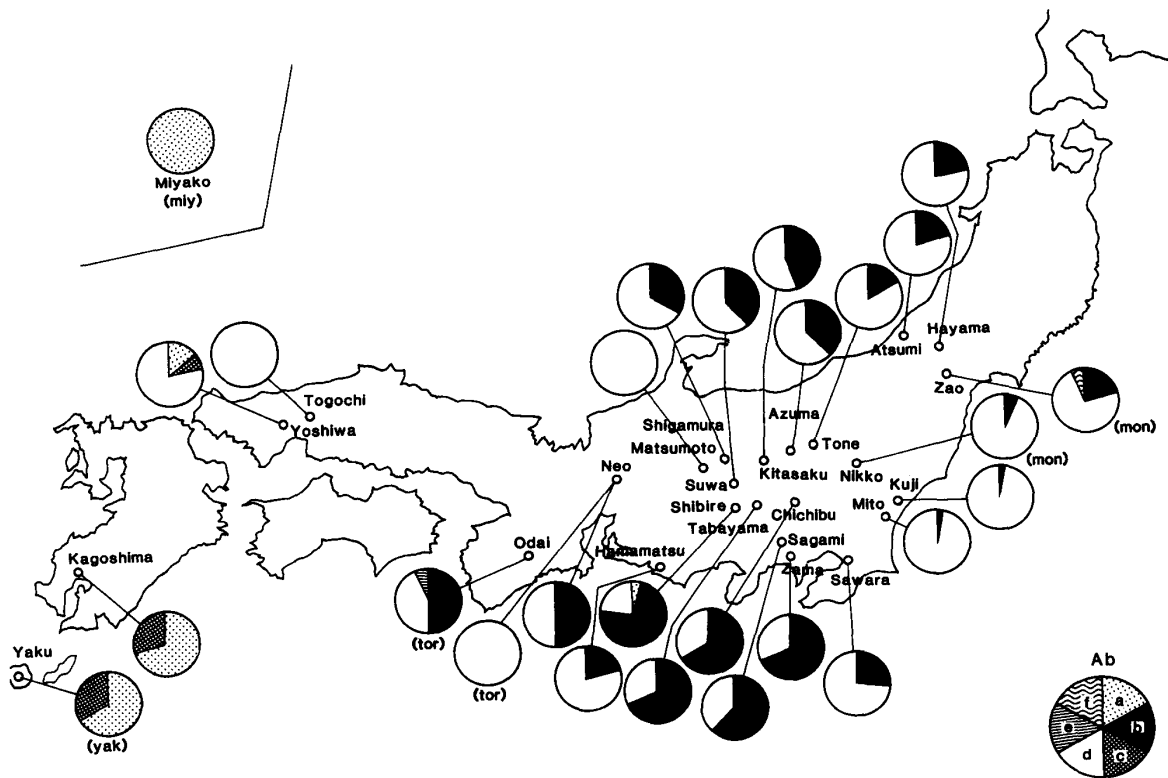


Fig. 20. Geographic distribution of Ab alleles among 27 populations of *Bufo japonicus*.

0.045. Allele *f* was also very low in frequency, being 0.059 in the Zao population of *mon*. There was only allele *a* in the Miyako population of *miy* (Table 5; Fig. 20).

## 22. Hb locus

The electrophoretic patterns of Hb obtained from 447 toads of 27 populations of *Bufo japonicus* showed six phenotypes, AA, BB, CC, DD, BD and CD, produced by four alleles, *a*, *b*, *c* and *d*.

All the 118 toads of seven populations of Mito, Tone, Sagami, Zama, Matsumoto, Shigamura and Kagoshima of *jap* and the Yaku population of *yak* showed only a BB band. The one toad of the Togocho population of *jap* showed a BD band. In seven populations including the Sawara, Azuma, Shimore, Suwa, Kitasaku and Neo populations of *jap*, and the Nikko population of *mon*, 41 of the 42 toads, nine of the 10 toads, 37 of the 47 toads, 12 of the 13 toads, 20 of the 21 toads, 13 of the 15 toads and three of the eight toads, respectively, showed a BB band, and the other one, one, 10, one, one, two and five toads showed a BD band. In eight populations including the Atsumi, Hayama, Kuji, Chichibu, Tabayama, Hamamatsu and Yoshiwa populations of *jap*, and the Zao population of *mon*, one of the 11 toads, two of the seven toads, 25 of the 37 toads, 21 of the 42 toads, six of the 20 toads, seven of the 13 toads, one of the six toads and nine of the 17 toads, respectively, showed a BB band, four, one, two, five, two, two, two and one, respectively, showed a DD band, and the remaining six, four, 10, 16, 12, four, three and seven, respectively, showed a BD band.

