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Speciation of Three Allied Genera, *Buergeria, Rhacophorus* and *Polypedates,* Elucidated by the Method of Electrophoretic Analyses

By

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(With 12 Text-figures)

ABSTRACT

Speciation of three allied genera, *Buergeria, Rhacophorus* and *Polypedates,* distributed in the Far East, was biochemically examined by the electrophoretic method. Electrophoretic patterns of 16 enzymes extracted from the skeletal muscles and livers and three blood proteins were analyzed in 257 frogs belonging to 14 populations, including one of *Buergeria buergeri,* four of *B. japonica,* two of *Rhacophorus arboreus,* two of *Rh. schlegelii,* three of *Rh. viridis,* one of *Rh. taipeianus* and one of *Polypedates leucomystax.* These frogs had 25 loci in total.

While 10 enzymes, ADA, ADH, AK, CK, Fum, α–GDH, GPI, ME, MPI and PGM, and three blood proteins, Ab, Prot–C and Hb, had a single locus, the other six enzymes, AAT, IDH, LDH, MDH, Pep and SOD, had two loci. At each of the 25 loci, there were 3~33 phenotypes, 10.6 on the average, produced by 3~17 alleles, 7.5 on the average. The mean proportions of heterozygous loci per individual in each of the 10 populations of *Buergeria* and *Rhacophorus* which were 5~58 in sample size were 4.0~23.0%, 12.0% on the average, when examined at the 25 loci controlling 16 enzymes and three blood proteins. These 10 populations were 24~68%, 44.8% on the average, in mean proportion of polymorphic loci per population and 1.28~2.12, 1.64 on the average, in mean number of alleles per locus.

Genetic distances were estimated from gene frequencies by the method of NEI (1972, 1975). Those among different populations of *B. japonica, Rh. arboreus* and *Rh. schlegelii* were 0.003~0.270, and those among different subspecies of *Rh. viridis* were 0.277 ~0.865. The genetic distances among four species, *Rh. arboreus, Rh. schlegelii, Rh. viridis* and *Rh. taipeianus,* were 0.301~0.854, while those between *Buergeria buergeri* and four populations of *B. japonica* were 2.045~2.243. The genetic distances between *Polypedates* and *Buergeria* and between *Polypedates* and *Rhacophorus* were 3.073~4.572 and 1.183 ~1.445, respectively. The genetic distances between *Buergeria* and *Rhacophorus* were very large, being from 2.782 to unlimited number owing to nonexistence of common alleles between them. A dendrogram was drawn for the species and populations of *Buergeria, Rhacophorus* and *Polypedates* on the basis of genetic distances by the UPGMA clustering method (SNEATH and SOKAL, 1973; NEI, 1975).

INTRODUCTION

STEJNEGER (1907) has described the following seven *Polypedates* species as rhacophorids distributed in Japan and adjacent territory: *P. schlegelii* GÜNTHER, *P. viridis* HALLOWELL, *P. owstoni* (new species), *P. buergeri* (SCHLEGEL), *P. eiffingeri* (BOETTGER), *P. japonicus* (HALLOWELL) and *P. leucomystax* (GRAVENHORST). OKADA (1931) has divided these species into two genera, *Polypedates* including *buergeri, japonicus* and *eiffingeri,* and *Rhacophorus* including *schlegelii schlegelii* (GÜNTHER), *schlegelii arborea* (OKADA et KAWANO), *schlegelii intermedia* (OKADA et KAWANO), *viridis* (HALLOWELL), *owstoni* (STEJNEGER) and *leucomystax* (GRAVENHORST). KAWAMURA (1962) placed *Rh. schlegelii arborea* to the position of a new species, as it differs distinctly from *Rh. s. schlegelii* in morphological and ecological characters, and moreover, it is completely isolated from this subspecies by various isolating mechanisms. NAKAMURA and UENO (1963) united *Polypedates* with *Rhacophorus,* and described the following six species and two subspecies as Japanese rhacophorids: *Rh. schlegelii, Rh. arboreus* (OKADA et KAWANO), *Rh. viridis viridis, Rh. viridis amamiensis* INGER, *Rh. viridis owstoni, Rh. eiffingeri* (BOETTGER), *Rh. buergeri* (SCHLEGEL) and *Rh. japonicus* (HALLOWELL). LIEM (1970) placed *Rh. eiffingeri* as *Chirixalus eiffingeri, Rh. buergeri* and *Rh. japonicus* as *Buergeria buergeri* and *B. japonica,* and *Rh. leucomystax* as *Polypedates leucomystax.*

The present study was planned to elucidate biochemically the genetic relationship between different genera, species, subspecies or populations in rhacophorids by the method of electrophoresis, as a similarity in morphological and ecological characters does not always correspond to an evolutional alliance.

MATERIALS AND METHODS

In the years from 1978 to 1986, 257 mature male and female frogs of *Buergeria, Rhacophorus* and *Polypedates* were collected from 14 stations (Fig. 1). The three genera used in the present study can be easily distinguished from one another by the following characters. The specimens of *Buergeria* are brown in dorsal color, and their females lay eggs enveloped with colorless, transparent gelatine, while those of *Rhacophorus* are green in dorsal color, and their females lay white foamy egg masses. In contrast to these genera, the specimens of *Polypedates* are brown in dorsal color, and their females lay white foamy egg masses.

1. *Buergeria buergeri* (SCHLEGEL)

One population including 40 mature males and females collected from Togochi-cho, Hiroshima Prefecture.

2. *Buergeria japonica* (HALLOWELL)

Two populations of Amami Island, Kagoshima Prefecture, each of which includes 30 frogs. Another population including 58 frogs collected from Nakanoshima Island of the Tokara Archipelago, Kagoshima Prefecture. The 118 frogs in total were all mature males and females. One more population including two

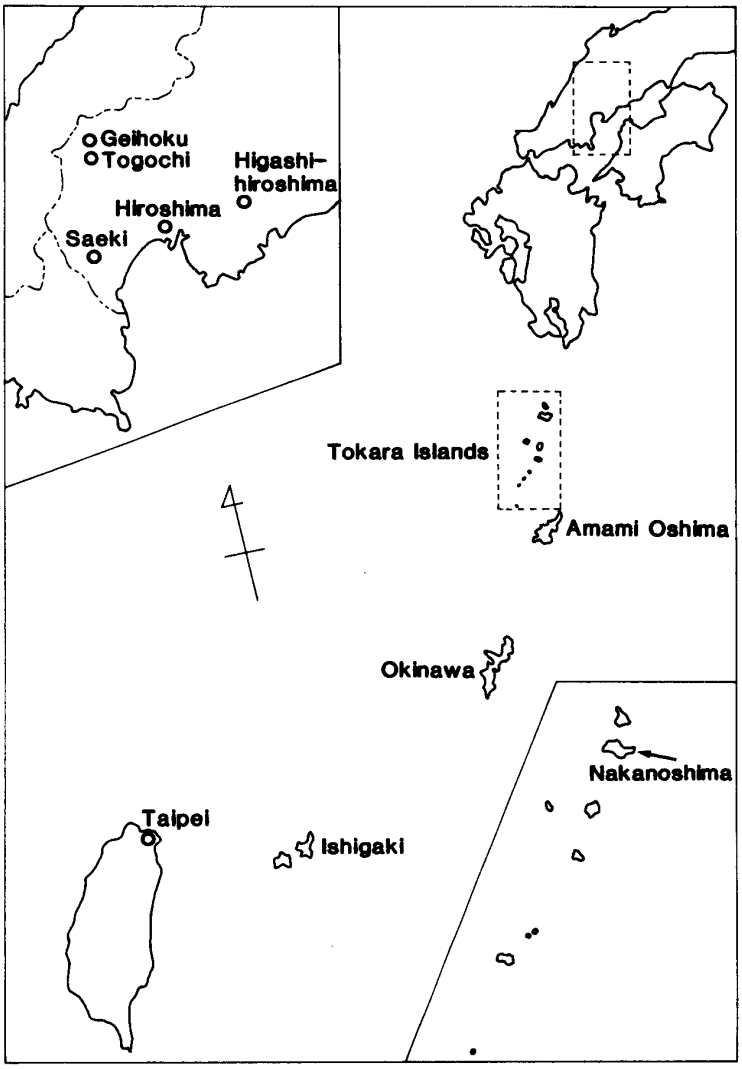

Fig. 1. Map showing localities of 13 population samples of *Buergeria* and *Rhacophorus*.

mature male and female collected from Yona, Okinawa Prefecture.

3. *Rhacophorus arboreus* (OKADA et KAWANO)

Two populations of Geihoku-cho and Saeki-cho, Hiroshima Prefecture, consisting of 34 and eight mature male and female frogs, respectively.

4. *Rhacophorus schlegelii* (GÜNTHER)

Two populations of Hiroshima and Higashihiroshima including 17 and 20 mature male and female frogs, respectively.

5. *Rhacophorus viridis amamiensis* INGER

A mature male collected from Amami Island, Kagoshima Prefecture.

6. *Rhacophorus viridis viridis* (HALLOWELL)

Nine mature males and females collected from Yona, Okinawa Prefecture.

7. *Rhacophorus viridis owstoni* (STEJNEGER)

Five mature males and females collected from Ishigaki Island, Okinawa Prefecture.

8. *Rhacophorus taipeianus* LIANG and WANG

One mature male collected from Taipei, Taiwan.

9. *Polypedates leucomystax* (GRAVENHORST)

Two mature males collected from Thailand (Tai).

In all of these frogs, 16 enzymes extracted from the skeletal muscles and livers and three blood proteins were analyzed by the method of horizontal starch-gel electrophoresis. It was found that the genes at 25 loci control these enzymes and blood proteins. The kinds of enzymes and blood proteins as well as their loci are as follows.

1. Enzymes extracted from skeletal muscles (13 enzymes, 18 loci)
   1) Aspartate aminotransferase (AAT-A, AAT-B)
   2) Adenosine deaminase (ADA)
   3) Adenylate kinase (AK)
   4) Creatine kinase (CK)
   5) α-Glycerophosphate dehydrogenase (α-GDH)
   6) Glucose phosphate isomerase (GPI)
   7) Isocitrate dehydrogenase (IDH-A, IDH-B)
   8) Lactate dehydrogenase (LDH-A, LDH-B)
   9) Malate dehydrogenase (MDH-A, MDH-B)
   10) Malic enzyme (ME)
   11) Mannose phosphate isomerase (MPI)
   12) Phosphoglucomutase (PGM)
   13) Superoxide dismutase (SOD-A, SOD-B)
2. Enzymes extracted from livers (3 enzymes, 4 loci)
   14) Alcohol dehydrogenase (ADH)
   15) Fumarase (Fum)
   16) Peptidase (Pep-B, Pep-D)
3. Blood proteins (3 proteins, 3 loci)
   17) Albumin (Ab)
   18) Protein-C (Prot-C)
   19) Hemoglobin (Hb)

The buffer systems and the kinds and loci of the enzymes and blood proteins analyzed by them are as follows.

1. Tris-citrate buffer, pH 6.0 : α-GDH, LDH-A, LDH-B, MDH-A, MDH-B (3 kinds, 5 loci)
2. Tris-citrate buffer, pH 7.0 : AAT-A, AAT-B, ADA, AK, IDH-A, IDH-B, ME, MPI (6 kinds, 8 loci)
3. Tris-borate-EDTA buffer, pH 8.0 : ADH, CK, Fum, GPI, Pep-B, Pep-D, PGM, SOD-A, SOD-B, Ab, Prot-C (9 kinds, 11 loci)
4. Tris-borate-EDTA buffer, pH 8.6 : Hb (1 kind, 1 locus)

The electrophoretic method and the buffer systems used in the present study have been previously described in detail by NISHIOKA, OHTANI and SUMIDA (1980). Staining of enzymes was principally performed by the agar overlay method, according to HARRIS and HOPKINSON (1976). The blood proteins were stained

with amido-black.

A locus was considered to be consisting of multiple alleles, when the alleles exist at a frequency of more than 1%. Average heterozygosity was first calculated at each locus and then estimated at all the loci of each population.

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

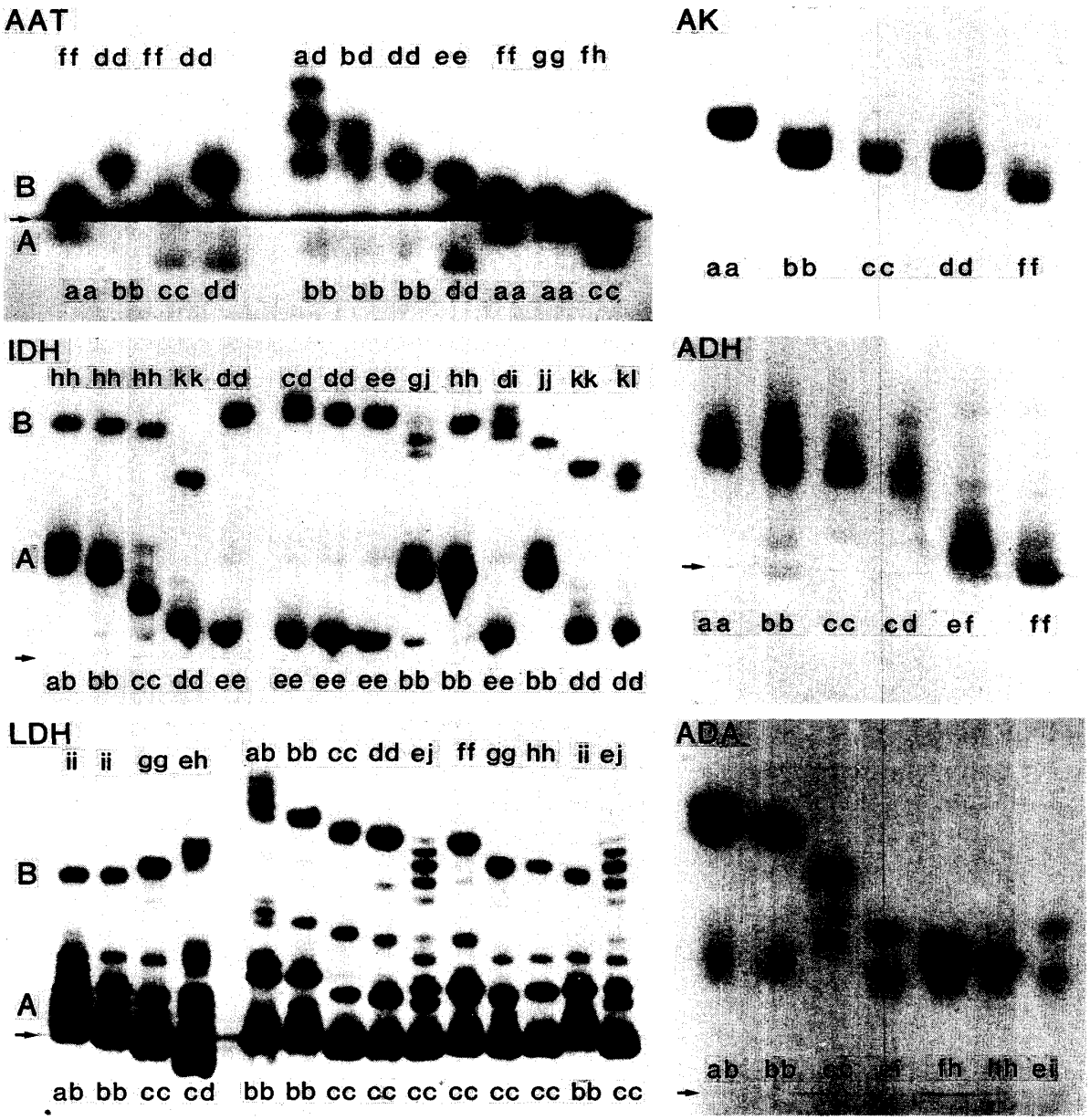

Fig. 2. Electrophoretic patterns of six enzymes, AAT, AK, ADH, ADA, IDH and LDH, in 14 populations of *Buergeria*, *Rhacophorus* and *Polypedates*.

OBSERVATION

I. Electrophoretic patterns and multiple alleles

The electrophoretic patterns of 16 enzymes extracted from skeletal muscles and livers and three blood proteins were examined in 257 frogs of 14 populations belonging to *Buergeria, Rhacophorus* and *Polypedates*. It was found that 25 loci participate in controlling these enzymes and blood proteins. The electrophoretic bands corresponding to multiple alleles at each locus were named A, B, C, ····· in the order of mobility from fast to slow, and the alleles were shown by *a, b, c,* ····· (Figs. 2~4).

