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Differentiation of *Rana limnocharis* and Two Allied Species Elucidated by Electrophoretic Analyses

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ABSTRACT

The genetic relationships among six populations of *Rana limnocharis* distributed in Japan and Taiwan, two populations of *R. cancrivora* distributed in Philippine and Thailand and one population of *Platymantis papuensis* distributed in New Guinea were examined by electrophoretic analyses of 17 enzymes and two blood proteins extracted from 97 frogs. These enzymes and blood proteins were controlled by genes at 29 loci, where 3.9 alleles produced 4.6 phenotypes on the average. The genetic relationships among the nine populations were examined by estimating the genetic distance according to the method of NEI (1975). The phylogenetic relationships were shown by a dendrogram drawn by the UPGMA clustering method.

It was found that *Platymantis papuensis* first diverged from the others, and then *Rana cancrivora* and *Rana limnocharis* were differentiated from each other. In *Rana limnocharis*, the Iriomote population seems to have first diverged from all the other populations.

INTRODUCTION

Rana limnocharis is widely distributed in southern and eastern Asia. In Japan, it is found everywhere west of the central part of Honshu, Shikoku, Kyushu and Ryukyu. According to STEJNEGER (1907), *R. limnocharis* is the commonest species of frog in Taiwan and adjacent islands. INGER (1954) reported on the distribution of *R. limnocharis vittigera* WIEGMANN and *R. cancrivora cancrivora* GRAVENHORST in the Philippines. According to INGER, the latter is the only species which may be confused with *R. limnocharis* in the Philippines. ZWEIFEL and TYLER (1982) have reported that *Platymantis papuensis* MEYER is an abundant species of the forested lowlands over much of New Guinea. This species belongs to Ranidae and is somewhat similar to *Rana limnocharis* in appearance.

It is an unaccountable matter that the nomenclator of *Rana limnocharis* has been indefinite. According to the "Amphibian Species of the World" edited by FROST (1985), the nomenclator is BOIE (1835). This had been adopted by LIU and HU (1961). However, STEJNEGER (1907) and NAKAMURA and UENO (1963) described that the nomenclator is WIEGMAN (1835). In contrast, according to OKADA (1966), *Rana limnocharis* was first named by GRAVENHORST (1829).

The present study was made to clarify the genetic relationships among six

populations of *Rana limnocharis* distributed in Japan and Taiwan, two populations of *R. cancrivora* distributed in the Philippines and Thailand, and one population of *Platymantis papuensis* distributed in New Guinea.

MATERIALS AND METHODS

A total of 97 mature males and females belonging to six populations of *Rana limnocharis* GRAVENHORST, two populations of *Rana cancrivora* GRAVENHORST and one population of *Platymantis papuensis* MEYER was used in the present study. The collecting stations and the number and sex ratio of the frogs from each station are shown in Table 1 and Fig. 1.

Seventeen enzymes extracted from the skeletal muscles or livers and two blood proteins of the 97 frogs were analyzed by the horizontal starch-gel electrophoresis described in detail by NISHIOKA, OHTANI and SUMIDA (1980). The buffer systems used in electrophoresis are shown in Table 2. The detection of each enzyme was made by the agar overlay method of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. The detection of blood proteins was made with amido-black staining.

A locus was considered to be polymorphic when each of multiple alleles at this locus existed in a frequency of more than 1%. The genetic variation of each local population was shown by the proportion of polymorphic loci and the average heterozygosity (LEWONTIN, 1974). The genetic relationships among local popula-

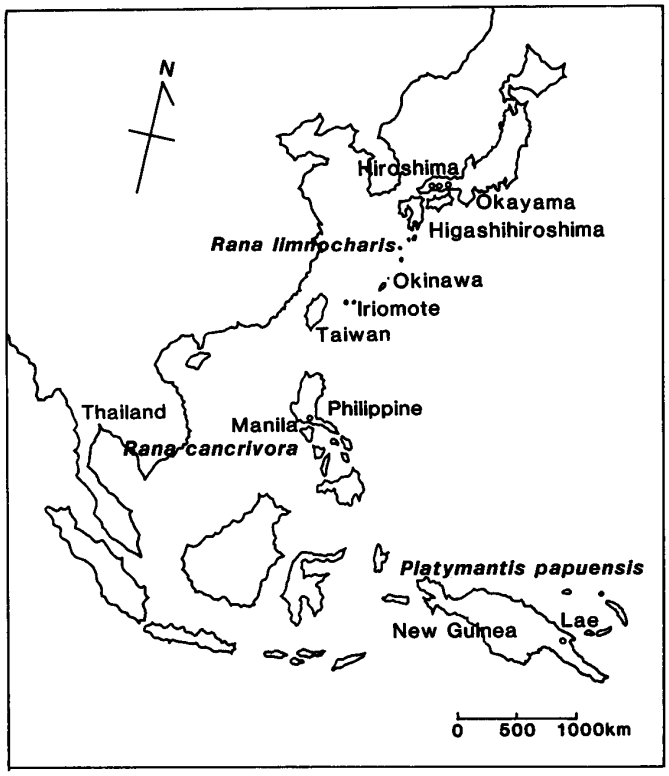

Fig. 1. Map showing localities of nine populations used in this study.

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

Species	Locality	No. of frogs		
		Total	Male	Female
Rana limnocharis	Okayama	5	1	4
〃	Higashihiroshima	16	11	5
〃	Hiroshima	20	16	4
〃	Okinawa	18	10	8
〃	Iriomote	5	5	0
〃	Taiwan	8	6	2
Rana cancrivora	Philippine	8	3	5
〃	Thailand	10	6	4
Platymantis papuensis	New Guinea	7	3	4
Total		97	61	36

TABLE 2
Enzymes and blood proteins analyzed and buffer systems used in the present study

Enzyme or blood protein	Abbreviation	E.C.No.	Sample	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	3.5.4.4	〃	〃
Adenylate kinase	AK	2.7.4.3	〃	〃
Creatine kinase	CK	2.7.3.2	〃	T-B-E pH 8.0
Fumarase	Fum	4.2.1.2	Liver	〃
α-Glycerophosphate dehydrogenase	α-GDH	1.1.1.8	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	5.3.1.9	〃	T-B-E pH 8.0
Hexokinase	HK	2.7.1.1	Liver	〃
Isocitrate dehydrogenase	IDH	1.1.1.42	Skeletal muscle	T-C pH 7.0
Lactate dehydrogenase	LDH	1.1.1.27	〃	T-C pH 6.0
Malate dehydrogenase	MDH	1.1.1.37	〃	〃
Malic enzyme	ME	1.1.1.40	〃	〃
Mannose phosphate isomerase	MPI	5.3.1.8	〃	T-C pH 7.0
Peptidase	Pep	3.4.3.1	Liver	T-B-E pH 8.0
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	Skeletal muscle	T-C pH 7.0
Superoxide dismutase	SOD	1.15.1.1	〃	T-B-E pH 8.0
Sorbitol dehydrogenase	SORDH	1.1.1.14	Liver	T-C pH 7.0
Serum albumin	Ab	—	Blood serum	T-B-E pH 8.0
Hemoglobin	Hb	—	Erythrocyte	T-B-E pH 8.6

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

tions were evaluated by estimating the genetic identity (I) and the genetic distance (D) according to the method of NEI (1975). On the basis of the genetic distances among these populations, their systematic relationships were shown by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975).

OBSERVATION

I. Electrophoretic patterns and multiple alleles

The electrophoretic patterns of 17 enzymes extracted from the skeletal muscles and livers and two blood proteins in 97 frogs of nine populations belonging to *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis* were examined. The results showed that these enzymes and blood proteins were controlled by genes at 29 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 shown by *a, b, c,* --- (Figs. 2 and 3).

At the four loci of AAT-B, AK, CK and SOD-A, there were two phenotypes produced by two alleles. At the five loci of AAT-A, α-GDH, MDH-A, SOD-B and Hb-I, there were three phenotypes produced by three alleles. At the loci of Pep-A and IDH-A, there were four phenotypes produced by three alleles and five phenotypes produced by three alleles, respectively. At the five loci of IDH-B, LDH-A, ME-A, 6-PGD and Hb-II, four phenotypes produced by four alleles were observed. At the LDH-B locus, there were five phenotypes produced by four alleles. At the three loci of HK, Pep-D and SORDH, there were six phenotypes produced by four alleles. At the two loci of MDH-B and Pep-B, there were five phenotypes produced by five alleles. At the Fum locus, six phenotypes were produced by five alleles. At the three loci of ME-B, MPI and

