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Intraspecific Differentiation of *Rana narina* Elucidated by Crossing Experiments and Electrophoretic Analyses of Enzymes and Blood Proteins

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(With 11 Text-figures)

ABSTRACT

There are six populations of *Rana narina* in four islands of the Southwest Islands of Japan. In each of Iriomote and Ishigaki Isls., the dwarf- and giant-type populations are sympatrically distributed, while the middle- and giant-type populations are found in Okinawa and Amami Isls., respectively. The frogs of the dwarf-type population are smaller in body size and lay distinctly fewer eggs, as compared with those of the giant-type population. The frogs of the middle-type population are intermediate between those of the dwarf- and giant-type populations in body size and egg number. The phylogenetic differentiation of the six populations was examined by crossing experiments among them as well as by electrophoretic analyses of enzymes and blood proteins extracted from them.

The dwarf-type populations of Ishigaki and Iriomote Isls. seem to be completely isolated from the giant-type populations of these two islands, and from the giant-type population of Amami Isl. or the middle-type population of Okinawa Isl. by gametic isolation or hybrid inviability by the gastrula stage. There seem to be no reproductively isolating mechanisms between the dwarf-type populations of Ishigaki and Iriomote Isls. as well as between the giant-type populations of these two islands. While all the hybrids between a female of the middle-type population of Okinawa Isl. and males of the giant-type population of Iriomote Isl. died by the blastula stage, a few of the hybrids between females of the giant-type population of Ishigaki or Iriomote Isl. and males of the middle-type population of Okinawa Isl. or the giant-type population of Amami Isl. developed into feeding tadpoles or metamorphosed frogs.

The results of electrophoretic analyses of enzymes and blood proteins showed that the genetic distances between the dwarf-type populations of Iriomote and Ishigaki Isls. and the giant-type populations of these two islands are 0.714~0.809, while that between the giant-type populations of Ishigaki and Iriomote Isls., and that between the dwarf-type populations of these two islands are 0.012 and 0.137, respectively. The genetic distances between the giant-type populations of Iriomote and Ishigaki Isls. and the middle-type population of Okinawa Isl. or the giant-type population of Amami Isl. are 0.456~0.471, while those between the dwarf-type populations of the former two islands and the middle-type and giant-type populations of the latter two islands are 0.809~1.079.

It is believed that the dwarf-type populations of Iriomote and Ishigaki Isls. have

evolved into a species level during the period of time when they were geographically isolated from the other populations. The giant-type populations of these two islands entered there as newcomers and became sympatric with the dwarf-type populations. The giant-type population of Iriomote or Ishigaki Isl., middle-type population of Okinawa Isl. and giant-type population of Amami Isl. seem to have evolved into a subspecies level.

INTRODUCTION

Rana narina was named and described by STEJNEGER (1901, 1907) on a specimen collected from Okinawa Isl. According to OKADA (1931), this species is distributed in Amami Isl., Okinawa Isl. and Taiwan. NAKAMURA and UENO (1963) have described that this species is found in Amami Isl., Tokunoshima Isl., Okinawa Isl., Ishigaki Isl., Iriomote Isl. and Taiwan. NISHIOKA and UEDA (1982) have preliminarily reported that *Rana narina* is divided into three types at least according to differences in various features, such as the size and shape of the body and the size and number of the eggs. They have made crossings between different populations to confirm the existence of reproductively isolating mechanisms. From differences in morphological characters and the results of crossings, it became evident that six populations belonging to three types are distributed in four islands; the giant-type and dwarf-type populations in each of Ishigaki and Iriomote Isls., the middle-type population in Okinawa Isl. and the giant-type population in Amami Isl.

In order to elucidate the phylogenetic relationships among the six populations distributed in the four islands belonging to the Southwest Islands of Japan, the present authors made crossing experiments and electrophoretic analyses of enzymes and blood proteins in the frogs of the six populations.

MATERIALS AND METHODS

During the 10 years from 1975 to 1984, a total of 162 *Rana narina* STEJNEGER consisting of 62 females and 100 males were collected from Ryukyu Islands and used as materials in the present studies (Fig. 1). They were divided into six populations.

The dates of collection, the stations and environment, and the conditions of collected frogs were as follow.

1. Giant-type population from Ishigaki Isl. (Is–G). 18 frogs (♀13, ♂5).

a. In May, 1976, three females were collected. On Dec. 25, a female with ovarian eggs was collected at 80 m above sea level, in Mt. Omoto, 19°C in atmospheric temperature.

b. On Feb. 12 and 13, 1978, eight females were collected at the places between 100 m and 350 m above sea level, in Mt. Omoto, 15°C in atmospheric and water temperatures. Two of these females had ovarian eggs, while the six

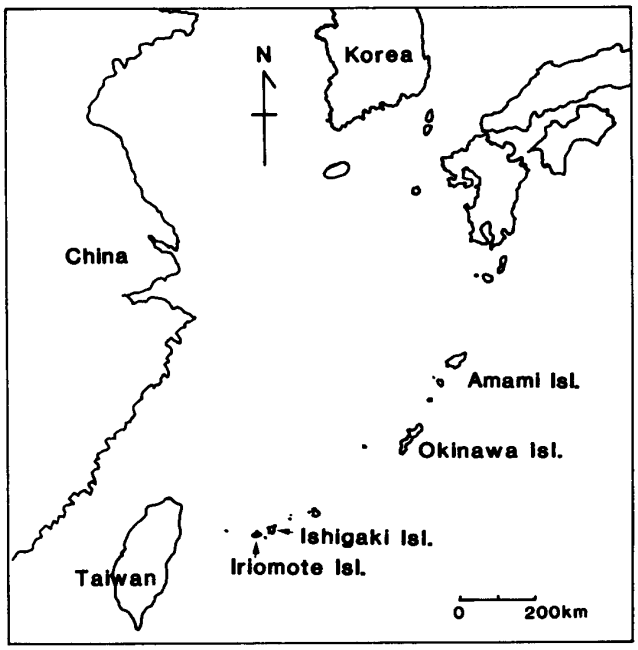

Fig. 1. Map showing localities of the six populations of *Rana narina*.

others had no eggs.

c. On Jan. 20 and 21, 1982, a female without eggs and five males were collected at the places between 200 m and 350 m above sea level, in Mt. Omoto, 16°C in atmospheric temperature and 15°C in water temperature.

2. Dwarf-type population from Ishigaki Isl. (Is–D). 98 frogs (♀25, ♂73).

a. On Dec. 25, 1976, five females with ovarian eggs and two males were collected at the places between 350 m and 400 m above sea level, in Mt. Omoto, 19°C in atmospheric temperature and 15.5°C in water temperature.

b. On Feb. 12 and 13, 1978, six females without eggs and one male were collected at the places between 300 m and 400 m above sea level, in Mt. Omoto, 15°C in atmospheric and water temperatures.

c. On Jan. 20 and 21, 1982, 14 females and 70 males were collected at the places between 270 m and 400 m above sea level, in Mt. Omoto, 16°C in atmospheric temperature and 15°C in water temperature. Of the 14 females, 10 had no eggs and four had mature eggs in their oviducts.

3. Giant-type population from Iriomote Isl. (Ir–G). 13 frogs (♀2, ♂11).

a. In May, 1976, one female and five males were collected at the beach along a branch of the Nakama.

b. On Jan. 22, 1982, one female without eggs and six males were collected at the beach of a branch of the Nakama, 10~20 m above sea level, 23°C in atmospheric temperature and 18°C in water temperature.

4. Dwarf-type population from Iriomote Isl. (Ir–D). Four frogs (♀3, ♂1).

a. On Jan. 22, 1982, three females and one male were collected at the beach of a

branch of the Nakama, 40~100 m above sea level, 23°C in atmospheric temperature and 18°C in water temperature. Of the three females, one had mature eggs in her oviducts and the other two had no eggs.

5. Middle-type population from Okinawa Isl. (Ok–M). Seven frogs (♀4, ♂3).

a. On Feb. 10, 1978, one female without eggs and two males were collected at the rocky beach of a mountain stream situated 80~100 m above sea level in Yona, Kunigami-mura, Kunigami-gun, 19°C in atmospheric temperature and 18°C in water temperature.
b. On Jan. 23, 1982, one female without eggs and one male were collected at the beach of a mountain stream situated in a virgin forest of Kunigami-mura, Kunigami-gun, 300~400 m above sea level, 18°C in atmospheric temperature and 16°C in water temperature.
c. On Feb. 11 and 12, 1984, two females were collected at an experimental plantation of Ryukyu University situated in Yona, Kunigami-mura, Kunigami-gun.

6. Giant-type population from Amami Isl. (Am–G). 22 frogs (♀15, ♂7).

All the frogs were collected and sent to our laboratory by Dr. Hiroshi SUZUKI, Institute for Tropical Medicine, Nagasaki University.

a. On May 16, 1975, two females and five males were collected from Uken-mura.
b. On June 1~3 1984, 10 females and two males were collected from Uken-mura. On the same days, three females were collected from Sumiyo-mura.

By using females and males of the six populations collected from the four islands, Ishigaki, Iriomote, Okinawa and Amami, crossings were made to confirm the existence of reproductively isolating mechanisms between different populations. The mature eggs contained in the oviducts of some females were used in fertilization as soon as possible after collection. Ovulation was accelerated in females with ovarian eggs by injecting emulsion of bullfrog's pituitaries. Insemination was always performed by the artificial method. Tadpoles were fed on boiled spinach or chard. Frogs were fed on crickets.

In order to examine the biochemical differentiation of the six populations, 19 kinds of enzymes extracted from the skeletal muscles and livers and three kinds of blood proteins were analyzed by the horizontal starch-gel electrophoretic method, which has been described in detail by NISHIOKA, OHTANI and SUMIDA (1980). The enzymes and blood proteins analyzed in the present study and the buffer systems used in electrophoresis are shown in Table 1. The detection of each enzyme was performed according to BREWER (1970) and HARRIS and HOPKINSON (1976) by using the agar overlay method which was slightly modified. The detection of blood proteins was made by amido-black staining.

The locus was considered to be consisting of multiple alleles, when the alleles exist at a frequency of more than 1%. In order to show quantitatively the genetic variability of each population, the mean proportions of polymorphic loci per

TABLE 1
Enzymes and blood proteins analyzed in the present study

Enzyme or blood protein	Abbreviation	Sample	Buffer system
Aspartate aminotransferase	AAT	Skeletal Muscle	T-C pH 7.0
Adenosine deaminase	ADA	〃	〃
Adenylate kinase	AK	〃	〃
Aldolase	ALD	〃	〃
Creatine kinase	CK	〃	T-B-E pH 8.0
Esterase	Est	〃	〃
Fumarase	Fum	Liver	T-C pH 7.0
α-Glycerophosphate dehydrogenase	α-GDH	Skeletal muscle	T-C pH 6.0
Glucose phosphate isomerase	GPI	〃	T-B-E pH 8.0
Hexokinase	HK	Liver	T-C pH 7.0
Isocitrate dehydrogenase	IDH	Skeletal muscle	〃
Lactate dehydrogenase	LDH	〃	T-C pH 6.0
Malate dehydrogenase	MDH	〃	〃
Malic enzyme	ME	〃	T-C pH 7.0
Mannose phosphate isomerase	MPI	〃	〃
Peptidase	Pep	Liver	T-B-E pH 8.0
6-Phosphogluconate dehydrogenase	6-PGD	Skeletal muscle	T-C pH 7.0
Phosphoglucomutase	PGM	〃	T-B-E pH 8.0
Superoxide dismutase	SOD	〃	〃
Serum albumin	Ab	Blood serum	〃
Serum protein-C	Prot-C	〃	〃
Hemoglobin	Hb	Erythrocytes	T-B-E pH 8.6

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

population and of heterozygous loci per individual were used (LEWONTIN and HUBBY, 1966; LEWONTIN, 1974).

The genetic relationships among the six populations of *Rana narina* were assumed by calculating the genetic identity (I) and genetic distance (D) according to the method of NEI (1972). A dendrogram for these populations was drawn from the genetic distances by the unweighted pair-group arithmetic average (UPGMA) clustering method, to clarify their phylogenetic connections (SNEATH and SOKAL, 1973; NEI, 1975).