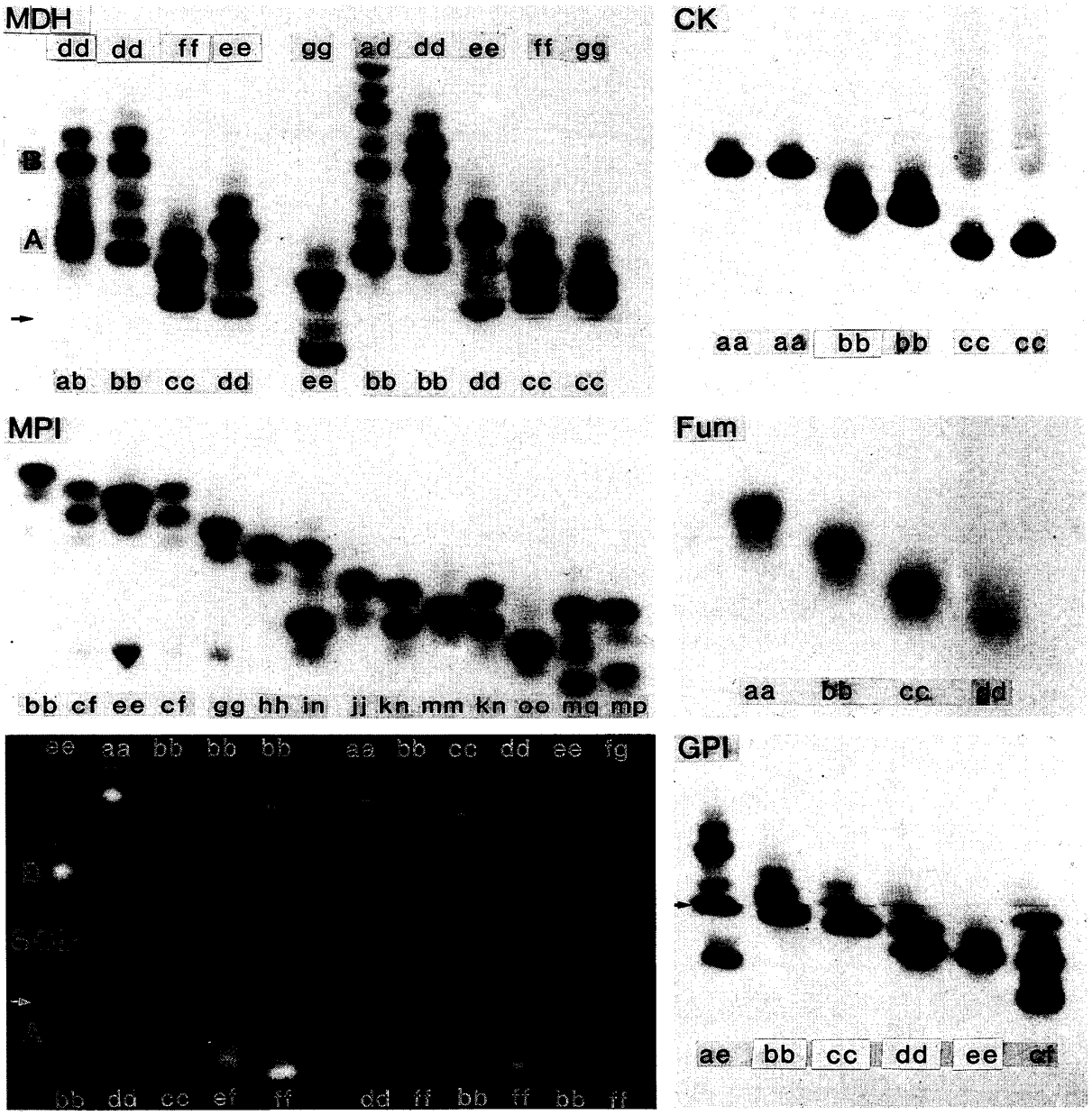

Fig. 3. Electrophoretic patterns of six enzymes, MDH, MPI, SOD, CK, Fum and GPI, in 14 populations of *Buergeria, Rhacophorus* and *Polypedates*.

When the numbers of phenotypes and alleles were examined at the 25 loci, it was found that there were 3~33 phenotypes produced by 3~17 alleles (Table 1). Of these phenotypes, the smallest was three which were produced by three alleles, *a*, *b* and *c* at the CK locus in all the populations. At the two loci of AAT-A and LDH-A, there were four or five phenotypes produced by four alleles, *a*, *b*, *c* and *d*. At the three loci of IDH-A, MDH-A and Fum, there were six or seven

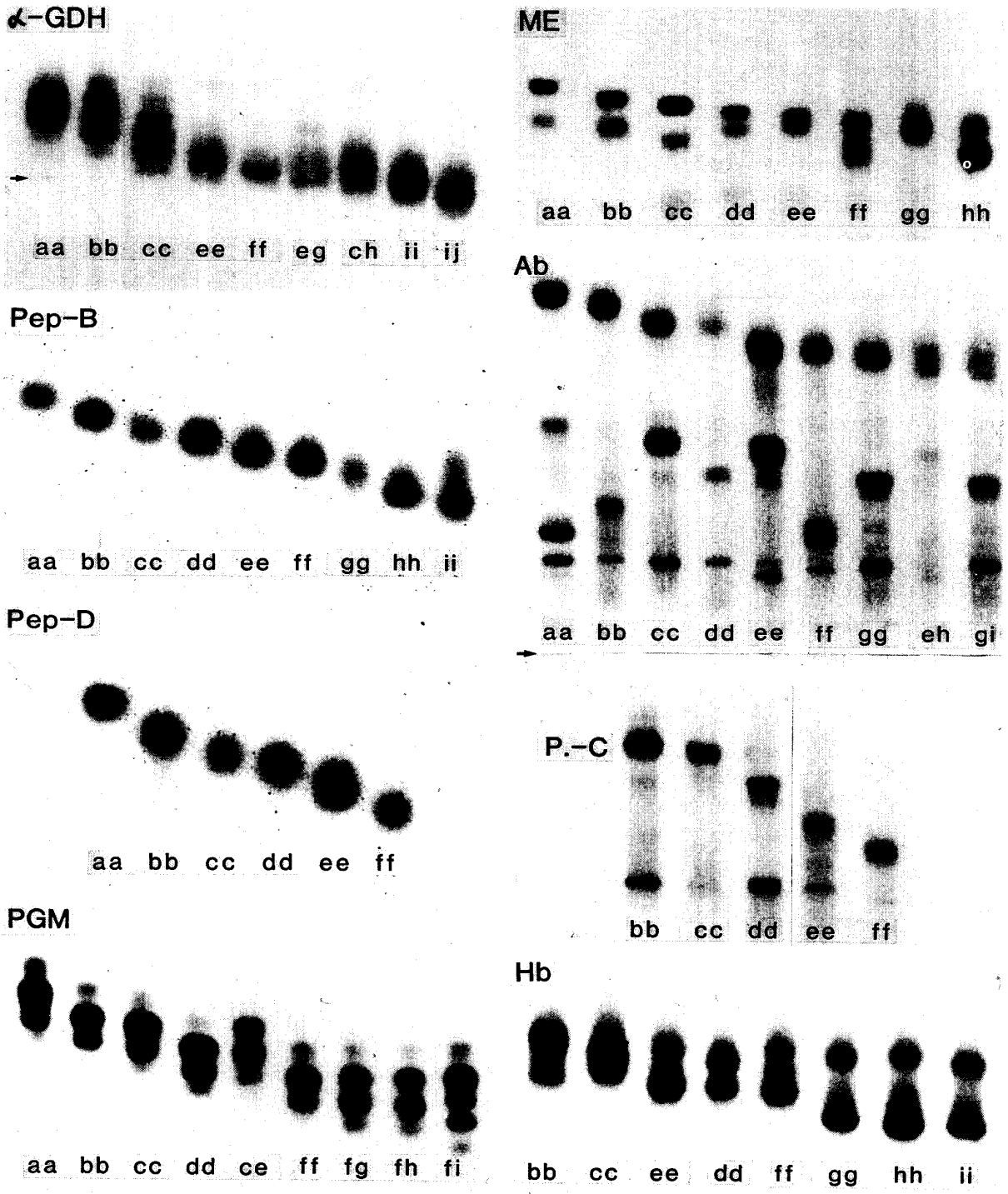

Fig. 4. Electrophoretic patterns of five enzymes, α-GDH, Pep-B, Pep-D, PGM and ME, and three blood proteins, Ab, Prot-C and Hb, in 14 populations of *Buergeria*, *Rhacophorus* and *Polypedates*.

TABLE 1

Number of phenotypes and alleles at 25 loci in two species of *Buergeria*, four species of *Rhacophorus* and one species of *Polypedates*

Enzyme or blood protein	Locus	No. of phenotypes	No. of alleles
1) Aspartate aminotransferase	AAT-A	5	4
	AAT-B	12	8
2) Adenosine deaminase	ADA	11	9
3) Alcohol dehydrogenase	ADH	7	6
4) Adenylate kinase	AK	7	6
5) Creatine kinase	CK	3	3
6) Fumarase	Fum	7	5
7) α-Glycerophosphate dehydrogenase	α-GDH	14	10
8) Glucose phosphate isomerase	GPI	12	6
9) Isocitrate dehydrogenase	IDH-A	6	5
	IDH-B	14	12
10) Lactate dehydrogenase	LDH-A	4	4
	LDH-B	12	10
11) Malate dehydrogenase	MDH-A	6	5
	MDH-B	7	7
12) Malic enzyme	ME	15	8
13) Mannose phosphate isomerase	MPI	33	17
14) Peptidase	Pep-B	17	9
	Pep-D	12	7
15) Phosphoglucomutase	PGM	14	9
16) Superoxide dismutase	SOD-A	7	6
	SOD-B	10	8
17) Serum albumin	Ab	14	9
18) Serum protein-C	Prot-C	7	6
19) Hemoglobin	Hb	9	9
Average		10.6	7.5

phenotypes produced by five alleles, *a, b, c, d* and *e*. At the four loci of ADH, AK, SOD-A and Prot-C, there were seven phenotypes produced by six alleles, *a, b, c, d, e* and *f*. At the GPI locus, 12 phenotypes produced by six alleles were observed. At the two loci of MDH-B and Pep-D, seven and 12 phenotypes produced by seven alleles, *a, b, c, d, e, f* and *g*, were observed, respectively. At the three loci of AAT-B, ME and SOD-B, there were 12, 15 and 10 phenotypes produced by eight alleles, *a, b, c, d, e, f, g* and *h*, respectively. At the five loci of ADA, Pep-B, PGM, Ab and Hb, there were 11, 17, 14, 14 and nine phenotypes produced by nine alleles, *a, b, c, d, e, f, g, h* and *i*, respectively. At the two loci of α-GDH and LDH-B, there were 14 and 12 phenotypes produced by 10 alleles, *a, b, c, d, e, f, g, h, i* and *j*, respectively. At the IDH-B locus, 14 phenotypes produced by 12 alleles, *a, b, c, d, e, f, g, h, i, j, k* and *l*, were observed. The MPI locus was most polymorphic with 33 phenotypes being produced by 17 alleles, *a* ~*q*. At these 25 loci, there were 10.6 phenotypes produced by 7.5 alleles on the average.

II. Gene frequency

1. Aspartate aminotransferase (AAT)

The analysis of electrophoretic patterns of AAT in the 257 frogs of the 14 populations belonging to *Buergeria, Rhacophorus* and *Polypedates* showed that AAT was controlled by genes at two loci, AAT-A and AAT-B.

At the AAT-A locus, there were five phenotypes produced by four alleles, *a, b, c* and *d*. No variations were observed in the 13 populations except the Geihoku of *Rh. arboreus*. In this population, 29 and one of the 34 frogs showed homozygous bands, AA and CC, respectively, while the remaining four showed a heterozygous band, AC. Alleles *a* and *c* were 0.912 and 0.088 in frequency, respectively. In each of the other 13 populations, all the frogs showed a single homozygous band. Eight frogs of the Saeki population of *Rh. arboreus* and five *Rh. v. owstoni* had only allele *a*. All the 120 frogs of the Amami-I and -II, Tokara and Okinawa populations of *Buergeria japonica* had only allele *b*, while 37 frogs of the Higashihiroshima and Hiroshima populations of *Rh. schlegelii*, 10 frogs of *Rh. v. viridis* and *Rh. v. amamiensis* and a frog of *Rh. taipeianus* had only allele *c*. All the 40 frogs of the Togochi population of *B. buergeri* and two frogs of the Tai population of *P. leucomystax* had only allele *d* (Table 2; Fig. 5).

At the AAT-B locus, there were 12 phenotypes produced by eight alleles, *a*–*h*. Of the 40 frogs of the Togochi population of *B. buergeri*, 32 showed a homozygous band, DD, one showed a homozygous band, EE, and seven showed a heterozygous band, DE. Alleles *d* and *e* were 0.888 and 0.113 in frequency, respectively. Of the 30 frogs of the Amami-I population of *B. japonica*, 26 showed a homozygous band, DD, and four showed a heterozygous band, BD. Alleles *d* and *b* were 0.933 and 0.067 in frequency, respectively. Of the 30 frogs of the Amami-II population of *B. japonica*, 27 showed a homozygous band, DD, while two and one showed heterozygous bands, BD and AD, respectively. Alleles *d, b* and *a* were 0.950, 0.033 and 0.017 in frequency, respectively. One of the two frogs of the Okinawa population showed a homozygous band, DD, while the other showed a heterozygous band, BD. Alleles *b* and *d* were 0.250 and 0.750 in frequency, respectively. All the 58 frogs of the Tokara population had only allele *d*. Of the 34 frogs of the Geihoku population of *Rh. arboreus*, 31 and one showed homozygous bands, FF and GG, respectively, while two showed a heterozygous band, FG. Alleles *f* and *g* were 0.941 and 0.059 in frequency, respectively. Of the eight frogs of the Saeki population, five and three showed homozygous and heterozygous bands, FF and FG, respectively. Alleles *f* and *g* were 0.813 and 0.188 in frequency, respectively. Of the 20 frogs of the Higashihiroshima population of *Rh. schlegelii*, 18 showed a homozygous band, FF, and one and one showed heterozygous bands, DF and EF, respectively. Alleles *f, d* and *e* were 0.950, 0.025 and 0.025 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 12 and five showed homozygous and heterozygous bands, FF and FH, respectively. Alleles *f* and *h* were 0.853 and 0.147 in frequency, respectively. A total of 16 frogs including nine

TABLE 2

Gene frequencies at 25 loci in 14 populations of two species of *Buergeria*, four species of *Rhacophorus* and one species of *Polypedates*

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
AAT-A	*a*						0.912	1.000				1.000			
	b		1.000	1.000	1.000	1.000									
	c						0.088		1.000	1.000	1.000		1.000	1.000	
	d	1.000													1.000
AAT-B	*a*			0.017											
	b		0.067	0.033		0.250									
	c														1.000
	d	0.888	0.933	0.950	1.000	0.750			0.025						
	e	0.113							0.025						
	f						0.941	0.813	0.950	0.853	1.000	1.000	1.000	1.000	
	g						0.059	0.188							
	h									0.147					
ADA	*a*	0.125													
	b	0.875													
	c		1.000	1.000	1.000	1.000									
	d														0.500
	e									0.033					0.250
	f						0.029		0.200	0.167					
	g														0.250
	h						0.971	1.000	0.775	0.733	1.000	1.000	1.000	1.000	
	i								0.025	0.067					
ADH	*a*				0.224										
	b	1.000													

TABLE 2 Continued-2

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
ADH	*c*		0.983	0.900	0.776	1.000									
	d		0.017	0.100											
	e											0.200			
	f						1.000	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000
AK	*a*	1.000													
	b		1.000	1.000	1.000	1.000									
	c								0.684	0.400					
	d											1.000			
	e														1.000
	f						1.000	1.000	0.316	0.600	1.000		1.000	1.000	
CK	*a*						1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b		1.000	1.000	1.000	1.000									
	c	1.000													
Fum	*a*						0.309	0.750			0.111				
	b						0.691	0.250	1.000	1.000	0.889	1.000	1.000	1.000	
	c	0.913	0.067	0.117	0.190										
	d	0.088	0.933	0.883	0.810	1.000									
	e														1.000
α-GDH	*a*		1.000	1.000	1.000	1.000									
	b										0.556		1.000		0.250
	c	0.088									0.444	0.800		1.000	
	d														0.750
	e						0.735	0.875	0.825	0.941					