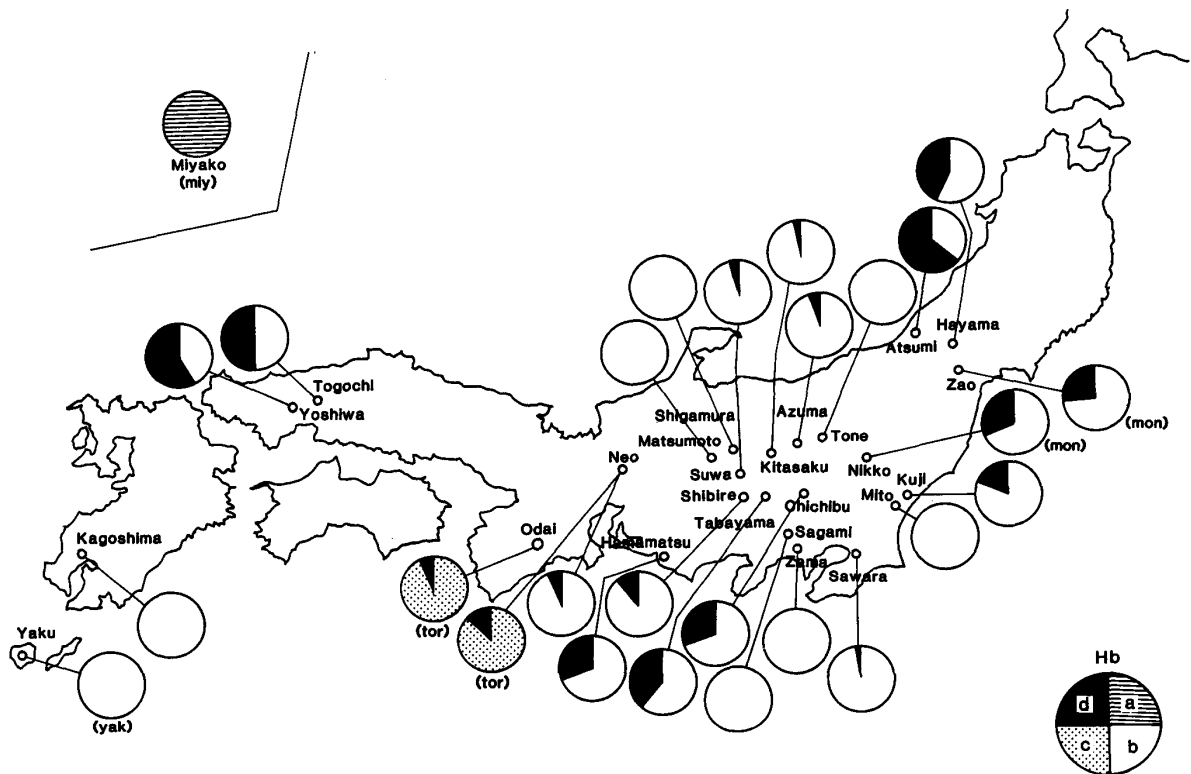


Fig. 21. Geographic distribution of Hb alleles among 27 populations of *Bufo japonicus*.

In the Neo and Odai populations of *tor*, three of the four and eight of the nine toads, respectively, showed a CC band, and the remaining one toad of each population showed a CD band. All the six toads of the Miyako population of *miy* showed an AA band.

In three populations of Atsumi, Yoshiwa and Togochi of *jap*, allele *b* was 0.364~0.500 in frequency, and allele *d* was 0.500~0.636. In the 11 populations of Hayama, Kuji, Sawara, Azuma, Chichibu, Shibre, Tabayama, Suwa, Kitasaku, Hamamatsu and Neo of *jap*, and the two populations of *mon*, allele *b* was high in frequency, being 0.571~0.988, while allele *d* was 0.012~0.429. All the remaining seven populations of *jap* and the Yaku population of *yak* had only allele *b*. In the Neo and Odai populations of *tor*, allele *c* was 0.875 and 0.944 in frequency, respectively, and allele *d* was 0.125 and 0.056, respectively. The Miyako population of *miy* had only allele *a* (Table 5; Fig. 21).

### III. Genetic differentiation

#### 1. Fixation index

The fixation index ( $F_{st}$ ) was calculated according to WRIGHT (1978) at 22 loci in the 525 toads belonging to the 40 populations (Table 6). When the gene frequencies at a definite locus are the same in all the 40 populations, fixation index is zero, while this is 1.000 when there is a characteristic allele at a definite locus in one or more populations. The results of examination of fixation indexes in the 40

TABLE 6  
Fixation index at 22 loci in 40 populations of *Bufo japonicus*

Locus	Fixation index (Fst)	
	40 populations	39 populations
AAT-A	0.458	0.451
AAT-B	0.140	0.137
ADA	0.382	0.161
AK	0.082	0.082
CK	0.139	0.138
$\alpha$ -GDH	0.247	0.116
GPI	0.108	0.110
IDH-A	0.922	0.050
IDH-B	0.733	0.730
LDH-A	0.806	0.114
LDH-B	0.350	0.346
MDH-A	0.070	0.065
MDH-B	0.101	0.061
ME-A	0.343	0.342
ME-B	0.446	0.420
MPI	0.411	0.383
Pep-A	0.614	0.040
PGM	0.175	0.141
SOD-A	0.122	0.122
SOD-B	0.067	0.067
Ab	0.421	0.366
Hb	0.547	0.477

populations showed that they can be arranged from lower to higher values. Three loci of SOD-B, MDH-A and AK were 0.067~0.082 in fixation index and indicated a slight genetic differentiation. Six loci of MDH-B, GPI, SOD-A, CK, AAT-B and PGM were 0.101~0.175 in fixation index, and eight loci of  $\alpha$ -GDH, ME-A, LDH-B, ADA, MPI, Ab, ME-B and AAT-A were 0.247~0.458. Five loci of Hb, Pep-A, IDH-B, LDH-A and IDH-A were 0.547~0.922 in fixation index. These values of the 22 loci show various degrees of genetic differentiation.

When the fixation indexes were examined in 39 populations other than the Miyako population of *miy* which was remarkably differentiated, there were large differences in fixation index between the 40 and 39 populations at the four loci of IDH-A, LDH-A, Pep-A and ADA, which were characteristic of *miy*. While the fixation indexes of IDH-A, LDH-A, Pep-A and ADA were 0.922, 0.806, 0.614 and 0.382, respectively, in the 40 populations, they were diminished to 0.050, 0.114, 0.040 and 0.161, respectively, in the 39 populations. These results show that the Miyako population of *miy* had differentiated from the other 39 populations of *B. japonicus* in a very old age, as observed in the genealogical tree of the 40 populations (Table 6; Fig. 22).

TABLE 7  
Estimates of genetic variabilities at 20 loci in 40 populations of *Bufo japonicus*

Species	Locality	Sample size	Average heterozygosity (%)	Proportion of polymorphic loci (%)	Mean number of alleles per locus
<i>B. j. jap.</i>	Hakodate	4	10.0( 7.3)	20.0	1.25
〃	Namioka	7	5.7( 7.1)	35.0	1.35
〃	Hirosaki	4	6.3( 5.3)	20.0	1.20
〃	Iwaki	1	10.0( 7.5)	10.0	1.10
〃	Wakuya	9	11.1(12.2)	45.0	1.50
〃	Atsumi	11	8.8( 8.3)	40.0	1.50
〃	Hayama	7	13.7(13.3)	40.0	1.45
<i>B. j. mon.</i>	Zao	17	10.6(11.8)	55.0	1.70
〃	Nikko	10	11.0(11.0)	35.0	1.40
<i>B. j. jap.</i>	Kuji	37	11.1(12.8)	60.0	1.95
〃	Mito	42	14.8(15.3)	65.0	1.95
〃	Minori	3	15.0(11.1)	30.0	1.35
〃	Sawara	42	12.4(12.9)	55.0	1.90
〃	Kashiwa	8	13.8(14.5)	45.0	1.55
〃	Azuma	10	14.5(14.5)	50.0	1.65
〃	Tone	3	13.3( 9.7)	25.0	1.30
〃	Chichibu	42	11.3(11.6)	50.0	1.75
〃	Sagami	22	13.9(12.5)	55.0	1.70
〃	Zama	26	12.5(12.2)	50.0	1.55
〃	Shibire	47	11.2(14.1)	55.0	1.75
〃	Tabayama	20	9.8( 9.5)	40.0	1.50
〃	Suwa	13	9.6( 9.7)	40.0	1.50
〃	Kitasaku	21	12.0(13.2)	45.0	1.60
〃	Matsumoto	2	15.0(11.3)	30.0	1.30
〃	Shigamura	3	10.0( 7.2)	20.0	1.20
〃	Hamamatsu	18	8.4(11.6)	50.0	1.65
〃	Neo	15	10.5(11.5)	55.0	1.65
<i>B. j. tor.</i>	Neo	4	5.0( 5.6)	20.0	1.25
〃	Odai	10	6.0( 6.0)	30.0	1.35
<i>B. j. jap.</i>	Hiei	3	21.7(14.4)	35.0	1.45
〃	Kurama	1	15.0( 7.5)	15.0	1.15
〃	Arashi	9	16.1(15.0)	55.0	1.70
〃	Yoshiwa	19	8.7(12.1)	40.0	1.65
〃	Togochi	4	5.0( 9.7)	25.0	1.35
〃	Hiroshima	1	10.0( 5.0)	10.0	1.10
〃	Omogo	3	6.8( 8.3)	20.0	1.30
〃	Ono	1	10.0( 5.0)	10.0	1.10
〃	Kagoshima	15	6.3( 5.1)	20.0	1.25
<i>B. j. yak.</i>	Yaku	5	1.0( 0.9)	5.0	1.05
<i>B. j. miy.</i>	Miyako	6	10.8(11.1)	35.0	1.40
Average (Total)		13.1 (525)	10.7(10.1)	36.0	1.46