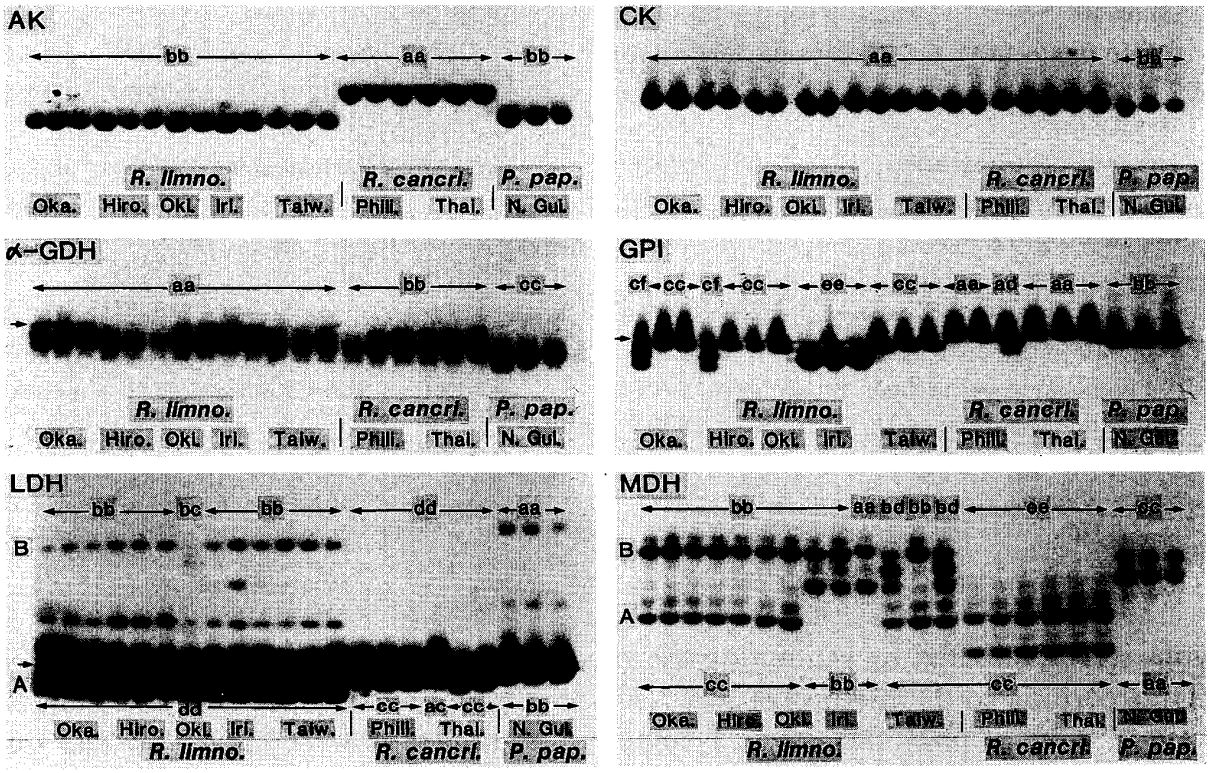

Fig. 2. Electrophoretic patterns of six enzymes, AK, CK, α-GDH, GPI, LDH and MDH, in eight populations of *Rana limnocharis, R. cancrivora* and *Platymantis papuensis.*

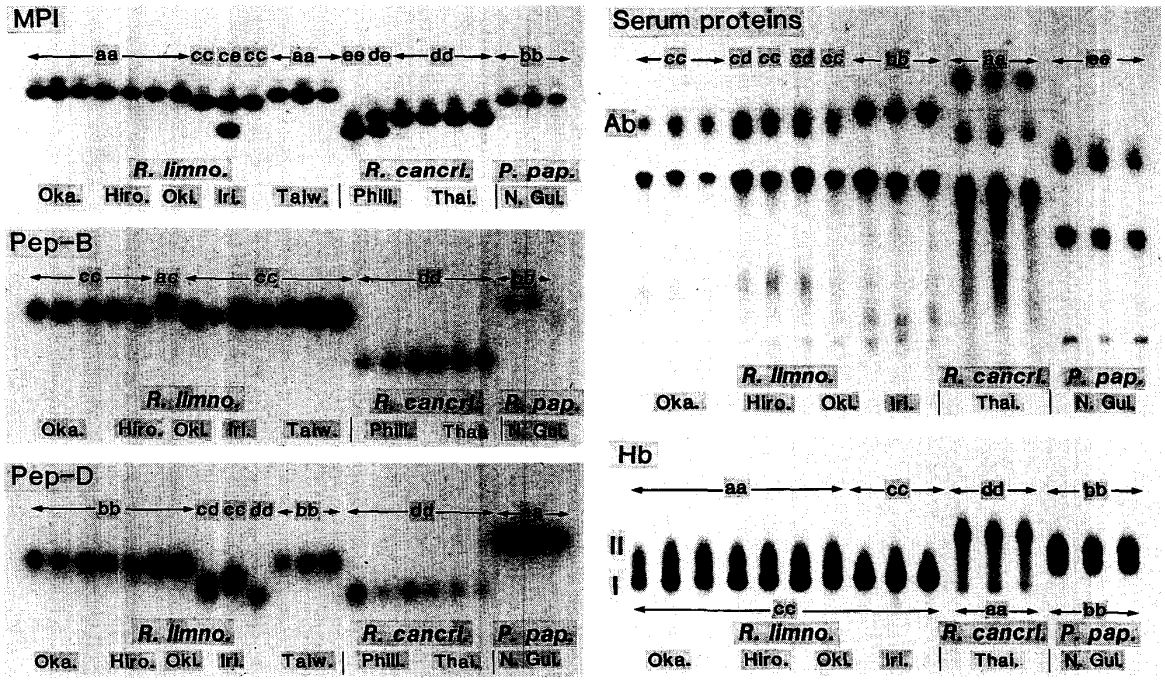

Fig. 3. Electrophoretic patterns of three enzymes, MPI, Pep-B and Pep-D, and two blood proteins, Ab and Hb, in eight populations of *Rana limnocharis*, *R. cancrivora* and *Platymantis papuensis*.

Ab, there were seven phenotypes produced by five alleles. At the ADA locus and the two loci of GPI and Pep-C, there were six phenotypes produced by six alleles and seven phenotypes produced by six alleles, respectively. At these 29 loci, there were 4.6 phenotypes produced by 3.9 alleles on the average (Table 3).

II. Gene frequency

1. Aspartate aminotransferase (AAT)

In the 97 frogs of the nine populations belonging to *Rana limnocharis*, *Rana cancrivora* and *Platymantis papuensis*, the electrophoretic analyses of AAT showed that this was controlled by genes at two loci, AAT-A and AAT-B.

a. AAT-A

At the AAT-A locus, there were three phenotypes, AA, BB and CC, produced by alleles *a*, *b* and *c*, respectively. In six populations of Okayama, Higashihiroshima, Hiroshima, Okinawa, Iriomote and Taiwan of *Rana limnocharis*, all the 72 frogs showed a homozygous BB band, while all the 18 frogs of the Philippine and Thailand populations belonging to *Rana cancrivora* showed a homozygous CC band. All the seven frogs of the New Guinea population belonging to *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 4).

b. AAT-B

At the AAT-B locus, there were two phenotypes, AA and BB, produced by alleles *a* and *b*, respectively. All the 72 frogs of the six populations belonging to

TABLE 3
Number of phenotypes and alleles at 29 loci in nine populations of *Rana limnocharis, R. cancrivora* and *Platymantis papuensis*

Locus	No. of Phenotypes	No. of alleles
AAT-A	3	3
AAT-B	2	2
ADA	6	6
AK	2	2
CK	2	2
Fum	6	5
α-GDH	3	3
GPI	7	6
HK	6	4
IDH-A	5	3
IDH-B	4	4
LDH-A	4	4
LDH-B	5	4
MDH-A	3	3
MDH-B	5	5
ME-A	4	4
ME-B	7	5
MPI	7	5
Pep-A	4	3
Pep-B	5	5
Pep-C	7	6
Pep-D	6	4
6-PGD	4	4
SOD-A	2	2
SOD-B	3	3
SORDH	6	4
Ab	7	5
Hb-I	3	3
Hb-II	4	4
Average	4.6	3.9

Rana limnocharis showed a homozygous AA band, while all the 25 frogs of the two populations belonging to *Rana cancrivora* and the one population of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 4).

2. Adenosine deaminase (ADA)

The electrophoretic patterns at the ADA locus in the 97 frogs of the nine populations belonging to the three species showed that there were six phenotypes, AA, CC, DD, EE, BE and DF, produced by six alleles, *a, b, c, d, e* and *f*. In *Rana limnocharis,* all the 44 frogs of the Okayama, Higashihiroshima, Okinawa and

TABLE 4

Gene frequencies at 29 loci in nine populations of *Rana limnocharis*, *R. cancrivora* and *Platymantis papuensis*

species		*R. limnocharis*						*R. cancrivora*		*P. papuensis*
Locality		Okayama	Higashi-hiroshima	Hiroshima	Okinawa	Iriomote	Taiwan	Philippine	Thailand	New Guinea
Sample size		5	16	20	18	5	8	8	10	7
Locus	Allele									
1) AAT-A	*a*									1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000			
	c							1.000	1.000	
2) AAT-B	*a*	1.000	1.000	1.000	1.000	1.000	1.000			
	b							1.000	1.000	1.000
3) ADA	*a*									1.000
	b			0.075						
	c							1.000	1.000	
	d						0.688			
	e	1.000	1.000	0.925	1.000	1.000				
	f						0.313			
4) AK	*a*							1.000	1.000	
	b	1.000	1.000	1.000	1.000	1.000	1.000			1.000
5) CK	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	b									1.000
6) Fum	*a*									1.000
	b							1.000		
	c	0.800	0.844	0.900	0.611	1.000	1.000			
	d								1.000	
	e	0.200	0.156	0.100	0.389					
7) α-GDH	*a*	1.000	1.000	1.000	1.000	1.000	1.000			
	b							1.000	1.000	
	c									1.000
8) GPI	*a*							0.938	1.000	
	b									1.000
	c	0.700	0.906	0.750	0.472		1.000			
	d							0.063		
	e					1.000				
	f	0.300	0.094	0.250	0.528					
9) HK	*a*				0.278					
	b	1.000	1.000	1.000	0.722	1.000	1.000	0.188	0.050	
	c							0.813	0.950	
	d									1.000

Continued

species		*R. limnocharis*						*R. cancrivora*		*P. papuensis*
Locality		Okayama	Higashi-hiroshima	Hiroshima	Okinawa	Iriomote	Taiwan	Philippine	Thailand	New Guinea
Sample size		5	16	20	18	5	8	8	10	7
Locus	Allele									
10) IDH-A	*a*	0.200	0.500	0.475	0.889	1.000	1.000	1.000	1.000	0.071
	b	0.800	0.500	0.525	0.111					
	c									0.929
11) IDH-B	*a*							1.000	1.000	
	b						1.000			
	c	1.000	1.000	1.000	1.000	1.000				
	d									1.000
12) LDH-A	*a*								0.100	
	b									1.000
	c							1.000	0.900	
	d	1.000	1.000	1.000	1.000	1.000	1.000			
13) LDH-B	*a*									1.000
	b	1.000	1.000	1.000	0.694	1.000	1.000			
	c				0.306					
	d							1.000	1.000	
14) MDH-A	*a*									1.000
	b					1.000				
	c	1.000	1.000	1.000	1.000		1.000	1.000	1.000	
15) MDH-B	*a*					0.200				
	b	1.000	1.000	1.000	1.000	0.800	0.750			
	c									1.000
	d						0.250			
	e							1.000	1.000	
16) ME-A	*a*							0.750	1.000	
	b									1.000
	c							0.250		
	d	1.000	1.000	1.000	1.000	1.000	1.000			
17) ME-B	*a*									1.000
	b							0.188		
	c	1.000	1.000	1.000	1.000		0.500	0.813	1.000	
	d					1.000				
	e						0.500			
18) MPI	*a*	1.000	1.000	1.000	1.000		1.000			
	b									1.000
	c					0.900				
	d							0.438	1.000	
	e					0.100		0.563		