TABLE 2 Continued–3

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
α-GDH	*f*						0.265	0.125							
	g								0.175	0.059					
	h											0.200			
	i	0.863													
	j	0.050													
GPI	*a*											0.100			
	b		1.000	0.867	1.000	1.000						0.400			
	c						0.926	1.000	0.675	0.735	1.000		1.000	1.000	1.000
	d	0.988		0.133			0.074			0.059					
	e								0.325	0.088		0.500			
	f	0.013								0.118					
IDH-A	*a*						0.015								
	b						0.985	1.000	0.950	0.971	1.000		1.000	1.000	
	c								0.050	0.029		1.000			1.000
	d	1.000													
	e		1.000	1.000	1.000	1.000									
IDH-B	*a*														0.250
	b														0.500
	c		0.033				0.074	0.063							
	d		0.933	0.950	1.000										
	e					1.000									
	f														0.250
	g										0.111				
	h						0.926	0.938	0.925	0.765		1.000			

TABLE 2 Continued-4

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
IDH-B	*i*		0.033	0.050											
	j								0.075	0.235	0.889		1.000	1.000	
	k	0.550													
	l	0.450													
LDH-A	*a*				0.017										
	b	1.000	1.000	1.000	0.983	1.000									
	c						1.000	1.000	1.000	1.000	0.944	1.000	1.000	1.000	1.000
	d										0.056				
LDH-B	*a*	0.088													
	b	0.900													
	c												1.000		
	d						1.000	1.000	0.550	0.471					
	e										0.313				
	f													1.000	
	g	0.013							0.450	0.529		1.000			1.000
	h										0.563				
	i		1.000	1.000	1.000	1.000									
	j										0.125				
MDH-A	*a*			0.033											
	b	0.025	1.000	0.967	1.000	1.000									
	c						1.000	1.000			1.000	1.000	1.000	1.000	1.000
	d	0.975													
	e								1.000	1.000					

TABLE 2 Continued–5

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
MDH-B	*a*		0.033	0.017											
	b														0.750
	c														0.250
	d		0.967	0.983	1.000	1.000									
	e	1.000													
	f													1.000	
	g						1.000	1.000	1.000	1.000	1.000	1.000	1.000		
ME	*a*											0.900			
	b						0.147	0.313	0.125	0.033	1.000	0.100			
	c													1.000	1.000
	d						0.853	0.688							
	e								0.675	0.667			1.000		
	f	0.063													
	g	0.850							0.200	0.300					
	h	0.088	1.000	1.000	1.000	1.000									
MPI	*a*														0.500
	b						0.015		0.075	0.029		0.700			
	c						0.044	0.063	0.025	0.029	0.889				
	d														0.500
	e						0.912	0.875	0.900	0.941				1.000	
	f			0.017			0.015				0.111				
	g						0.015	0.063				0.300	1.000		
	h		0.367	0.333	0.026										
	i	0.013													

TABLE 2 Continued–6

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
MPI	*j*	0.150	0.583	0.483	0.974										
	k	0.063													
	l					0.250									
	m	0.350													
	n	0.213	0.050	0.167		0.750									
	o	0.150													
	p	0.038													
	q	0.025													
Pep–B	*a*								0.175	0.059					
	b								0.325	0.235				0.500	
	c														1.000
	d						0.897	0.938	0.450	0.500	1.000	1.000		0.500	
	e		0.267	0.400	0.983								1.000		
	f						0.103	0.063							
	g		0.350	0.333	0.017	1.000			0.050	0.206					
	h	0.963	0.383	0.267											
	i	0.038													
Pep–D	*a*								0.028	0.147		0.900		1.000	
	b						0.779	0.643	0.861	0.765	1.000	0.100			1.000
	c		0.167	0.133	0.069		0.221	0.357	0.111	0.088					
	d												1.000		
	e		0.783	0.800	0.862										
	f	0.925	0.050	0.067	0.069	1.000									
	g	0.075													

TABLE 2 Continued-7

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
PGM	*a*						0.897	0.875	0.175	0.324	1.000		1.000	1.000	
	b						0.103	0.125				1.000			0.750
	c		0.033	0.083					0.825	0.529					
	d		0.967	0.900	1.000	1.000									0.250
	e									0.147					
	f	0.963													
	g	0.013		0.017											
	h	0.013													
	i	0.013													
SOD-A	*a*														1.000
	b		1.000	1.000	1.000	1.000									
	c								0.450	0.300					
	d	1.000													
	e								0.050	0.067					
	f						1.000	1.000	0.500	0.633	1.000	1.000	1.000	1.000	
SOD-B	*a*	1.000													
	b						1.000	1.000	0.950	1.000	1.000		1.000		0.250
	c		0.483	0.533	1.000										
	d											1.000			
	e		0.517	0.467		1.000									
	f								0.050					0.500	
	g													0.500	
	h														0.750

TABLE 2 Continued–8

Species		*B. buerg.*	*B. japonica*				*Rh. arboreus*		*Rh. schlegelii*		*Rh.v.virid.*	*Rh.v.owst.*	*Rh.v.amam.*	*Rh. taip.*	*P. leuc.*
Locality		Togochi	Amami-I	Amami-II	Tokara	Okinawa	Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
Sample size		40	30	30	58	2	34	8	20	17	9	5	1	1	2
Locus	Alle.														
Ab	*a*											1.000	1.000		
	b										1.000				
	c													1.000	1.000
	d						0.338	0.313							
	e	0.963					0.662	0.688		0.059					
	f		0.967	1.000	1.000										
	g								0.975	0.882					
	h	0.038	0.033			1.000			0.025	0.029					
	i									0.029					
Prot–C	*a*														1.000
	b													1.000	
	c	1.000													
	d						1.000	1.000	0.975	1.000					
	e								0.025		1.000		1.000		
	f		1.000	1.000	1.000	1.000						1.000			
Hb	*a*														1.000
	b		0.067												
	c	1.000													
	d		0.933	1.000	1.000	1.000									
	e													1.000	
	f											1.000			
	g										1.000		1.000		
	h						1.000	1.000							
	i								1.000	1.000					

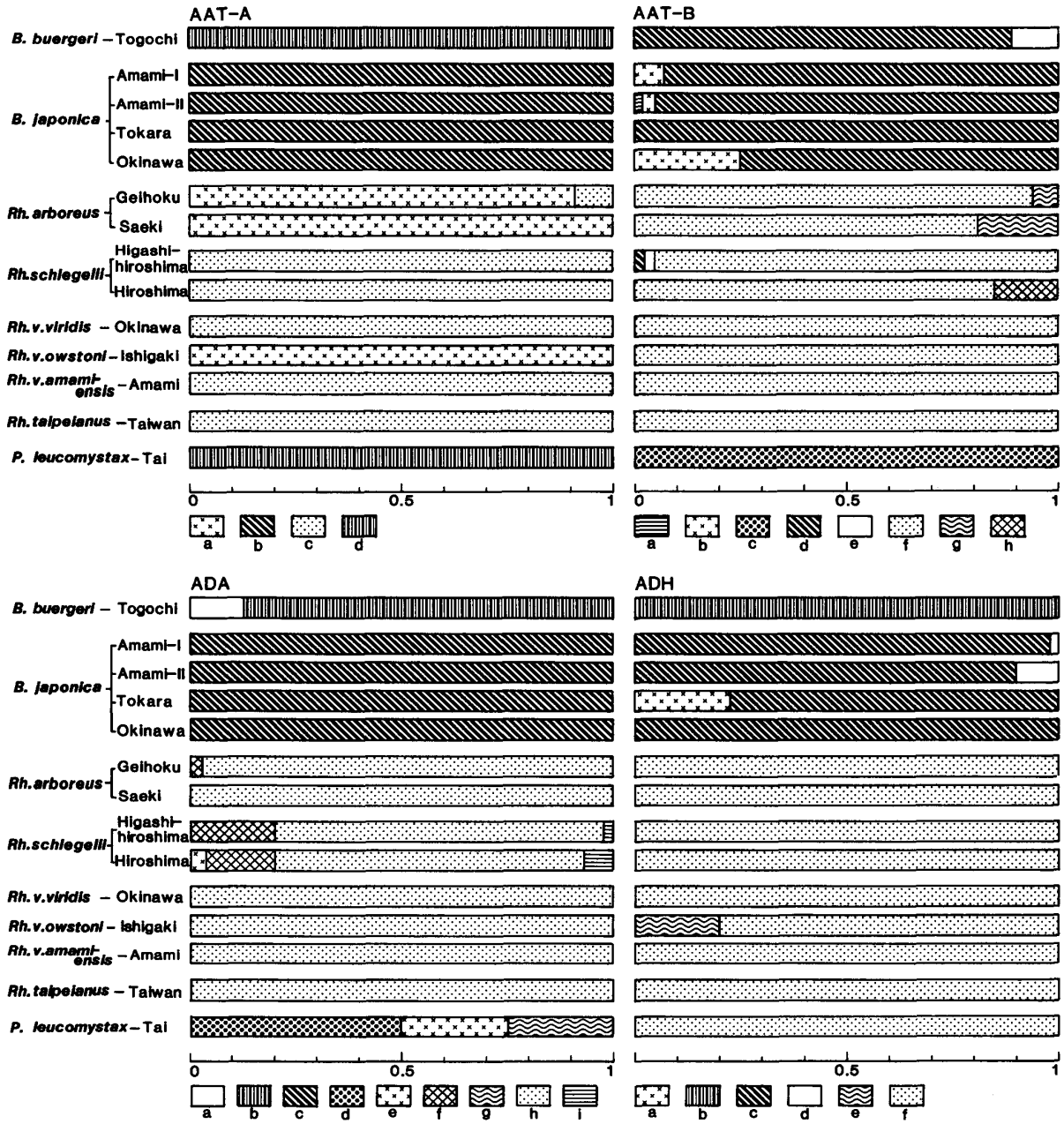

Fig. 5. Gene frequencies at four loci, AAT-A, AAT-B, ADA and ADH, in 14 populations of *Buergeria*, *Rhacophorus* and *Polypedates*.

Rh. v. viridis, five *Rh. v. owstoni*, one *Rh. v. amamiensis* and one *Rh. taipeianus* had only allele *f*. Two *P. leucomystax* had only allele *c* (Table 2; Fig. 5).

2. Adenosine deaminase (ADA)

The electrophoretic patterns of the 255 frogs of the 14 populations elucidated that there were 11 phenotypes produced by nine alleles, *a*~*i*, at the ADA locus. In the four populations of *B. japonica*, the Amami-I and -II, Tokara and Okinawa populations, all the 120 frogs showed a homozygous band, CC. They had only allele *c*. All the 15 frogs of *Rh. v. viridis*, *Rh. v. owstoni* and *Rh. v. amamiensis* and

one *Rh. taipeianus* showed a homozygous band, HH. They had only allele *h*. Of the 40 frogs of the Togochi population of *B. buergeri*, 30 and 10 showed homozygous and heterozygous bands, BB and AB, respectively. Alleles *b* and *a* were 0.875 and 0.125 in frequency, respectively. All the eight frogs of the Saeki population of *Rh. arboreus* had only allele *h*, while 32 and two of the 34 frogs of the Geihoku population showed homozygous and heterozygous bands, HH and FH, respectively. In this population, alleles *h* and *f* were 0.971 and 0.029 in frequency, respectively. In *Rh. schlegelii*, 12 and one of the 20 frogs of the Higashihiroshima population showed homozygous bands, HH and FF, respectively, while six and one showed heterozygous bands, HF and HI, respectively. In this population, alleles *h*, *f* and *i* were 0.775, 0.200 and 0.025 in frequency, respectively. Of the 15 frogs of the Hiroshima population, nine showed a homozygous band, HH, while four, one and one showed heterozygous bands, HF, FI and EI, respectively. Alleles *h*, *f*, *i* and *e* were 0.733, 0.167, 0.067 and 0.033 in frequency, respectively. Two frogs of the Tai population of *P. leucomystax* showed homozygous and heterozygous bands, DD and EG. In this population, alleles *d*, *e* and *g* were 0.500, 0.250 and 0.250 in frequency, respectively (Table 2; Fig. 5).

3. Alcohol dehydrogenase (ADH)

The electrophoretic patterns of the 257 frogs of the 14 populations indicated that there were seven phenotypes produced by six alleles, *a*~*f*, at the ADH locus. All of the 40 *B. buergeri* had only allele *b*, and a total of 88 frogs including 42 *Rh. arboreus*, 37 *Rh. schlegelii* and nine *Rh. v. viridis* had only allele *f*. A total of four frogs including one *Rh. v. amamiensis*, one *Rh. taipeianus* and two *P. leucomystax* had also only allele *f*. In *B. japonica*, 29 and one of the 30 frogs of the Amami-I population showed homozygous and heterozygous bands, CC and CD, respectively. In this population, alleles *c* and *d* were 0.983 and 0.017 in frequency, respectively. Of the 30 frogs of the Amami-II population, 24 and six showed homozygous and heterozygous bands, CC and CD, respectively. Alleles *c* and *d* were 0.900 and 0.100 in frequency, respectively. Of the 58 frogs of the Tokara population, 36 and four showed homozygous bands, CC and AA, respectively, and 18 showed a heterozygous band, AC. Alleles *c* and *a* were 0.776 and 0.224 in frequency, respectively. Two frogs of the Okinawa population showed a homozygous band, CC. Of the five frogs of the Ishigaki population of *Rh. v. owstoni*, three and two showed homozygous and heterozygous bands, FF and EF, respectively. Alleles *f* and *e* were 0.800 and 0.200 in frequency, respectively (Table 2; Fig. 5).

4. Adenylate kinase (AK)

The electrophoretic patterns of the 254 frogs of the 14 populations indicated that seven phenotypes produced by six alleles, *a*~*f*, at the AK locus. It was found that all of the 40 frogs of *B. buergeri* and all of the 120 frogs of the four populations of *B. japonica* showed homozygous bands, AA and BB, respectively. All of the 42 frogs of the two populations of *Rh. arboreus*, nine frogs of *Rh. v. viridis* and two frogs of *Rh. v. amamiensis* and *Rh. taipeianus* showed a homozygous band, FF. All of the five frogs

of *Rh. v. owstoni* and two frogs of *P. leucomystax* showed homozygous bands, DD and EE, respectively. In contrast, eight and one of the 19 frogs of the Higashihiroshima population of *Rh. schlegelii* showed homozygous bands, CC and FF, respectively, and the remaining 10 showed a heterozygous band, CF. Alleles *c* and *f* were 0.684 and 0.316 in frequency, respectively. Of the 15 frogs of the Hiroshima population, five and two showed homozygous bands, FF and CC, respectively, and eight showed a heterozygous band, CF. Alleles *f* and *c* were 0.600 and 0.400 in frequency, respectively (Table 2; Fig. 6).