Parentheses show expected values.



## 2. Average heterozygosity

The average heterozygosity of each of the 40 populations was calculated on the 20 loci analyzed in 525 toads. The lowest was 1.0% in the Yaku population of *yak*. In 17 populations including the Togoichi population of *jap*, the Neo population of *tor*, the Namioka population of *jap*, the Odai population of *tor* and the Hirosaki, Kagoshima, Omogo, Hamamatsu, Yoshiwa, Atsumi, Suwa, Tabayama, Hakodate, Iwaki, Shigamura, Hiroshima and Ono populations of *jap*, the rates of average heterozygosity were successively ranged from 5.0% to 10.0%. In eight populations including the Neo population of *jap*, the Zao population of *mon*, the Miyako population of *miy*, the Nikko population of *mon* and the Kuji, Wakuya, Shibire and Chichibu populations of *jap*, they were from 10.5% to 11.3%. In nine populations of Kitasaku, Sawara, Zama, Tone, Hayama, Kashiwa, Sagami, Azuma and Mito of *jap*, they became successively higher from 12.0% to 14.8%. In four populations of Minori, Matsumoto, Kurama and Arashi of *jap*, they were high, being 15.0 and 16.1%. The highest rate of average heterozygosity was 21.7% in the Hiei population of *jap*. The rates of average heterozygosity in the 40 populations were 10.7% on the average. There were no noticeable differences between these rates and the expected values, except populations consisting of one, two or three samples (Table 7).

## 3. Proportion of polymorphic loci

The proportion of polymorphic loci which contain plural alleles at the rate of more than 1% was estimated in each of the 40 populations. The Yaku population of *yak* was 5%, the lowest in the proportion of polymorphic loci. Twelve populations including the Iwaki, Hiroshima, Ono, Kurama, Hakodate, Hirosaki and Shigamura populations of *jap*, the Neo population of *tor*, and the Omogo, Kagoshima, Tone and Togoichi populations of *jap* successively ranged from 10% to 25%. Fifteen populations including the Minori and Matsumoto populations of *jap*, the Odai population of *tor*, the Namioka and Hiei populations of *jap*, the Nikko population of *mon*, the Miyako population of *miy* and the Atsumi, Hayama, Tabayama, Suwa, Yoshiwa, Wakuya, Kashiwa and Kitasaku populations of *jap* ranged from 30% to 45%. Eleven populations including the Azuma, Chichibu, Zama, Hamamatsu populations of *jap*, the Zao population of *mon* and the Sawara, Sagami, Shibire, Neo, Arashi and Kuji populations of *jap* ranged from 50% to 60%. The highest proportion of polymorphic loci was 65% in the Mito population of *jap*. The proportions of polymorphic loci in the 40 populations were 36.0% on the average (Table 7).

## 4. Mean number of alleles per locus

The mean number of alleles at each of the 20 loci which control enzymes in 525 toads of the 40 populations was examined. The results showed that the smallest mean number was 1.05 in the Yaku population of *yak*. Somewhat larger mean numbers of alleles of 1.10~1.25 were found in nine populations, the Iwaki,

TABLE 8  
Genetic identity(I) and genetic distance(D)