OBSERVATION

I. External characters of mature frogs in six populations of Rana narina

1. Giant-type population from Ishigaki Isl. (Is-G)

Four females and five males were 91.0~115.5 mm, 98.7 mm on the average, and 70.0~77.0 mm, 73.4 mm on the average, in body length, respectively. The body was stout, although the snout was acuter than that of the frog of the dwarf-type population. The four legs were comparatively short. When the hind leg was

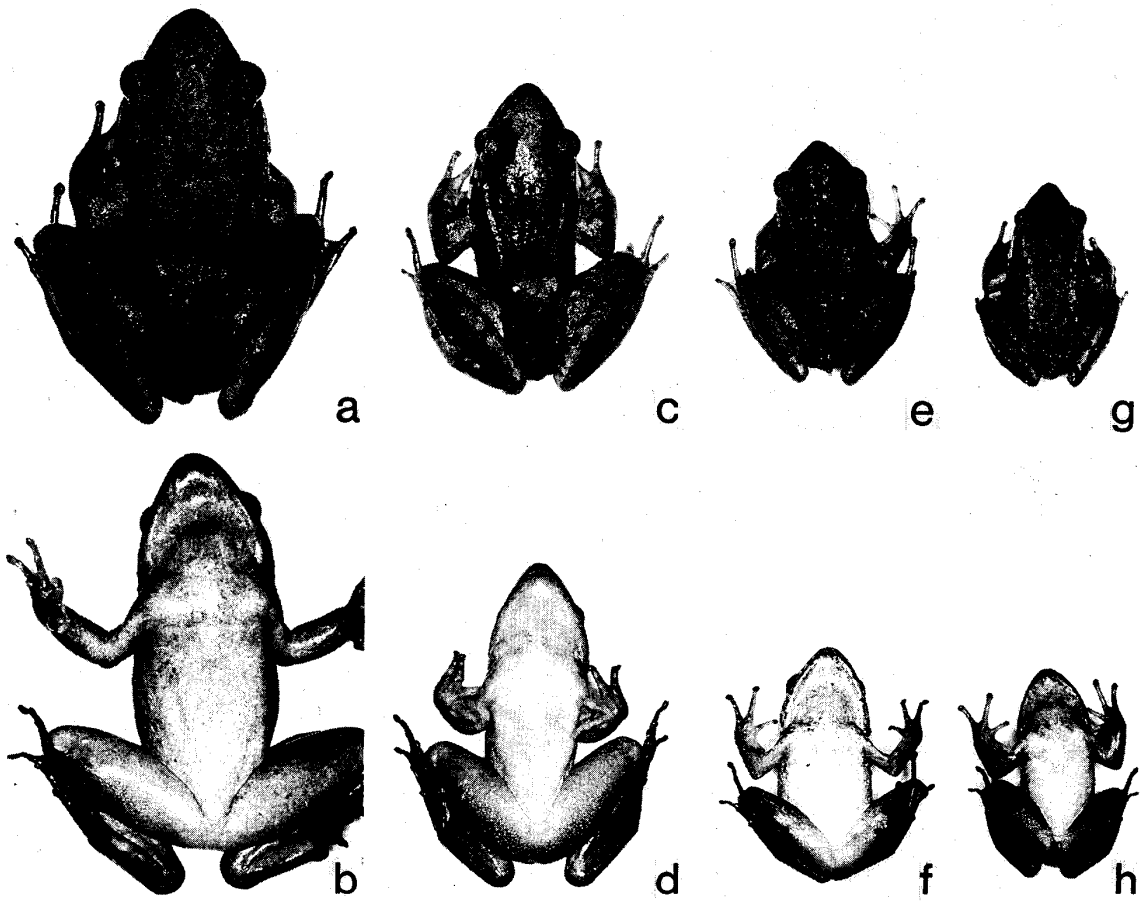

Fig. 2. Two populations of *Rana narina* from Ishigaki Isl. ×0.5
a, b. A female of the giant-type population, Is–G 78♀, No. 5
c, d. A male of the giant-type population, Is–G 76♂, No. 2
e, f. A female of the dwarf-type population, Is–D 76♀, No. 5
g, h. A male of the dwarf-type population, Is–D 82♂, No. 5

extended along the body axis from the hip joint, the tibio-tarsal articulation reached the site between the nostril and the eye. The margin of the upper jaw was whitish and remarkably prominent in contrast to that of the frogs of the dwarf-type population. The dorsal surface was grayish brown, light yellowish-brown or exceptionally yellowish green. It was speckled in a few individuals. Some individuals had no tubercles on the dorsal surface, while others had a few of them. The dorso-lateral fold was like a string of beads, and the beads were generally more longer than those of the frogs of the dwarf-type population, in which it appeared to be a dotted line. The belly was beige and the lateral skin of the trunk was covered with numerous indistinct tubercles. In two females which were green in dorsal surface, the throats showed dark-brown, marvelous markings. There were two or three dark bars on the forearm and some irregular markings on the upper arm, while there were four or five dark bars on the lower legs and two or four dark bars on the thigh. These dark bars were more obscure in outline as compared with those of the frogs of the dwarf-type population (Tables 2, 3; Fig. 2a~d).

TABLE 2

Measurements of various body sites of the females and males in six populations of *Rana narina*

Population	Dwarf-type				Giant-type				Middle-type		Giant-type	
	Ishigaki Isl.		Iriomote Isl.		Ishigaki Isl.		Iriomote Isl.		Okinawa Isl.		Amami Isl.	
No. of frogs	♀14	♂10	♀2	♂1	♀4	♂5	♀1	♂4	♀2	♂3	♀12	♂2
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Body length	56.1	48.9	53.5	45.0	98.7	73.4	98.0	68.3	69.5	52.2	86.5	65.8
Head length	18.2	16.1	17.9	15.5	34.1	24.2	33.0	23.2	22.3	17.8	29.1	22.7
Head width	19.7	16.7	17.9	16.0	32.8	23.5	32.8	23.0	22.6	17.1	28.6	20.3
Snout length	6.4	6.1	6.5	5.8	12.1	9.5	12.0	9.6	8.1	7.0	10.3	8.2
Distance between nostrils	5.4	5.1	5.5	5.1	9.6	6.9	9.0	7.0	6.9	5.8	10.3	8.2
Diameter of eye	8.4	7.1	8.3	7.0	13.4	10.2	13.0	9.6	9.5	7.0	11.6	9.1
Distance between orbitals	4.3	4.0	4.8	4.2	8.6	5.2	7.2	5.7	6.2	3.9	7.5	5.5
Diameter of tympanum	3.2×3.2	3.2×3.2	3.7×3.1	3.5×3.0	6.0×5.3	5.0×4.6	6.2×5.1	4.8×4.6	5.0×5.0	3.5×3.5	5.2×5.0	3.9×3.9
Arm length	33.1	29.1	31.7	27.0	58.9	42.8	55.0	40.2	42.8	36.6	55.3	42.3
Hind-leg length	89.3	75.9	88.5	71.5	157.6	115.8	150.2	105.1	130.8	98.4	162.3	116.5
Outer metatarsal tubercle	dot, 5 none, 9	dot, 6 none, 4	dot, 2	none, 1	none, 4	none, 5	none, 1	none, 4	dot, 1 none, 1	dot, 1 none, 2	dot, 9 none, 3	dot, 1 none, 1
Length of inner metatarsal tubercle	2.7	2.5	3.0	2.5	4.9	3.6	4.8	3.8	2.9	2.5	4.2	2.8
Foot length	26.0	22.6	26.0	21.5	44.2	34.0	44.2	32.1	37.2	27.4	47.3	37.0

dot, No. of frogs having the dot-like outer metatarsal tubercle

none, No. of frogs having no outer metatarsal tubercle

TABLE 3

Relative size of various body sites of the females and males in six populations of *Rana narina*

Population	Dwarf-type				Giant-type				Middle-type		Giant-type	
	Ishigaki Isl.		Iriomote Isl.		Ishigaki Isl.		Iriomote Isl.		Okinawa Isl.		Amami Isl.	
No. of frogs	♀14	♂10	♀2	♂1	♀4	♂5	♀1	♂4	♀2	♂3	♀12	♂2
Female body length /Male body length	1.15		1.19		1.34		1.43		1.33		1.31	
Head length/Body length	0.32	0.33	0.33	0.34	0.35	0.33	0.34	0.34	0.32	0.34	0.34	0.34
Head width/Head length	1.08	1.04	1.00	1.03	0.96	0.97	0.99	0.99	1.01	0.96	0.98	0.89
Snout length/Head length	0.35	0.38	0.36	0.37	0.35	0.39	0.36	0.41	0.36	0.39	0.35	0.36
Distance between nostrils/Head length	0.30	0.32	0.31	0.33	0.28	0.29	0.27	0.30	0.31	0.33	0.35	0.36
Diameter of eye /Body length	0.15	0.15	0.16	0.16	0.14	0.14	0.13	0.14	0.14	0.13	0.13	0.14
Diameter of tympanum /Head length	0.18× 0.18	0.20× 0.20	0.21× 0.17	0.23× 0.19	0.18× 0.16	0.21× 0.19	0.19× 0.15	0.21× 0.20	0.22× 0.22	0.20× 0.20	0.18× 0.17	0.17× 0.17
Arm length/Body length	0.59	0.60	0.59	0.60	0.60	0.58	0.56	0.59	0.62	0.70	0.64	0.64
Hind-leg length /Body length	1.59	1.55	1.65	1.59	1.60	1.58	1.53	1.54	1.88	1.89	1.88	1.77
L. of inner metatarsal tubercle/Body length	0.05	0.05	0.06	0.06	0.05	0.05	0.05	0.06	0.04	0.05	0.05	0.04
Foot length/Body length	0.46	0.46	0.48	0.48	0.45	0.46	0.45	0.47	0.54	0.52	0.55	0.56

2. Dwarf-type population from Ishigaki Isl. (Is–D)

Fourteen females and ten males were 51.5~60.0 mm, 56.1 mm on the average, and 46.0~50.5 mm, 48.9 mm on the average, in body length, respectively. The snout was roundish and the body was stout. The four legs were comparatively short. When the hind leg was extended along the body axis from the hip joint, the tibio-tarsal articulation reached the site between the nostril and the eye, as found in the frogs of giant-type population. Their motion was not so quick as that of the frogs of the foregoing population. The frogs of the dwarf-type population, therefore, could easily be caught by hand at night. The eyes were prominent, as they were large and somewhat projecting. The dorsal surface was light yellowish-brown in most of the frogs collected, while this color was mingled with yellowish-green parts to some extent. There were numerous conical tubercles on the dorsal skin. In some frogs, each of these tubercles was surrounded with a blackish-brown spot. The tubercles were generally conspicuous, as their tips were whitish. The dorso-lateral fold appeared to be a dotted line which was curved inside from the posterior margin of the tympanum. The belly was brownish white. There were small, closely arranged brown spots on the throat of male, while they were fewer on the throat of females. There were numerous tubercles on the surface of hind leg, while no tubercles were found on the foreleg. Two to four dark bars and some small irregular markings were found on the forearm and upper arm, respectively. In contrast, three or four and four to six distinct dark bars were found on the lower leg and thigh, respectively. The lateral part of the trunk was densely covered with small tubercles whose tips were white and conspicuous (Tables 2, 3; Fig. 2e~h).

3. Giant-type population from Iriomote Isl. (Ir–G)

One female and four males were 98.0 mm and 65.0~70.0 mm, 68.3 mm on the average, in body length, respectively. They were nearly the same as the males and females of the giant-type population from Ishigaki Isl. in external characters (Tables 2, 3; Fig. 3a~d).

4. Dwarf-type population from Iriomote Isl. (Ir–D)

Two females were 53.0 mm and 54.0 mm, 53.5 mm on the average, in body length, while one male was 45.0 mm. The dorsal surfaces of these three frogs were yellowish green. These frogs were nearly the same as those of the dwarf-type population from Ishigaki Isl. in the other characters, except that the diameter of the eye and the length of the tarsus were somewhat larger than those of the frogs belonging to the dwarf-type population from Ishigaki Isl. (Tables 2, 3; Fig. 3e~d).