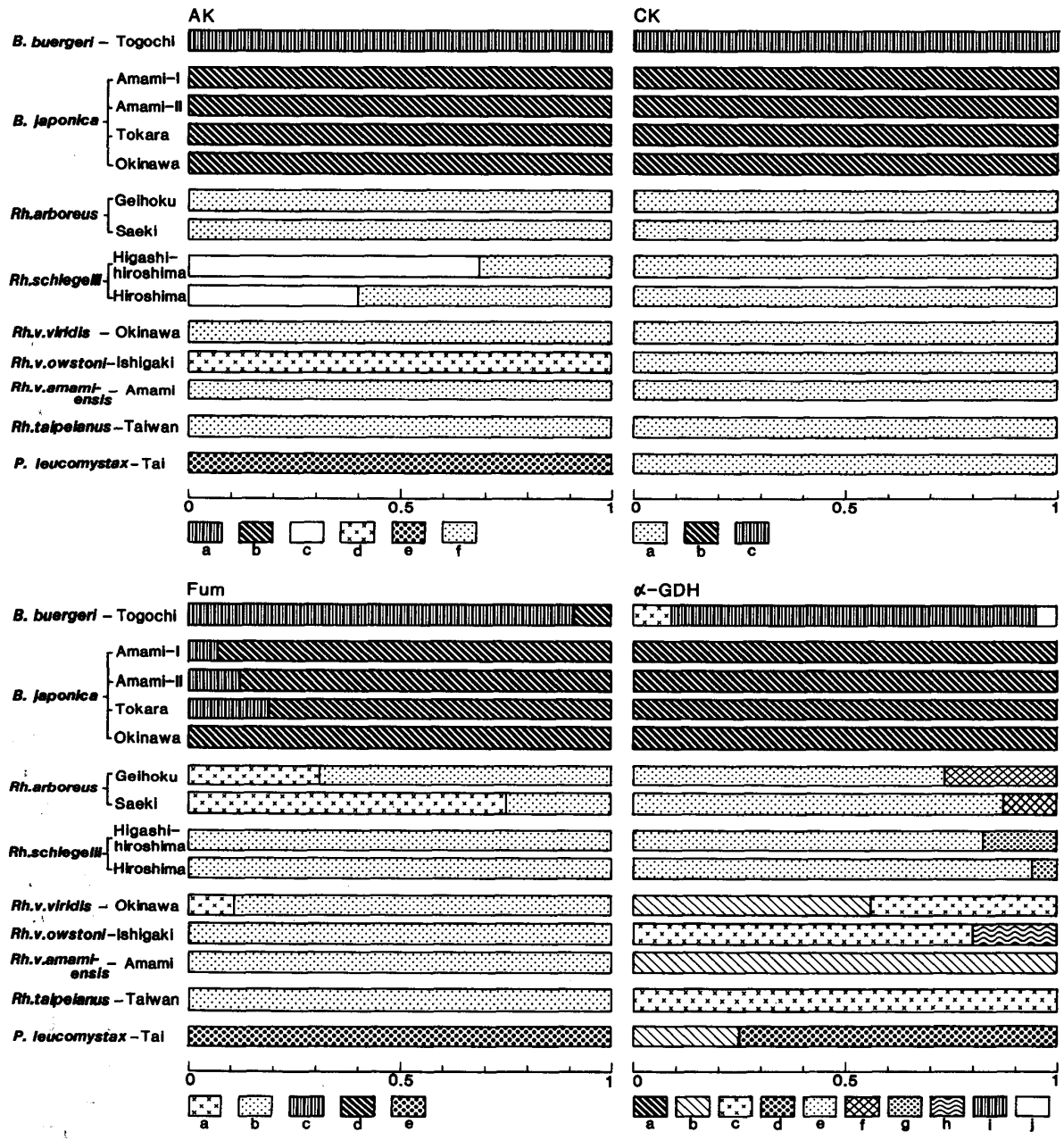

Fig. 6. Gene frequencies at four loci, AK, CK, Fum and α–GDH, in 14 populations of *Buergeria, Rhacophorus* and *Polypedates*.

5. Creatine kinase (CK)

The electrophoretic patterns of the 257 frogs of the 14 populations indicated that there were three phenotypes produced by three alleles, *a, b* and *c,* at the CK locus. All of the 40 frogs of *B. buergeri* and the 120 frogs of the four populations of *B. japonica* showed homozygous bands, CC and BB, respectively. A total of 97 frogs including 42 *Rh. arboreus,* 37 *Rh. schlegelii,* nine *Rh. v. viridis,* five *Rh. v. owstoni,* one *Rh. v. amamiensis,* one *Rh. taipeianus* and two *P. leucomystax* showed a homozygous band, AA (Table 2; Fig. 6).

6. Fumarase (Fum)

The electrophoretic patterns of the 257 frogs of the 14 populations indicated that there were seven phenotypes produced by five alleles, *a~e,* at the Fum locus. Of the 40 frogs of *B. buergeri,* 33 and seven showed homozygous and heterozygous bands, CC and CD, respectively. Alleles *c* and *d* were 0.913 and 0.088 in frequency, respectively. In *B. japonica,* 26 and four of the 30 frogs of the Amami-I population showed homozygous and heterozygous bands, DD and CD, respectively. Alleles *d* and *c* were 0.933 and 0.067 in frequency, respectively. Of the 30 frogs of the Amami-II population, 24 and one showed homozygous bands, DD and CC, respectively, and the remaining five showed a heterozygous band, CD. Alleles *d* and *c* were 0.883 and 0.117 in frequency, respectively. Of the 58 frogs of the Tokara population, 38 and two showed homozygous bands, DD and CC, respectively, and 18 showed a heterozygous band, CD. Alleles *d* and *c* were 0.810 and 0.190 in frequency, respectively. Two frogs of the Okinawa population showed a homozygous band, DD. In a total of 120 frogs of these four populations of *B. japonica,* alleles *d* and *c* were 0.863 and 0.138 in frequency, respectively. In *Rh. arboreus,* four and 17 of the 34 frogs of the Geihoku population showed homozygous bands, AA and BB, respectively, and the remaining 13 showed a heterozygous band, AB. Alleles *a* and *b* were 0.309 and 0.691 in frequency, respectively. Of the eight frogs of the Saeki population, four and four were homozygous and heterozygous bands, AA and AB, respectively. Alleles *a* and *b* were 0.750 and 0.250 in frequency, respectively. In a total of 42 frogs of *Rh. arboreus,* alleles *a* and *b* were 0.393 and 0.607 in frequency, respectively. All of the 44 frogs including 37 frogs of the two populations of *Rh. schlegelii,* five *Rh. v. owstoni,* one *Rh. v. amamiensis* and one *Rh. taipeianus* showed a single homozygous band, BB. Of nine *Rh. v. viridis,* seven and two showed homozygous and heterozygous bands, BB and AB, respectively. Alleles *b* and *a* were 0.889 and 0.111 in frequency, respectively. Two *P. leucomystax* showed a homozygous band, EE (Table 2; Fig. 6).

7. α–Glycerophosphate dehydrogenase (α–GDH)

The electrophoretic patterns of the 257 frogs of the 14 populations indicated that there were 14 phenotypes produced by 10 alleles, *a~j,* at the α–GDH locus. Of the 40 frogs of *B. buergeri,* 29 showed a homozygous band, II, while seven and four

showed heterozygous bands, CI and IJ, respectively. Alleles *i*, *c* and *j* were 0.863, 0.088 and 0.050 in frequency, respectively. A total of 120 frogs of the four populations of *B. japonica* showed a homozygous band, AA. In *Rh. arboreus,* 19 and three of the 34 frogs of the Geihoku population showed homozygous bands, EE and FF, respectively, and the remaining 12 showed a heterozygous band, EF. Alleles *e* and *f* were 0.735 and 0.265 in frequency, respectively. Of the eight of the Saeki population, six and two showed homozygous and heterozygous bands, EE and EF, respectively. Alleles *e* and *f* were 0.875 and 0.125 in frequency, respectively. In *Rh. schlegelii,* 13 and seven of the 20 frogs of the Higashihiroshima population showed homozygous and heterozygous bands, EE and EG, respectively. Alleles *e* and *g* were 0.825 and 0.175 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 15 and two showed homozygous and heterozygous bands, EE and EG, respectively. Alleles *e* and *g* were 0.941 and 0.059 in frequency, respectively. In *Rh. v. viridis,* four and three of the nine frogs of the Okinawa population showed homozygous bands, BB and CC, respectively, and the remaining two showed a heterozygous band, BC. Alleles *b* and *c* were 0.556 and 0.444 in frequency, respectively. Of five *Rh. v. owstoni,* three and two showed homozygous and heterozygous bands, CC and CH, respectively. Alleles *c* and *h* were 0.800 and 0.200 in frequency, respectively. One *Rh. v. amamiensis* showed a homozygous band, BB, one *Rh. taipeianus* showed a homozygous band, CC, and two *P. leucomystax* showed homozygous and heterozygous bands, DD and BD (Table 2; Fig. 6).

8. Glucose phosphate isomerase (GPI)

The electrophoretic patterns of the 257 frogs of the 14 populations indicated that there were 12 phenotypes produced by six alleles, *a*~*f,* at the GPI locus. In *B. buergeri,* 39 and one of the 40 frogs showed homozygous and heterozygous bands, DD and DF, respectively. Alleles *d* and *f* were 0.988 and 0.013 in frequency, respectively. In *B. japonica,* all of the 90 frogs of three populations, the Amami-I, Tokara and Okinawa populations, showed a homozygous band, BB, while 23 and one of the 30 frogs of the Amami-II population showed homozygous bands, BB and DD, respectively, and the remaining six showed a heterozygous band, BD. Alleles *b* and *d* were 0.867 and 0.133 in frequency, respectively. In *Rh. arboreus,* 30 and one of the 34 frogs of the Geihoku population showed homozygous bands, CC and DD, respectively, and the remaining three showed a heterozygous band, CD. Alleles *c* and *d* were 0.926 and 0.074 in frequency, respectively. All of the eight frogs of the Saeki population showed a homozygous band, CC. In *Rh. schlegelii,* 10 and three of the 20 frogs of the Higashihiroshima population showed homozygous bands, CC and EE, respectively, and the remaining seven showed a heterozygous band, CE. Alleles *c* and *e* were 0.675 and 0.325 in frequency, respectively. Of the 17 frogs of the Hiroshima population, nine and one showed homozygous bands, CC and FF, respectively, and two, three and two of the remaining seven showed heterozygous bands, CD, CE and CF, respectively. Alleles *c, d, e* and *f* were 0.735, 0.059, 0.088 and 0.118 in frequency, respectively.

All of the 13 frogs including nine *Rh. v. viridis,* one *Rh. v. amamiensis,* one *Rh. taipeianus* and two *P. leucomystax* showed a homozygous band, CC. Of five *Rh. v. owstoni,* one and one showed homozygous bands, BB and EE, respectively, and one and two of the remaining three showed heterozygous bands, AE and BE, respectively. Alleles *b*, *e* and *a* were 0.400, 0.500 and 0.100 in frequency, respectively (Table 2; Fig. 8).

9. Isocitrate dehydrogenase (IDH)

The electrophoretic patterns of the 257 frogs of the 14 populations showed that IDH was controlled by two loci, IDH-A and IDH-B. At IDH-A locus, there were six phenotypes produced by five alleles, *a*~*e*. All of the 40 frogs of *B. buergeri* showed a homozygous band, DD, while 120 frogs of the four populations of *B. japonica* showed a homozygous band, EE. In *Rhacophorus* and *Polypedates,* a total of 11 frogs, including nine *Rh. v. viridis,* one *Rh. v. amamiensis* and one *Rh. taipeianus,* showed a homozygous band, BB, while seven frogs, including five *Rh. v. owstoni* and two *P. leucomystax,* showed a homozygous band, CC. In *Rh. arboreus,* 33 and one of the 34 frogs of the Geihoku population showed homozygous and heterozygous bands, BB and AB, respectively. Alleles *b* and *a* were 0.985 and 0.015 in frequency, respectively. All of the eight frogs of the Saeki population showed a homozygous band, BB. In *Rh. schlegelii,* 18 and two of the 20 frogs of the Higashihiroshima population showed homozygous and heterozygous bands, BB and BC, respectively. Alleles *b* and *c* were 0.950 and 0.050 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 16 and one showed homozygous and heterozygous bands, BB and BC, respectively. Alleles *b* and *c* were 0.971 and 0.029 in frequency, respectively (Table 2; Fig. 7).

At the IDH-B locus, there were 14 phenotypes produced by 12 alleles, *a*~*l*. In *B. buergeri,* 12 and eight of the 40 frogs of the Togochi population showed homozygous bands, KK and LL, respectively, and the remaining 20 showed a heterozygous band, KL. Alleles *k* and *l* were 0.550 and 0.450 in frequency, respectively. In *B. japonica,* 26 of the 30 frogs of the Amami-I population showed a homozygous band, DD, and two and two of the remaining four showed heterozygous bands, CD and DI, respectively. Alleles *d*, *c* and *i* were 0.933, 0.033 and 0.033 in frequency, respectively. Of the 30 frogs of the Amami-II population, 27 and three showed homozygous and heterozygous bands, DD and DI, respectively. Alleles *d* and *i* were 0.950 and 0.050 in frequency, respectively. All of the 58 frogs of the Tokara population showed a homozygous band, DD, while two frogs of the Okinawa population showed a homozygous band, EE. In *Rh. arboreus,* 29 and five of the 34 frogs of the Geihoku population showed homozygous and heterozygous bands, HH and CH, respectively. Alleles *h* and *c* were 0.926 and 0.074 in frequency, respectively. Of the eight frogs of the Saeki population, seven and one showed homozygous and heterozygous bands, HH and CH, respectively. Alleles *h* and *c* were 0.938 and 0.063 in frequency, respectively. In *Rh. schlegelii,* 17 and three of the 20 frogs of the Higashihiroshima population showed homozygous and heterozygous bands, HH and HJ, respectively. Alleles *h*

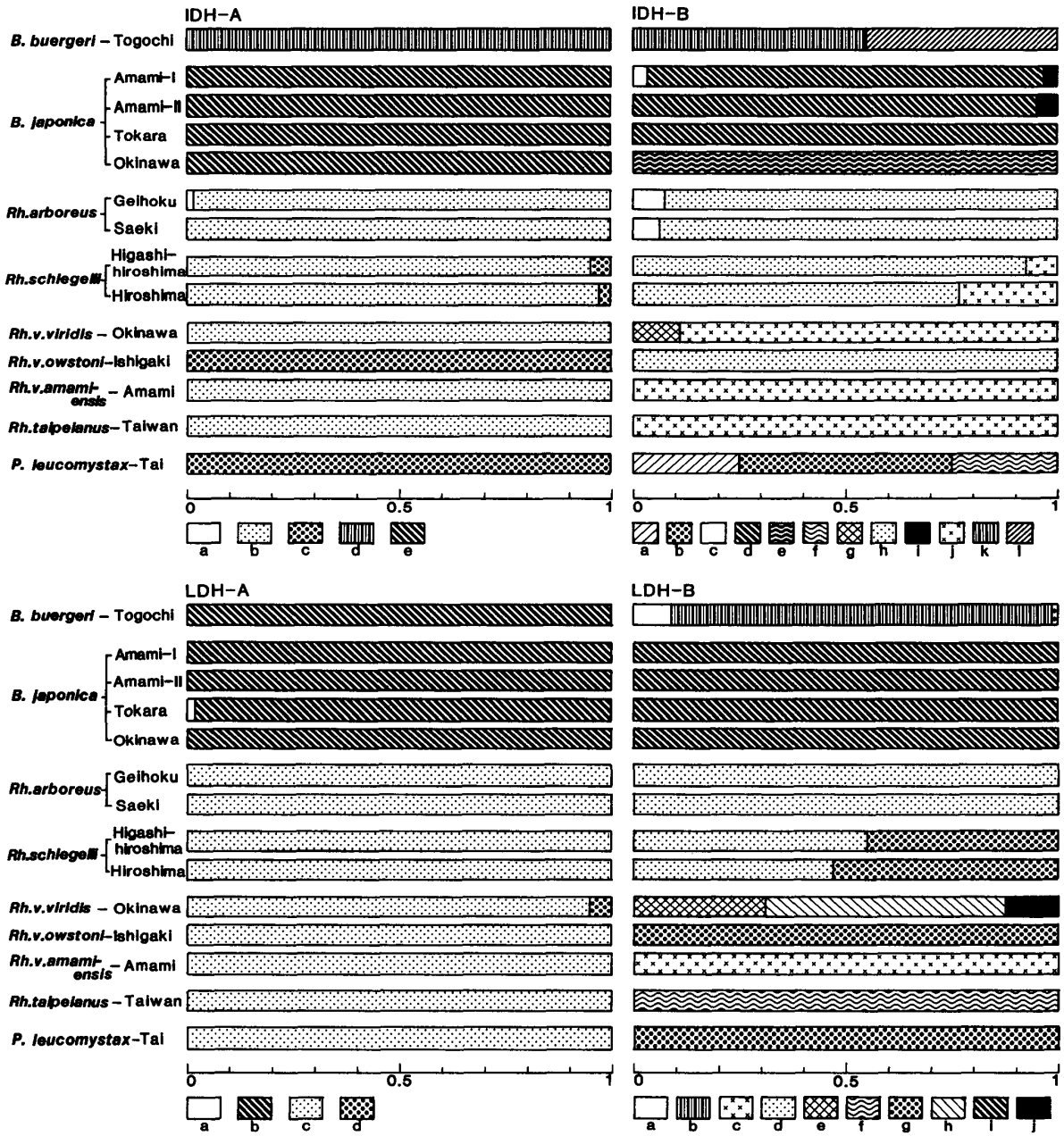

Fig. 7. Gene frequencies at four loci, IDH-A, IDH-B, LDH-A and LDH-B, in 14 populations of *Buergeria, Rhacophorus* and *Polypedates*.

and *j* were 0.925 and 0.075 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 10 and one showed homozygous bands, HH and JJ, respectively, and the remaining six showed a heterozygous band, HJ. Alleles *h* and *j* were 0.765 and 0.235 in frequency, respectively. In *Rh. v. viridis,* seven and two of the nine frogs showed homozygous and heterozygous bands, JJ and GJ, respectively. Alleles *j* and *g* were 0.889 and 0.111 in frequency, respectively. While one *Rh. v. amamiensis* showed a homozygous band, JJ, five *Rh. v. owstoni* showed a homozygous band, HH, and one *Rh. taipeianus* showed a homozygous

band, JJ. Of two *P. leucomystax,* one and one showed heterozygous bands, AB and BF, respectively. Alleles *b, a* and *f* were 0.500, 0.250 and 0.250 in frequency, respectively (Table 2; Fig. 7).