Species	Locality		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>B. j. jap.</i>	Hakodate	1	—	.937	.935	.913	.957	.939	.975	.972	.963	.973	.972	.974	.990	.974	.990	.951	.974
"	Namioka	2	.065	—	.997	.966	.983	.992	.972	.985	.978	.983	.977	.921	.958	.962	.944	.965	.976
"	Hirosaki	3	.067	.003	—	.968	.984	.994	.971	.986	.979	.984	.975	.914	.958	.965	.947	.967	.975
"	Iwaki	4	.091	.035	.033	—	.984	.974	.947	.960	.971	.963	.946	.880	.931	.940	.928	.918	.971
"	Wakuya	5	.044	.018	.016	.016	—	.988	.982	.990	.987	.990	.982	.930	.971	.968	.965	.958	.990
"	Atsumi	6	.063	.008	.006	.027	.012	—	.979	.988	.984	.989	.980	.921	.965	.966	.954	.965	.981
"	Hayama	7	.025	.029	.030	.054	.018	.021	—	.991	.985	.988	.989	.953	.989	.974	.981	.965	.989
<i>B. j. mon.</i>	Zao	8	.029	.015	.014	.041	.010	.012	.009	—	.989	.997	.992	.955	.985	.985	.978	.980	.988
"	Nikko	9	.038	.022	.022	.030	.013	.016	.015	.011	—	.991	.980	.941	.977	.981	.975	.966	.995
<i>B. j. jap.</i>	Kuji	10	.028	.018	.016	.038	.010	.011	.012	.003	.009	—	.933	.959	.986	.985	.981	.980	.988
"	Mito	11	.028	.024	.025	.055	.018	.020	.011	.008	.020	.007	—	.967	.989	.977	.981	.975	.980
"	Minori	12	.026	.082	.089	.128	.073	.082	.048	.046	.061	.042	.034	—	.974	.968	.968	.964	.943
"	Sawara	13	.010	.043	.042	.072	.029	.036	.011	.015	.024	.014	.011	.026	—	.983	.996	.969	.983
"	Kashiwa	14	.026	.039	.036	.062	.032	.035	.026	.015	.019	.015	.023	.033	.017	—	.984	.978	.978
"	Azuma	15	.010	.057	.054	.075	.035	.047	.019	.022	.026	.019	.020	.033	.004	.017	—	.961	.980
"	Tone	16	.050	.035	.034	.086	.043	.035	.036	.020	.034	.021	.025	.036	.032	.022	.039	—	.959
"	Chichibu	17	.027	.024	.025	.029	.010	.020	.011	.012	.005	.012	.020	.058	.017	.022	.020	.042	—
"	Sagami	18	.018	.053	.053	.069	.031	.037	.011	.021	.023	.017	.015	.032	.006	.027	.010	.044	.018
"	Zama	19	.022	.066	.067	.078	.042	.052	.021	.035	.036	.031	.023	.045	.010	.037	.012	.071	.027
"	Shibire	20	.025	.052	.053	.064	.033	.044	.018	.029	.022	.030	.037	.069	.020	.036	.021	.065	.011
"	Tabayama	21	.018	.047	.047	.052	.026	.040	.015	.022	.013	.022	.030	.055	.016	.025	.016	.056	.006
"	Suwa	22	.018	.044	.045	.049	.024	.038	.017	.023	.014	.021	.031	.060	.018	.026	.017	.060	.006
"	Kitasaku	23	.015	.034	.033	.048	.020	.028	.014	.010	.009	.008	.014	.032	.008	.007	.009	.030	.009
"	Matsumoto	24	.029	.047	.051	.048	.027	.046	.023	.033	.027	.034	.034	.066	.021	.037	.023	.071	.012
"	Shigamura	25	.025	.057	.055	.061	.034	.045	.018	.031	.023	.032	.036	.071	.019	.038	.020	.074	.014
"	Hamamatsu	26	.023	.037	.035	.045	.021	.030	.016	.017	.013	.018	.029	.065	.018	.021	.018	.053	.007
"	Neo	27	.032	.037	.036	.047	.022	.026	.010	.021	.013	.022	.029	.073	.019	.032	.023	.057	.007
<i>B. j. tor.</i>	Neo	28	.135	.189	.186	.184	.130	.158	.113	.130	.146	.131	.122	.185	.122	.165	.124	.205	.137
"	Odai	29	.094	.160	.156	.154	.105	.144	.093	.102	.112	.107	.106	.156	.097	.124	.092	.174	.103
<i>B. j. jap.</i>	Hiei	30	.080	.151	.148	.146	.098	.121	.078	.100	.106	.095	.092	.129	.073	.116	.075	.166	.090
"	Kurama	31	.060	.134	.131	.128	.082	.106	.065	.081	.087	.076	.078	.120	.062	.100	.057	.143	.073
"	Arashi	32	.086	.139	.135	.135	.087	.106	.071	.089	.097	.083	.080	.130	.072	.115	.074	.152	.087
"	Yoshiwa	33	.111	.183	.183	.185	.123	.155	.093	.119	.125	.119	.111	.157	.102	.146	.102	.182	.116
"	Togochi	34	.109	.183	.178	.182	.121	.150	.094	.116	.127	.116	.111	.167	.100	.144	.096	.183	.116
"	Hiroshima	35	.110	.178	.175	.172	.115	.150	.098	.115	.128	.116	.110	.165	.102	.145	.102	.189	.118
"	Omogo	36	.122	.195	.196	.202	.139	.157	.103	.134	.150	.131	.120	.169	.106	.166	.107	.200	.133
"	Ono	37	.199	.271	.269	.253	.193	.223	.161	.200	.205	.199	.184	.243	.172	.237	.175	.268	.191
"	Kagoshima	38	.157	.227	.223	.225	.161	.187	.128	.159	.168	.158	.145	.202	.134	.190	.136	.227	.158
<i>B. j. yak.</i>	Yaku	39	.157	.227	.224	.225	.161	.184	.133	.159	.179	.157	.144	.202	.135	.197	.140	.232	.166
<i>B. j. miy.</i>	Miyako	40	.386	.471	.457	.476	.438	.440	.406	.422	.426	.416	.419	.448	.382	.395	.369	.480	.409

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

Hiroshima, Ono, Kurama, Hirosaki and Shigamura populations of *jap*, the Neo population of *tor* and the Hakodate and Kagoshima populations of *jap*. The mean numbers of alleles in 11 populations, the Tone, Matsumoto, Omogo, Namioka and Minori populations of *jap*, the Odai population of *tor*, the Togochi population of *jap*, the Nikko population of *mon*, the Miyako population of *miy* and the Hayama and Hiei populations of *jap* successively increased from 1.30 to 1.45. They ranged from 1.50 to 1.65 in 11 other populations of Wakuya, Atsumi, Tabayama, Suwa, Kashiwa, Zama, Kitasaku, Azuma, Hamamatsu, Neo and Yoshiwa of *jap*. The mean numbers of alleles in six other populations, the Zao population of *mon* and the Sagami, Arashi, Chichibu, Shibire and Sawara populations of *jap* were 1.70~1.90. The largest mean number of alleles per locus was 1.95 in the Kuji and Mito populations of *jap*. The mean numbers of alleles per locus in the 40 populations were 1.46 on the average (Table 7).