5. Middle-type population from Okinawa Isl. (Ok–M)

Two females were 67.0 mm and 72.0 mm, 69.5 mm on the average, in body length, while three males were 50.0 mm, 53.0 mm and 53.5 mm, 52.2 mm on the

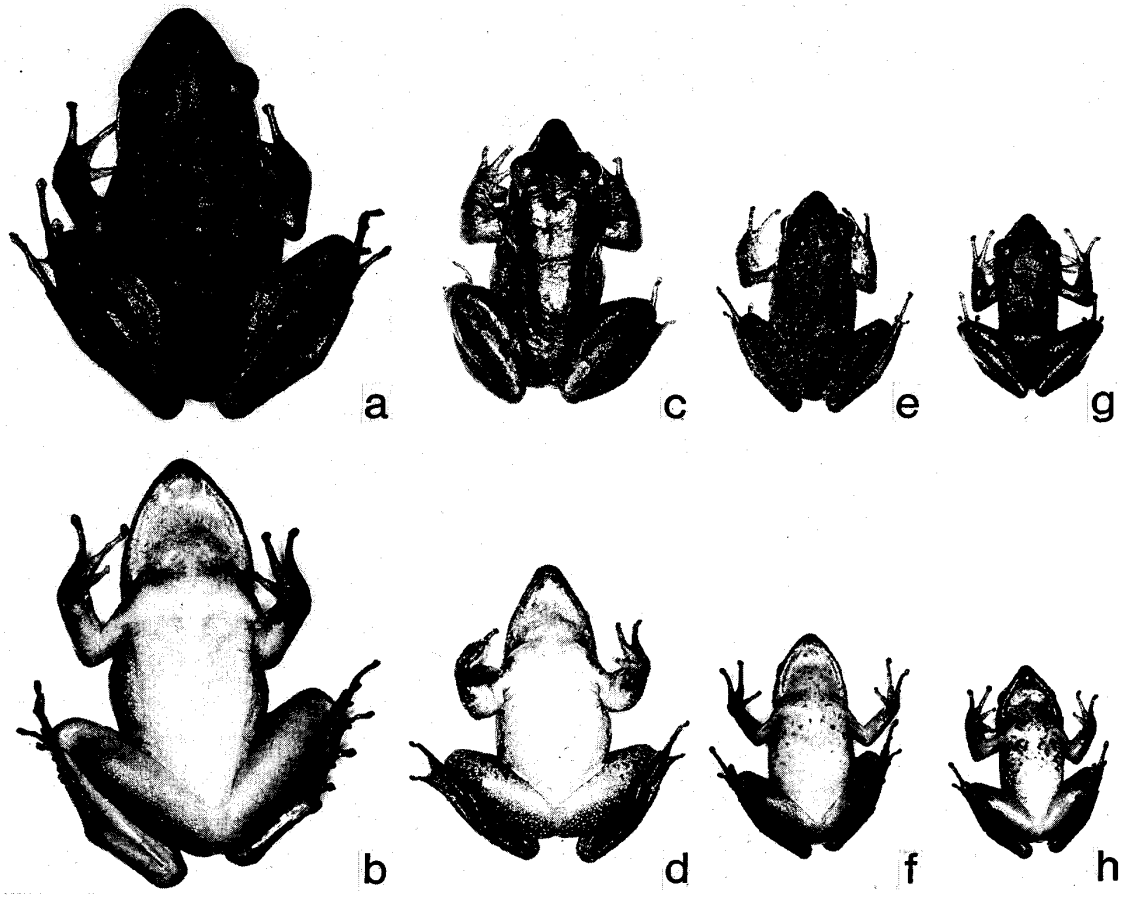

Fig. 3. Two populations of *Rana narina* from Iriomote Isl. ×0.5
a, b. A female of the giant-type population, Ir-G 76♀, No. 1
c, d. A male of the ginat-type population, Ir-G 76♂, No. 1
e, f. A female of the dwarf-type population, Ir-D 82♀, No. 1
g, h. A male of the dwarf-type population, Ir-D 82♂, No. 1

average. They had a slender body and long legs. When the hind leg was extended along the body axis from the hip joint, the tibio-tarsal articulation reached 10~12 mm beyond the snout. The tarsus was distinctively long, as compared with those of the frogs of the other populations. The length of the tarsus between the upper end of fingers and the inner metatarsal tubercle was 54% of the body length, in contrast to those which were 46~48% in the foregoing four populations. Thus, the frogs of this population were the quickest in motion and they were very difficult to catch in even winter night.

The iris was pale yellow, in contrast to light yellow-brown in the frogs of the other five populations. The dorsal surface was light brown and had numerous tubercles. There were no distinct markings. The dorso-lateral fold appeared to be a string of beads. Although the breaks of the string were more numerous than those of the frogs belonging to the giant-type population from Ishigaki Isl., they were not so numerous as those of a dotted line found in the dwarf-type populations. The space between the two dorso-lateral folds was narrow. The narrowest space was 43% of the head width in contrast to 54%, 53% and 49% of

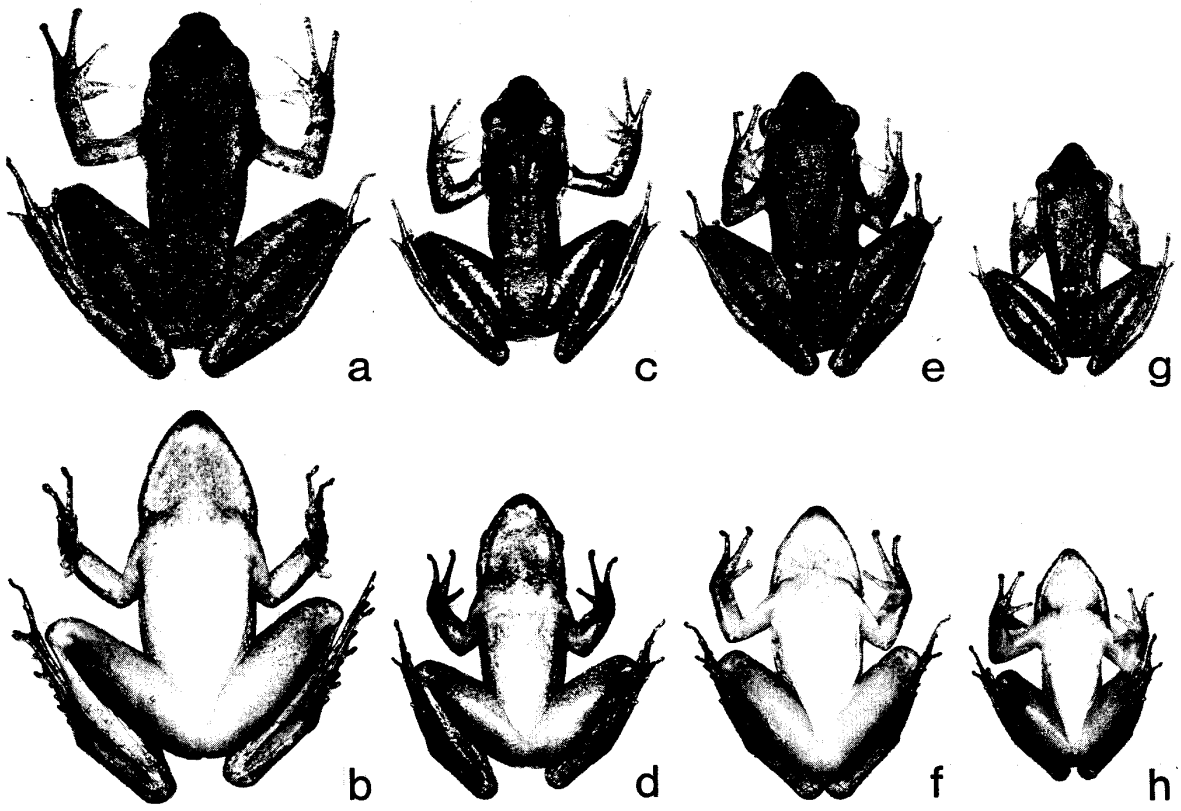

Fig. 4. Two populations of *Rana narina* from Amami and Okinawa Isls. ×0.5
a, b. A female of the giant-type population from Amami Isl., Am-G 84♀, No. 3
c, d. A male of the giant-type population from Amami Isl., Am-G 84♂, No. 2
e, f. A female of the middle-type population from Okinawa Isl., Ok-M 78♀, No. 1
g, h. A male of the middle-type population from Okinawa Isl., Ok-M 78♂, No. 1

those in the frogs belonging to the dwarf-type from Ishigaki Isl., the giant-type from Ishigaki Isl. and the giant-type from Amami Isl., respectively. There were numerous distinct tubercles on the lateral surface of the trunk. The belly was yellowish white, and the throat was covered with light-brown, marble marking. It was remarkable that the frogs of this population had numerous tubercles on the forelegs, in contrast to the frogs of the other populations. Two to four and two dark bars were found on the forearm and upper arm, respectively. In contrast, three to five dark bars were found on each of the lower leg and thigh (Tables 2, 3; Fig. 4e~h).

6. Giant-type population from Amami Isl. (Am-G)

Twelve females were 68.0~98.0 mm, 86.5 mm on the average, in body length, while two males were 64.5 mm and 67.0 mm, 65.8 mm on the average. They were slender in body shape and had long four legs, just as the frogs belonging to the middle-type population from Okinawa Isl. The snout was acute and the margin of the upper jaw was distinctly whitish. The dorsal surface was light yellowish-brown in most of the frogs, while it was green in the others. On the dorsal surface, there were only a few tubercles and no distinct black spots. The

dorso-lateral fold was a dotted line. On the lateral skin of the trunk, there were numerous tubercles. The belly was beige and had black spots. Dark brown marble markings came in sight on the throat of females, when these were kept in the dark. There were two to five distinct dark bars on the forearm and four to seven distinct dark bars on each of lower leg and thigh (Tables 2, 3; Fig. 4a~d).

II. Crossings among the six populations of Rana narina

1. Females used in crossings

a. Dwarf-type populations

Nine females from Ishigaki Isl., Is-D 76♀, Nos. 1~5 and Is-D 82♀, Nos. 7~10, used in 1977~1982, were 51.0~59.0 mm, 54.9 mm on the average, in body length. They laid a comparatively small number of large, pale yellow eggs. The eggs were 3.2~4.2 mm, 3.67 mm on the average, in diameter (Fig. 5a). The number of eggs was 45~136, 88.0 on the average. The gelatinous envelopes were 7.2~10.7 mm, 8.87 mm on the average, in diameter, when they were measured five hours after insemination. The gelatinous envelope of each egg consisted of three layers (Table 4).

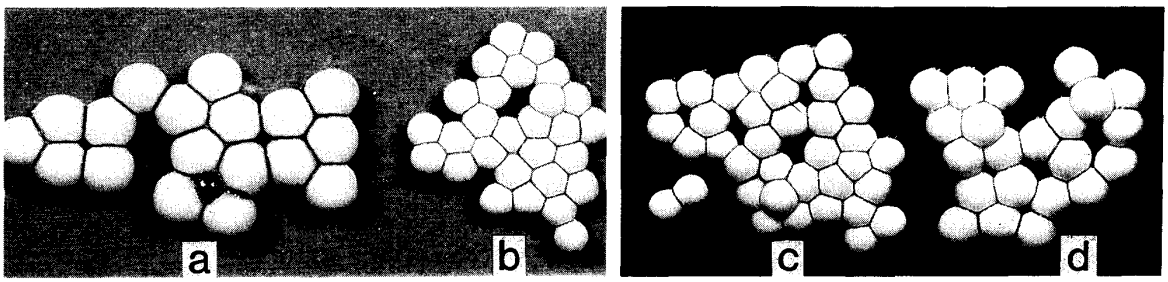

Fig. 5. Eggs of females from three populations of *Rana narina*. ×1.4
a. A female of the dwarf-type population of Ishigaki Isl., Is-D 76♀, No. 5
b. A female of the giant-type population of Ishigaki Isl., Is-G 76♀, No. 2
c. A female of the giant-type population of Ishigaki Isl., Is-G 78♀, No. 9
d. A female of the middle-type population of Okinawa Isl., Ok-M 78♀, No. 1

One female from Iriomote Isl., Ir-D 82♀, No. 1, used in 1982, was 54.0 mm in body length. She laid 67 pale yellow eggs which were 2.8~2.9 mm, 2.87 mm on the average, in diameter. These eggs were somewhat smaller than those of the foregoing females. When measured five hours after insemination, the gelatinous envelopes were 7.5~8.0 mm, 7.75 mm on the average, in diameter. It was evident that the envelope of each egg consisted of three layers (Table 4).

b. Giant-type populations

Nine females from Ishigaki Isl., Is-G 76♀, Nos. 1 and 2 and Is-G 78♀, Nos. 3~9, used in 1977~1980, were 91.0~101.0 mm, 94.5 mm on the average, in body length. They laid numerous small, pale yellow eggs. These eggs were 2.6~2.9 mm, 2.71 mm on the average, in diameter (Fig. 5b, c). Eight of the nine females laid 686~1623 eggs, 1211.5 eggs on the average. In the remaining female, No. 7,

TABLE 4
Number and size of the eggs of females in six populations of *Rana narina*

Population	Date of crossing	Individual no.	Body length (mm)	No. of eggs	Mean diameter of eggs (mm)	Mean diameter of gelatinous envelopes (mm)
Is-D	Jan. 20, '77	Is-D 76♀, No. 1	51.5	86	—	—
		Is-D 76♀, No. 2	54.5	97	—	—
	Feb. 25, '77	Is-D 76♀, No. 3	51.0	61	3.88	9.52
		Is-D 76♀, No. 4	52.0	63	4.12	10.64
		Is-D 76♀, No. 5	58.0	45	4.18	10.66
	Feb. 24, '78	Is-D 76♀, No. 5	58.5	71	3.48	7.83
	Jan. 26, '82	Is-D 82♀, No. 7	57.5	93	3.24	8.34
		Is-D 82♀, No. 8	59.0	136	3.60	8.43
		Is-D 82♀, No. 9	52.0	121	3.21	7.23
		Is-D 82♀, No. 10	55.0	107	3.61	8.30
Is-G	Feb. 25, '77	Is-G 76♀, No. 1	92.5	1345	2.88	5.87
		Is-G 76♀, No. 2	93.0	686	2.73	5.25
	Feb. 24, '78	Is-G 78♀, No. 3	91.0	955	2.66	5.28
		Is-G 78♀, No. 4	93.5	1188	2.76	5.43
	Apr. 4, '79	Is-G 78♀, No. 5	98.5	1226	2.61	5.22
		Is-G 78♀, No. 6	91.0	1131	2.60	5.25
	Apr. 10, '80	Is-G 78♀, No. 7	101.0	164	2.66	5.53
		Is-G 78♀, No. 8	97.0	1538	2.71	5.60
		Is-G 78♀, No. 9	93.0	1623	2.74	5.74
Ir-D	Jan. 26, '82	Ir-D 82♀, No. 1	54.0	67	2.87	7.75
Ir-G	Jan. 20, '77	Ir-G 76♀, No. 1	98.0	1350	—	—
Am-G	Jan. 20, '77	Am-G 75♀, No. 1	98.5	1452	—	—
Ok-M	Apr. 4, '79	Ok-M 78♀, No. 1	72.0	237	3.60	5.57
	Apr. 10, '80	Ok-M 78♀, No. 1	72.5	149	3.63	5.90

Is-D, Dwarf-type population of Ishigaki Isl.
Is-G, Giant-type population of Ishigaki Isl.
Ir-D, Dwarf-type population of Iriomote Isl.
Ir-G, Giant-type population of Iriomote Isl.
Am-G, Giant-type population of Amami Isl.
Ok-M, Middle-type population of Okinawa Isl.

ovulation was incomplete and only 164 eggs were laid. The gelatinous envelopes were 5.2~5.9 mm, 5.46 mm on the average, in diameter five hours after insemination. The envelope of each egg consisted of two layers (Table 4).