10. Lactate dehydrogenase (LDH)

The electrophoretic patterns of the 257 frogs of the 14 populations showed that there were two loci, LDH-A and LDH-B, controlling LDH. At the LDH-A locus, four phenotypes produced by four alleles, *a*~*d,* were observed. A total of 158 frogs including 40 of *B. buergeri* and 118 of the four populations of *B. japonica* showed a homozygous band, BB, except two frogs of the Tokara population which showed a heterozygous band, AB. In the Tokara population, alleles *b* and *a* were 0.983 and 0.017 in frequency, respectively. In *Rhacophorus* and *Polypedates,* a total of 96 frogs, including 42 of the two populations of *Rh. arboreus,* 37 of the two populations of *Rh. schlegelii,* eight of nine *Rh. v. viridis,* five *Rh. v. owstoni,* one *Rh. v. amamiensis,* one *Rh. taipeianus* and two *P. leucomystax,* showed a homozygous band, CC, except one *Rh. v. viridis* which showed a heterozygous band, CD. Alleles *c* and *d* in this subspecies were 0.944 and 0.056 in frequency, respectively (Table 2; Fig. 7).

At the LDH-B locus, there were 12 phenotypes produced by 10 alleles, *a*~*j.* In *B. buergeri,* 32 of the 40 frogs showed a homozygous band, BB, and seven and one of the other eight showed heterozygous bands, AB and BG, respectively. Alleles *b, a* and *g* were 0.900, 0.088 and 0.013 in frequency, respectively. A total of 120 frogs of the four populations of *B. japonica* showed a homozygous band, II. A total of 42 frogs of the two populations of *Rh. arboreus* showed a homozygous band, DD. In *Rh. schlegelii,* eight and six of the 20 frogs of the Higashihiroshima population showed homozygous bands, DD and GG, respectively, and the remaining six showed a heterozygous band, DG. Alleles *d* and *g* were 0.550 and 0.450 in frequency, respectively. In the Hiroshima population, three and four of the 17 frogs showed homozygous bands, DD and GG, respectively, and the remaining 10 showed a heterozygous band, DG. Alleles *d* and *g* were 0.471 and 0.529 in frequency, respectively. All of the five *Rh. v. owstoni* showed a homozygous band, GG, one *Rh. v. amamiensis* showed a homozygous band, CC, one *Rh. taipeianus* showed a homozygous band, FF, and two *P. leucomystax* showed a homozygous band, GG. In *Rh. v. viridis,* three of the eight frogs showed a homozygous band, HH, and three and two of the other five showed heterozygous bands, EH and EJ, respectively. Alleles *h, e* and *j* were 0.563, 0.313 and 0.125 in frequency, respectively (Table 2; Fig. 7).

11. Malate dehydrogenase (MDH)

The electrophoretic patterns of MDH in the 257 frogs of the 14 populations indicated that there were two loci, MDH-A and MDH-B, controlling MDH. At the MDH-A locus, six phenotypes produced by five alleles, *a*~*e,* were observed. In *B. buergeri* which belonged to the Togochi population, 38 of the 40 frogs showed a homozygous band, DD, and the other two showed a heterozygous band, BD.

Alleles *d* and *b* were 0.975 and 0.025 in frequency, respectively. In *B. japonica,* all of the 90 frogs belonging to three of the four populations showed a homozygous band, BB, while 28 and two of the 30 frogs belonging to the Amami-II population showed homozygous and heterozygous bands, BB and AB, respectively. In the Amami-II population, alleles *b* and *a* were 0.967 and 0.033 in frequency, respectively. In *Rh. schlegelii,* all of the 37 frogs of the two populations showed a homozygous band, EE, produced by allele *e.* All of the 42 frogs of the two populations in *Rh. arboreus,* 15 frogs belonging to the three subspecies of *Rh. viridis,* one *Rh. taipeianus* and two *P. leucomystax* showed a homozygous band, CC (Table 2; Fig. 8).

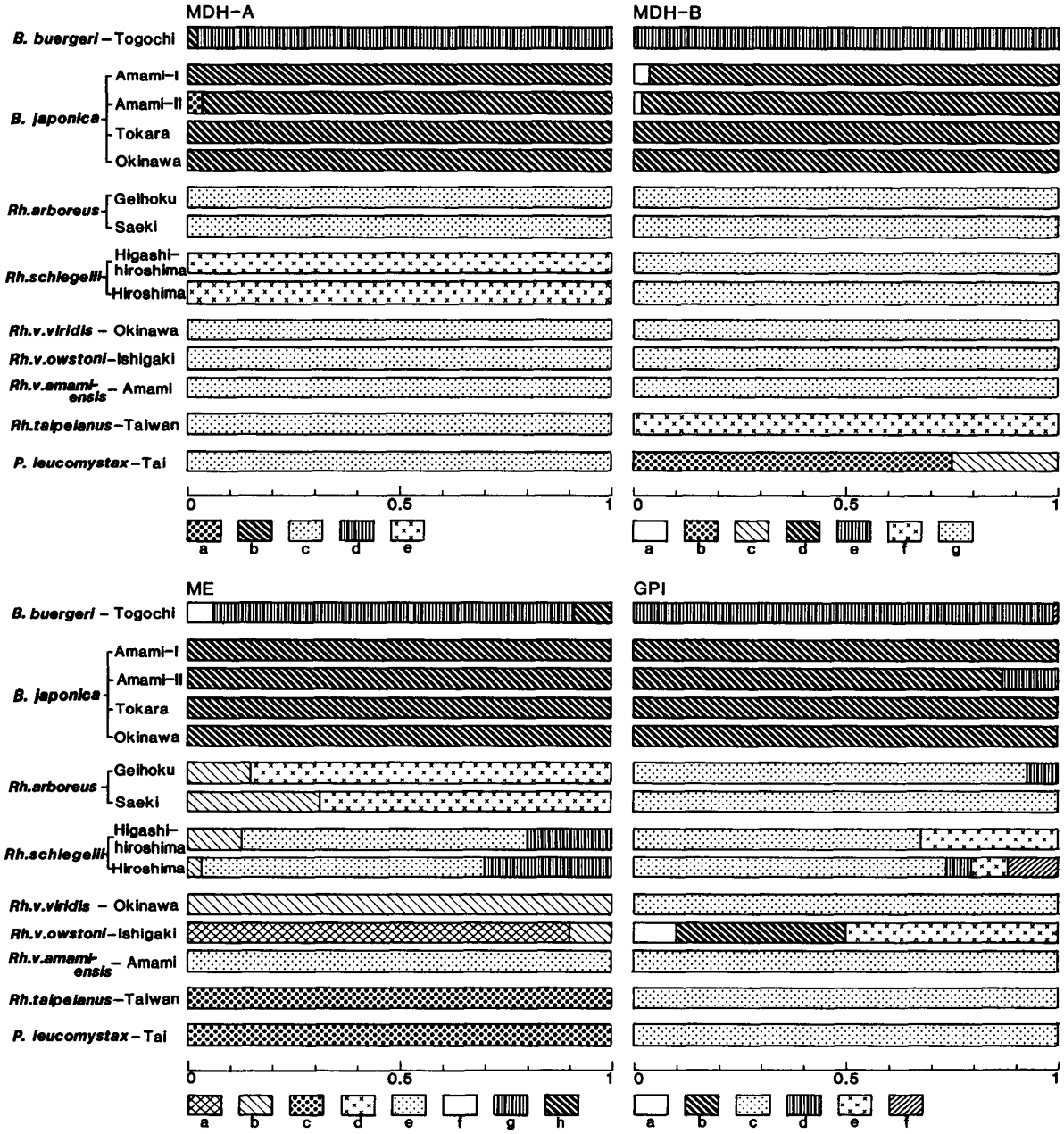

Fig. 8. Gene frequencies at four loci, MDH-A, MDH-B, ME and GPI, in 14 populations of *Buergeria, Rhacophorus* and *Polypedates.*

At the MDH-B locus, seven phenotypes produced by seven alleles, *a*~*g*, were observed. In *B. buergeri*, all of the 40 frogs showed a homozygous band, EE. In *B. japonica*, 28 and two of the 30 frogs of the Amami-I population showed homozygous and heterozygous bands, DD and AD, respectively. Alleles *d* and *a* were 0.967 and 0.033 in frequency, respectively. Of the 30 frogs of the Amami-II population, 29 and one showed homozygous and heterozygous bands, DD and AD, respectively. Alleles *d* and *a* were 0.983 and 0.017 in frequency, respectively. All of the 60 frogs of the remaining two populations showed a homozygous band, DD. All of the 42 frogs of the two populations in *Rh. arboreus*, 37 frogs of the two populations in *Rh. schlegelii* and 15 frogs in the three subspecies of *Rh. viridis* showed a homozygous band, GG. One frog of *Rh. taipeianus* showed a homozygous band, FF. One and one of the two *P. leucomystax* showed homozygous and heterozygous bands, BB and BC, respectively. Alleles *b* and *c* were 0.750 and 0.250 in frequency, respectively (Table 2; Fig. 8).

12. Malic enzyme (ME)

The electrophoretic patterns of ME in the 255 frogs of the 14 populations indicated that there were 15 phenotypes produced by eight alleles, *a*~*h*. In *B. buergeri*, 29 and one of the 40 frogs showed homozygous bands, GG and FF, respectively, and three and seven of the other 10 showed heterozygous bands, FG and GH, respectively. Alleles *g*, *f* and *h* were 0.850, 0.063 and 0.088 in frequency, respectively. All of the 120 frogs of the four populations in *B. japonica* showed a homozygous band, HH. In *Rh. arboreus*, 24 and 10 of the 34 frogs of the Geihoku population showed homozygous and heterozygous bands, DD and BD, respectively. Alleles *d* and *b* were 0.853 and 0.147 in frequency, respectively. Of the eight frogs of the Saeki population, three and five showed homozygous and heterozygous bands, DD and BD, respectively. Alleles *d* and *b* were 0.688 and 0.313 in frequency, respectively. In *Rh. schlegelii*, nine and one of the 20 frogs of the Higashihiroshima population showed homozygous bands, EE and GG, respectively, and four, one and five of the other 10 frogs showed heterozygous bands, BE, BG and EG, respectively. Alleles *e*, *g* and *b* were 0.675, 0.200 and 0.125 in frequency, respectively. Of the 15 frogs of the Hiroshima population, six and one showed homozygous bands, EE and GG, respectively, and one and seven of the remaining eight frogs showed heterozygous bands, BE and EG, respectively. Alleles *e*, *g* and *b* were 0.667, 0.300 and 0.033 in frequency, respectively. All of the nine *Rh. v. viridis* showed a homozygous band, BB. One *Rh. v. amamiensis* showed a homozygous band, EE. Of the five *Rh. v. owstoni*, four showed a homozygous band, AA, and the remainder showed a heterozygous band, AB. Alleles *a* and *b* were 0.900 and 0.100 in frequency, respectively. One *Rh. taipeianus* and two *P. leucomystax* showed a homozygous band, CC (Table 2; Fig. 8).

13. Mannose phosphate isomerase (MPI)

The electrophoretic patterns of MPI in the 257 frogs of the 14 populations indicated that there were 33 phenotypes produced by 17 alleles, *a*~*q*. In *B.*

buergeri, three, seven, three and two of the 40 frogs showed homozygous bands, JJ, MM, NN and OO, respectively, and the other 25 frogs showed various heterozygous bands. Each of the heterozygous bands, JM, KM, MO and NO, was shown by three frogs, each of heterozygous bands, JN, MN and MP, was shown by two frogs, and each of heterozygous bands, IN, JO, KN, KO, MQ, NP and NQ, was shown by one frog. Alleles *m, n, j* and *o* were 0.350, 0.213, 0.150 and 0.150 in frequency, respectively, while the other *k, p, q* and *i* were 0.063, 0.038, 0.025 and 0.013 in frequency, respectively. In *B. japonica,* three and 11 of the 30 frogs of the Amami-I population showed homozygous bands, HH and JJ, respectively, and three and 13 of the other 16 frogs showed heterozygous bands, HN and HJ, respectively. Alleles *j, h* and *n* were 0.583, 0.367 and 0.050 in frequency, respectively. Of the 30 frogs of the Amami-II population, five, nine and one showed homozygous bands, HH, JJ and NN, respectively, and one, three,

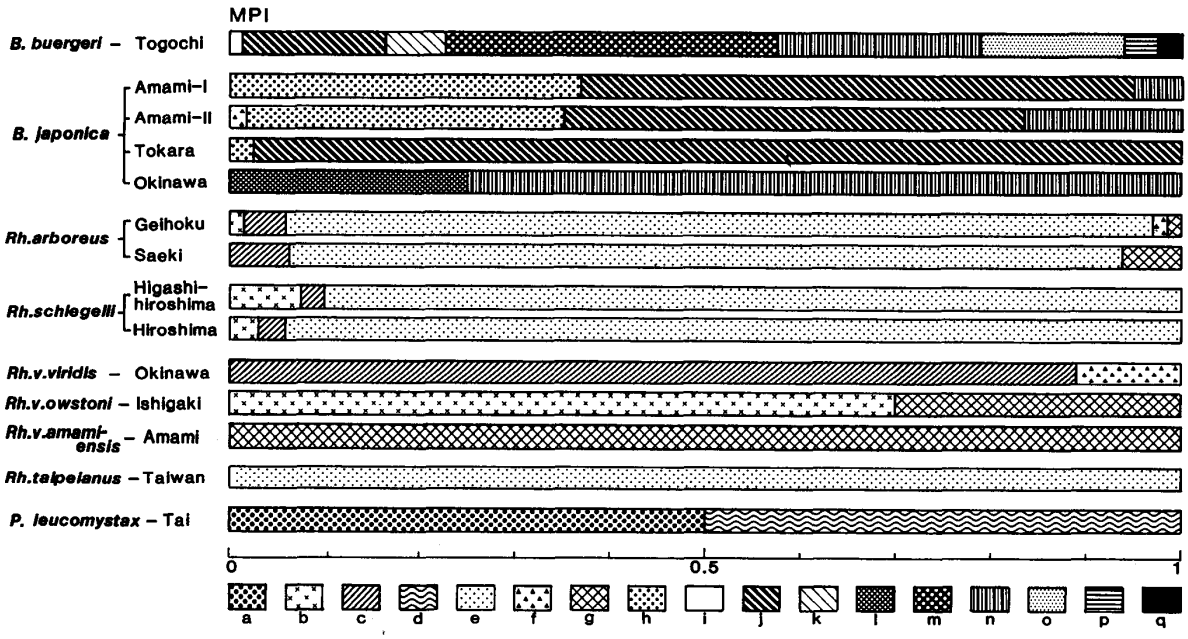

Fig. 9. Gene frequencies at the MPI locus in 14 populations of *Buergeria, Rhacophorus* and *Polypedates.*

six and five of the other 15 frogs showed heterozygous bands, FH, HN, HJ and JN, respectively. Alleles *j, h, n* and *f* were 0.483, 0.333, 0.167 and 0.017 in frequency, respectively. Of the 58 frogs of the Tokara population, 55 showed a homozygous band, JJ, and the other three showed a heterozygous band, HJ. Alleles *j* and *h* were 0.974 and 0.026 in frequency, respectively. Of the two frogs of the Okinawa population, one and one showed homozygous and heterozygous bands, NN and LN, respectively. Alleles *n* and *l* were 0.750 and 0.250 in frequency, respectively (Table 2; Fig. 9).