among 40 populations of *Bufo japonicus*

18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
.983	.979	.975	.982	.982	.985	.972	.975	.977	.968	.874	.910	.923	.942	.918	.895	.897	.896	.885	.819	.855	.854	.680
.949	.936	.950	.954	.957	.967	.954	.945	.964	.964	.828	.852	.860	.875	.871	.832	.833	.837	.823	.762	.797	.797	.624
.949	.935	.948	.954	.956	.968	.950	.946	.966	.965	.830	.855	.863	.877	.873	.833	.837	.840	.822	.764	.800	.799	.633
.933	.925	.938	.949	.952	.953	.953	.941	.956	.954	.832	.857	.864	.880	.874	.831	.833	.842	.817	.776	.799	.799	.621
.970	.959	.967	.975	.976	.980	.973	.967	.980	.978	.878	.901	.906	.922	.916	.885	.886	.891	.871	.824	.851	.852	.645
.964	.949	.957	.961	.963	.973	.955	.956	.970	.974	.853	.866	.886	.899	.899	.857	.861	.861	.855	.800	.830	.832	.644
.989	.979	.982	.985	.983	.986	.978	.982	.984	.990	.894	.911	.925	.937	.931	.911	.910	.906	.902	.851	.880	.876	.666
.979	.966	.971	.979	.978	.990	.968	.969	.983	.979	.878	.903	.905	.922	.915	.888	.891	.892	.874	.819	.853	.853	.656
.977	.965	.979	.987	.986	.991	.973	.977	.987	.987	.865	.894	.900	.916	.907	.883	.881	.880	.860	.814	.846	.836	.653
.983	.969	.970	.978	.979	.992	.966	.969	.982	.978	.877	.899	.910	.927	.920	.888	.891	.890	.877	.820	.854	.854	.660
.985	.977	.964	.971	.970	.986	.967	.964	.971	.971	.885	.899	.913	.925	.923	.895	.895	.896	.887	.832	.865	.866	.658
.968	.956	.933	.947	.942	.969	.936	.931	.937	.930	.831	.855	.879	.887	.878	.855	.846	.848	.845	.784	.817	.817	.639
.994	.990	.980	.984	.982	.992	.980	.981	.982	.981	.885	.908	.929	.940	.930	.903	.905	.903	.899	.842	.875	.874	.682
.973	.963	.964	.976	.974	.993	.964	.963	.979	.969	.848	.883	.891	.905	.891	.864	.866	.865	.847	.789	.827	.821	.674
.990	.988	.979	.984	.983	.991	.977	.981	.982	.978	.883	.912	.928	.945	.928	.903	.909	.903	.898	.839	.873	.869	.692
.957	.932	.937	.946	.941	.971	.931	.929	.948	.944	.815	.841	.847	.866	.859	.833	.833	.828	.818	.765	.797	.793	.619
.982	.973	.989	.994	.994	.991	.988	.986	.993	.994	.872	.902	.914	.930	.917	.890	.891	.888	.875	.826	.854	.847	.664
—	.992	.979	.985	.981	.988	.973	.982	.979	.984	.895	.908	.939	.947	.942	.914	.914	.910	.912	.860	.887	.887	.678
.008	—	.976	.980	.979	.982	.981	.983	.976	.977	.906	.919	.949	.954	.948	.920	.921	.921	.921	.865	.896	.898	.694
.021	.024	—	.995	.992	.981	.984	.991	.992	.992	.879	.910	.923	.940	.922	.902	.905	.899	.889	.838	.867	.860	.682
.016	.020	.005	—	.996	.991	.986	.992	.994	.992	.879	.914	.924	.938	.922	.900	.899	.899	.881	.831	.862	.855	.679
.019	.021	.008	.004	—	.990	.989	.990	.995	.992	.884	.918	.932	.948	.929	.903	.906	.903	.891	.833	.865	.860	.638
.012	.019	.019	.009	.010	—	.981	.981	.990	.984	.874	.905	.919	.931	.919	.890	.890	.892	.876	.819	.854	.851	.675
.027	.019	.016	.014	.011	.020	—	.985	.986	.984	.874	.904	.920	.931	.915	.890	.890	.892	.882	.830	.857	.853	.676
.018	.017	.009	.008	.010	.019	.015	—	.988	.990	.886	.915	.932	.945	.929	.904	.906	.905	.895	.843	.875	.869	.693
.021	.025	.008	.006	.005	.010	.014	.012	—	.993	.876	.911	.920	.937	.920	.892	.896	.895	.879	.821	.856	.851	.684
.016	.023	.008	.008	.008	.016	.016	.010	.007	—	.883	.906	.928	.939	.929	.903	.905	.898	.895	.846	.872	.865	.679
.111	.099	.129	.129	.123	.134	.135	.121	.132	.125	—	.980	.938	.943	.951	.952	.956	.957	.946	.911	.937	.944	.631
.097	.085	.094	.090	.086	.100	.101	.089	.093	.099	.020	—	.931	.946	.939	.945	.947	.953	.920	.881	.915	.914	.645
.063	.052	.080	.079	.071	.085	.083	.070	.084	.075	.064	.072	—	.974	.992	.966	.963	.971	.963	.934	.959	.967	.690
.055	.047	.062	.064	.053	.072	.071	.057	.065	.063	.058	.055	.026	—	.981	.965	.974	.973	.964	.907	.936	.943	.685
.059	.054	.081	.082	.074	.085	.089	.074	.084	.073	.050	.063	.008	.019	—	.979	.978	.982	.976	.948	.972	.978	.670
.090	.084	.104	.106	.102	.117	.117	.101	.115	.102	.049	.057	.035	.035	.021	—	.989	.991	.969	.965	.985	.978	.634
.090	.082	.100	.107	.099	.116	.116	.099	.110	.100	.045	.054	.037	.026	.022	.011	—	.982	.981	.951	.973	.970	.648
.095	.082	.107	.106	.102	.115	.115	.099	.111	.107	.044	.048	.030	.027	.018	.009	.018	—	.960	.947	.975	.979	.648
.092	.082	.117	.127	.116	.133	.125	.111	.129	.111	.055	.083	.038	.037	.024	.031	.019	.041	—	.947	.963	.970	.645
.151	.145	.177	.185	.183	.200	.186	.170	.197	.167	.093	.127	.069	.098	.054	.036	.050	.054	.055	—	.981	.974	.594
.119	.109	.142	.148	.145	.157	.154	.134	.156	.137	.066	.089	.042	.067	.029	.015	.027	.025	.038	.019	—	.992	.632
.120	.107	.151	.156	.151	.161	.159	.140	.161	.145	.058	.090	.034	.059	.022	.022	.030	.021	.031	.026	.008	—	.635
.389	.366	.382	.387	.381	.393	.392	.367	.380	.387	.460	.438	.372	.379	.401	.455	.434	.433	.439	.521	.458	.454	—

distance(D) is given below.

#### IV. Genetic distance and dendrogram

##### 1. Genetic distance

The genetic relationships among the 40 populations of *Bufo japonicus* distributed in Japan were assumed by estimating genetic distances by the method of Nei (1975) on the basis of gene frequencies at 20 loci controlling 14 enzymes extracted from 525 toads. The results showed that the largest genetic distance among the populations of *jap* in eastern Japan was 0.091 between the Hakodate(1) and Iwaki(4) populations, while that among the populations of *jap* in western Japan was 0.098 between the Kurama(31) and Ono(37) populations. In each of eastern and western Japan, there were no remarkably large differences between two populations of *jap*. On the other hand, the smallest genetic distance between the eastern and western populations of *jap* was 0.047 between the Zama(19) and Kurama(31) populations, while the largest was 0.271 between the Namioka(2) and

Ono(37) populations. The genetic differences between the populations of eastern Japan and Kyushu districts were especially large (Table 8).