19) Pep-A	*a*	0.800	0.531	0.575	0.583			0.375		
	b	0.200	0.469	0.425	0.417	1.000	1.000	0.625	1.000	
	c									1.000
20) Pep-B	*a*			0.050						
	b									1.000
	c	1.000	1.000	0.950	1.000	1.000	1.000			
	d							0.938	0.900	
	e							0.063	0.100	
21) Pep-C	*a*				0.167					
	b	0.500	0.719	0.950	0.833	1.000	0.250			
	c	0.500	0.281	0.050			0.750			
	d							0.125		
	e							0.875	1.000	
	f									1.000
22) Pep-D	*a*									1.000
	b	1.000	1.000	1.000	0.611		1.000			
	c				0.389	0.600				
	d					0.400		1.000	1.000	
23) 6-PGD	*a*	1.000	1.000	1.000	1.000		1.000			
	b					1.000				
	c							1.000	1.000	
	d									1.000
24) SOD-A	*a*									1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
25) SOD-B	*a*	1.000	1.000	1.000	1.000	1.000	1.000			
	b									1.000
	c							1.000	1.000	
26) SORDH	*a*									1.000
	b	0.900	0.563	0.900	1.000	0.800	1.000			
	c	0.100	0.438	0.100						
	d					0.200		1.000	1.000	
27) Ab	*a*						—	—	1.000	
	b					0.900	—	—		
	c	0.800	0.594	0.525	0.500	0.100	—	—		
	d	0.200	0.406	0.475	0.500		—	—		
	e						—	—		1.000
28) Hb-I	*a*						—	—	1.000	
	b						—	—		1.000
	c	1.000	1.000	1.000	1.000	1.000	—	—		
29) Hb-II	*a*	1.000	1.000	1.000	1.000		—	—		
	b						—	—		1.000
	c					1.000	—	—		
	d						—	—	1.000	

—, No sample

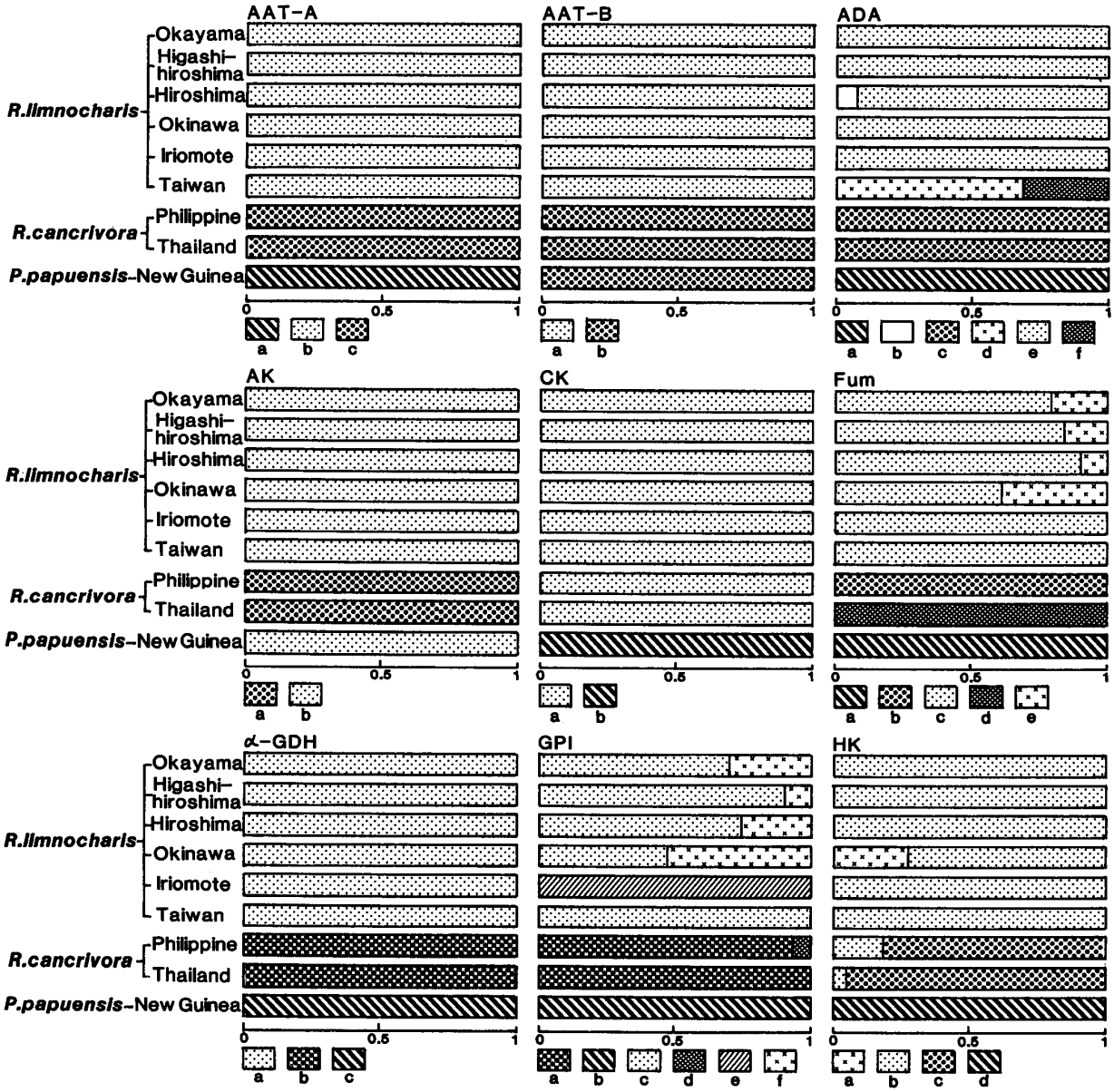

Fig. 4. Gene frequencies at nine loci, AAT-A, AAT-B, ADA, AK, CK, Fum, α-GDH, GPI and HK, in nine populations of *Rana limnocharis*, *R. cancrivora* and *Platymantis papuensis*.

Iriomote populations showed a homozygous EE band, produced by allele *e*, while 17 and three of the 20 frogs of the Hiroshima population showed EE and BE bands, respectively. Alleles *b* and *e* were 0.075 and 0.925 in frequency, respectively. Of the eight frogs of the Taiwan population, three and five showed DD and DF bands, respectively. Alleles *d* and *f* were 0.688 and 0.313 in frequency, respectively. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous CC band. All the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 4).

It was found that in *Rana limnocharis* excluding the Taiwan population, allele *e* was overwhelmingly abundant, while there were alleles *d* and *f* in the Taiwan population. *Rana cancrivora* and *Platymantis papuensis* had only alleles *c* and *a*,

respectively.

3. Adenylate kinase (AK)

The electrophoretic patterns at the AK locus in the 97 frogs of the nine populations belonging to the three species showed that there were two phenotypes, AA and BB, produced by two alleles, *a* and *b*. All the 79 frogs of the seven populations belonging to *Rana limnocharis* and *Platymantis papuensis* showed a homozygous BB band, while all the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous AA band (Table 4; Fig. 4).

4. Creatine kinase (CK)

The electrophoretic patterns at the CK locus in the 97 frogs of the nine populations belonging to the three species showed that there were two homozygous bands, AA and BB, produced by two alleles, *a* and *b*. All the 90 frogs of the six populations belonging to *Rana limnocharis* and the two populations belonging to *Rana cancrivora* showed a homozygous AA band, while all the seven frogs belonging to *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 4).

5. Fumarase (Fum)

The electrophoretic patterns at the Fum locus in the 97 frogs of the nine populations belonging to the three species showed that there were six phenotypes, AA, BB, CC, DD, EE and CE, produced by five alleles, *a, b, c, d* and *e*. All the 13 frogs of the Iriomote and Taiwan populations belonging to *Rana limnocharis* showed a homozygous CC band. In the Okayama, Higashihiroshima and Hiroshima populations of the same species, three of the five frogs, 11 of the 16 frogs and 16 of the 20 frogs, respectively, showed a homozygous CC band, while the other two, five and four, respectively, showed a heterozygous CE band. In these three populations, allele *c* was 0.800, 0.844 and 0.900 in frequency, while allele *e* was 0.200, 0.156 and 0.100. In the Okinawa population, seven, three and eight of the 18 frogs showed CC, EE and CE bands, respectively. In this population, alleles *c* and *e* were 0.611 and 0.389 in frequency, respectively. All the eight frogs of the Philippine population belonging to *Rana cancrivora* showed a homozygous BB band, while all the 10 frogs of the Thailand population showed a homozygous DD band. All the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 4).

It was found that in the six populations of *Rana limnocharis,* allele *c* was the most abundant, while there were only alleles *b, d* and *a* in the Philippine and Thailand populations of *Rana cancrivora* and the population of *Platymantis papuensis,* respectively (Table 4; Fig. 4).

6. α–Glycerophosphate dehydrogenase (α–GDH)

The electrophoretic patterns at the α–GDH locus in the 97 frogs of the nine populations belonging to the three species showed that there were three phenotypes, AA, BB and CC, produced by three alleles, *a, b* and *c*.

All the 72 frogs of the six populations belonging to *Rana limnocharis* showed a homozygous AA band. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous BB band, while all the seven frogs of *Platymantis papuensis* showed a homozygous CC band (Table 4; Fig. 4).

7. Glucose phosphate isomerase (GPI)

The electrophoretic patterns at the GPI locus in the 97 frogs of the nine populations belonging to the three species showed that there were seven phenotypes, AA, BB, CC, EE, FF, AD and CF, produced by six alleles, *a, b, c, d, e* and *f*.