In *Rh. arboreus,* 28 of the 34 frogs of the Geihoku population showed a homozygous band, EE, and three, one, one and one showed heterozygous bands, CE, BE, EF and EG, respectively. Alleles *e, b, c, f* and *g* were 0.912, 0.015, 0.044, 0.015 and 0.015 in frequency, respectively. Six of the eight frogs of the Saeki

population showed a homozygous band, EE, and the other two showed heterozygous bands, CE and EG. Alleles *e*, *c* and *g* were 0.875, 0.063 and 0.063 in frequency, respectively. In *Rh. schlegelii*, one and 17 of the 20 frogs of the Higashihiroshima population showed homozygous bands, BB and EE, respectively, and the other two showed heterozygous bands, BE and CE. Alleles *e*, *b* and *c* were 0.900, 0.075 and 0.025 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 15 showed a homozygous band, EE, and the other two showed heterozygous bands, BE and CE. Alleles *e*, *b* and *c* were 0.941, 0.029 and 0.029 in frequency, respectively. Of the nine *Rh. v. viridis*, seven showed a homozygous band, CC, and the other two showed a heterozygous band, CF. Alleles *c* and *f* were 0.889 and 0.111 in frequency, respectively. Two and three of the five *Rh. v. owstoni* showed homozygous and heterozygous bands, BB and BG, respectively. Alleles *b* and *g* were 0.700 and 0.300 in frequency, respectively. One *Rh. v. amamiensis* and one *Rh. taipeianus* showed homozygous bands, GG and EE, respectively. Two *P. leucomystax* showed a heterozygous band, AD. Each of alleles *a* and *d* was 0.500 in frequency (Table 2; Fig. 9).

14. Peptidase (Pep)

The electrophoretic patterns of Pep in the 257 frogs of the 14 populations indicated that there were several loci controlling Pep. However, two loci, Pep-B and Pep-D, could be analyzed. At the Pep-B locus, 17 phenotypes produced by nine alleles, *a*~*i*, were observed. In *B. buergeri*, 37 and three of 40 frogs showed homozygous and heterozygous bands, HH and HI, respectively. Alleles *h* and *i* were 0.963 and 0.038, respectively. Of the 30 frogs of the Amami-I population in *B. japonica*, one, two and six showed homozygous bands, EE, GG and HH, respectively, and 10, four and seven of the remaining 21 frogs showed heterozygous bands, EG, EH and GH, respectively. Alleles *e*, *g* and *h* were 0.267, 0.350 and 0.383 in frequency, respectively. Of the 30 frogs of the Amami-II population, four, three and two showed homozygous bands, EE, GG and HH, respectively, and nine, seven and five of the remaining 21 frogs showed heterozygous bands, EG, EH and GH, respectively. Alleles *e*, *g* and *h* were 0.400, 0.333 and 0.267 in frequency, respectively. Of the 58 frogs of the Tokara population, 56 and two showed homozygous and heterozygous bands, EE and EG, respectively. Alleles *e* and *g* were 0.983 and 0.017 in frequency, respectively. Two frogs of the Okinawa population showed a homozygous band, GG (Table 2; Fig. 10).

In *Rh. arboreus*, 28 and one of the 34 frogs of the Geihoku population showed homozygous bands, DD and FF, respectively, and the remaining five showed a heterozygous band, DF. Alleles *d* and *f* were 0.897 and 0.103 in frequency, respectively. Of the eight frogs of the Saeki population, seven showed a homozygous band, DD, and the remainder showed a heterozygous band, DF. Alleles *d* and *f* were 0.938 and 0.063 in frequency, respectively. In *Rh. schlegelii*, one, four and four of the 20 frogs of the Higashihiroshima population showed homozygous bands, AA, BB and DD, respectively, and five, four, one and one of the remaining 11 frogs showed heterozygous bands, AD, BD, BG and DG,

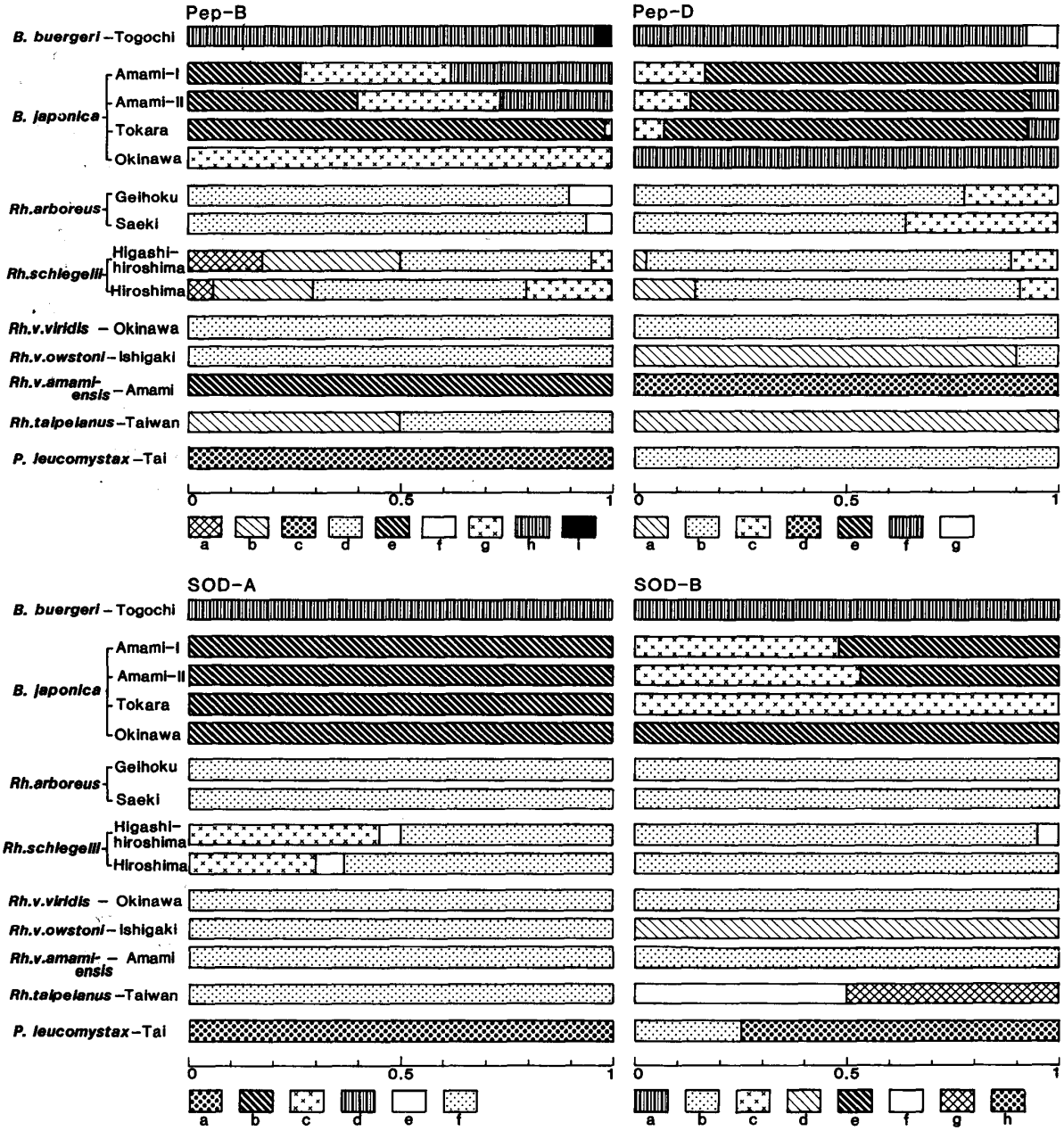

Fig. 10. Gene frequencies at four loci, Pep-B, Pep-D, SOD-A and SOD-B, in 14 populations of *Buergeria, Rhacophorus* and *Polypedates*.

respectively. Alleles *a, b, d* and *g* were 0.175, 0.325, 0.450 and 0.050 in frequency, respectively. One, five and one of the 17 frogs of the Hiroshima population showed homozygous bands, BB, DD and GG, respectively, and two, three, three and two of the other 10 frogs showed heterozygous bands, AD, BD, BG and DG, respectively. Alleles *a, b, d* and *g* were 0.059, 0.235, 0.500 and 0.206 in frequency, respectively. Nine *Rh. v. viridis* and five *Rh. v. owstoni* showed a homozygous band, DD. One *Rh. v. amamiensis* showed a homozygous band, EE, while two *P. leucomystax* showed a homozygous band, CC. One *Rh. taipeianus* showed a heterozygous band, BD (Table 2; Fig. 10).

At the Pep-D locus, there were 12 phenotypes produced by seven alleles, *a*~*g*.

Of the 40 *B. buergeri,* 35 and one showed homozygous bands, FF and GG, respectively, and the remaining four showed a heterozygous band, FG. Alleles *f* and *g* were 0.925 and 0.075 in frequency, respectively. In *B. japonica,* 20 and one of the 30 frogs of the Amami-I population showed homozygous bands, EE and CC, respectively, and six, two and one of the other nine frogs showed heterozygous bands, CE, CF and EF, respectively. Alleles *e, c* and *f* were 0.783, 0.167 and 0.050 in frequency, respectively. Of the 30 frogs of the Amami-II population, 21 showed a homozygous band, EE, and five, three and one of the other nine frogs showed heterozygous bands, CE, CF and EF, respectively. Alleles *e, c* and *f* were 0.800, 0.133 and 0.067 in frequency, respectively. Of the 58 frogs of the Tokara population, 44 showed a homozygous band, EE, and six, two and six of the other 14 frogs showed heterozygous bands, CE, CF and EF, respectively. Alleles *e, c* and *f* were 0.862, 0.069 and 0.069 in frequency, respectively. Two frogs of the Okinawa population showed a homozygous band, FF (Table 2; Fig. 10).

In *Rh. arboreus,* 21 and two of the 34 frogs of the Geihoku population showed homozygous bands, BB and CC, respectively, and the remaining 11 showed a heterozygous band, BC. Alleles *b* and *c* were 0.779 and 0.221 in frequency, respectively. Of the seven frogs of the Saeki population, two and five showed homozygous and heterozygous bands, BB and BC, respectively. Alleles *b* and *c* were 0.643 and 0.357 in frequency, respectively. In *Rh. schlegelii,* 13 of the 18 frogs of the Higashihiroshima population showed a homozygous band, BB, and one and four of the other five frogs showed heterozygous bands, AB and BC, respectively. Alleles *b, a* and *c* were 0.861, 0.028 and 0.111 in frequency, respectively. Of the 17 frogs of the Hiroshima population, nine showed a homozygous band, BB, and five and three of the other eight frogs showed heterozygous bands, AB and BC, respectively. Alleles *b, a* and *c* were 0.765, 0.147 and 0.088 in frequency, respectively. Nine *Rh. v. viridis* and two *P. leucomystax* showed a homozygous band, BB, and one *Rh. v. amamiensis* and one *Rh. taipeianus* showed homozygous bands, DD and AA, respectively. Of the five *Rh. v. owstoni,* four and one showed homozygous and heterozygous bands, AA and AB, respectively. Alleles *a* and *b* were 0.900 and 0.100 in frequency, respectively (Table 2; Fig. 10).

15. Phosphoglucomutase (PGM)

The electrophoretic patterns of PGM in the 257 frogs of the 14 populations indicated that there were 14 phenotypes produced by nine alleles, *a*~*i,* at the PGM locus. In *B. buergeri,* 37 of the 40 frogs showed a homozygous band, FF, and the remaining three showed heterozygous bands, FG, FH and FI. Alleles *f, g, h* and *i* were 0.963, 0.013, 0.013 and 0.013 in frequency, respectively. In *B. japonica,* 28 and two of the 30 frogs of the Amami-I population showed homozygous and heterozygous bands, DD and CD, respectively. Alleles *d* and *c* were 0.967 and 0.033 in frequency, respectively. Of the 30 frogs of the Amami-II population, 25 and one showed homozygous bands, DD and CC, respectively, and three and one of the other four frogs showed heterozygous bands, CD and DG, respectively.

Alleles *d*, *c* and *g* were 0.900, 0.083 and 0.017 in frequency, respectively. All of the 58 frogs of the Tokara population and two frogs of the Okinawa population showed a homozygous band, DD (Table 2; Fig. 11).

In *Rh. arboreus*, 29 and two of the 34 frogs of the Geihoku population showed homozygous bands, AA and BB, respectively, and the remaining three showed a heterozygous band, AB. Alleles *a* and *b* were 0.897 and 0.103 in frequency, respectively. Of the eight frogs of the Saeki population, six and two showed homozygous and heterozygous bands, AA and AB, respectively. Alleles *a* and *b* were 0.875 and 0.125 in frequency, respectively. In *Rh. schlegelii*, 13 of the 20 frogs of the Higashihiroshima population showed a homozygous band, CC, and the other seven showed a heterozygous band, AC. Alleles *c* and *a* were 0.825 and 0.175 in frequency, respectively. Of the 17 frogs of the Hiroshima population, three and four showed homozygous bands, AA and CC, respectively, and five and five of the other 10 frogs showed heterozygous bands, AC and CE, respectively. Alleles *c*, *a* and *e* were 0.529, 0.324 and 0.147 in frequency, respectively. Nine *Rh. v. viridis*, one *Rh. v. amamiensis* and one *Rh. taipeianus* all showed a homozygous band, AA, produced by allele *a*, while five *Rh. v. owstoni* showed a homozygous band, BB. Two *P. leucomystax* showed homozygous and heterozygous bands, BB and BD (Table 2; Fig. 11).

16. Superoxide dismutase (SOD)

The electrophoretic patterns of SOD in the 257 frogs of the 14 populations indicated that there were two loci, SOD-A and SOD-B, controlling SOD. At the SOD-A locus, 245 frogs analyzed showed seven phenotypes produced by six alleles, *a*~*f*. All of the 40 frogs of *B. buergeri* showed a homozygous band, DD, produced by allele *d*, while all of the 120 frogs of the four populations in *B. japonica* showed a homozygous band, BB, produced by allele *b*. All of the 58 frogs including 42 frogs of the two populations of *Rh. arboreus*, nine *Rh. v. viridis*, five *Rh. v. owstoni*, one *Rh. v. amamiensis* and one *Rh. taipeianus* showed a homozygous band, FF. In *Rh. schlegelii*, two and two of the 10 frogs of the Higashihiroshima population showed homozygous bands, CC and FF, respectively, and five and one of the other six frogs showed heterozygous bands, CF and EF, respectively. Alleles *c*, *e* and *f* were 0.450, 0.050 and 0.500 in frequency, respectively. Of the 15 frogs of the Hiroshima population, one and five showed homozygous bands, CC and FF, respectively, and seven and two of the other nine frogs showed heterozygous bands, CF and EF, respectively. Alleles *c*, *e* and *f* were 0.300, 0.067 and 0.633 in frequency, respectively. Two *P. leucomystax* showed a homozygous band, AA (Table 2; Fig. 10).