The genetic distance between the two populations of *tor* was remarkably small, being 0.020, although their stations are fairly separated from each other. The smallest genetic distance between *tor* and the 27 populations of *jap* and *mon* distributed in eastern Japan was 0.085 between the Odai(29) and Zama(19) populations, while the largest was 0.205 between the Neo(28) and Tone(16) populations. The genetic distances between the Neo population and the 27 populations of eastern Japan were 0.099~0.205, 0.141 on the average. Those between the Odai population and the latter 27 populations were 0.085~0.174, 0.112 on the average. The genetic distances between the Neo population and the nine populations of *jap* distributed in western Japan were 0.044~0.093, 0.058 on the average, while those between the Odai population and the latter nine populations were 0.048~0.127, 0.072 on the average. These figures clearly indicate that *tor* is closely related to *jap* distributed in western Japan (Table 8).

Of the genetic distances between *mon* and the populations of *jap* distributed in eastern Japan, the smallest was 0.003 between the Zao(8) and Kuji(10) populations, while the largest was 0.061 between the Nikko(9) and Minori(12) populations. Of the genetic distances between *mon* and the populations of *jap* distributed in western Japan, the smallest was 0.081 between the Zao(8) and Kurama(31) populations, while the largest was 0.205 between the Nikko(9) and Ono(37) populations. It was found that the western *jap* were larger than the eastern *jap* in genetic distance from *mon*. Of the genetic distances between *mon* and *tor*, the smallest was 0.102 between the Zao(8) and Odai(29) populations, while the largest was 0.146 between the Nikko(9) and Neo(28) populations. These figures were similar to those of the genetic distances between *mon* and the populations of *jap* distributed in western Japan (Table 8).

Of the genetic distances between *yak* and the western and eastern populations of *jap*, the smallest was 0.008 between the Yaku(39) and Kagoshima(38) populations, while the largest was 0.232 between the Yaku and Tone(16) populations. The genetic distances (0.107~0.232) between the Yaku and the eastern populations were distinctly larger than those (0.008~0.059) between the Yaku and the western populations. Of the genetic distances between *yak* and *tor*, that between the Yaku and Neo(28) populations was 0.058, and that between the Yaku and Odai(29) populations was 0.090. These genetic distances were smaller than those between the Yaku and the eastern populations of *jap* (Table 8).

The genetic distances between *miy* and the other 39 populations were very large. Of the genetic distances between *miy* and *jap*, the smallest was 0.366 between the Miyako(40) and Zama(19) populations, while the largest was 0.521 between the Miyako and Ono(37) populations. The genetic distance between *miy* and *yak* was 0.454. Those between the Miyako of *miy* and the two populations of *tor* were 0.438 and 0.460, while those between the Miyako of *miy* and the two populations of *mon* were 0.422 and 0.426, respectively. It was quite clear that *miy* is genetically far isolated from any other subspecies (Table 8).

## 2. Dendrogram

Dendrograms were drawn for the 40 populations of the five subspecies of *Bufo japonicus* on the basis of genetic distances among these populations by seven methods. Phylogenetic relationships of these populations were assumed from a dendrogram drawn by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975), which is the most commonly used. The results showed that the toads distributed in Japan had evolved into the eastern and western groups, except *miy* which seems to have come from the mainland of eastern Asia. The eastern group was divided into almost three subgroups. Two populations belonging to one of these subgroups adapted to the environment of high mountains and became *mon*, while all the other populations belonging to the three subgroups became *jap* which were somewhat differentiated from each other. On the other hand, the western group was divided into almost two subgroups. Two populations belonging to one of these two subgroups adapted early to the environment of high mountains and became *tor*. A part of the other subgroup was isolated in a small island and became *yak*, while the remaining part became *jap* which genetically differs somewhat from those distributed in eastern Japan (Fig. 22).

The six kinds of dendrograms drawn by the furthest neighbor method, flexible method, centroid method, median method, nearest neighbor method and Ward method did not remarkably differ from that drawn by the UPGMA method.

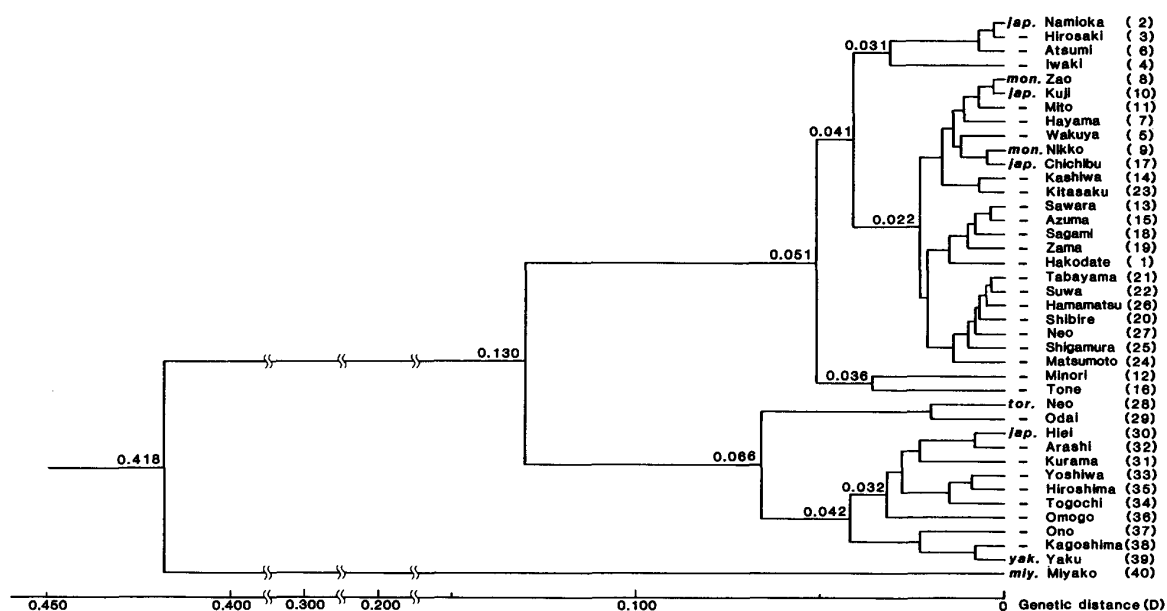


Fig. 22. Dendrogram for 40 populations of *Bufo japonicus* based on genetic distances.