In *Rana limnocharis,* three of the five frogs of the Okayama population and four of the 18 frogs of the Okinawa population showed a homozygous CC band, one and five others showed a homozygous FF band and the remaining one and nine showed a heterozygous CF band. In these two populations, allele *c* was 0.700 and 0.472 in frequency, while allele *f* was 0.300 and 0.528. In the Higashihiroshima and Hiroshima populations, 13 of the 16 frogs and 10 of the 20 frogs, respectively, showed a homozygous CC band, while the other three and 10, respectively, showed a heterozygous CF band. In these two populations, allele *c* was 0.906 and 0.750 in frequency, while allele *f* was 0.094 and 0.250. All the five frogs of the Iriomote population showed a homozygous EE band, while all the eight frogs of the Taiwan population showed a homozygous CC band. In the Philippine population of *Rana cancrivora,* seven and one of the eight frogs showed AA and AD bands, respectively. In this population, alleles *a* and *d* were 0.938 and 0.063 in frequency, respectively. All the 10 frogs of the Thailand population showed a homozygous AA band. All the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 4).

It was found that in the Okayama, Higashihiroshima, Hiroshima and Taiwan populations of *Rana limnocharis,* allele *c* was most abundant, being 0.700~1.000 in frequency. In the Okinawa population, alleles *c* and *f* were nearly the same in frequency, being 0.472 and 0.528, respectively. In the Iriomote population, there was only allele *e*. In the two populations of *Rana cancrivora,* allele *a* was overwhelmingly abundant, being 0.938 and 1.000 in frequency. The population of *Platymantis papuensis* had only allele *b* (Table 4; Fig. 4).

8. Hexokinase (HK)

The electrophoretic patterns at the HK locus in the 97 frogs of the nine populations belonging to the three species showed that there were six phenotypes, AA, BB, CC, DD, AB and BC, produced by four alleles, *a, b, c* and *d*. In *Rana limnocharis,* all the 54 frogs of five populations other than the Okinawa population showed a homozygous BB band, while two, 10 and six of the 18 frogs of the Okinawa population showed AA, BB and AB bands, respectively. In this population, alleles *a* and *b* were 0.278 and 0.722 in frequency, respectively. In *Rana cancrivora,* five of the eight frogs of the Philippine population and nine of the 10 frogs of the Thailand population showed a homozygous CC band, while the other three and one, respectively, showed a heterozygous BC band. In these two

populations, allele *b* was 0.188 and 0.050 in frequency, while allele *c* was 0.813 and 0.950. All the seven frogs of *Platymantis papuensis* showed a homozygous DD band.

It was found that the six populations of *Rana limnocharis* had almost allele *b* alone, the two populations of *Rana cancrivora* had mostly allele *c*, and *Platymantis papuensis* had only allele *d* (Table 4; Fig. 4).

9. Isocitrate dehydrogenase (IDH)

The electrophoretic patterns of IDH in the 97 frogs of the nine populations belonging to the three species showed that they were controlled by genes at two loci, IDH-A and IDH-B.

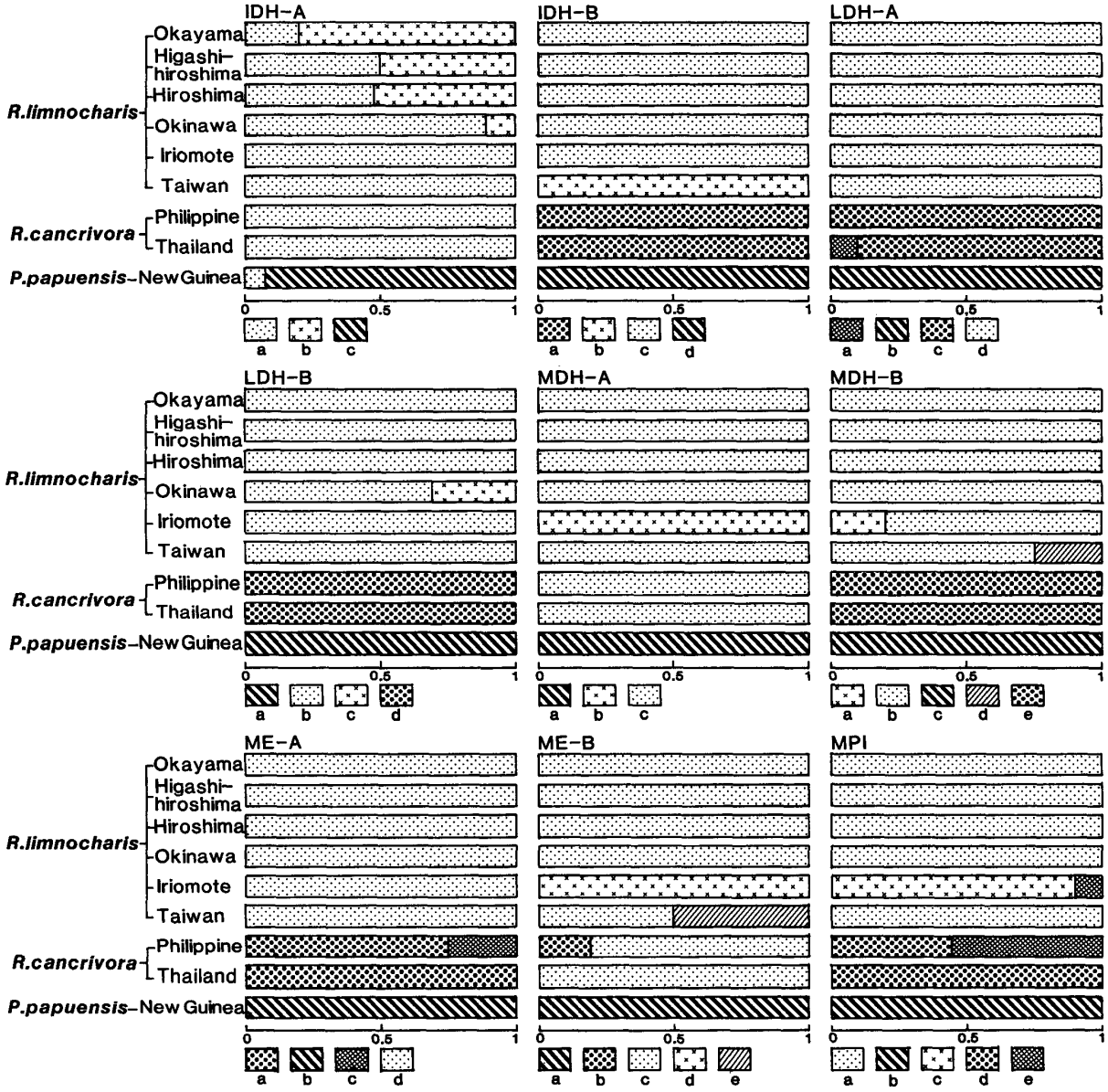

Fig. 5. Gene frequencies at nine loci, IDH-A, IDH-B, LDH-A, LDH-B, MDH-A, MDH-B, ME-A, ME-B and MPI, in nine populations of *Rana limnocharis*, *R. cancrivora* and *Platymantis papuensis*.

a. IDH-A

At the IDH-A locus, five phenotypes, AA, BB, CC, AB and AC, produced by three alleles, *a, b* and *c,* were observed. In *Rana limnocharis,* three and two of the five frogs of the Okayama population showed BB and AB bands, respectively. In this population, alleles *a* and *b* were 0.200 and 0.800 in frequency, respectively. In the Higashihiroshima and Hiroshima populations, four of the 16 frogs and five of the 20 frogs, respectively, showed a homozygous AA band, four and six, respectively, showed a homozygous BB band, and the remaining eight and nine, respectively, showed a heterozygous AB band. In these two populations, allele *a* was 0.500 and 0.475 in frequency, and allele *b* was 0.500 and 0.525. In the Okinawa population, 14 and four of the 18 frogs showed AA and AB bands, respectively. In this population, alleles *a* and *b* were 0.889 and 0.111 in frequency, respectively. All the 13 frogs of the Iriomote and Taiwan populations showed a homozygous AA band. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous AA band. Of the seven frogs of *Platymantis papuensis,* six and one showed CC and AC bands, respectively. In this population, alleles *a* and *c* were 0.071 and 0.929 in frequency, respectively.

It was found that in *Rana limnocharis,* allele *b* was most abundant in the Okayama population, alleles *a* and *b* were nearly the same in frequency in the Higashihiroshima and Hiroshima populations, and allele *a* was overwhelmingly abundant in the Okinawa, Iriomote and Taiwan populations. The two populations of *Rana cancrivora* had only allele *a,* while *Platymantis papuensis* had mostly allele *c* (Table 4; Fig. 5).

b. IDH-B

At the IDH-B locus, four phenotypes, AA, BB, CC and DD, produced by four alleles, *a, b, c* and *d,* were observed. All the 64 frogs of the five populations other than the Taiwan in *Rana limnocharis* showed a homozygous CC band, while all the eight frogs of the Taiwan population showed a homozygous BB band. All the 18 frogs of the two populations belonging to *Rana cancrivora* and all the seven frogs of *Platymantis papuensis* showed AA and DD bands, respectively (Table 4; Fig. 5).

10. Lactate dehydrogenase (LDH)

The electrophoretic patterns of LDH in the 97 frogs of the nine populations of the three species showed that they were controlled by genes at two loci, LDH-A and LDH-B.

a. LDH-A

At the LDH-A locus, there were four phenotypes, BB, CC, DD and AC, produced by four alleles, *a, b, c* and *d.* All the 72 frogs of the six populations belonging to *Rana limnocharis* showed a homozygous DD band. In *Rana cancrivora,* all the eight frogs of the Philippine population showed a homozygous CC band, while eight and two of the 10 frogs of the Thailand population showed CC and AC

bands, respectively. In this population, alleles *a* and *c* were 0.100 and 0.900 in frequency, respectively. All the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 5).

b. LDH-B

At the LDH-B locus, five phenotypes, AA, BB, CC, DD and BC, produced by four alleles, *a, b, c* and *d,* were observed. All the 54 frogs of the five populations other than the Okinawa population in *Rana limnocharis* showed a homozygous BB band, while eight, one and nine of the 18 frogs of the Okinawa population showed BB, CC and BC bands, respectively. In this population, alleles *b* and *c* were 0.694 and 0.306 in frequency, respectively. All the 18 frogs of the two populations belonging to *Rana cancrivora* and all the seven frogs of *Platymantis papuensis* showed DD and AA bands, respectively (Table 4; Fig. 5).