One female from Iriomote Isl., Ir-G 76♀, No. 1, and one female from Amami Isl., Am-G 75♀, No. 1, were 98.0 mm and 98.5 mm in body length, respectively. These two females laid 1350 and 1452 pale yellow eggs, respectively. The eggs were small and nearly the same in diameter as those of the females of the giant-type population from Ishigaki Isl. (Table 4).

c. Middle-type population

One female from Okinawa Isl., Ok-M 78♀, No. 1, was 72.0 mm in body length. In 1979, she laid 237 pale yellow eggs which were 3.5~3.7 mm, 3.60 mm on the average, in diameter (Fig. 5d). The gelatinous envelopes were 5.2~6.0 mm, 5.57 mm on the average, in diameter five hours after insemination. In 1980, this female was 72.5 mm in body length and laid 149 eggs which were 3.6~3.7 mm, 3.63 mm on the average, in diameter. The gelatinous envelopes were 5.4~6.2 mm, 5.90 mm on the average, in diameter five hours after insemination. The gelatinous envelope of each egg consisted of three layers (Table 4).

2. Males used in crossings

a. Dwarf-type populations

Five males from Ishigaki Isl., Is-D 76♂, Nos. 1 and 2, Is-D 78♂, No. 3 and Is-D 82♂, Nos. 4 and 5, used in 1977~1982 were 45.0~50.5 mm, 47.2 mm on the average, in body length. Their testes were 7.0~10.0 mm in length and 4.5~5.5 mm in width. All of them were pale yellow in color.

One male from Iriomote Isl., Ir-D 82♂, No. 1, used in 1982 was 45.0 mm in

TABLE 5

Size of the testes of males in six populations of *Rana narina*

Population	Date of crossing	Individual no.	Body length (mm)	Size of testes	
				Left (mm)	Right (mm)
Is-D	Jan. 20, '77	Is-D 76♂, No. 1	45.0	8.5×5.0	8.5×5.0
	Feb. 25, '77	Is-D 76♂, No. 2	47.0	8.5×5.0	8.5×5.0
	Feb. 24, '78	Is-D 78♂, No. 3	46.5	8.0×5.5	—
	Jan. 26, '82	Is-D 82♂, No. 4	47.0	7.0×4.5	7.0×4.5
	Jan. 26, '82	Is-D 82♂, No. 5	50.5	10.0×4.5	—
	Feb. 9, '82	Is-D 82♂, No. 5	50.5	—	10.0×5.5
Is-G	Jan. 26, '82	Is-G 82♂, No. 1	72.5	12.0×6.0	11.5×6.0
Ir-D	Jan. 26, '82	Ir-D 82♂, No. 1	45.0	8.5×4.0	—
	Feb. 9, '82	Ir-D 82♂, No. 1	45.0	—	8.5×4.0
Ir-G	Apr. 4, '79	Ir-G 76♂, No. 1	63.0	9.0×4.5	—
		Ir-G 76♂, No. 2	65.0	10.5×4.5	—
	Apr. 10, '80	Ir-G 76♂, No. 3	72.0	11.0×5.5	11.5×6.0
		Ir-G 76♂, No. 4	74.0	12.0×6.0	11.5×6.0
	Jan. 26, '82	Ir-G 82♂, No. 5	70.0	11.5×5.5	11.5×6.0
	Feb. 9, '82	Ir-G 82♂, No. 5	70.0	11.5×5.5	11.5×6.0
Am-G	Jan. 20, '77	Am-G 75♂, No. 1	66.0	—	—
Ok-M	Feb. 24, '78	Ok-M 78♂, No. 1	53.0	8.0×4.0	—
	Apr. 4, '79	Ok-M 78♂, No. 2	53.5	6.5×3.0	—
	Jan. 26, '82	Ok-M 82♂, No. 3	50.0	—	6.0×3.0
	Feb. 9, '82	Ok-M 82♂, No. 3	50.0	7.0×3.0	—

body length. The testes were 8.5 mm in length and 4.0 mm in width. They were pale yellow (Table 5).

b. Giant-type populations

One male from Ishigaki Isl., Is-G 82♂, No. 1, used in 1982 was 72.5 mm in body length. The testes were 11.5 mm and 12.0 mm in length and 6.0 mm in width. They were pale yellow.

Five males from Iriomote Isl., Ir-G 76♂, Nos. 1~4 and Ir-G 82♂, No. 5, used in 1979~1982 were 63.0~74.0 mm, 68.8 mm on the average, in body length. Their testes were pale yellow and 9.0~12.0 mm in length and 4.5~6.0 mm in width.

One male from Amami Isl., Am-G 75♂, No. 1, used in 1977 was 66.0 mm in body length. The size of the testes was not measured (Table 5).

c. Middle-type population

Three males from Okinawa Isl., Ok-M 78♂, Nos. 1 and 2 and Ok-M 82♂, No. 3, used in 1978~1982 were 50.0~53.5 mm, 52.2 mm on the average, in body length. Their testes were pale yellow, 6.0~8.0 mm in length and 3.0~4.0 mm in width (Table 5).

3. Results of crossings

a. Females of the dwarf-type population from Ishigaki Isl. and males of the other five populations

i) Control matings

A total of 10 control matings were made in 1977, 1978 and 1982. They included five matings in 1977, Is-D 76♀, Nos. 1~5×Is-D 76♂, Nos. 1 and 2, one mating in 1978, Is-D 76♀, No. 5×Is-D 78♂, No. 3, and four matings in 1982, Is-D 82♀, Nos. 7~10×Is-D 82♂, Nos. 4 and 5. Eight hours after insemination, 42.9~80.0% of the eggs in each control series, 194 (67.4%) of 288 eggs in total, cleaved normally, and only nine eggs did abnormally. Of the normally cleaved eggs, 12, 23, 36 and 25 died of various abnormalities at the blastula, gastrula, neurula and hatching stages, respectively. Eventually, 25.4~37.9%, 98 eggs (34.0%) in total, hatched normally. After 27 of the normally hatched tadpoles died of ill-development of the body and abnormalities of the gills and some other organs, without taking food, 9.7~28.6% of the eggs in each control series, 51 (17.7%) in total, completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 26.3% of the normally cleaved eggs (Table 6). These juvenile frogs immediately after metamorphosis were 7.0~8.2 mm, 7.34 mm on the average, in body length. They required 42~55 days from fertilization to completion of metamorphosis.

ii) Crossing experiments

In 1982, four crossings, Is-D 82♀, Nos. 7~10×Ir-D 82♂, No. 1, were made. Eight hours after insemination, 35.7~68.4% of the eggs in each experimental

series, 54 (54.5%) of the 99 eggs in total, cleaved normally, and only three eggs did abnormally. Of the normally cleaved eggs, six, 15 and five died of abnormalities at the gastrula, neurula and hatching stages, respectively, while 16.7~36.8%, 28 eggs (28.3%) in total, hatched normally. After four tadpoles died of ill-development by metamorphosis, 16.7~29.8% of the eggs in each experimental series, 24 tadpoles (24.2%) in total, completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 44.4% of the normally cleaved eggs. The frogs immediately after metamorphosis were 7.0~7.2 mm, 7.12 mm on the average, in body length. They required 41~57 days from fertilization to completion of metamorphosis (Table 6).

Crossings were attempted in 1977 between two females belonging to the dwarf-type population from Ishigaki Isl., Is-D 76♀, Nos. 1 and 2, and one male belonging to the giant-type population from Amami Isl., Am-G 75♂, No. 1, and in 1982 between four females belonging to the dwarf-type population from Ishigaki Isl., Is-D 82♀, Nos. 7~10, and one male belonging to the giant-type population from Ishigaki Isl., Is-G 82♂, No. 1, or one male belonging to the giant-type population from Iriomote Isl., Ir-G 82♂, No. 5. The results showed that no cleaved eggs were obtained in these crossings. Only five of the 109 eggs of five females belonging to the dwarf-type population from Ishigaki Isl. mated in 1978 and 1982 with two males of the middle-type population from Okinawa Isl. cleaved abnormally and died (Table 6).

b. A female of the dwarf-type population from Iriomote Isl. and males of four other populations

i) Control mating

A mating was made in 1982 between a female, Ir-D 82♀, No. 1, and a male, Ir-D 82♂, No. 1. Eight hours after insemination, 25 (73.5%) of the 34 eggs of the female cleaved normally. After nine and one died of abnormalities at the neurula and hatching stages, respectively, 15 (44.1%) hatched normally. While four tadpoles died of ill-development or edema by metamorphosis, 11 (32.4%) completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 44.0% of the normally cleaved eggs. These frogs were 6.8~8.5 mm, 7.53 mm on the average, in body length. They required 38~50 days from fertilization to metamorphosis (Table 6).

ii) Crossing experiments

In 1982, two crossings, Ir-D 82♀, No. 1×Is-D 82♂, Nos. 4 and 5, were made. The results showed that four of 10 eggs of the female cleaved normally. After one of the normally cleaved eggs died of incomplete invagination at the gastrula stage, the other three grew normally and completed metamorphosis. All of them were 7.2 mm in body length. They required 42~51 days from fertilization to metamorphosis. No normally cleaved eggs were obtained from crossings between Ir-D 82♀, No. 1×Ir-G 82♂, No. 5, between Ir-D 82♀, No. 1×Is-G 82♂, No. 1, and between Ir-D 82♀, No. 1×Ok-M 82♂, No. 3 (Table 6).

TABLE 6

Developmental capacity of the hybrids between different populations in *Rana narina*

Parents		No. of eggs	No. of normal cleavages	No. of gastrulae		No. of neurulae		No. of normally hatched tadpoles	No. of normally feeding tadpoles	No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal			
Is-D, Nos. 1~10	Is-D, Nos. 1~5	288	194 (67.4%)	159 (55.2%)	23 (8.0%)	123 (42.7%)	36 (12.5%)	98 (34.0%)	71 (24.7%)	51 (17.7%)
Is-D, Nos. 7~10	Ir-D, No. 1	99	54 (54.5%)	48 (48.5%)	6 (6.1%)	33 (33.3%)	15 (15.2%)	28 (28.3%)	27 (27.3%)	24 (24.2%)
Is-D, Nos. 7~10	Is-G, No. 1	87	0							
Is-D, Nos. 7~10	Ir-G, No. 5	75	0							
Is-D, Nos. 1, 2	Am-G, No. 1	111	0							
Is-D, Nos. 5, 7~10	Ok-M, Nos. 1, 3	109	0							
Ir-D, No. 1	Ir-D, No. 1	34	25 (73.5%)	25 (73.5%)	0	16 (47.1%)	9 (26.5%)	15 (44.1%)	13 (38.2%)	11 (32.4%)
	Is-D, Nos. 4, 5	10	4 (40.0%)	3 (30.0%)	1 (10.0%)	3 (30.0%)	0	3 (30.0%)	3 (30.0%)	3 (30.0%)
	Ir-G, No. 5	8	0							
	Is-G, No. 1	7	0							
	Ok-M, No. 3	8	0							
Ok-M, No. 1	Ok-M, No. 2	61	19 (31.1%)	10 (16.4%)	3 (4.9%)	9 (14.8%)	1 (1.6%)	9 (14.8%)	7 (11.5%)	5 (8.2%)
Ok-M, No. 1	Ir-G, Nos. 1, 2, 4	179	131 (73.2%)	0	0					
Is-G, Nos. 1~4	Is-D, Nos. 2, 3	1304	737 (56.5%)	0	712 (54.6%)	0	0			
Is-G, Nos. 3~6	Ok-M, Nos. 1, 2	1051	739 (70.3%)	676 (64.3%)	41 (3.9%)	219 (20.8%)	457 (43.5%)	28 (2.7%)	2 (0.2%)	0
Is-G, Nos. 5~9	Ir-G, Nos. 1~4	1543	1487 (96.4%)	1310 (84.9%)	89 (5.8%)	1173 (76.0%)	137 (8.9%)	1036 (67.1%)	802 (52.0%)	654 (42.4%)
Ir-G, No. 1	Am-G, No. 1	150	76 (50.7%)	62 (41.3%)	14 (9.3%)	22 (14.7%)	40 (26.7%)	13 (8.7%)	6 (4.0%)	5 (3.3%)
	Is-D, No. 1	200	0							
Am-G, No. 1	Am-G, No. 1	239	197 (82.4%)	180 (75.3%)	12 (5.0%)	164 (68.6%)	16 (6.7%)	152 (63.6%)	131 (54.8%)	117 (49.0%)
	Is-D, No. 1	166	113 (68.1%)	0	68 (41.0%)	0	0			

c. A female of the middle-type population from Okinawa Isl. and males of the giant-type population from Iriomote Isl.

i) Control mating

A mating was made in 1979 between a female, Ok–M 78♀, No. 1, and a male, Ok–M 78♂, No. 2. Of the 61 eggs of the female, 19 (31.1%) cleaved normally. After six, three and one of the normally cleaved eggs became abnormal and died at the blastula, gastrula and neurula stages, respectively, the remaining nine (14.8%) hatched normally. While four of these nine tadpoles died of edema and ill-development, the other five (8.2%) completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 26.3% of the normally cleaved eggs (Table 6).

ii) Crossing experiments

In 1979 and 1980, a female, Ok–M 78♀, No. 1, was crossed with three males, Ir–G 76♂, Nos. 1, 2 and 4. The results showed that 131 (73.2%) of the 179 eggs of the female cleaved normally, and eight others did abnormally. While the normally cleaved eggs became normal blastula, all of them died by the gastrula stage without showing any sign of invagination (Table 6).

d. Females of the giant-type population from Ishigaki Isl. and males of three other populations

i) Crossings with males of the dwarf-type population from Ishigaki Isl.