At the SOD-B locus, 257 frogs analyzed showed 10 phenotypes produced by eight alleles, *a*~*h*. In *B. buergeri*, all of the 40 frogs showed a homozygous band, AA, produced by allele *a*. In *B. japonica*, nine and 10 of the 30 frogs of the Amami-I population showed homozygous bands, CC and EE, respectively, and the remaining 11 frogs showed a heterozygous band, CE. Alleles *c* and *e* were 0.483 and 0.517 in frequency, respectively. Of the 30 frogs of the Amami-II

population, 10 and eight showed homozygous bands, CC and EE, respectively, and the remaining 12 showed a heterozygous band, CE. Alleles *c* and *e* were 0.533 and 0.467 in frequency, respectively. All of the 58 frogs of the Tokara population showed a homozygous band, CC, while two frogs of the Okinawa population showed a homozygous band, EE. All of the 42 frogs of the two populations in *Rh. arboreus* showed a homozygous band, BB. In *Rh. schlegelii,* 18 and two of the 20 frogs of the Higashihiroshima population showed homozygous and heterozygous bands, BB and BF, respectively. Alleles *b* and *f* were 0.950 and 0.050 in frequency, respectively. In the Hiroshima population, all of the 17 frogs

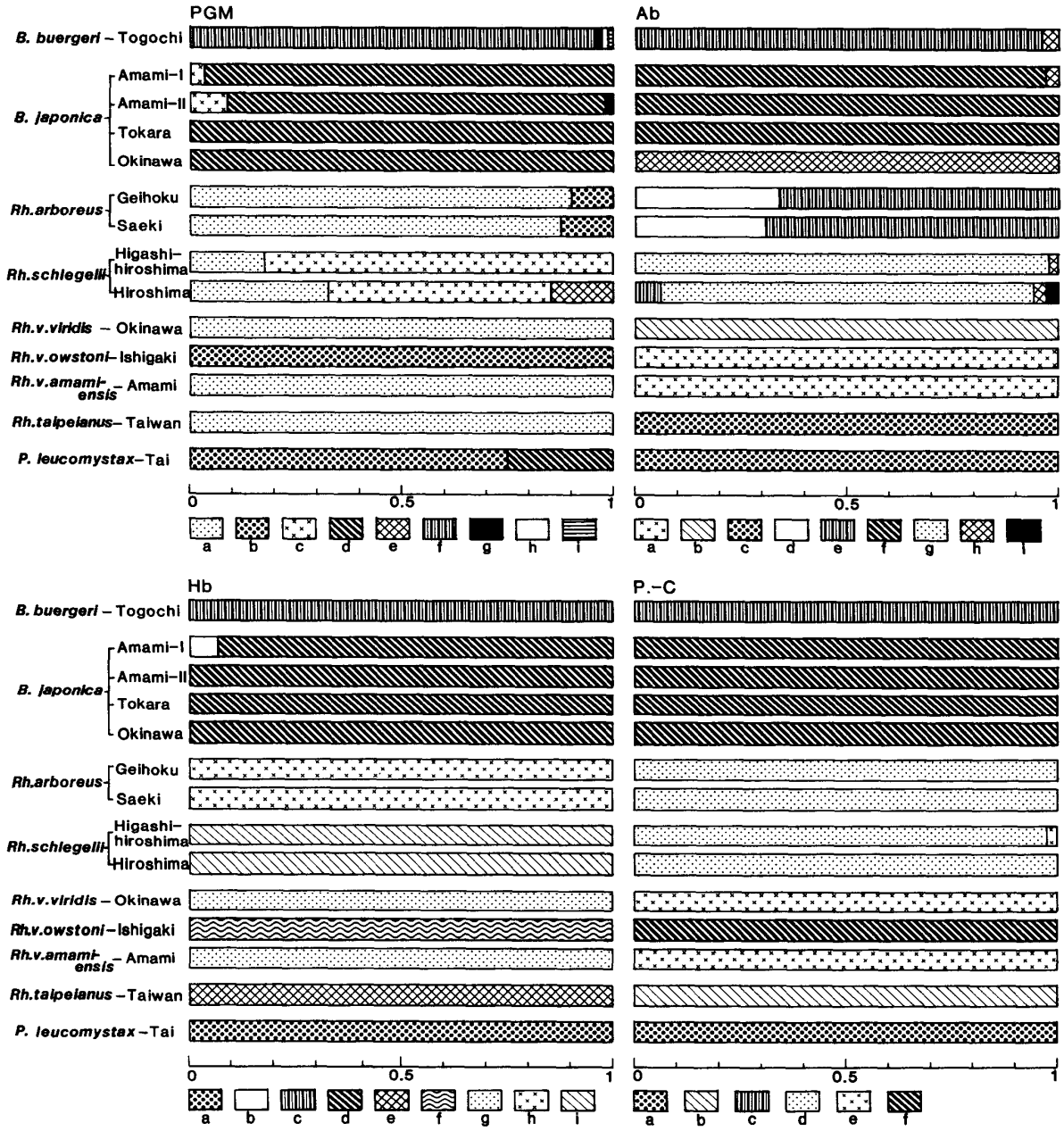

Fig. 11. Gene frequencies at four loci, PGM, Ab, Hb and Prot-C, in 14 populations of *Buergeria*, *Rhacophorus* and *Polypedates*.

showed a homozygous band, BB, produced by allele *b*. Nine *Rh. v. viridis* and one *Rh. v. amamiensis* showed a homozygous band, BB, while five *Rh. v. owstoni* showed a homozygous band, DD. One *Rh. taipeianus* showed a heterozygous band, FG. Two *P. leucomystax* showed homozygous and heterozygous bands, HH and BH (Table 2; Fig. 10).

17. Albumin (Ab)

The electrophoretic patterns of Ab in the 243 frogs of the 14 populations indicated that there were 14 phenotypes produced by nine alleles, *a*~*i*. In *B. buergeri,* 37 and three of the 40 frogs showed homozygous and heterozygous bands, EE and EH, respectively. Alleles *e* and *h* were 0.963 and 0.038 in frequency, respectively. In *B. japonica,* two frogs of the Okinawa population showed a homozygous band, HH, while 102 of the 104 frogs of the other three populations showed a homozygous band, FF. The remaining two frogs belonging to the Amami-I population showed a heterozygous band, FH. Thus, alleles *f* and *h* in the Amami-I population were 0.967 and 0.033 in frequency, respectively.

In *Rh. arboreus,* three and 14 of the 34 frogs of the Geihoku population showed homozygous bands, DD and EE, respectively, and the other 17 frogs showed a heterozygous band, DE. Alleles *e* and *d* were 0.662 and 0.338 in frequency, respectively. Of the eight frogs of the Saeki population, one and four showed homozygous bands, DD and EE, respectively, and the remaining three showed a heterozygous band, DE. Alleles *e* and *d* were 0.688 and 0.313 in frequency, respectively. In *Rh. schlegelii,* 19 and one of the 20 frogs of the Higashihiroshima population showed homozygous and heterozygous bands, GG and GH, respectively. Alleles *g* and *h* were 0.975 and 0.025 in frequency, respectively. Of the 17 frogs of the Hiroshima population, 13 showed a homozygous band, GG, and two, one and one of the other four frogs showed heterozygous bands, EG, GH and GI, respectively. Alleles *g, e, h* and *i* were 0.882, 0.059, 0.029 and 0.029 in frequency, respectively. Nine *Rh. v. viridis* showed a homozygous band, BB, while five *Rh. v. owstoni* and one *Rh. v. amamiensis* showed a homozygous band, AA. One *Rh. taipeianus* and two *P. leucomystax* showed a homozygous band, CC (Table 2; Fig. 11).

18. Protein-C (Prot-C)

The electrophoretic patterns of Prot-C in the 257 frogs of the 14 populations indicated that there were seven phenotypes produced by six alleles, *a*~*f,* at the Prot-C locus. In *B. buergeri,* all of the 40 frogs showed a homozygous band, CC, while all of the 120 frogs of the four populations in *B. japonica* showed a homozygous band, FF. While all of the 42 *Rh. arboreus* and 36 of the 37 *Rh. schlegelii* showed a homozygous band, DD, only one frog of the Higashihiroshima population showed a heterozygous band, DE. Nine *Rh. v. viridis* and one *Rh. v. amamiensis* showed a homozygous band, EE. Five *Rh. v. owstoni,* one *Rh. taipeianus* and two *P. leucomystax* showed homozygous bands, FF, BB and AA, respectively (Table 2; Fig. 11).

19. Hemoglobin (Hb)

The electrophoretic patterns of Hb in the 257 frogs of the 14 populations indicated that there were nine phenotypes produced by nine alleles, *a*~*i*. In *B. buergeri*, all of the 40 frogs showed a homozygous band, CC. In *B. japonica*, 118 of the 120 frogs of the four populations showed a homozygous band, DD, while the remaining two belonging to the Amami-I population showed a homozygous band, BB. Alleles *d* and *b* in the Amami-I population were 0.933 and 0.067 in frequency, respectively. In *Rh. arboreus*, all of the 42 frogs of the two populations showed a homozygous band, HH, while all of the 37 frogs of the two populations in *Rh. schlegelii* showed a homozygous band, II. Nine *Rh. v. viridis* and one *Rh. v. amamiensis* showed a homozygous band, GG, five *Rh. v. owstoni* showed a homozygous band, FF, one *Rh. taipeianus* showed a homozygous band, EE, and two *P. leucomystax* showed a homozygous band, AA (Table 2; Fig. 11).

III. Genetic variation

1. Proportion of heterozygous loci

The proportion of heterozygous loci per individual shows the degree of differentiation at all the loci in each individual. When the mean proportion of heterozygous loci per individual was estimated at 25 loci controlling 16 enzymes

TABLE 3

Estimates of genetic variabilities at 25 loci in 14 populations of two species of *Buergeria*, four species of *Rhacophorus* and one species of *Polypedates*

Species	Population	Sample size	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
B. buergeri	Togochi	40	11.4 (11.9)	56.0	2.00
B. japonica	Amami-I	30	10.4 (11.1)	48.0	1.64
	Amami-II	30	11.6 (12.8)	48.0	1.72
	Tokara	58	4.0 (4.1)	24.0	1.28
	Okinawa	2	4.0 (3.0)	8.0	1.08
Rh. arboreus	Geihoku	34	11.1 (12.1)	56.0	1.68
	Saeki	8	14.1 (11.6)	40.0	1.44
Rh. schlegelii	Higashi-hiroshima	20	18.3 (19.8)	68.0	2.00
	Hiroshima	17	23.0 (22.2)	60.0	2.12
Rh. v. viridis	Okinawa	9	6.3 (7.0)	24.0	1.28
Rh. v. owstoni	Ishigaki	5	9.6 (8.0)	24.0	1.28
Rh. v. amamiensis	Amami	1	0	0	1.00
Rh. taipeianus	Taiwan	1	8.0 (4.0)	8.0	1.08
P. leucomystax	Tai	2	16.0 (13.0)	28.0	1.36
Average (Total)		18.4 (257)	10.6 (10.0)	35.1	1.50

Parentheses show an expected value.

and three blood proteins in each of the 14 populations of *Buergeria, Rhacophorus* and *Polypedates,* it was 0~23.0%, 10.6% on the average. In *Rh. v. amamiensis,* it was zero, as a single sample of this species showed a homozygous band in electrophoretic pattern at each of the 25 loci. Although three other populations including the Okinawa population of *B. japonica* (two frogs), *P. leucomystax* (two frogs) and *Rh. taipeianus* (one frog) were 4.0%, 16.0% and 8.0% in proportion of heterozygous loci, respectively, these populations were one or two in sample size. The remaining 10 populations, 5~58 in sample size, were 4.0~23.0%, 12.0% on the average, in the mean proportion of heterozygous loci per individual. Of these populations, the Tokara of *B. japonica* was the lowest in mean proportion of heterozygous loci, being 4.0%, while the two populations of *Rh. schlegelii* were the highest, being 18.3% and 23.0% (Table 3).

2. Proportion of polymorphic loci

The proportion of polymorphic loci at which each of the alleles was contained at the rate of more than 1% was estimated in each of the 14 populations. These 14 populations were 0~68%, 35.1% on the average, in the proportion of polymorphic loci. *Rh. v. amamiensis* was zero, as there was only a single specimen and showed a homozygous band at each of the 25 loci. The Okinawa population of *B. japonica, Rh. taipeianus* and *P. leucomystax* were 8%, 8% and 28% in the proportion of polymorphic loci, although they were only two, one and two in sample size, respectively. The 10 populations other than those which were one or two in sample size were 24~68%, 44.8% on the average. The Tokara population of *B. japonica,* the Okinawa population of *Rh. v. viridis* and the Ishigaki population of *Rh. v. owstoni* were low in the proportion of polymorphic loci, each population being 24%, while the two populations of *Rh. schlegelii* were the highest, being 60% and 68% (Table 3).

3. Mean number of alleles per locus

The mean number of alleles at each locus was 1.00~2.12, 1.50 on the average. *Rh. v. amamiensis* was the smallest, being 1.00 in this population, which was one in sample size and showed a homozygous band at each of the 25 loci. Two frogs of the Okinawa population of *B. japonica* and one *Rh. taipeianus* were 1.08, while two *P. leucomystax* was 1.36 in spite of the small sample size of this species. The 10 populations other than the foregoing four were 1.28~2.12, 1.64 on the average, in mean number of alleles per locus. Each of three populations, *Rh. v. viridis, Rh. v. owstoni* and the Tokara population of *B. japonica,* was 1.28. The two populations of *Rh. schlegelii* were 2.00 and 2.12, while the single population of *B. buergeri* was 2.00 (Table 3).

IV. Genetic distance and dendrogram

1. Genetic distance

On the basis of gene frequencies at the 25 loci examined in 257 frogs of the 14

populations of *Buergeria, Rhacophorus* and *Polypedates,* the genetic identities between different populations of the same subspecies or species, between different subspecies and between different species or genera were estimated. From these genetic identities, genetic distances were calculated according to NEI (1972, 1975) (Table 4).

a. Populational difference

The genetic distances among three of the four populations, the Amami-I, Amami-II and Tokara of *Buergeria japonica,* were 0.003~0.038, while those between these three populations and the remaining Okinawa population were 0.175~0.270.

The genetic distances between the Geihoku and Saeki populations of *Rhacophorus arboreus,* and between the Higashihiroshima and Hiroshima populations of *Rhacophorus schlegelii* were 0.014 and 0.017, respectively.

b. Subspecific difference

The genetic distance between two of the three subspecies of *Rhacophorus viridis, Rh. v. viridis* and *Rh. v. amamiensis,* was 0.277, while those between these two subspecies and *Rh. v. owstoni* were very large, being 0.819 and 0.865.

c. Specific difference

The smallest genetic distances between different species were found between *Rhacophorus arboreus* and *Rh. schlegelii,* being 0.301~0.387. The genetic distances between these two mainland species, *Rhacophorus arboreus* and *Rh. schlegelii,* and a subspecies of an island species, *Rh. v. viridis,* were 0.392~0.533, while those between these two mainland species and another island subspecies, *Rh. v. amamiensis,* were 0.548~0.618. Those between the two mainland *Rhacophorus* species and the remaining subspecies, *Rh. v. owstoni,* were somewhat larger than the foregoing, being 0.684~0.844.

The genetic distances between *Rh. taipeianus* and the two mainland *Rhacophorus* species were 0.580~0.713. Those between *Rh. taipeianus* and two subspecies of *Rh. viridis, Rh. v. viridis* and *Rh. v. amamiensis,* were 0.476 and 0.559, respectively, while that between *Rh. taipeianus* and *Rh. v. owstoni* was very large, being 0.854. These data show that the subspecies, *Rh. v. owstoni,* electrophoretically differs from the other two subspecies, *Rh. v. viridis* and *Rh. v. amamiensis.* At the same time, they show that *Rh. taipeianus* is more closely related to *Rh. arboreus, Rh. schlegelii, Rh. v. viridis* and *Rh. v. amamiensis* than *Rh. v. owstoni.*

The genetic distances between *Buergeria buergeri* and *B. japonica* were remarkably larger than those between different species of *Rhacophorus.* They were 2.045~2.243.

d. Generic difference

The genetic distances between *Polypedates leucomystax* and four species of *Rhacophorus, Rh. arboreus, Rh. schlegelii, Rh. viridis* and *Rh. taipeianus,* were compara-

TABLE 4

Genetic identity (I) and genetic distance (D) among 14 populations of two

Species / Locality		*B. buerg.*	*B. japonica*			
Species	Locality	Togochi	Amami-I	Amami-II	Tokara	Okinawa
B. buergeri	Togochi	—	0.117	0.123	0.106	0.129
B. japonica	Amami-I	2.142	—	0.997	0.963	0.839
	Amami-II	2.094	0.003	—	0.969	0.836
	Tokara	2.243	0.038	0.031	—	0.763
	Okinawa	2.045	0.175	0.179	0.270	—
Rh. arboreus	Geihoku	3.433	6.331	6.317	7.317	∞
	Saeki	3.506	5.884	6.136	6.840	∞
Rh. schlegelii	Higashi-hiroshima	4.646	5.487	5.130	6.484	5.461
	Hiroshima	3.999	5.287	5.049	7.721	4.526
Rh. v. viridis	Okinawa	6.361	∞	9.386	∞	∞
Rh. v. owstoni	Ishigaki	5.598	2.782	2.811	2.820	2.825
Rh. v. amamiensis	Amami	∞	4.481	4.066	3.215	∞
Rh. taipeianus	Taiwan	5.566	∞	∞	∞	∞
P. leucomystax	Tai	3.073	4.510	4.572	4.515	4.520

Genetic identity (I) is given above the diagonal and genetic distance (D) is given below.