11. Malate dehydrogenase (MDH)

The electrophoretic patterns of MDH in the 97 frogs of the nine populations belonging to the three species showed that they were controlled by genes at two loci, MDH-A and MDH-B.

a. MDH-A

At the MDH-A locus, three phenotypes, AA, BB and CC, produced by three alleles, *a, b* and *c,* were observed. All the 85 frogs of the five populations other than the Iriomote population in *Rana limnocharis* and two populations of *Rana cancrivora* showed a homozygous CC band, while all the five frogs of the Iriomote population showed a homozygous BB band. All the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 5).

b. MDH-B

At the MDH-B locus, five phenotypes, AA, BB, CC, EE and BD, produced by five alleles, *a, b, c, d* and *e,* were observed. All the 59 frogs of the four populations other than the Iriomote and Taiwan populations in *Rana limnocharis* showed a homozygous BB band. Of the five frogs of the Iriomote population, one and four showed AA and BB bands, respectively. In this population, alleles *a* and *b* were 0.200 and 0.800 in frequency, respectively. Of the eight frogs of the Taiwan population, four and four showed BB and BD bands, respectively. In this population, alleles *b* and *d* were 0.750 and 0.250 in frequency, respectively. All the 18 frogs of the two populations of *Rana cancrivora* showed a homozygous EE band. All the seven frogs of *Platymantis papuensis* showed a homozygous CC band (Table 4; Fig. 5).

12. Malic enzyme (ME)

The electrophoretic patterns of ME in the 97 frogs of the nine populations of the three species showed that they were controlled by genes at two loci, ME-A and ME-B.

a. ME-A

At the ME-A locus, four phenotypes, AA, BB, DD and AC, produced by four alleles, *a, b, c* and *d,* were observed. All the 72 frogs of the six populations belonging to *Rana limnocharis* showed a homozygous DD band. Of the eight frogs of the Philippine population of *Rana cancrivora,* four and four showed AA and AC bands, respectively. Alleles *a* and *c* were 0.750 and 0.250 in frequency, respectively. All the 10 frogs of the Thailand population showed a homozygous AA band. All the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 5).

b. ME-B

At the ME-B locus, seven phenotypes, AA, BB, CC, DD, EE, BC and CE, produced by five alleles, *a, b, c, d* and *e,* were observed. In *Rana limnocharis,* all the 59 frogs of the four populations other than the Iriomote and Taiwan populations showed a homozygous CC band, while all the five frogs of the Iriomote population showed a homozygous DD band. Of the eight frogs of the Taiwan population, two, two and four showed CC, EE and CE bands, respectively. Both alleles *c* and *e* were 0.500 in frequency in this population. Of the eight frogs of the Philippine population belonging to *Rana cancrivora,* one, six and one showed BB, CC and BC bands, respectively. Alleles *b* and *c* were 0.188 and 0.813, respectively. All the 10 frogs of the Thailand population showed a homozygous CC band, while all the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4).

It was found that in the Okayama, Higashihiroshima, Hiroshima and Okinawa populations of *Rana limnocharis* and two populations of *Rana cancrivora,* allele *c* was overwhelmingly abundant. In the Taiwan population of *Rana limnocharis,* alleles *c* and *e* were the same in frequency. The Iriomote population of *Rana limnocharis* and the population of *Platymantis papuensis* had only alleles *d* and *a,* respectively (Table 4; Fig. 5).

13. Mannose phosphate isomerase (MPI)

The electrophoretic patterns at the MPI locus in the 97 frogs of the nine populations belonging to the three species showed that there were seven phenotypes, AA, BB, CC, DD, EE, CE and DE, produced by five alleles, *a, b, c, d* and *e.* All the 67 frogs of the five populations other than the Iriomote population in *Rana limnocharis* showed a homozygous AA band. Of the five frogs of the Iriomote population, four and one showed CC and CE bands, respectively. In this population, alleles *c* and *e* were 0.900 and 0.100 in frequency, respectively. In *Rana cancrivora,* two, three and three of the eight frogs of the Philippine population showed DD, EE and DE bands, respectively. In this population, alleles *d* and *e* were 0.438 and 0.563 in frequency, respectively. On the other hand, all the 10 frogs of the Thailand population showed a homozygous DD band. All the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 5).

14. Peptidase (Pep)

The electrophoretic patterns of Pep in the 97 frogs of the nine populations belonging to the three species showed that this enzyme was controlled by genes at four loci, Pep-A, Pep-B, Pep-C and Pep-D.

a. Pep-A

At the Pep-A locus, four phenotypes, AA, BB, CC and AB, produced by three alleles, *a, b* and *c,* were observed. In *Rana limnocharis,* three and two of the five frogs of the Okayama population showed AA and AB bands, respectively. Alleles *a* and *b* were 0.800 and 0.200 in frequency, respectively. In the Higashihiroshima, Hiroshima and Okinawa populations, four of the 16 frogs, seven of the 20 frogs and six of the 18 frogs, respectively, showed an AA band, three, four and three, respectively, showed a homozygous BB band, and the remaining nine, nine and nine, respectively, showed a heterozygous AB band. In these three populations, alleles *a* and *b* were 0.531~0.583 and 0.417~0.469 in frequency, respectively. All the 13 frogs of the Iriomote and Taiwan populations showed a homozygous BB band. In *Rana cancrivora,* two, four and two of the eight frogs of the Philippine population showed AA, BB and AB bands, respectively. In this population, alleles *a* and *b* were 0.375 and 0.625 in frequency, respectively. All the 10 frogs of the Thailand population showed a homozygous BB band. In *Platymantis papuensis,* all the seven frogs showed a homozygous CC band (Table 4; Fig. 6).

It was found that there were alleles *a* and *b* in the Okayama, Higashihiroshima, Hiroshima and Okinawa populations of *Rana limnocharis* and the Philippine population of *Rana cancrivora,* while there was only allele *b* in the Iriomote and Taiwan populations of *Rana limnocharis* and the Thailand population of *Rana cancrivora. Platymantis papuensis* had only allele *c.*

b. Pep-B

At the Pep-B locus, five phenotypes, BB, CC, DD, AC and DE, produced by five alleles, *a, b, c, d* and *e,* were observed. All the 52 frogs of the five populations other than the Hiroshima population in *Rana limnocharis* showed a homozygous CC band. Of the 20 frogs of the Hiroshima population, 18 and two showed CC and AC bands, respectively. In this population, alleles *a* and *c* were 0.050 and 0.950 in frequency, respectively. In the Philippine and Thailand populations of *Rana cancrivora,* seven of the eight frogs and eight of the 10 frogs showed a homozygous DD band and the other one and two showed a heterozygous DE band, respectively. In these two populations, allele *d* was 0.938 and 0.900 in frequency, while allele *e* was 0.063 and 0.100. All the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 6). It was found that in *Rana limnocharis,* allele *c* was overwhelmingly abundant, while allele *d* was the most abundant in the two populations of *Rana cancrivora,* and there was only allele *b* in *Platymantis papuensis.*