In 1977, two crossings were made between two females, Is–G 76♀, Nos. 1 and 2, and a male, Is–D 76♂, No 2. In 1978, two more crossings were made between two females, Is–G 78♀, Nos. 3 and 4, and a male, Is–D 78♂, No. 3. It was found that 32.5~95.5% eggs in each series, 737 (56.5%) of 1304 eggs in total, cleaved normally, and 23 did abnormally. Of the normally cleaved eggs, 25 became abnormal at the blastula stage, and the other 712 (54.6%) began to invaginate. However, all the eggs stopped their development and died without completing gastrulation (Table 6; Fig. 6d).

ii) Crossings with males of the middle-type population from Okinawa Isl.

In 1978, two matings were made between two females, Is–G 78♀, Nos. 3 and 4, and a male, Ok–M 78♂, No. 1, and in 1979, two other matings were made between two females, Is–G 78♂, Nos. 5 and 6, and a male, Ok–M 78♂, No. 2. The results showed that 28.9~83.8% of eggs in each series, 739 (70.3%) of the 1051 eggs in total, cleaved normally and six others did abnormally. Of the normally cleaved eggs, 22, 41, 457 and 191 became abnormal and died at the blastula, gastrula, neurula and hatching stages, respectively. Although 0~4.2%, 28 eggs in total, hatched normally, almost all the tadpoles died of ill-development of the external gills, edema and some other abnormalities. Only two tadpoles began to eat, but they died of malnutrition without making metamorphosis (Table 6; Fig. 6b).

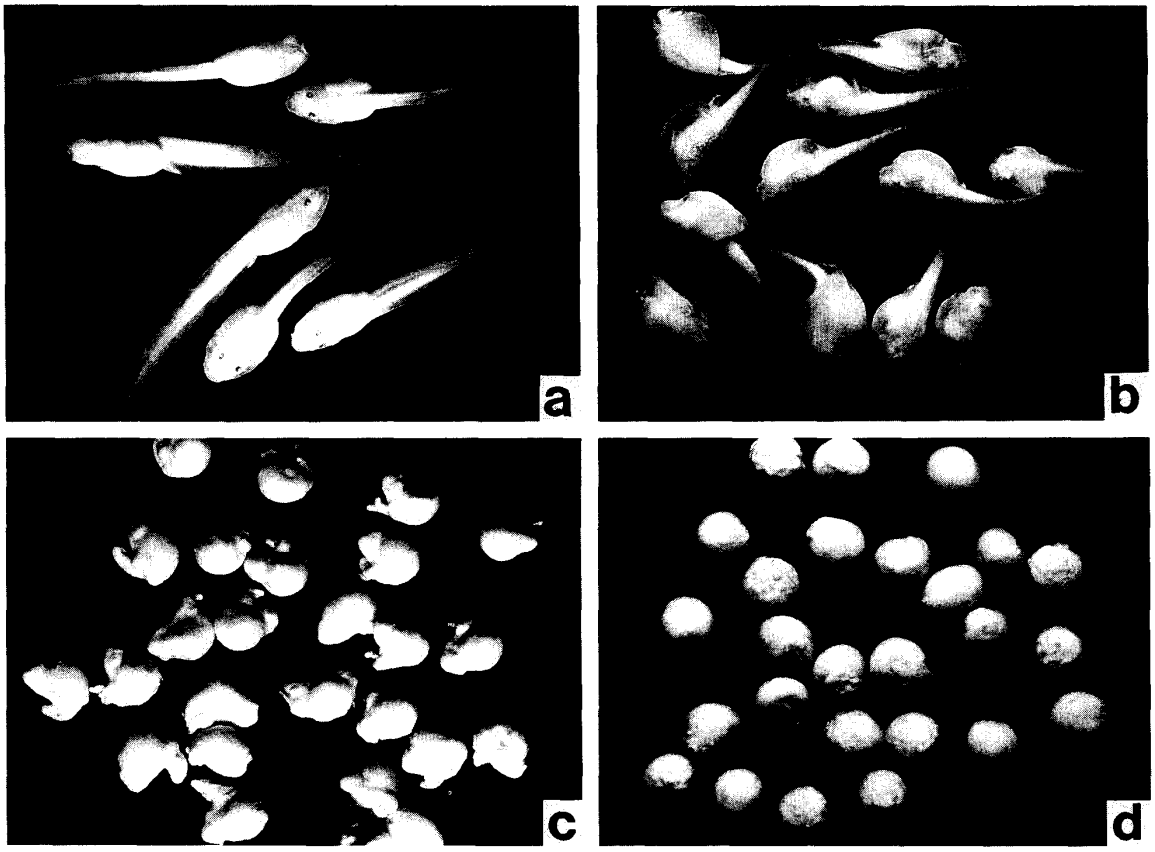

Fig. 6. Normal and abnormal tadpoles and embryos produced from crossings between different populations of *Rana narina*. ×2

a. Normal tadpoles produced from Is-G 78♀, No. 5×Ir-G 76♂, No. 1
b. Abnormal tadpoles produced from Is-G 78♀, No. 3×Ok-M 78♂, No. 1
c. Abnormal tail-bud embryos produced from Ir-G 76♀, No. 1×Am-G 75♂, No, 1
d. Abnormal gastrulae produced from Is-G 76♀, No. 1×Is-D 76♂, No. 2

iii) Crossings with males of the giant-type population from Iriomote Isl.

In 1979 and 1980, five crossings were made between five females, Is-G 78♀, Nos. 5~9, and four males, Ir-G 76♂, Nos. 1~4. Six hours after insemination, 92.7~99.0% in each series, 1487 (96.4%) of the 1543 eggs in total, cleaved normally. Of the normally cleaved eggs, 88, 89, 137, 91 and 46 died of various abnormalities at the blastula, gastrula, neurula, tail-bud and hatching stages, respectively, while 17.1~97.6%, 1036 eggs (67.1%) in total, hatched normally. Of the normally hatched tadpoles, 234 died of ill-development of the gills and edema without taking any food and 148 other tadpoles died of malnutrition and edema without making metamorphosis. Eventually, 15.2~71.4 % in each series, 654 tadpoles (42.4%) in total, completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 44.0% of the normally cleaved eggs. They were 10.0~12.5 mm in body length immediately after metamorphosis. They required 64~77 days from fertilization to metamorphosis (Table 6). All the 52 mature frogs were males whose testes were filled with normal spermatozoa.

e. A female of the giant-type population from Iriomote Isl. and males of two other populations

In 1977, a female, Ir–G 76♀, No. 1, was crossed with a male, Am–G 75♂, No. 1. It was found that 76 (50.7%) of the 150 eggs of the female cleaved normally. Of these normally cleaved eggs, 14, 40, five and four became abnormal and died at the gastrula, neurula, tail-bud and hatching stages (Fig. 6c), respectively, and only 13 (8.7%) hatched normally. Seven of the tadpoles died of ill-development of gills and edema without taking food and another died during metamorphosis. The remaining five (3.3%) completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 6.6% of the normally cleaved eggs. No normally cleaved eggs were obtained from a crossing between a female, Ir–G 76♀, No. 1, and a male, Is–D 76♂, No. 1 (Table 6).

f. A female of the giant-type population from Amami Isl. and a male of the dwarf-type population from Ishigaki Isl.

i) Control mating

In 1977, a control mating was made between a female, Am–G 75♀, No. 1, and a male, Am–G 75♂, No. 1. The result showed that 197 (82.4%) of the 239 eggs of the female cleaved normally. While five, 12, 16, five and seven died of various abnormalities at the blastula, gastrula, neurula, tail-bud and hatching stages, respectively, 152 (63.6%) hatched normally. Of these tadpoles, 21 died of ill-development of the gills, edema and some other abnormalities during the tadpole stage, and 14 died before or during metamorphosis. Eventually, 117 (49.0%) completed metamorphosis and became juvenile frogs. This number of frogs corresponded to 59.4% of the normally cleaved eggs.

ii) Crossing experiment

In 1977, the same female was crossed with a male, Is–D 75♂, No. 1. It was found that 113 (68.1%) of the 166 eggs of the female cleaved normally. Of these normally cleaved eggs, 17 died by the blastula stage, and 28 showed sign of invagination. The remaining 68 eggs died of abnormalities at the early gastrula stage (Table 6).

III. Electrophoretic patterns and allelomorphs

Electrophoretic analyses of 16 enzymes extracted from skeletal muscles, three enzymes extracted from livers and three blood proteins were made in a total of 92 frogs belonging to the six populations of *Rana narina*. These frogs included seven (♀1, ♂6) of the giant-type population and three (♀2, ♂1) of the dwarf-type population collected from Iriomote Isl., eight (♀3, ♂5) of the giant-type population and 54 (♀8, ♂46) of the dwarf-type population collected from Ishigaki Isl., five (♀4, ♂1) of the middle-type population collected from Okinawa Isl. and 15 (♀13, ♂2) of the giant-type population collected from Amami Isl. The results of analyses showed that the enzymes and blood proteins were controlled by alleles at 30 loci. The bands corresponding to multiple alleles at each locus were

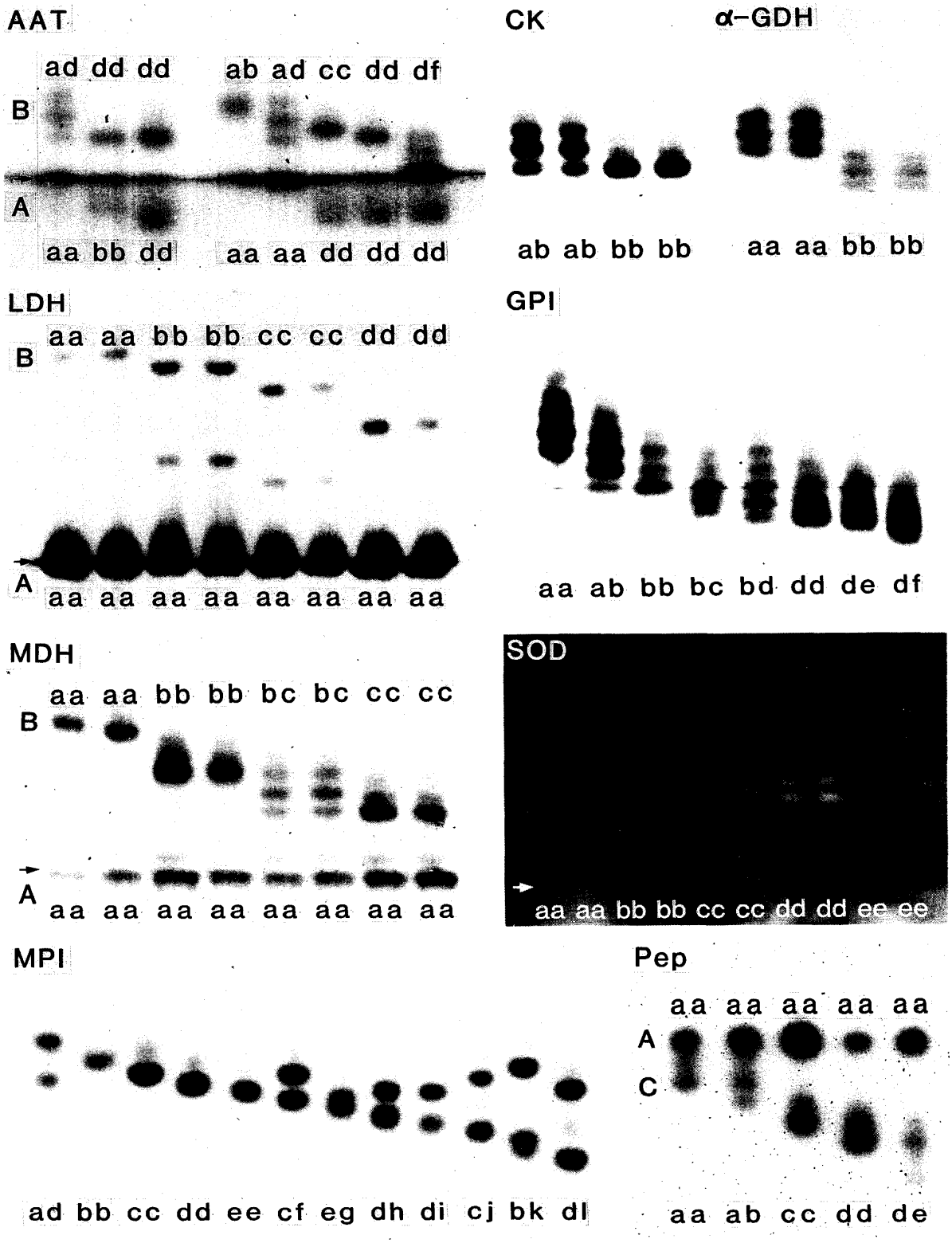

Fig. 7. Electrophoretic patterns of nine enzymes, AAT, CK, α–GDH, LDH, GPI, MDH, SOD, MPI and Pep, in the six populations of *Rana narina*.