## DISCUSSION

KAWAMURA, NISHIOKA and UEDA (1980) have reported that the Japanese toads

are almost completely isolated from European toads by hybrid sterility, although they are barely or considerably isolated from the latter by hybrid inviability. On the basis of hybrid sterility, *Bufo bufo japonicus* SCHLEGEL was changed into *Bufo japonicus japonicus* SCHLEGEL. Specifically, female hybrids produced from reciprocal crosses between *B. j. japonicus* and *B. bufo* are barely fertile and produced only a few mature triploid offspring raised from larger eggs, while male hybrids are almost completely sterile. The same authors changed *Bufo torrenticola* named by MATSUI (1976) into *Bufo japonicus torrenticola*, as this subspecies is neither isolated from *B. j. japonicus* by hybrid inviability nor by hybrid sterility. In reciprocal hybrids between those two subspecies, there is nearly an equal number of males and females. *Bufo japonicus miyakonis* described as *B. bufo miyakonis* by OKADA (1931, 1966) was considered to be identical with *B. bufo gargarizans* by NAKAMURA and UENO (1963). However, KAWAMURA, NISHIOKA and UEDA (1980) and KAWAMURA, NISHIOKA, UEDA and WU (1982) have considered that *B. j. miyakonis* is a subspecies of *B. japonicus*, as the male and female hybrids produced from reciprocal crossings between *B. j. japonicus* and *B. j. miyakonis* as well as those between *B. j. gargarizans* and *B. j. miyakonis* are quite normal in both fertilization rate and production of offspring. KAWAMURA, NISHIOKA, UEDA and WU (1982) and NISHIOKA and UEDA (1982) have found that diploid females of *B. j. miyakonis* often produce triploid offspring in mating with diploid males of *B. j. miyakonis*, *B. j. japonicus* and the hybrids between *B. j. japonicus* and *B. j. miyakonis*. Thereafter, KAWAMURA, NISHIOKA, UEDA, KONDO and WU (in preparation) have observed that the production of triploid toads from *B. j. miyakonis* was exceptional and have confirmed that four subspecies of *Bufo japonicus*, *japonicus*, *miyakonis*, *gargarizans* from China and *bankorensis* from Taiwan, are not reproductively isolated from one another.

As shown in the dendrogram drawn for the 40 populations of the five subspecies of *B. japonicus* by the UPGMA clustering method (Fig. 22), *B. japonicus* seems to have divided into the eastern and western groups after entered Japan, except *B. j. miyakonis* which seems to have entered into Miyako Island after making a certain degree of characteristic differentiation in China. The eastern and western groups of *B. japonicus* were roughly divided into five and two subgroups, respectively, after extending their geographical distribution all over Japan from the plains toward the mountainous districts.

Of the eastern group, the first of the five subgroups includes the Namioka, Hirosaki, Atsumi and Iwaki populations of *B. j. japonicus* and the second subgroup includes the Kuji, Mito, Hayama, Wakuya, Chichibu, Kashiwa and Kitasaku populations of *B. j. japonicus*, and the Zao and Nikko populations of *B. j. montanus*. The third subgroup includes the Sawara, Azuma, Sagami, Zama and Hakodate populations. It is curious that the Hakodate population belongs to the third subgroup, in spite of the most northern position of distribution. This seems to suggest that some toads of *B. j. japonicus* were artificially introduced into Hokkaido from the Kanto district surrounding Tokyo Bay. The fourth subgroup includes the Tabayama, Suwa, Hamamatsu, Shibire, Neo, Shigamura and Matsumoto

populations of *B. j. japonicus*. The fifth subgroup includes the Minori and Tone populations of *B. j. japonicus*.

The western group is divided into two subgroups. The first subgroup includes the Neo and Odai populations of *B. j. torrenticola*, while the second subgroup includes the Hiei, Arashi, Kurama, Yoshiwa, Hiroshima, Togochi, Omogo, Ono and Kagoshima populations of *B. j. japonicus*, and the Yaku population of *B. j. yakushimensis*.

The results of electrophoretic analyses of the enzymes and blood proteins extracted from 525 toads of 40 populations belonging to five subspecies of *Bufo japonicus* seem to have confirmed the correctness of the classification of *B. japonicus* established on the basis of the absence of hybrid sterility. The geographic distribution of alleles at the 22 loci in the 40 populations of *B. japonicus* shows some interesting aspects. Except the Hakodate and Miyako populations, the alleles at the AAT-A, IDH-B, ME-B and MPI loci show distinct gradients from east to west in the populations of *B. j. japonicus*, including *B. j. montanus*, *B. j. torrenticola* and *B. j. yakushimensis* (Figs. 3, 10, 16 and 17). The most northeastern group of the three populations of Namioka, Hirosaki and Iwaki of Honshu has exclusively allele *a* at the AAT-A locus and exclusively allele *b* at the IDH-B locus, while the most southwestern group of the three populations of Ono, Kagoshima and Yaku has exclusively allele *b* at the AAT-A locus and exclusively allele *c* at the IDH-B locus. A similar distribution of alleles is found at the ME-B and MPI loci. The most northeastern group of populations has exclusively allele *f* or a mixture of alleles *f* and *i* at the ME-B locus and exclusively allele *e* or a mixture of alleles *e* and *g* at the MPI locus, while the most southwestern group of populations has almost exclusively allele *b* at the ME-B locus and also almost exclusively allele *c* at the MPI locus. These two groups of populations may correspond to the classification of *B. j. formosus* and *B. j. japonicus*, as they completely differ from each other in the alleles at the four loci. However, they are merely both ends of a long gradient. There are numerous populations showing all degrees of intermediates in combination of alleles. Thus, it is difficult to define the difference between *B. j. formosus* and *B. j. japonicus* on the basis of the results of electrophoretic analyses.

There are a few loci having peculiar alleles in some populations of the Kinki, Chugoku or Kyushu district. The Arashi population of *B. j. japonicus* and the Odai population of *B. j. torrenticola* in the Kinki district have rarely alleles *e* and *a*, respectively, at the IDH-B locus. The Hiei, Kurama and Arashi populations of *B. j. japonicus* in the Kinki district, and the Yoshiwa, Togochi and Hiroshima populations of *B. j. japonicus* in the Chugoku district have rarely or abundantly allele *d* at the ME-B locus. The Kagoshima population of *B. j. japonicus* and the Yaku population of *B. j. yakushimensis* in the most southwestern district have exclusively a mixture of alleles *a* and *c* at the Ab locus, in contrast to the populations in the other districts.

It is remarkable that the Zao population of *B. j. montanus* is very similar to the neighboring populations of *B. j. japonicus* in the alleles at 20 of the 22 loci. The Zao population has rarely allele *e* at the ADA locus and also has rarely allele *f* at

the Ab locus, while the Nikko population of *B. j. montanus* has no peculiar allele. The Yaku population of *B. j. yakushimensis* has only one allele at each of 20 loci. This seems to be a characteristic of the inhabitants in a small island isolated for a long time. The Neo and Odai populations of *B. j. torrenticola* have peculiar alleles at three and four of the 22 loci, respectively. The Neo population of *B. j. torrenticola* has exclusively allele *g* at the ME-B locus, a low frequency of allele *b* at the SOD-A locus and a high frequency of allele *c* at the Hb locus, while the Odai population has a low frequency of allele *a* at the IDH-B, a high frequency of allele *g* at the ME-B locus, a very low frequency of allele *e* at the Ab locus and nearly exclusively allele *c* at the Hb locus. This seems to indicate that the Neo and Odai populations of *B. j. torrenticola* living in remote mountains have been more completely isolated from the surrounding populations of *B. j. japonicus* than those of *B. j. montanus*.