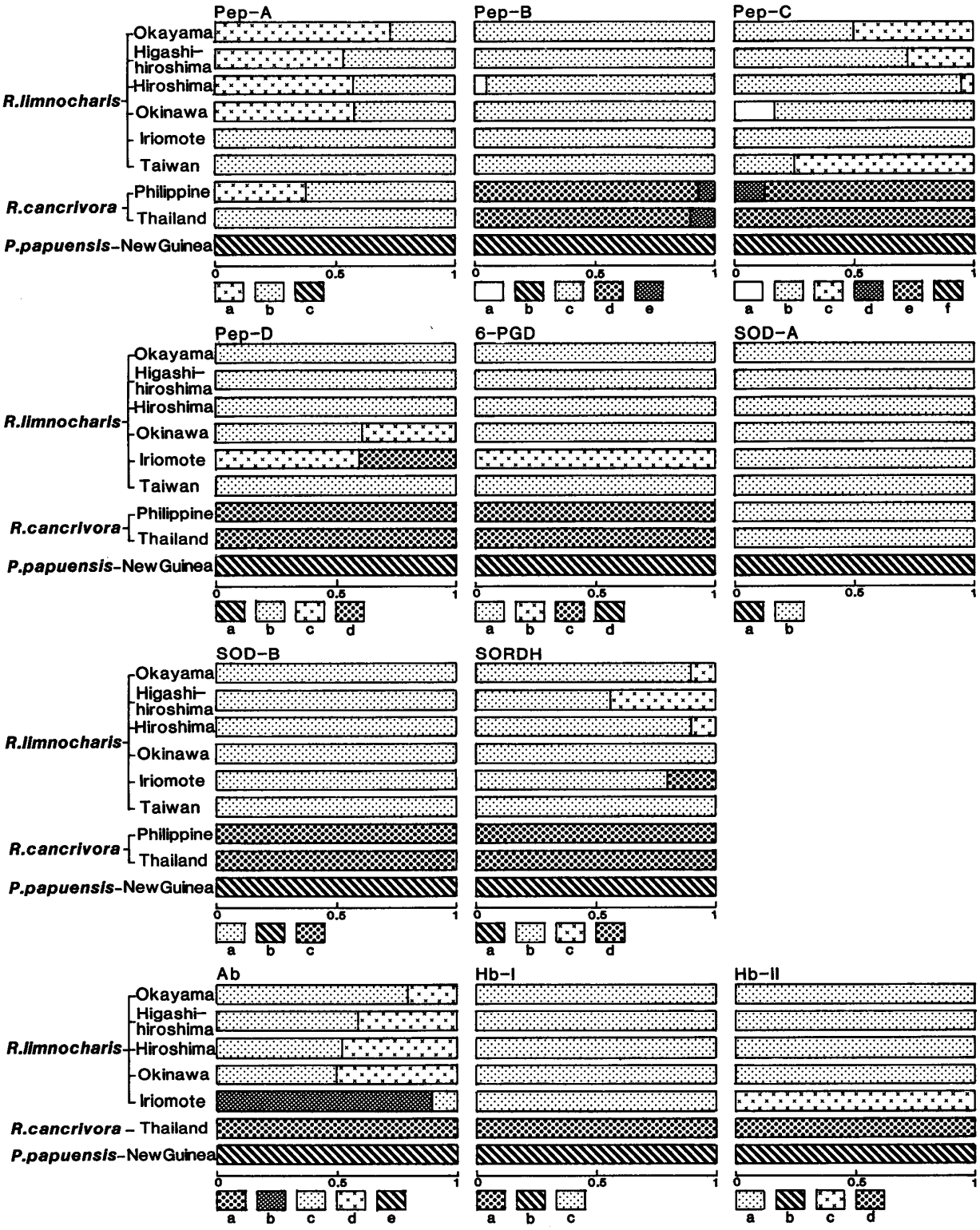

Fig. 6. Gene frequencies at 11 loci, Pep-A, Pep-B, Pep-C, Pep-D, 6-PGD, SOD-A, SOD-B, SORDH, Ab, Hb-I and Hb-II, in nine populations of *Rana limnocharis, R. cancrivora* and *Platymantis papuensis*.

c. Pep-C

At the Pep-C locus, seven phenotypes, BB, CC, EE, FF, AB, BC and DE, produced by six alleles, *a, b, c, d, e* and *f*, were observed. In the Okayama and

Higashihiroshima populations of *Rana limnocharis,* one of the five frogs and eight of the 16 frogs, respectively, showed a homozygous BB band, one and one, respectively, showed a homozygous CC band, and the remaining three and seven, respectively, showed a heterozygous BC band. In these two populations, allele *b* was 0.500 and 0.719 in frequency, while allele *c* was 0.500 and 0.281. In the Hiroshima population of the same species, 18 and two of the 20 frogs showed BB and BC bands, respectively. Alleles *b* and *c* were 0.950 and 0.050 in frequency, respectively. In the Okinawa population, 12 and six of the 18 frogs showed BB and AB bands, respectively. Alleles *a* and *b* were 0.167 and 0.833 in frequency, respectively. In the Iriomote population, all the five frogs showed a homozygous BB band, while in the Taiwan population, four and four of the eight frogs showed CC and BC bands, respectively. Alleles *b* and *c* were 0.250 and 0.750 in frequency, respectively. In the Philippine population of *Rana cancrivora,* six and two of the eight frogs showed EE and DE bands, respectively. Alleles *d* and *e* were 0.125 and 0.875 in frequency, respectively. In the Thailand population, all the 10 frogs showed a homozygous EE band. All the seven frogs of *Platymantis papuensis* showed a homozygous FF band (Table 4; Fig. 6).

It was found that in the Okayama population of *Rana limnocharis,* there were equal frequencies of alleles *b* and *c,* while allele *b* was the most abundant in the Higashihiroshima, Hiroshima, Okinawa and Iriomote populations, and allele *c* was the most abundant in the Taiwan population of the same species. In the two populations of *Rana cancrivora,* allele *e* was overwhelmingly abundant, and there was only allele *f* in *Platymantis papuensis.*

d. Pep-D

At the Pep-D locus, six phenotypes, AA, BB, CC, DD, BC and CD, produced by four alleles, *a, b, c* and *d,* were observed. All the 49 frogs of the four populations other than the Okinawa and Iriomote populations in *Rana limnocharis* showed a homozygous BB band. In the Okinawa population, seven, three and eight of the 18 frogs showed BB, CC and BC bands, respectively. In this population, alleles *b* and *c* were 0.611 and 0.389 in frequency, respectively. In the Iriomote population, two, one and two of the five frogs showed CC, DD and CD bands, respectively. Alleles *c* and *d* were 0.600 and 0.400 in frequency, respectively. In *Rana cancrivora,* all the 18 frogs of the two populations showed a homozygous DD band, while all the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 6).

It was found that the Okayama, Higashihiroshima, Hiroshima and Taiwan populations of *Rana limnocharis* had only allele *b,* the Okinawa population had alleles *b* and *c,* and the Iriomote population had alleles *c* and *d.* The two populations of *Rana cancrivora* had only allele *d,* while the seven frogs of *Platymantis papuensis* had only allele *a.*

15. 6-Phosphogluconate dehydrogenase (6-PGD)

The electrophoretic patterns at the 6-PGD locus in the 97 frogs of the nine

populations belonging to the three species showed that there were four phenotypes, AA, BB, CC and DD, produced by four alleles, *a, b, c* and *d*. In *Rana limnocharis*, all the 67 frogs of the five populations other than the Iriomote population showed a homozygous AA band, while all the five frogs of the Iriomote population showed a homozygous BB band. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous CC band, while all the seven frogs of *Platymantis papuensis* showed a homozygous DD band (Table 4; Fig. 6).

16. Superoxide dismutase (SOD)

The electrophoretic patterns of SOD in the 97 frogs of the nine populations belonging to the three species showed that they were controlled by genes at two loci, SOD-A and SOD-B.

a. SOD-A

At the SOD-A locus, two phenotypes, AA and BB, produced by two alleles, *a* and *b*, were observed. All the 90 frogs of the six populations of *Rana limnocharis* and the two populations of *Rana cancrivora* showed a homozygous BB band, while all the seven frogs of *Platymantis papuensis* showed a homozygous AA band (Table 4; Fig. 6).

b. SOD-B

At the SOD-B locus, three phenotypes, AA, BB and CC, produced by three alleles, *a, b* and *c*, were observed. All the 72 frogs of the six populations belonging to *Rana limnocharis* showed a homozygous AA band. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous CC band, while all the seven frogs of *Platymantis papuensis* showed a homozygous BB band (Table 4; Fig. 6).

17. Sorbitol dehydrogenase (SORDH)

The electrophoretic patterns at the SORDH locus in the 97 frogs of the nine populations belonging to the three species showed that there were six phenotypes, AA, BB, CC, DD, BC and BD, produced by four alleles, *a, b, c* and *d*. In the Okayama and Hiroshima populations of *Rana limnocharis*, four of the five frogs and 16 of the 20 frogs, respectively, showed a homozygous BB band, and the other one and four frogs, respectively, showed a heterozygous BC band. In these populations, alleles *b* and *c* were 0.900 and 0.100 in frequency, respectively. Of the 16 frogs of the Higashihiroshima population of *Rana limnocharis*, five, three and eight showed BB, CC and BC bands, respectively. Alleles *b* and *c* were 0.563 and 0.438 in frequency, respectively. Of the five frogs of the Iriomote population of the same species, three and two showed BB and BD bands, respectively. Alleles *b* and *d* were 0.800 and 0.200 in frequency, respectively. All the 26 frogs of the Okinawa and Taiwan populations showed a homozygous BB band. All the 18 frogs of the two populations belonging to *Rana cancrivora* showed a homozygous DD band, while all the seven frogs of *Platymantis papuensis* showed a homozygous AA

band (Table 4; Fig. 6).

It was found that allele *b* was overwhelmingly abundant in the five populations other than the Higashihiroshima population of *Rana limnocharis,* while alleles *b* and *c* were nearly the same in frequency in the Higashihiroshima population. There were only alleles *d* and *a* in the two populations of *Rana cancrivora* and the population of *Platymantis papuensis,* respectively.

18. Serum albumin (Ab)

The electrophoretic patterns at the Ab locus in 81 frogs of seven populations of the three species showed that there were seven phenotypes, AA, BB, CC, DD, EE, BC and CD, produced by five alleles, *a, b, c, d* and *e.* In *Rana limnocharis,* three and two of the five frogs of the Okayama population showed CC and CD bands, respectively. Alleles *c* and *d* were 0.800 and 0.200 in frequency, respectively. In the Higashihiroshima, Hiroshima and Okinawa populations, six of the 16 frogs, five of the 20 frogs and five of the 18 frogs, respectively, showed a homozygous CC band, three, four and five, respectively, showed a homozygous DD band, and the remaining seven, eleven and eight, respectively, showed a heterozygous CD band. In these three populations, allele *c* was 0.500~0.594 in frequency and allele *d* was 0.406~0.500. Of the five frogs of the Iriomote population, four and one showed BB and BC bands, respectively. In this population, alleles *b* and *c* were 0.900 and 0.100 in frequency, respectively. In *Rana cancrivora,* all the 10 frogs of the Thailand population showed a homozygous AA band. In *Platymantis papuensis,* all the seven frogs showed a homozygous EE band (Table 4; Fig. 6).

19. Hemoglobin (Hb)

The electrophoretic patterns of Hb in the 81 frogs of the seven populations belonging to the three species showed that they were controlled by genes at two loci, Hb-I and Hb-II.

a. Hb-I

At the Hb-I locus, three phenotypes, AA, BB and CC, produced by three alleles, *a, b* and *c,* were observed. All the 64 frogs of five populations of Okayama, Higashihiroshima, Hiroshima, Okinawa and Iriomote of *Rana limnocharis* showed a homozygous CC band. All the 10 frogs of the Thailand population of *Rana cancrivora* showed a homozygous AA band. In *Platymantis papuensis,* all the seven frogs showed a homozygous BB band (Table 4; Fig. 6).

b. Hb-II

At the Hb-II locus, four phenotypes, AA, BB, CC and DD, produced by four alleles, *a, b, c* and *d,* were observed. In *Rana limnocharis,* all the 59 frogs of the four populations other than the Iriomote population showed a homozygous AA band, while all the five frogs of the Iriomote population showed a homozygous CC band. In *Rana cancrivora,* all the 10 frogs of the Thailand population showed a homozygous DD band, while in *Platymantis papuensis,* all the seven frogs showed a

homozygous BB band (Table 4; Fig. 6).