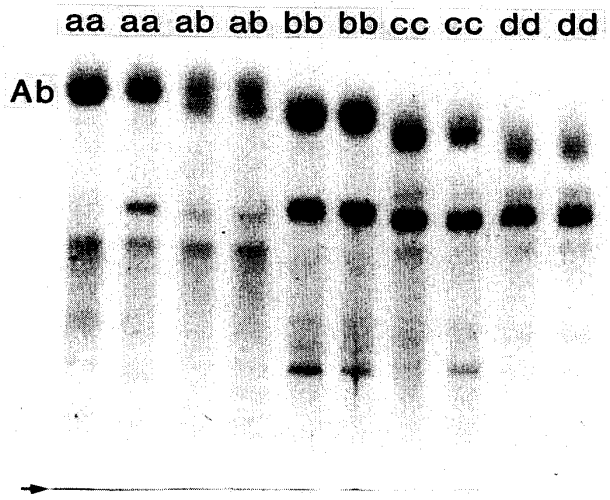

Fig. 8. A electrophoretic pattern of a blood protein, Ab, in the six populations of *Rana narina*.

TABLE 7
Number of phenotypes and alleles at 30 loci in six populations of *Rana narina*

Locus	No. of phenotypes	No. of alleles	Locus	No. of phenotypes	No. of alleles
AAT-A	5	4	MDH-B	4	3
AAT-B	7	6	ME	4	4
ADA	10	6	MPI	16	12
AK	1	1	Pep-A	1	1
ALD	3	2	Pep-B	1	1
CK	2	2	Pep-C	7	5
Est-1	8	6	Pep-D	4	3
Fum	2	2	6-PGD	3	3
α-GDH	3	2	PGM	8	5
GPI	8	6	SOD	6	5
HK	2	2	Ab	5	4
IDH-A	3	2	Hb-I	2	2
IDH-B	6	5	Hb-II	1	1
LDH-A	1	1	Prot-C	5	4
LDH-B	4	4			
MDH-A	1	1	Average	4.4	3.5

named A, B, C and so on in accordance with the order of mobility from fast to slow, and the alleles are shown by *a, b, c* and so on (Figs. 7, 8).

Of the 30 loci, those of five enzymes and one blood protein, AK, LDH-A, MDH-A, Pep-A, Pep-B and Hb-II showed a single phenotype produced by a single allele *a*. The four loci of CK, Fum, HK and Hb-I showed two kinds of phenotypes produced by two alleles, *a* and *b*, while the three loci of ALD, α-GDH and IDH-A showed three kinds of phenotypes produced by two alleles. The

locus of 6-PGD showed three kinds of phenotypes produced by three alleles, *a, b* and *c,* while the two loci of MDH-B and Pep-D showed four kinds of phenotypes produced by three alleles. The two loci of LDH-B and ME showed four kinds of phenotypes produced by four alleles, *a, b, c* and *d,* while the three loci of AAT-A, Ab and Prot-C showed five kinds of phenotypes produced by four alleles. The two loci of IDH-B and SOD showed six kinds of phenotypes, a locus of Pep-C showed seven kinds of phenotypes, and the locus of PGM showed eight phenotypes. All these phenotypes were produced by five alleles, *a, b, c, d* and *e.* The locus of AAT-B showed seven kinds of phenotypes, the two loci of Est-1 and GPI showed eight kinds of phenotypes, and the locus of ADA showed 10 kinds of phenotypes. All these phenotypes were produced by six alleles, *a, b, c, d, e* and *f.* The locus of MPI was most polymorphic and showed 16 kinds of phenotypes produced by 12 alleles, *a* ~ *l.* The numbers of phenotypes and alleles at the 30 loci were 4.4 and 3.5 on the average, respectively (Table 7).

IV. Gene frequency

Phenotypes and gene frequencies were examined at 24 of the 30 loci analyzed in *Rana narina,* except for six loci, AK, LDH-A, MDH-A, Pep-A, Pep-B and Hb-II, which consisted of a single allele.

1. AAT-A locus

Five phenotypes produced by four alleles, *a, b, c* and *d,* were observed by analyzing electrophoretic patterns at the AAT-A locus in the 92 frogs belonging to the six populations. All the seven and eight frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls., respectively, showed a homozygous band, DD, produced by allele *d,* while all the five and 15 frogs belonging to the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl., respectively, showed a homozygous band, CC, produced by allele *c.* All the 54 frogs belonging to the dwarf-type population from Ishigaki Isl. showed a homozygous band, BB, produced by allele *b.* Of the three frogs belonging to the dwarf-type population from Iriomote Isl., two showed a homozygous band, AA, while the remainder showed a heterozygous band, AB. Alleles *a* and *b* were 0.833 and 0.167 in frequency, respectively (Table 8; Fig. 9).

It was found that the giant-type populations from Iriomote and Ishigaki Isls. have only allele *d,* the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. have only allele *c,* and the dwarf-type population from Ishigaki Isl. has only allele *b,* while the dwarf-type population from Iriomote Isl. mostly has allele *a* (Table 8; Fig. 9).

2. AAT-B locus

Seven phenotypes produced by six alleles, *a, b, c, d, e* and *f,* were observed at the AAT-B locus in the 91 frogs belonging to the six populations. Of the seven frogs belonging to the giant-type population from Iriomote Isl., six showed a homozygous band, DD, while the remainder showed a heterozygous band, DF. Alleles *d*

TABLE 8

Gene frequencies at 24 loci in

Population		Iriomote Isl.		Ishigaki Isl.		Okinawa Isl.	Amami Isl.
		Giant-type	Dwarf-type	Giant-type	Dwarf-type	Middle-type	Giant-type
Sample size		7	3	8	54	5	15
Locus	Allele						
1) AAT-A	*a*		0.833				
	b		0.167		1.000		
	c					1.000	1.000
	d	1.000		1.000			
2) AAT-B	*a*		0.500				
	b		0.167				
	c			0.125			
	d	0.929	0.333	0.875	1.000	0.600	0.964
	e						0.036
	f	0.071				0.400	
3) ADA	*a*					0.100	
	b					0.300	0.567
	c		1.000		1.000		
	d						0.267
	e	1.000		1.000		0.400	0.100
	f					0.200	0.067
4) ALD	*a*	0.214	1.000		1.000		
	b	0.786		1.000		1.000	1.000
5) CK	*a*				0.046		
	b	1.000	1.000	1.000	0.954	1.000	1.000
6) Est-1	*a*	0.714		0.929			
	b		1.000		1.000	1.000	
	c	0.286		0.071			
	d						0.667
	e						0.233
	f						0.100
7) Fum	*a*	1.000	1.000	1.000	1.000	1.000	0.900
	b						0.100
8) α-GDH	*a*						0.600
	b	1.000	1.000	1.000	1.000	1.000	0.400
9) GPI	*a*					0.600	0.633
	b	0.857		1.000		0.400	0.367
	c	0.071					
	d	0.071	0.833		0.981		
	e		0.167				
	f				0.019		
10) HK	*a*		1.000		1.000		
	b	1.000		1.000		1.000	1.000
11) IDH-A	*a*	0.286	1.000		1.000		
	b	0.714		1.000		1.000	1.000
12) IDH-B	*a*	1.000	1.000	0.938	0.741	0.900	
	b				0.259		
	c					0.100	
	d						1.000
	e			0.063			
13) LDH-B	*a*						1.000
	b		1.000		1.000		
	c	1.000		1.000			
	d					1.000	

six populations of *Rana narina*

Population		Iriomote Isl.		Ishigaki Isl.		Okinawa Isl.	Amami Isl.
		Giant-type	Dwarf-type	Giant-type	Dwarf-type	Middle-type	Giant-type
Sample size		7	3	8	54	5	15
Locus	Allele						
14) MDH-B	*a*		1.000		1.000		
	b			0.125		1.000	
	c	1.000		0.875			1.000
15) ME	*a*				0.037		
	b	1.000	1.000	0.929	0.963		
	c					1.000	1.000
	d			0.071			
16) MPI	*a*						0.033
	b						0.267
	c	0.714	0.167	0.875			
	d					0.600	0.467
	e		0.833		0.917		
	f	0.214					
	g				0.083		
	h						0.167
	i					0.300	
	j	0.071		0.125			
	k						0.067
	l					0.100	
17) Pep-C	*a*	1.000		0.813			
	b			0.188		0.100	
	c					0.900	0.933
	d		1.000		0.991		0.067
	e				0.009		
18) Pep-D	*a*	0.071				0.700	0.933
	b	0.929	0.833	0.938	0.991	0.300	0.067
	c		0.167	0.063	0.009		
19) 6-PGD	*a*		0.167				
	b	1.000	0.667	1.000		1.000	1.000
	c		0.167		1.000		
20) PGM	*a*				0.009		
	b	0.143	1.000	0.313	0.796	0.100	
	c				0.037		
	d				0.157		
	e	0.857		0.688		0.900	1.000
21) SOD	*a*				0.333		
	b	1.000		1.000			1.000
	c					1.000	
	d		1.000				
	e				0.667		
22) Ab	*a*					0.900	1.000
	b	1.000		1.000		0.100	
	c				1.000		
	d		1.000				
23) Hb-I	*a*	1.000		1.000		1.000	1.000
	b		1.000		1.000		
24) Prot-C	*a*	1.000		1.000			
	b					1.000	0.900
	c		1.000		1.000		
	d						0.100

and *f* were 0.929 and 0.071 in frequency, respectively. One and two of the three frogs belonging to the dwarf-type population from Iriomote Isl. showed heterozygous bands, AB and AD, respectively. Alleles *a*, *b* and *d* were 0.500, 0.167 and 0.333 in frequency, respectively. Of the eight frogs belonging to the giant-type population from Ishigaki Isl., one and seven showed homozygous bands, CC and DD, respectively. Alleles *c* and *d* were 0.125 and 0.875 in frequency, respectively. All the 54 frogs belonging to the dwarf-type population from Ishigaki Isl. showed a homozygous band, DD. Of five frogs belonging to the middle-type population from Okinawa Isl., two and one showed homozygous bands, DD and FF, respectively, and the remaining two showed a heterozygous band, DF. Alleles *d* and *f* were 0.600 and 0.400 in frequency, respectively. Of the 14 frogs belonging to the giant-type population from Amami Isl., 13 showed a homozygous band, DD, and the remainder showed a heterozygous band, DE. Alleles *d* and *e* were 0.964 and 0.036 in frequency, respectively (Table 8; Fig. 9).

Thus, it was found that almost all of the frogs belonging to the giant-type population from Iriomote Isl., the giant-type and dwarf-type populations from Ishigaki Isl. and the giant-type population from Amami Isl. have allele *d*. In the dwarf-type population from Iriomote Isl. and the middle-type population from Okinawa Isl., alleles *a* and *d* are most abundantly found, respectively (Table 8; Fig. 9).

3. ADA locus

Ten phenotypes produced by six alleles, *a, b, c, d, e* and *f,* were observed at the ADA locus in the 92 frogs belonging to the six populations. All the seven and eight frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls., respectively, showed a homozygous band, EE, while all the three and 54 frogs belonging to the dwarf-type populations of the same islands showed a homozygous band, CC. Of the five frogs belonging to the middle-type population from Okinawa Isl., two showed homozygous bands, BB and EE, and three frogs showed heterozygous bands, AF, BE and EF. Alleles *a, b, e* and *f* were 0.100, 0.300, 0.400 and 0.200 in frequency, respectively. Of the 15 frogs belonging to the giant-type population from Amami Isl., six and one showed homozygous bands, BB and DD, respectively, and the other four, one, two and one showed heterozygous bands, BD, BF, DE and EF, respectively. Alleles *b, d, e* and *f* in this population were 0.567, 0.267, 0.100 and 0.067 in frequency, respectively. It was found that the giant-type populations from Iriomote and Ishigaki Isls. have only allele *e,* while the dwarf-type populations from the same islands have only allele *c*. In contrast, the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. have four kinds of alleles (Table 8; Fig. 9).