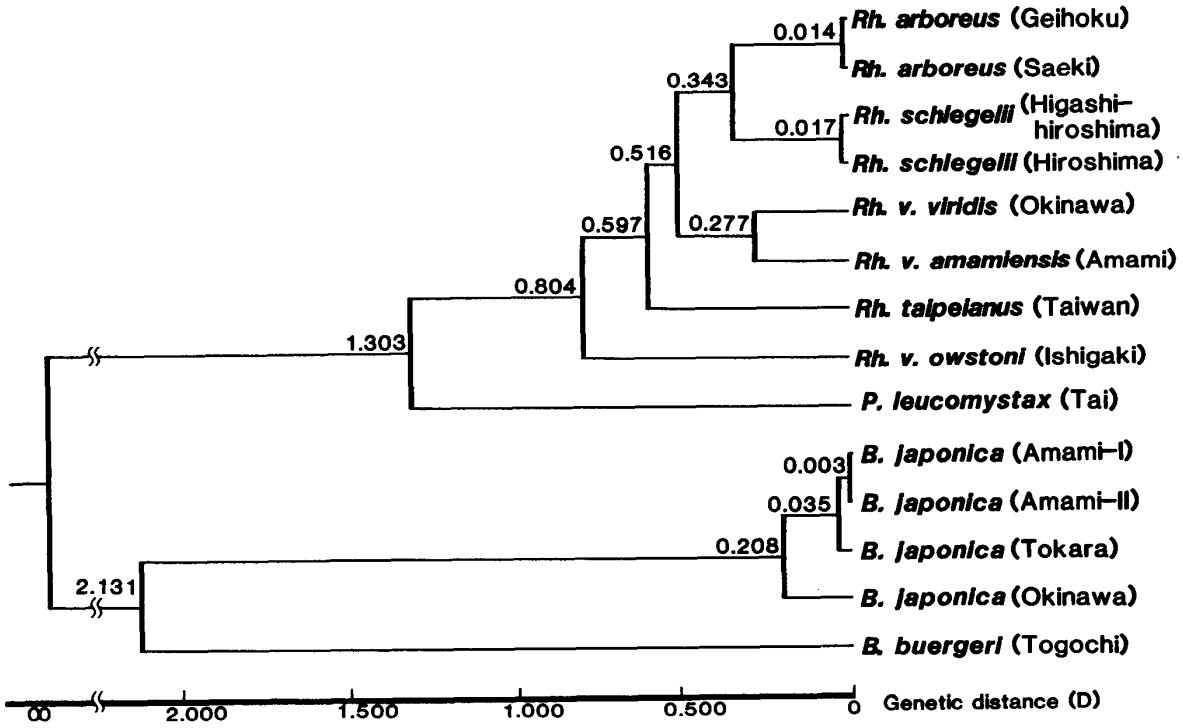

Fig. 12. Dendrogram for 14 populations of *Buergeria, Rhacophorus* and *Polypedates* based on genetic distances.

species of *Buergeria*, four species of *Rhacophorus* and one species of *Polypedates*

Rh. arboreus		*Rh. schlegelii*		*Rh. v. virid.*	*Rh. v. owst.*	*Rh. v. amami.*	*Rh. taip.*	*P. leuc.*
Geihoku	Saeki	Higashi-hirosh.	Hiro-shima	Okinawa	Ishigaki	Amami	Taiwan	Tai
0.032	0.030	0.010	0.018	0.002	0.004	0	0.004	0.046
0.002	0.003	0.004	0.005	0	0.062	0.011	0	0.011
0.002	0.002	0.006	0.006	0.000	0.060	0.017	0	0.010
0.001	0.001	0.002	0.000	0	0.060	0.040	0	0.011
0	0	0.004	0.011	0	0.059	0	0	0.011
—	0.987	0.707	0.740	0.676	0.505	0.577	0.560	0.276
0.014	—	0.679	0.713	0.657	0.487	0.553	0.533	0.273
0.347	0.387	—	0.984	0.587	0.430	0.539	0.490	0.253
0.301	0.339	0.017	—	0.622	0.431	0.578	0.534	0.258
0.392	0.420	0.533	0.476	—	0.441	0.758	0.621	0.282
0.684	0.720	0.844	0.841	0.819	—	0.421	0.426	0.297
0.551	0.592	0.618	0.548	0.277	0.865	—	0.572	0.236
0.580	0.629	0.713	0.627	0.476	0.854	0.559	—	0.306
1.288	1.298	1.376	1.353	1.267	1.213	1.445	1.183	—

tively small, being 1.183~1.445. The genetic distances between *Buergeria buergeri* and the two mainland species of *Rhacophorus, Rh. arboreus* and *Rh. schlegelii,* were 3.433~4.646, while those between *Buergeria buergeri* and *Rhacophorus viridis* or *Rh. taipeianus* were more larger, being 5.566~∞. Those between *Buergeria japonica* and *Rhacophorus viridis owstoni* were 2.782~2.825, while those between *Buergeria japonica* and the other two subspecies of *Rh. viridis, Rh. v. viridis* and *Rh. v. amamiensis,* were 3.215~∞. The genetic distances between *Buergeria japonica* and the two mainland species of *Rhacophorus, Rh. arboreus* and *Rh. schlegelii,* were 4.526~∞, while those between *Buergeria japonica* and *Rh. taipeianus* were all unlimited.

2. Dendrogram

A dendrogram was drawn for the species, subspecies and populations belonging to *Rhacophorus, Buergeria* and *Polypedates* on the basis of genetic distances by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975). As shown in Fig. 12, it was found that *Buergeria* is definitely divided from *Rhacophorus,* and that *B. buergeri* is comparatively distant from *B. japonica.* In *B. japonica,* the Okinawa population is somewhat remotely related with the other three populations.

In *Rhacophorus* and *Polypedates,* it was found that *P. leucomystax, Rh. v. owstoni* and *Rh. taipeianus* branched off one after another from the common ancestor. Thereafter, the common ancestors were divided into two groups, one of which

contains *Rh. arboreus* and *Rh. schlegelii,* while the other contains *Rh. v. viridis* and *Rh. v. amamiensis.*

DISCUSSION

1. Genetic variability

The present study has elucidated that most of the seven species of rhacophorids distributed in Japan and the adjacent territory are distinctly high in genetic variation. Of seven populations of *Buergeria* and *Rhacophorus* which are more than 17 in sample size, the two populations of *Rh. schlegelii* are the highest in the three parameters showing a genetic variation. They are 2.00 or 2.12 in mean number of alleles per locus, 18.3% or 23.0% in mean proportion of heterozygous loci per individual, and 68.0% or 60.0% in mean proportion of polymorphic loci per population. The Geihoku population of *Rh. arboreus,* the single population of *Buergeria buergeri,* and two populations of *B. japonica* are also remarkably high, being 1.64~2.00, 10.4~11.6% and 48.0~56.0% in the three parameters of genetic variation, respectively. NISHIOKA, OHTA and SUMIDA (1987) have reported that seven populations of *Rana tagoi* distributed in mainlands and islands of Japan are very high in degree of genetic variability. These populations are 1.64~2.50, 1.97 on the average, in mean number of alleles per locus, 10.2~21.4%, 16.1% on the average, in proportion of heterozygous loci per individual and 40.9~63.6%, 55.2% on the average, in proportion of polymorphic loci per population.

In addition to these species, genetic variability has been hitherto reported at least in 23 amphibian species whose enzymes were analyzed by the electrophoretic method (NISHIOKA, OHTA and SUMIDA, 1987). Of these species, six are more than 42% in mean proportion of polymorphic loci per population as follows: *Bufo cognatus* and *B. speciosus* (ROGERS, 1973), *B. viridis* (DESSAUER, NEVO and CHUANG, 1975), *Hyla versicolor* (RALIN and SELANDER, 1979), *Scaphiopus bombifrons* (SATTLER, 1980) and *Triturus alpestris* (KALEZIĆ and HEDGECOCK, 1979). Seven other species, *Rana ridibunda* (NEVO, 1976), *Bufo arenarum* (MATTHEWS, 1975), *Hyla arborea* (NEVO and YANG, 1979), *Hyla chrysoscelis* (RALIN and SELANDER, 1979), *Scaphiopus maltiplicatus* (SATTLER, 1980), *Taricha granulosa* (HEDGECOCK, 1976) and *Triturus vulgaris* (KALEZIĆ and HEDGECOCK, 1979), are 32.4~39.8% in mean proportion of polymorphic loci per population. The remaining 10 species, *Bufo americanus* (GUTTMAN, 1975), *Pelobates syriacus* (NEVO, 1976), *Scaphiopus holbrooki, S. couchi* and *S. hammondi* (SATTLER, 1980), *Taricha torosa* (HEDGECOCK, 1976), *T. rivularis* (HEDGECOCK, 1978), *Triturus cristatus* (KALEZIĆ and HEDGECOCK, 1979), *Eurycea lucifuga* (MERKLE and GUTTMAN, 1977), and *Plethodon yonahlossee* (GUTTMAN, KARLIN and LABANICK, 1978), are 8.2~27.8 in mean proportion of polymorphic loci per population.

It was found that the slow-motion amphibians settling down in ecologically variable, hilly localities such as *Buergeria, Rhacophorus* and *Rana tagoi* are generally high in degree of mean proportion of polymorphic loci per population. A similar phenomenon has been found in many species of *Bufo, Hyla, Scaphiopus* and *Triturus.*

Buergeria and *Rhacophorus* seem to have a comparatively narrow habitat, as they always cling to trees, herbs or rocks in mountain districts. DESSAUER, NEVO and CHUANG (1975) have described that the heterozygosity is high in organisms living in ecologically variable environments as compared to those living in relatively constant ones. It is evident that this interpretation is principally applicable to the rhacophorids observed in the present study. It seems also necessary to be added here that the high heterozygosity of a species, subspecies or population requires a considerably wide range of distribution. *Buergeria buergeri, Rhacophorus arboreus* and *Rh. schlegelii* are distributed in mainlands and comparatively large in the range of distribution. The Amami-I and -II populations of *Buergeria japonica* are evidently larger in range than the Tokara population of the same species.

2. Genetic distance

The genetic distances between different species, subspecies or local populations have been calculated from the results of electrophoretic analyses of enzymes and proteins in various amphibians by many investigators. In urodeles, GUTTMAN, KARLIN and LABANICK (1978) have reported that the genetic distances between *Plethodon longicrus* and *P. yonahlossee* are only 0.002~0.095, and that *P. longicrus* should be placed as a synonym of *P. yonahlossee*. The genetic distances among four species of *Plethodon, vehiculum, elongatus, dunni* and *gordoni,* are 0.001~1.708 (FEDER, WURST and WAKE, 1978). As the genetic distance between the latter two species is only 0.001 in contrast to the others which are 1.200~1.708, *P. gordoni* has been considered to be a somewhat localized color morph of *P. dunni.* The genetic distances among five species of *Plethodon, ouachitae, glutinosus, yonahlossee, fourchensis* and *caddoensis,* are 0.18~0.87 (DUNCAN and HIGHTON, 1979), while 26 species of *Plethodon* are 0.17~4.5 (HIGHTON and LARSON, 1979). On the other hand, LARSON and HIGHTON (1978) have found that the genetic distances between *P. welleri* and two populations, the northern and southern, of *P. dorsalis* are 0.31~0.58 and 1.30~1.62, respectively, while those between the northern and southern populations are 1.67~2.15. These authors have stated that the electrophoretic divergence of the two populations of *P. dorsalis* is greater than that observed in any species previously studied.

HEDGECOCK and AYALA (1974) have examined the genetic distances among three species and one subspecies of *Taricha, rivularis, granulosa, t. torosa* and *t. sierrae.* They have elucidated that those between geographic populations, between subspecies and between species are 0.005~0.053, 0.109~0.253, and 0.28~0.59, respectively. HEDGECOCK (1976) has also reported that the genetic distance between the two subspecies, *T. t. torosa* and *T. t. sierrae,* is 0.104~0.309. KALEZIĆ and HEDGECOCK (1979) analyzed the enzymes and proteins of *Triturus vulgaris, T. alpestris, T. cristatus dobrogicus* and *T. c. karelinii* and found that the genetic distances between local populations, between subspecies and between species are 0.031 ± 0.017, 0.347 and 0.906 ± 0.058, respectively. *Desmognathus fuscus* distributed in eastern North America are biochemically divided into three groups, northern populations, southern populations and an undescribed form. The

genetic distances between the northern and southern populations are 0.176~0.482, while those between the undescribed form and the two populations, northern and southern, are 0.456~0.762 and 0.337~0.432, respectively (TILLEY and SCHWERDTFEGER, 1981). The genetic distances among five species of *Hydromantes* are 0.121~1.836, while those among five populations of *H. shastae* are 0.003~0.275 (WAKE, MAXSON and WURST, 1978).

In anurans, the genetic distances among seven species of *Rana* are 0.35~1.59 (CASE, 1978a), while those between two species, *R. boylei and R. muscosa,* are 0.68~0.77 (CASE, 1978b). The genetic distances among three species of *Hyla* are 0.11~2.34 (CASE, HANELINE and SMITH, 1975). Those between two species of *Bufo, B. boreas* and *B. punctatus,* are 0.795~1.011 (FEDER, 1979), and those among three species of *Leiopelma* are 0.34~1.14 (DAUGHERTY, BELL, ADAMS and MAXSON, 1981). ROGER's pairwise coefficients of genetic similarity among 13 species of *Litoria* are 0.07~0.84 (DESSAUER, GARTSIDE and ZWEIFEL, 1977), while those among five species of *Scaphiopus* are 0.208~0.706 (SATTLER, 1980). In *Rana tagoi* dwelling along mountain streams of the mainlands and islands of Japan, NISHIOKA, OHTA and SUMIDA (1987) have reported that the genetic distances between two subspecies are 0.182~0.314, while those among six populations are 0.031~0.283.

The two genera, *Buergeria* and *Rhacophorus,* used in the present study were first described as one genus, *Polypedates.* While OKADA (1931) divided this into two genera, *Polypedates* and *Rhacophorus,* NAKAMURA and UENO (1963) united *Polypedates* with *Rhacophorus.* LIEM (1970) converted *Polypedates* into *Buergeria* and placed *Rh. leucomystax* as *Polypedates leucomystax.* These changes of rhacophorids in taxonomic position seem to be attributable to their morphological similarity.

The electrophoretic examination of the seven species of rhacophorids in the present study has elucidated that *Buergeria* and *Rhacophorus* are remotely separated in biochemical characters, and has shown that the classification of Japanese rhacophorids into two genera at least is proper. As the genetic distances between *B. buergeri* and *B. japonica* are 2.142~2.243, and those between *Rhacophorus arboreus* or *Rh. schlegelii* and *Rh. v. viridis* or *Rh. v. owstoni* are 0.392~0.844, it seems evident that the speciation of the two *Buergeria* species occurred at a far older age than that of the three *Rhacophorus* species, as shown in the dendrogram (Fig. 12). The genetic distances between two species of *Rhacophorus* distributed in Hiroshima Prefecture are 0.301~0.387, while those between the mainland species, *Rh. arboreus* or *Rh. schlegelii,* and the island species, *Rh. viridis,* distributed in Okinawa are 0.392~0.533, and those between the mainland *Rhacophorus* and the island *Rh. viridis* distributed in Ishigaki are 0.684~0.844. The genetic distance between the two subspecies, *Rh. v. viridis* and *Rh. v. owstoni,* is 0.819, which seems to be at a species level, as compared with those between the two mainland species of *Rhacophorus,* and between the two species of *Buergeria.* The two subspecies of *Rhacophorus* seem to be farther separated from each other than those between *Taricha t. torosa* and *T. t. sierrae* (HEDGECOCK and AYALA, 1974), between *Triturus cristatus dobrogicus* and *T. c. karelinii* (KALEZIĆ and HEDGECOCK, 1979) and between *Rana t. tagoi* and *R. t. yakushimensis* (NISHIOKA, OHTA and SUMIDA, 1987). The genetic distances of

Polypedates leucomystax from the two species of *Buergeria* and the two mainland species of *Rhacophorus* are 3.073~4.572 and 1.288~1.376, respectively, while those from the two island subspecies, *Rh. v. viridis* and *Rh. v. owstoni,* are 1.267 and 1.213, respectively. Thus, the separation of *leucomystax* from *Rhacophorus* in taxonomy made by LIEM (1970) seems to be biochemically proper, although only two specimens were examined by the electrophoretic method in the present study.

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