III. Genetic variation

1. Proportion of heterozygous loci

The degree of differentiation at all the 29 loci of each individual is shown by the proportion of heterozygous loci per individual. The mean proportion of heterozygous loci in each population was examined at the 26 loci controlling their 17 enzymes in 97 frogs of nine populations belonging to *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis*. The results showed that the mean proportion of heterozygous loci per individual was the lowest, being 0.5%, in the population of *Platymantis papuensis*. It was 1.9% in the Thailand population of *Rana cancrivora,* 3.8% in the Iriomote population of *Rana limnocharis,* and 8.2% in each of the Taiwan population of *Rana limnocharis* and the Philippine population of *Rana cancrivora*. In the Hiroshima, Okayama and Higashihiroshima populations of *Rana limnocharis,* they were 8.3%, 8.5% and 9.6%, respectively, while it was 12.6% in the Okinawa population of the same species. In the nine populations of the three species, they were 6.8% on the average.

These values of the mean proportions of heterozygous loci per individual were compared with the expected values calculated by the method of LEWONTIN (1974). It was found that there was scarcely any difference between the actual and expected values, as the expected values were 0.5~12.5%, 6.8% on the average, in contrast to the actual values which were 0.5~12.6%, 6.8% on the average (Table 5).

TABLE 5

Genetic variabilities at 26 loci in nine populations of *Rana limnocharis, R. cancrivora* and *Platymantis papuensis*

Species	Locality	Sample size	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)	Mean number of alleles per locus
Rana limnocharis	Okayama	5	8.5 (7.9)	23.1	1.23
〃	Higashi-hiroshima	16	9.6 (9.5)	23.1	1.23
〃	Hiroshima	20	8.3 (7.9)	30.8	1.31
〃	Okinawa	18	12.6 (12.5)	30.8	1.31
〃	Iriomote	5	3.8 (5.0)	15.4	1.15
〃	Taiwan	8	8.2 (6.5)	15.4	1.15
Rana cancrivora	Philippine	8	8.2 (9.2)	30.8	1.31
〃	Thailand	10	1.9 (1.8)	11.5	1.12
Platymantis papuensis	New Guinea	7	0.5 (0.5)	3.8	1.04
Average (Total)		10.8 (97)	6.8 (6.8)	20.5	1.21

Parentheses show expected values.

2. Proportion of polymorphic loci

At the 26 loci controlling the 17 enzymes in the 97 frogs of nine populations of *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis,* the mean proportion of polymorphic loci at which each of the alleles was contained at the rate of more than 1% was estimated in each population. It was the lowest, being 3.8%, in the population of *Platymantis papuensis* and the second lowest value was 11.5% in the Thailand population of *Rana cancrivora.* These were followed by 15.4% in each of the Iriomote and Taiwan populations of *Rana limnocharis,* 23.1% in each of the Okayama and Higashihiroshima populations of the same species, and 30.8% in each of the Hiroshima and Okinawa populations of *Rana limnocharis* and the Philippine population of *Rana cancrivora.* The mean proportions of polymorphic loci were 20.5% on the average in the nine populations of the three species (Table 5).

3. Mean number of alleles per locus

The mean number of alleles in each of the 26 loci which controlled the 17 enzymes in 97 frogs of the nine populations belonging to *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis* was counted. The results showed that it was the smallest, being 1.04, in the population of *Platymantis papuensis.* These were followed by 1.12 in the Thailand population of *Rana cancrivora,* 1.15 in each of the Iriomote and Taiwan populations of *Rana limnocharis,* 1.23 in each of the Okayama and Higashihiroshima populations of the same species, and 1.31 in each of the Hiroshima and Okinawa populations of *Rana limnocharis* and the Philippine population of *Rana cancrivora.* The mean numbers of alleles in the nine populations were 1.21 on the average (Table 5).

IV. Genetic distance and dendrogram

1. Genetic distance

Genetic distances (D) among different populations were estimated from gene frequencies at the 26 loci examined in 97 frogs of the nine populations belonging to *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis* (Nei, 1975).

Of the genetic distances among six populations of *Rana limnocharis,* the smallest was 0.009 between the Hiroshima and Higashihiroshima populations. The next was 0.015 between the Hiroshima and Okayama populations, followed by 0.016 between the Higashihiroshima and Okayama populations. The genetic distances between the Okinawa population and the Hiroshima, Higashihiroshima and Okayama populations were 0.030, 0.042 and 0.050, respectively. Those between the Taiwan population and the Higashihiroshima, Hiroshima, Okayama and Okinawa populations were 0.137, 0.143, 0.161 and 0.169, respectively, while those between the Iriomote population and the Okayama, Higashihiroshima, Hiroshima and Okinawa populations were 0.345, 0.307, 0.293 and 0.276, respectively. It was remarkable that the genetic distance between the Iriomote and Taiwan

TABLE 6

Genetic identity(I) and genetic distance(D) among nine populations of *Rana limnocharis*, *R. cancrivora* and *Platymantis papuensis*

Species	Locality		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
R. limnocharis	Okayama	(1)	—	0.985	0.985	0.952	0.708	0.852	0.195	0.180	0.041
〃	Higashihiroshima	(2)	0.016	—	0.991	0.958	0.735	0.872	0.211	0.204	0.042
〃	Hiroshima	(3)	0.015	0.009	—	0.971	0.746	0.867	0.208	0.200	0.042
〃	Okinawa	(4)	0.050	0.042	0.030	—	0.759	0.845	0.229	0.222	0.044
〃	Iriomote	(5)	0.345	0.307	0.293	0.276	—	0.679	0.185	0.185	0.042
〃	Taiwan	(6)	0.161	0.137	0.143	0.169	0.387	—	0.218	0.223	0.043
R. cancrivora	Philippine	(7)	1.637	1.555	1.568	1.472	1.687	1.524	—	0.935	0.043
〃	Thailand	(8)	1.715	1.589	1.609	1.507	1.687	1.502	0.067	—	0.042
P. papuensis	New Guinea	(9)	3.200	3.174	3.181	3.128	3.161	3.154	3.139	3.178	—

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

populations was large, being 0.387, in spite of the short distance between these two islands (Table 6).

The genetic distance between the Philippine and Thailand populations of *Rana cancrivora* was 0.067. Of the genetic distances between the populations of *Rana limnocharis* and those of *Rana cancrivora,* the smallest was 1.472 between the Okinawa and Philippine populations, followed by 1.502 between the Taiwan and Thailand populations, and 1.507 between the Okinawa and Thailand populations. The genetic distances between the Philippine population of *Rana cancrivora* and the Taiwan, Higashihiroshima and Hiroshima populations of *Rana limnocharis* were 1.524, 1.555 and 1.568, respectively. The genetic distances between the Thailand population of *Rana cancrivora* and the Higashihiroshima, Hiroshima and Okayama populations of *Rana limnocharis* were 1.589, 1.609 and 1.715, respectively. The genetic distance between each of the Philippine and Thailand populations of *Rana cancrivora* and the Iriomote population of *Rana limnocharis* was 1.687. The genetic distances between the population of *Platymantis papuensis* and the six populations of *Rana limnocharis* were 3.128~3.200. Those between the population of *Platymantis papuensis* and the Philippine and Thailand populations of *Rana cancrivora* were 3.139 and 3.178, respectively (Table 6).

These results showed that the Hiroshima, Higashihiroshima, Okayama and Okinawa populations of *Rana limnocharis* are very similar to one another in differentiation, as the genetic distances among them were 0.009~0.050. In contrast, these four populations evidently differ from the other two populations of Iriomote and Taiwan of the same species, as the genetic distances between these two groups were 0.137~0.345. It was found that the six populations of *Rana limnocharis* are distinctly separated from the two populations of *Rana cancrivora* in differentiation, and that the two populations of *Rana cancrivora* are further separated from the population of *Platymantis papuensis.*

2. Dendrogram

A dendrogram for the nine populations of *Rana limnocharis, Rana cancrivora* and *Platymantis papuensis* was drawn on the basis of the genetic distances among them by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975). This dendrogram shows that *Platymantis papuensis* first diverged from the others, and then *Rana cancrivora* and *Rana limnocharis* were differentiated from each other. In *Rana limnocharis,* the Iriomote population first diverged from all the other populations. The Taiwan population next diverged from the Okayama, Higashihiroshima, Hiroshima and Okinawa populations which were very closely related to each other (Fig. 7).

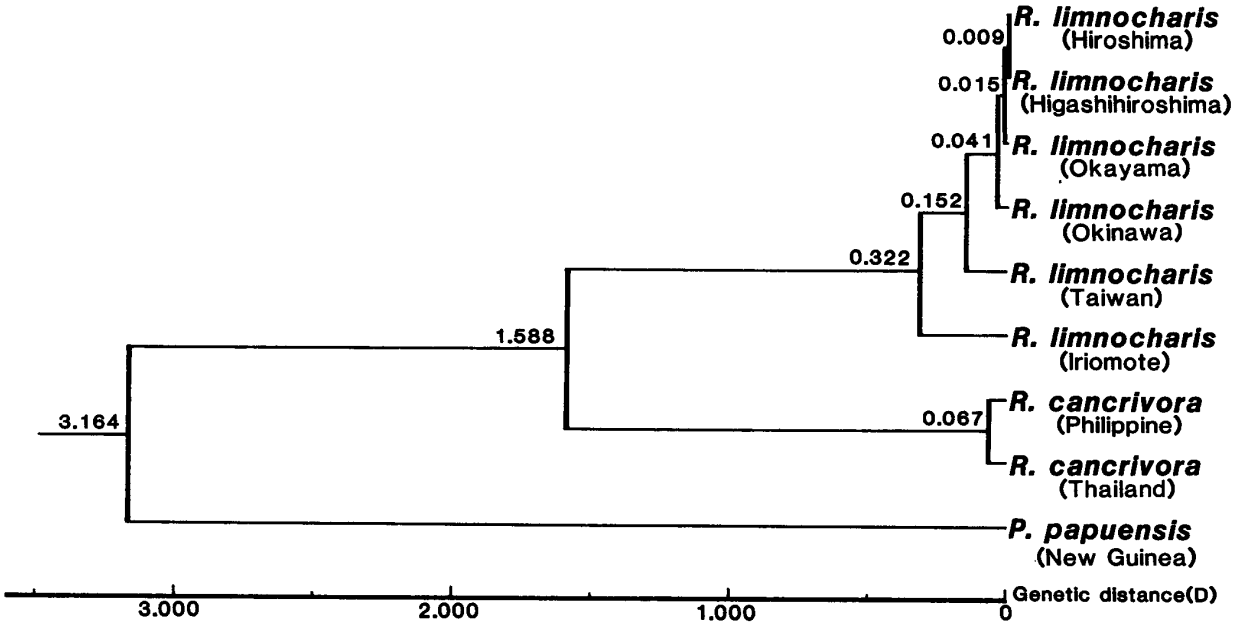

Fig. 7. Dendrogram for nine populations of *Rana limnocharis, R. cancrivora* and *Platymantis papuensis* based on genetic distances.