4. ALD locus

Three phenotypes produced by two alleles, *a* and *b,* were observed at the ALD locus in the 92 frogs belonging to the six populations. All the three and 54 frogs belonging to the dwarf-type populations from Iriomote and Ishigaki Isls.,

respectively, showed a homozygous band, AA, produced by allele *a*. All the eight, 15 and five frogs belonging to the giant-type population from Ishigaki Isl., the giant-type population from Amami Isl. and the middle-type population from Okinawa Isl., respectively, showed a homozygous band, BB, produced by allele *b*. Of the seven frogs belonging to the giant-type population from Iriomote Isl., four showed a homozygous band, BB, and the other three showed a heterozygous band, AB. In this population, alleles *a* and *b* were 0.214 and 0.786 in frequency, respectively (Table 8; Fig. 9).

It was found that the dwarf-type populations from Iriomote and Ishigaki Isls. have only allele *a*, while the giant-type population from Ishigaki Isl., the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. have only allele *b*. The giant-type population from Iriomote Isl. has mostly allele *b* and slightly allele *a* (Fig. 9).

5. CK locus

Two phenotypes produced by two alleles, *a* and *b*, were observed at the CK locus in the 92 frogs belonging to the six populations. All the 38 frogs belonging to the five populations other than the dwarf-type population from Ishigaki Isl. showed a homozygous band, BB, produced by allele *b*. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 49 showed a homozygous band, BB, and the other five showed a heterozygous band, AB. In this population, alleles *a* and *b* were 0.046 and 0.954 in frequency, respectively (Table 8; Fig. 9).

6. Est-1 locus

Eight phenotypes produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*, were observed at the Est-1 locus in the 91 frogs belonging to the six populations. Of the seven frogs belonging to each of the giant-type populations from Iriomote and Ishigaki Isls., three and six, respectively, showed a homozygous band, AA, and the other four and one, respectively, showed a heterozygous band, AC. In these two populations, allele *a* was 0.714 and 0.929 in frequency, respectively, and allele *c* was 0.286 and 0.071, respectively. All the three, 54 and five frogs belonging to the dwarf-type population from Iriomote Isl., the dwarf-type population from Ishigaki Isl. and the middle-type population from Okinawa Isl. showed a homozygous band, BB, produced by allele *b*. Of the 15 frogs belonging to the giant-type population from Amami Isl., eight and two showed homozygous bands, DD and EE, respectively, and two, two and one showed heterozygous bands, DE, DF and EF, respectively. In this population, alleles *d*, *e* and *f* were 0.667, 0.233 and 0.100 in frequency, respectively (Table 8; Fig. 9).

It was found that the giant-type populations from Iriomote and Ishigaki Isls. have mostly allele *a*, while the dwarf-type populations of these two islands and the middle-type population from Okinawa Isl. have only allele *b*. The giant-type population from Amami Isl. has mostly allele *d* (Table 8; Fig. 9).

7. Fum locus

Two phenotypes produced by two alleles, *a* and *b*, were observed at the Fum locus in the 92 frogs belonging to the six populations. All the 77 frogs belonging to the five populations other than the giant-type population from Amami Isl. showed a homozygous band, AA, produced by allele *a*. Of the 15 frogs belonging to the giant-type population from Amami Isl., 12 showed a homozygous band, AA, and the other three showed a heterozygous band, AB. In this population, alleles *a* and *b* were 0.900 and 0.100 in frequency, respectively (Table 8; Fig. 9).

8. α–GDH locus

Three phenotypes produced by two alleles, *a* and *b*, were observed at the α–GDH locus in the 92 frogs belonging to the six populations. All the 77 frogs belonging to the five populations other than the giant-type population from Amami Isl. showed a homozygous band, BB, produced by allele *b*. Of the 15 frogs belonging to the giant-type population from Amami Isl., five and two showed homozygous bands, AA and BB, respectively, and the other eight showed a heterozygous band, AB. In this population, alleles *a* and *b* were 0.600 and 0.400 in frequency, respectively (Table 8; Fig. 9).

9. GPI locus

Eight phenotypes produced by six alleles, *a*, *b*, *c*, *d*, *e* and *f*, were observed at the GPI locus in the 92 frogs belonging to the six populations. Of the seven frogs belonging to the giant-type population from Iriomote Isl., five showed a homozygous band, BB, and the other two showed heterozygous bands, BC and BD. In this population, alleles *b*, *c* and *d* were 0.857, 0.071 and 0.071 in frequency, respectively. All the eight frogs belonging to the giant-type population from Ishigaki Isl. showed a homozygous band, BB, produced by allele *b*. Of the three frogs belonging to the dwarf-type population from Iriomote Isl., two and one showed a homozygous band, DD, and a heterozygous band, DE, respectively. Alleles *d* and *e* were 0.833 and 0.167 in frequency, respectively. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 52 showed a homozygous band, DD, and the other two showed a heterozygous band, DF. Alleles *d* and *f* were 0.981 and 0.019 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl., one showed a homozygous band, AA, and the other four showed a heterozygous band, AB. Alleles *a* and *b* were 0.600 and 0.400 in frequency, respectively. Of the 15 frogs belonging to the giant-type population from Amami Isl., six and two showed homozygous bands, AA and BB, respectively, and the other seven showed a heterozygous band, AB. Alleles *a* and *b* were 0.633 and 0.367 in frequency, respectively (Table 8; Fig. 9).

It was found that the giant-type populations from Iriomote and Ishigaki Isls. have mostly allele *b*, while the dwarf-type populations from these two islands have mostly allele *d*. The middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. have alleles *a* and *b*, although allele *a* is

somewhat higher than allele *b* in frequency (Table 8; Fig. 9).

10. HK locus

Two phenotypes produced by two alleles, *a* and *b*, were observed at the HK locus in the 92 frogs belonging to the six populations. All the seven, eight, five and 15 frogs belonging to the giant-type population from Iriomote Isl., the giant-type population from Ishigaki Isl., the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl., respectively, showed a homozygous band, BB, produced by allele *b*. All the three and 54 frogs belonging to the dwarf-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, AA, produced by allele *a*. It was found that all the dwarf-type populations have only allele *a*, while the giant-type and middle-type populations have only allele *b* (Table 8; Fig. 9).

11. IDH–A locus

Three phenotypes produced by two alleles, *a* and *b*, were observed at the IDH–A locus in the 92 frogs belonging to the six populations. Of the seven frogs belonging to the giant-type population from Iriomote Isl., three showed a homozygous band, BB, and the other four showed a heterozygous band, AB. Alleles *a* and *b* were 0.286 and 0.714 in frequency, respectively. All the eight, five and 15 frogs belonging to the giant-type population from Ishigaki Isl., the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl., respectively, showed a homozygous band, BB, produced by allele *b*. The three and 54 frogs belonging to the dwarf-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, AA, produced by allele *a*. It was found that all the frogs belonging to the dwarf-type populations have only allele *a*, while almost all the giant-type and middle-type populations have allele *b* (Table 8; Fig. 9).

12. IDH–B locus

Six phenotypes produced by five alleles, *a*, *b*, *c*, *d* and *e*, were observed at the IDH–B locus in the 92 frogs belonging to the six populations. The seven and three frogs belonging to the giant-type and dwarf-type populations from Iriomote Isl., respectively, showed a homozygous band, AA, produced by allele *a*. Of the eight frogs belonging to the giant-type population from Ishigaki Isl., seven showed a homozygous band, AA, and the remainder showed a heterozygous band, AE. Alleles *a* and *e* were 0.938 and 0.063 in frequency, respectively. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 32 and six showed homozygous bands, AA and BB, respectively, and the remaining 16 showed a heterozygous band, AB. Alleles *a* and *b* were 0.741 and 0.259 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl., four showed a homozygous band, AA, and the remainder showed a heterozygous band, AC. Alleles *a* and *c* were 0.900 and 0.100 in frequency, respectively. All the 15 frogs belonging to the giant-type population from Amami

Isl. showed a homozygous band, DD, produced by allele *d* (Table 8; Fig. 9).

It was found that almost all the frogs collected from Iriomote, Ishigaki and Okinawa Islands have allele *a*, while all the frogs collected from Amami Isl. have allele *d* (Table 8; Fig. 9).

13. LDH-B locus

Four phenotypes produced by four alleles, *a*, *b*, *c* and *d*, were observed at the LDH-B locus in the 92 frogs belonging to the six populations. All the seven and eight frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, CC, produced by allele *c*. All the three and 54

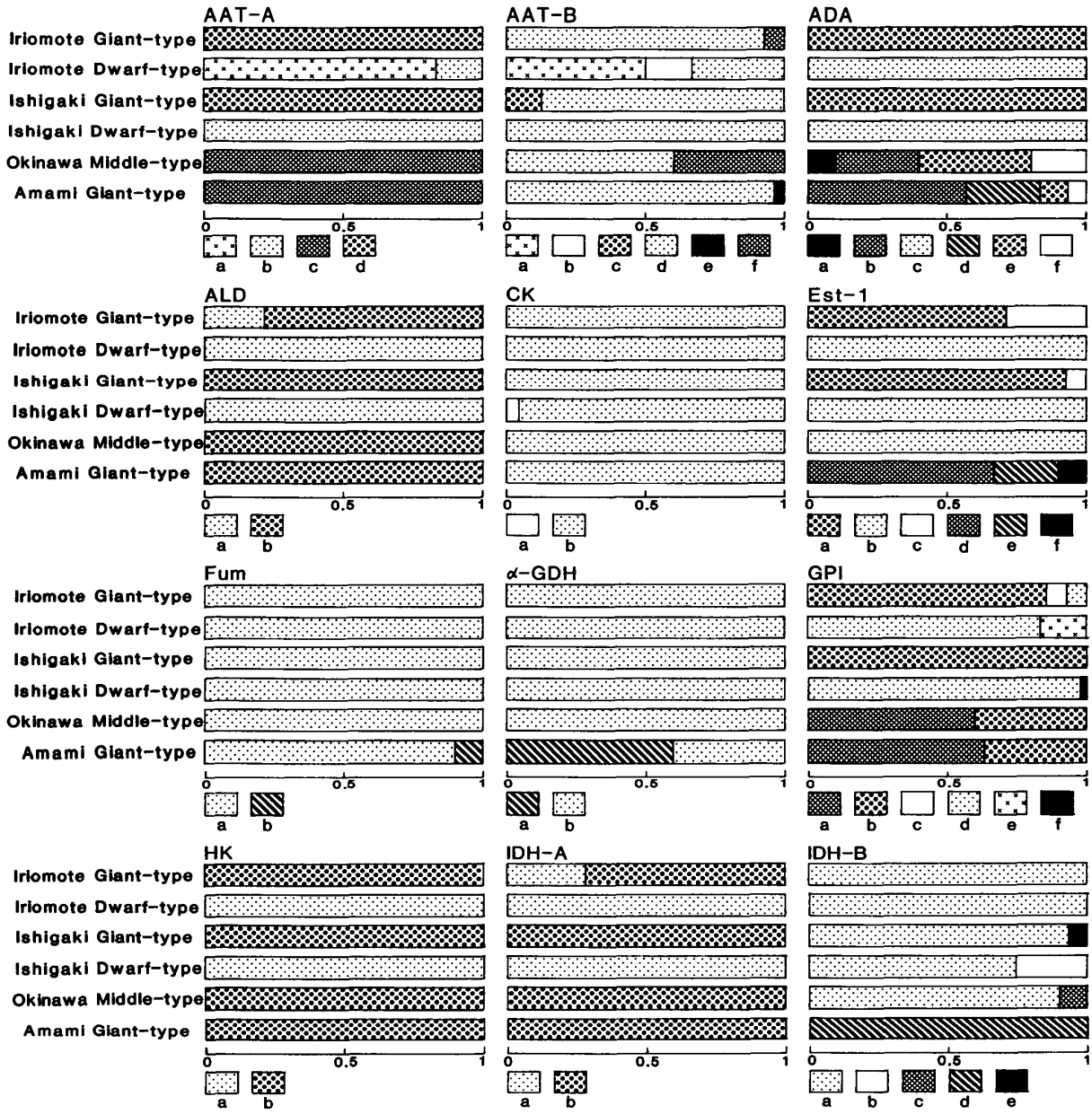

Fig. 9. Gene frequencies at 12 loci, AAT-A, AAT-B, ADA, ALD, CK, Est-1, Fum, α-GDH, GPI, HK, IDH-A and IDH-B, in the six populations of *Rana narina*.

frogs belonging to the dwarf-type populations from these two islands showed a homozygous band, BB, produced by allele *b*. All the five frogs belonging to the middle-type population from Okinawa Isl. showed a homozygous band, DD, produced by allele *d*. All the 15 frogs belonging to the giant-type population from Amami Isl. showed a homozygous band, AA, produced by allele *a* (Table 8; Fig. 10).