The Miyako population of *B. j. miyakonis* differs from the populations of the other subspecies of *B. japonicus* in having numerous peculiar alleles. This population has exclusively allele *e* at the ADA locus, while only the Zao population of *B. j. montanus* has rarely the same allele *e*. The Miyako population only has allele *b* in frequency of 0.500 at the  $\alpha$ -GDH locus and has exclusively allele *a* at the IDH-A locus, while only the Mito and Kashiwa populations of *B. j. japonicus* have rarely allele *a*. Only the Miyako population has exclusively allele *c* at the LDH-A locus, rarely allele *a* at the MDH-A locus, a low frequency of allele *b* at the MDH-B locus, exclusively allele *c* at the ME-B locus, alleles *b* and *f* in a low and high frequencies, respectively, at the MPI locus, and exclusively allele *a* at the Hb locus. The Miyako population has also allele *a* in a high frequency at the Pep-A locus, while six of the other 39 populations of *B. japonicus* have allele *a* in a very low frequency.

In the present study, it has been clarified that the genetic distances among the 39 populations of *B. japonicus* other than the Miyako population are distinctly small. The genetic distances between the eastern and western populations of *B. j. japonicus* are 0.047~0.271. Those between the two populations of *B. j. torrenticola* and the 27 eastern populations of *B. j. japonicus* and *B. j. montanus* are 0.085~0.205, while those between the former and the nine western populations of *B. j. japonicus* are 0.044~0.127. The genetic distances between the two populations of *B. j. montanus* and the eastern populations of *B. j. japonicus* are 0.003~0.061, while those between the former and the western populations of *B. j. japonicus* are 0.081~0.205. The genetic distances between the Yaku population of *B. j. yakushimensis* and the western and eastern populations of *B. j. japonicus* are 0.008~0.232. The genetic distances between the Miyako population of *B. j. miyakonis* and the 39 populations belonging to *B. j. japonicus*, *B. j. montanus*, *B. j. torrenticola* and *B. j. yakushimensis* are very large, being 0.366~0.521.

NISHIOKA, SUMIDA, UEDA and WU (1990) have reported in another paper of this volume on the genetic relationships among 13 *Bufo* species and subspecies. According to their report, the genetic distances between the China population of *B. j. gargarizans* and 10 other populations belonging to *B. j. montanus*, *B. j. japonicus*,



*B. j. yakushimensis* and *B. j. torrenticola* are 0.273~0.383, while those between the Taiwan population of *B. j. gargarizans* and the latter 10 populations are 0.235~0.356. The genetic distance between the Miyako population of *B. j. miyakonis* and the China population of *B. j. gargarizans* is small, being 0.112, while that between the Miyako population of *B. j. miyakonis* and the Taiwan population of *B. j. gargarizans* is 0.366. The genetic distance between the China and Taiwan populations of *B. j. gargarizans* is 0.168. The Taiwan populations of *B. j. gargarizans* and *B. j. bankorensis* are very similar to each other, as the genetic distance between them is only 0.020. LIU and HU (1961) have placed *B. j. bankorensis* as a synonym of *B. j. gargarizans*. In contrast, it seems evident that *B. j. miyakonis* is not a synonym of *B. j. gargarizans*, but it is a separate subspecies of *B. japonicus*.

Genetic distances have been calculated in many species of anurans and urodeles. In *Rana tagoi* distributed widely in Japan, NISHIOKA, OHTA and SUMIDA (1987) have reported that the genetic distances among six populations of *R. t. tagoi* are 0.031~0.283, while those between these six populations and the Yaku population of *R. tagoi yakushimensis* are 0.182~0.335. According to NISHIOKA, SUMIDA, OHTA and SUZUKI (1987), the genetic distances among four populations of *Buergeria japonica* are 0.003~0.270. In *Rana limnocharis* distributed in the central and southwestern parts of Japan, NISHIOKA and SUMIDA (1990) have reported that the genetic distances between three populations found in the western part of the Honshu of Japan and the Okinawa population distributed in the middle of the Ryukyu Islands are 0.030~0.050, while those between the former three populations and the Iriomote population distributed at the western end of the Ryukyu Islands are 0.293~0.345. In *Hyla japonica* distributed widely in Japan and the adjacent territory, NISHIOKA, SUMIDA and BORKIN (1990) have reported that the genetic distances among 11 populations are 0.012~0.201.

HEDGECOCK and AYALA (1974) and KALEZIĆ and HEDGECOCK (1979) have calculated the genetic distances between consubspecific populations, between subspecies and between species in American and European newts, respectively. According to HEDGECOCK and AYALA, these three degrees of genetic distances among three populations of *Taricha rivularis*, two populations of *T. granulosa*, three populations of *T. t. torosa* and one population of *T. t. sierrae* are  $0.029 \pm 0.010$ ,  $0.145 \pm 0.027$ , and  $0.466 \pm 0.021$ . On the other hand, KALEZIĆ and HEDGECOCK have described that the three degrees of genetic distances among three populations of *Triturus vulgaris*, two populations of *T. alpestris*, two populations of *T. cristatus dobrogicus* and one population of *T. c. karelinii* are  $0.031 \pm 0.017$ , 0.347, and  $0.906 \pm 0.058$ .

On the basis of electrophoretic examinations, it is believed that the common ancestor of *B. japonicus* appeared in the East and was divided into two groups, one of which was divided again into two parts. One part became *B. j. gargarizans* in China and Taiwan, while the other part became *B. j. miyakonis* after entering into Miyako Island situated near China and Taiwan. The other group produced four subspecies in Japan. The two populations of *B. j. montanus* seem to have been

produced on the plateau surrounding Mt. Zao and near Nikko. *Bufo j. yakushimensis* seems to have differentiated in Yaku Island by geographic isolation. *Bufo j. torrenticola* seems probably to have been produced on a large plateau surrounding Mt. Odaigahara by almost complete geographic isolation. After acquisition of genetic characters to adapt to mountain torrents, *B. j. torrenticola* became able to live in some other mountain torrents without losing its specific characters. However, if the subspecies was carried down stream and began to live in the same territory with *B. j. japonicus*, the two subspecies would easily cross-breed and produce hybrids, as there are no isolating mechanisms such as sexual isolation, hybrid inviability and hybrid sterility, between them.

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