DISCUSSION

The present study on the genetic differentiation of *Rana limnocharis* and two allied species elucidated by electrophoretic analyses shows that the three parameters showing genetic variation are very low. In the six populations of *R. limnocharis,* the mean proportion of heterozygous loci per individual is 8.5%, the mean proportion of polymorphic loci per population is 23.1%, and the mean number of alleles per locus is 1.23. These values are also very low in *R. cancrivora* and *Platymantis papuensis.* The three parameters in two populations of *R. cancrivora* are 5.1%, 21.2% and 1.22, while those in one population of *P. papuensis* are 0.5%, 3.8% and 1.04, although the sample size of the latter species is only seven. In addition to these species, there are several species having low values of the three parameters in Japan and adjacent territory. The Sakhalin population of *Hyla*

japonica is 4.4%, 15.4%, and 1.15 in the three parameters (NISHIOKA, SUMIDA and BORKIN, 1990). Two populations of *Rhacophorus viridis* are 8.0%, 24.0% and 1.28 in the three parameters (NISHIOKA, SUMIDA, OHTA and SUZUKI, 1987). In some districts other than Far East, various amphibian species which are remarkably low in the three parameters showing genetic variation have been reported. *Pelobates syriacus* is 2.3%, 9% and 1.09 in mean proportion of heterozygous loci per individual, mean proportion of polymorphic loci per population and mean number of alleles per locus, respectively (NEVO, 1976). The mean proportion of heterozygous loci per individual and mean proportion of polymorphic loci per population are 3.9% and 23.8%, respectively, in *Scaphiopus holbrooki,* and 2.6% and 19.0%, respectively, in *S. couchi* (SATTLER, 1980). There are many urodeles which are low in the three parameters showing genetic variation. In the three parameters, mean proportion of heterozygous loci per individual, mean proportion of polymorphic loci per population and mean number of alleles per locus, *Taricha torosa* is 4.6%, 20.5% and 1.22 (HEDGECOCK, 1976), *T. rivularis* is 6.8%, 20.9% and 1.24 (HEDGECOCK, 1978), *Triturus cristatus* is 5.2%, 27.8% and 1.28 (KALEZIĆ and HEDGECOCK, 1979), *Eurycea lucifuga* is 3.9%, 13.9% and 1.14 (MERKLE and GUTTMAN, 1977) and *Plethodon yanahlossee* is 2.3%, 8.2% and 1.14 (GUTTMAN, KARLIN and LABANICK, 1978), respectively.

These low values in the three parameters showing genetic variation are in distinct contrast to those found in several anurans and urodeles. The seven populations of *Rana tagoi* which are distributed in the mountainous districts of Japan are remarkably high in these three parameters, being 16.1%, 55.2% and 1.97 (NISHIOKA, OHTA and SUMIDA, 1987). In the three parameters showing genetic variation, *Buergeria buergeri* is 11.4%, 56.0% and 2.00, *Rhacophorus schlegelii* is 20.7%, 64.0% and 2.06, *Rhacophorus arboreus* is 12.6%, 48.0% and 1.56 (NISHIOKA, SUMIDA, OHTA and SUZUKI, 1987), the Hiroshima population of *Hyla japonica* is 15.4%, 69.2% and 2.04, the Taiwan population of *Hyla chinensis* is 15.9%, 50.0% and 1.62 (NISHIOKA, SUMIDA and BORKIN, 1990), and the Mito population of *Bufo j. japonicus* is 14.8% or 15.4%, 65.0% or 63.2% and 1.95 (KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI, 1990; NISHIOKA, SUMIDA, UEDA and WU, 1990). In European amphibians, *Triturus alpestris* is 13.5%, 49.2% and 1.62 in the three parameters showing genetic variation (KALEZIĆ and HEDGECOCK, 1979), and *Bufo viridis* is 13.4%, 42.3% and 1.65 (DESSAUER, NEVO and CHUANG, 1975) and 14.1%, 47.0% and 1.69 (NEVO, 1976).

NISHIOKA, OHTA and SUMIDA (1987) have assumed that the high degrees of genetic variability observed in *Rana tagoi* and some other species seem to be attributable to the selection for heterozygosity operating as an adaptive strategy in the ecologically variable environment, as suggested in *Bufo viridis* by DESSAUER, NEVO and CHUANG (1975). In contrast, the low degrees of genetic variability found in *Rana limnocharis, Rana cancrivora, Platymantis papuensis* and some other species, like the Sakhalin population of *Hyla japonica, Rhacophorus viridis, Pelobates syriacus, Scaphiopus holbrooki, Scaphiopus couchi* and several urodeles seem to be an adaptation to ecologically stabilized environment.

The genetic distances between three populations of the Okayama–Hiroshima area and the Okinawa population of *Rana limnocharis* are 0.030~0.050, while those between the three populations and the population collected from Iriomote Island are 0.293~0.345. While the genetic distances between the three populations and the Taiwan population are 0.137~0.161, and that between the population from Okinawa Island and the Taiwan population is 0.169, that between the Iriomote and Taiwan populations is 0.387. The genetic distances between all the six populations of *R. limnocharis* and the two populations of *R. cancrivora* are 1.472 ~1.715. These results show that the Iriomote population of *Rana limnocharis* has been genetically differentiated far from the other populations, probably owing to long-term geographic isolation. The Iriomote population may be genetically given a position as a subspecies. However, it is remarkable that the genetic distances of subspecies are not generally definite.

According to HEDGECOCK and AYALA (1974), the genetic distances between populations, between subspecies and between species confirmed among three populations of *Taricha rivularis,* two populations of *T. granulosa,* three populations of *T. t. torosa* and one population of *T. t. sierrae* distributed mainly in the coastal area of California are 0.029 ± 0.010, 0.145 ± 0.027 and 0.466 ± 0.021, respectively. Among three populations of *Triturus vulgaris,* two populations of *T. alpestris,* two populations of *T. cristatus dobrogicus* and one population of *T. cristatus karelinii* distributed in the mainland of Yugoslavia, KALEZIĆ and HEDGECOCK (1979) have confirmed that the genetic distances between populations, between subspecies and between species are 0.031 ± 0.017, 0.347 and 0.906 ± 0.058, respectively.

The genetic distances among 39 populations of the four subspecies of *Bufo japonicus* other than *B. j. miyakonis* distributed in Miyako Island are 0.003~0.231, while those between *B. j. miyakonis* and the above 39 populations are 0.366~0.521 (KAWAMURA, NISHIOKA, SUMIDA and RYUZAKI, 1990). The genetic distances among seven populations of *Bufo j. japonicus* are 0.008~0.241, while those between the seven populations of *B. j. japonicus* and *B. j. gargarizans* from China and Taiwan are 0.235~0.383. The genetic distances between the seven populations of *B. j. japonicus* and *B. b. bufo* from Europe are 0.895~1.047 (NISHIOKA, SUMIDA, UEDA and WU, 1990).

The genetic distances among seven populations of *Hyla japonica* distributed in Japan and Korea are 0.012~0.201, while those between the seven populations of *H. japonica* and *H. chinensis* from Taiwan are 1.177~1.360, and those between the former seven populations and *H. hallowelli* are 0.974~1.131 (NISHIOKA, SUMIDA and BORKIN, 1990). The genetic distances among four populations of *R. t. tagoi* collected from the western half of Honshu, Shikoku and Kyushu of Japan are 0.031~0.152, while those between these four populations and *R. t. yakushimensis* collected from Yaku Island are 0.225~0.335 (NISHIOKA, OHTA and SUMIDA, 1987). The genetic distances between two populations of *Rhacophorus arboreus* and two populations of *Rh. schlegelii* are 0.301~0.387 (NISHIOKA, SUMIDA, OHTA and SUZUKI, 1987). These two species are completely isolated from each other by gametic isolation and hybrid inviability (KAWAMURA, 1962), although they are so similar to

each other in external characters that *Rh. arboreus* was first named as *Polypedates schlegelii* var. *arborea* by OKADA and KAWANO (1924).

The genetic distances between populations, between subspecies and between species obtained in the foregoing 15 species are 0.030~0.241, 0.145~0.521 and 0.301~1.715, respectively. These values seem to show that the nomenclature based on external morphology is correct on the whole, although there are some exceptions.

However, it is remarkable that there is a species in which the genetic distances between populations are extremely large. In *Rana narina,* there are six populations, including two dwarf-type, three giant-type and one middle-type populations. The genetic distances between the two dwarf-type populations and among the three giant-type populations are 0.012~0.462, while those between the two dwarf-type and the three giant-type populations are 0.714~1.079. The genetic distances between the middle-type and the two dwarf-type populations are 0.809 and 0.865, while those between the middle-type and the three giant-type populations are 0.232~0.471. On the basis of genetic distances, each of the dwarf-, middle- and giant-type populations seems to be a real species, as stated previously. In fact, the hybrids between the females of the two dwarf-type populations and the males of the three giant-type populations or the middle-type population, and the reciprocal hybrids between these populations are all lethal at the early developmental stage. In contrast, the two dwarf-type populations or the three giant-type populations are not reproductively isolated from each other (NISHIOKA, UEDA and SUMIDA, 1987).

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