14. MDH-B locus

Four phenotypes produced by three alleles, *a*, *b* and *c*, were observed at the MDH-B locus in the 92 frogs belonging to the six populations. All the three and

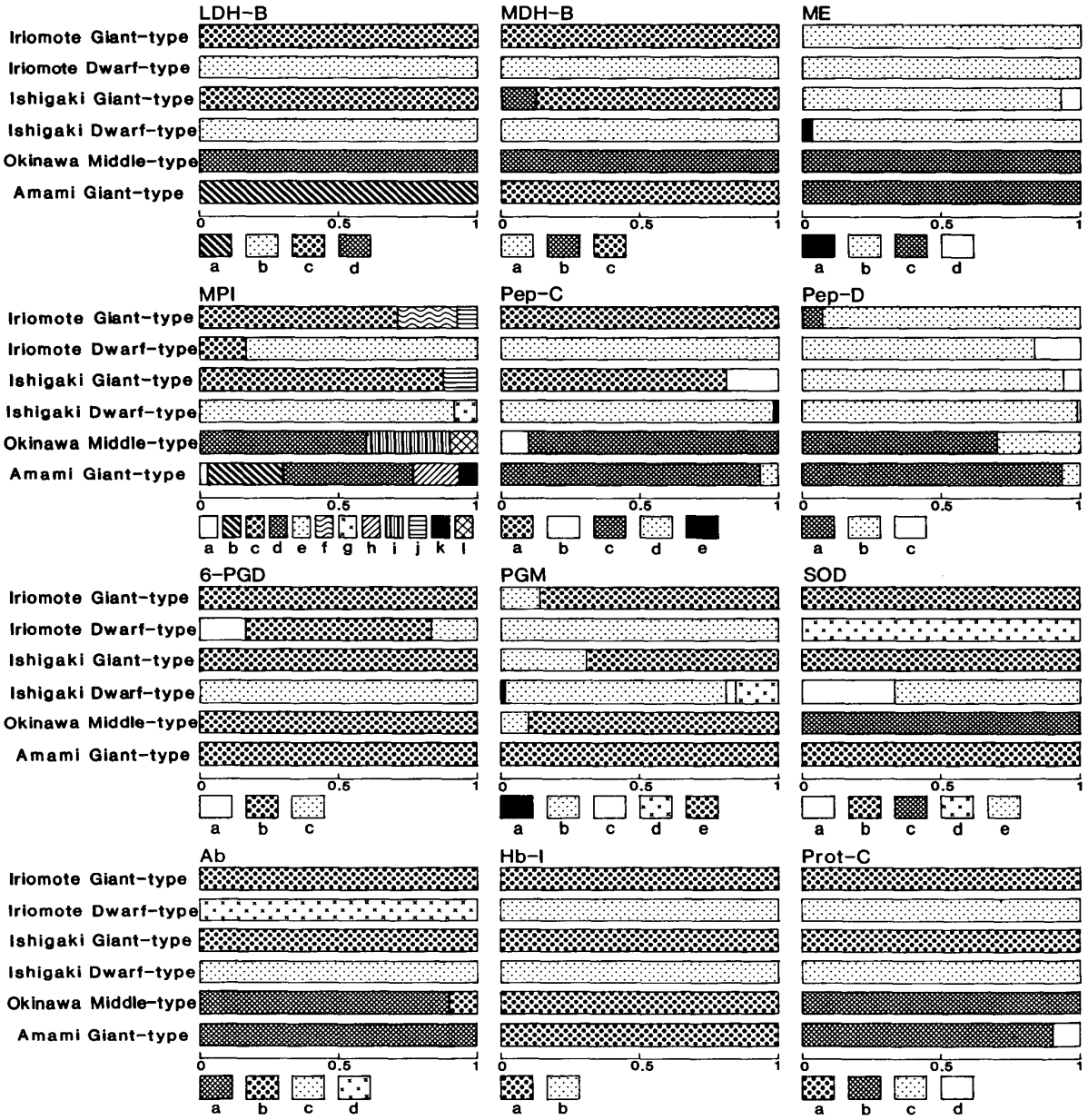

Fig. 10. Gene frequencies at 12 loci, LDH-B, MDH-B, ME, MPI, Pep-C, Pep-D, 6-PGD, PGM, SOD, Ab, Hb-I and Prot-C, in the six populations of *Rana narina*.

54 frogs belonging to the dwarf-type populations from Iriomote and Ishigaki Isls., respectively, showed a homozygous band, AA, produced by allele *a*. All the seven frogs belonging to the giant-type population from Iriomote Isl. showed a homozygous band, CC, produced by allele *c*. Of the eight frogs belonging to the giant-type population from Ishigaki Isl., six showed a homozygous band, CC, and the other two showed a heterozygous band, BC. In this population, alleles *b* and *c* were 0.125 and 0.875 in frequency, respectively. All the five frogs belonging to the middle-type population from Okinawa Isl. showed a homozygous band, BB, produced by allele *b*, while all the 15 frogs belonging to the giant-type population from Amami Isl. showed a homozygous band, CC, produced by allele *c* (Table 8; Fig. 10).

It was found that the giant-type populations from Iriomote, Ishigaki and Amami Isls. have only allele *c*, except that the population from Ishigaki Isl. slightly contained allele *b* in addition to allele *c*. The dwarf-type populations from Iriomote and Ishigaki Isls. have only allele *a*, and the middle-type population from Okinawa Isl. has only allele *b* (Table 8; Fig. 10).

15. ME locus

Four phenotypes produced by four alleles, *a*, *b*, *c* and *d*, were observed at the ME locus in the 91 frogs belonging to the six populations. All the seven frogs belonging to the giant-type population and three frogs belonging to the dwarf-type population from Iriomote Isl. showed a homozygous band, BB, produced by allele *b*. All the five frogs belonging to the middle-type population from Okinawa Isl. and the 15 frogs belonging to the giant-type population from Amami Isl. showed a homozygous band, CC, produced by allele *c*. Of the seven frogs belonging to the giant-type population from Ishigaki Isl., six showed a homozygous band, BB, and the remainder showed a heterozygous band, BD. Alleles *b* and *d* were 0.929 and 0.071 in frequency, respectively. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 50 showed a homozygous band, BB, and the other four showed a heterozygous band, AB. Alleles *a* and *b* were 0.037 and 0.963 in frequency, respectively (Table 8; Fig. 10). It was found that the giant-type and dwarf-type populations from Iriomote Isl. have only allele *b*. The middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. have only allele *c*. The giant-type and dwarf-type populations from Ishigaki Isl. have a slight amount of alleles *d* and *a*, respectively, in addition to an overwhelming majority of allele *b* (Table 8; Fig. 10).

16. MPI locus

Sixteen phenotypes produced by 12 alleles, *a* ~ *l*, were observed at the MPI locus in the 92 frogs belonging to the six populations. Of the seven frogs belonging to the giant-type population from Iriomote Isl., three showed a homozygous band, CC, and the other three and one showed heterozygous bands, CF and CJ, respectively. Alleles *c*, *f* and *j* were 0.714, 0.214 and 0.071 in frequency, respectively. Of the three frogs belonging to the dwarf-type population from

Iriomote Isl., two showed a homozygous band, EE, and the remainder showed a heterozygous band, CE. Alleles *c* and *e* were 0.167 and 0.833, respectively. Of the eight frogs belonging to the giant-type population from Ishigaki Isl., six showed a homozygous band, CC, and the other two showed a heterozygous band, CJ. Alleles *c* and *j* were 0.875 and 0.125 in frequency, respectively. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 45 showed a homozygous band, EE, and the other nine showed a heterozygous band, EG. Alleles *e* and *g* were 0.917 and 0.083 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl., one showed a homozygous band, DD, and the other three and one showed heterozygous bands, DI and DL, respectively. Alleles *d*, *i* and *l* were 0.600, 0.300 and 0.100 in frequency, respectively. Of the 15 frogs belonging to the giant-type population from Amami Isl., one and three showed homozygous bands, BB and DD, respectively, and the other one, three, two, one, three and one showed heterozygous bands, AD, BD, BH, BK, DH and DK, respectively. Alleles *a*, *b*, *d*, *h* and *k* were 0.033, 0.267, 0.467, 0.167 and 0.067, respectively (Table 8; Fig. 10).

It was found that allele *c* is abundant in the giant-type populations from Iriomote and Ishigaki Isls., while allele *e* is abundant in the dwarf-type populations of the same islands. Allele *d* is considerably abundant in the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. (Fig. 10).

17. Pep–C locus

Seven phenotypes produced by five alleles, *a*, *b*, *c*, *d* and *e*, were observed at the Pep–C locus in the 92 frogs belonging to the six populations. All the seven frogs belonging to the giant-type population from Iriomote Isl. showed a homozygous band, AA, produced by allele *a*, while all the three frogs belonging to the dwarf-type population showed a homozygous band, DD, produced by allele *d*. Of the eight frogs belonging to the giant-type population from Ishigaki Isl., five showed a homozygous band, AA, and the other three showed a heterozygous band, AB. Alleles *a* and *b* were 0.813 and 0.188 in frequency, respectively, in this population. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 53 showed a homozygous band, DD, and the remainder showed a heterozygous band, DE. Alleles *d* and *e* were 0.991 and 0.009 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl. and 15 frogs belonging to the giant-type population from Amami Isl., four and 13 showed a homozygous band, CC, respectively, and the remaining one and two showed heterozygous bands, BC and CD, respectively. In the middle-type population from Okinawa Isl., alleles *b* and *c* were 0.100 and 0.900 in frequency, respectively, while alleles *c* and *d* were 0.933 and 0.067 in frequency, respectively, in the giant-type population from Amami Isl. (Table 8; Fig. 10).

It was found that alleles *a* and *d* are most abundant in the giant-type populations and dwarf-type populations, respectively, from Iriomote and Ishigaki Isls. In the middle-type population from Okinawa Isl. and the giant-type

population from Amami Isl., allele *c* is most abundant (Fig. 10).

18. Pep–D locus

Four phenotypes produced by three alleles, *a, b* and *c,* were observed at the Pep–D locus in the 92 frogs belonging to the six populations. Of the seven frogs belonging to the giant-type population from Iriomote Isl., six showed a homozygous band, BB, and the remainder showed a heterozygous band, AB. Alleles *a* and *b* were 0.071 and 0.929 in frequency, respectively. Of the three frogs belonging to the dwarf-type population from Iriomote Isl., eight frogs belonging to the giant-type population from Ishigaki Isl., and 54 frogs belonging to the dwarf-type population from Ishigaki Isl., two, seven and 53, respectively, showed a homozygous band, BB, while one frog of each of the three populations showed a heterozygous band, BC. Alleles *b* and *c* in these three populations were 0.833 and 0.167, 0.938 and 0.063, and 0.991 and 0.009 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl., three and one showed homozygous bands, AA and BB, respectively and the remainder showed a heterozygous band, AB. Alleles *a* and *b* were 0.700 and 0.300 in frequency, respectively. Of the 15 frogs belonging to the giant-type population from Amami Isl., 13 showed a homozygous band, AA, and the other two showed a heterozygous band, AB. Alleles *a* and *b* were 0.933 and 0.067 in frequency, respectively (Table 8; Fig. 10)

It was found that allele *b* is very abundant in the populations from Iriomote and Ishigaki Isls., while allele *a* is also very abundant in the populations from Okinawa and Amami Isls. (Fig. 10).

19. 6–PGD locus

Three phenotypes produced by three alleles, *a, b* and *c,* were observed at the 6–PGD locus in the 92 frogs belonging to the six populations. All the 35 frogs including seven of the giant-type population from Iriomote Isl., eight of the giant-type population from Ishigaki Isl., five of the middle-type population from Okinawa Isl. and 15 of the giant-type population from Amami Isl., showed a homozygous band, BB, produced by allele *b.* All the 54 frogs belonging to the dwarf-type population from Ishigaki Isl. showed a homozygous band, CC, produced by allele *c.* Of the three frogs belonging to the dwarf-type population from Iriomote Isl., two showed a homozygous band, BB, and the remainder showed a heterozygous band, AC. Alleles *a, b* and *c* were 0.167, 0.667 and 0.167 in frequency, respectively (Table 8; Fig. 10).

20. PGM locus

Eight phenotypes produced by five alleles, *a, b, c, d* and *e,* were observed at the PGM locus in the 92 frogs belonging to the six populations. Of the seven and eight frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls., five and three showed a homozygous band, EE, respectively, and the remaining two and five showed a heterozygous band, BE, respectively. In these

two populations, alleles *b* and *e* were 0.143 and 0.857, and 0.313 and 0.688 in frequency, respectively. All the three frogs belonging to the dwarf-type population from Iriomote Isl. showed a homozygous band, BB, produced by allele *b*. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., 38, one and two showed homozygous bands, BB, CC and DD, respectively, and one, 10 and two showed heterozygous bands, AD, BD and CD, respectively. Alleles *a*, *b*, *c* and *d* were 0.009, 0.796, 0.037 and 0.157 in frequency, respectively. Of the five frogs belonging to the middle-type population from Okinawa Isl., four showed a homozygous band, EE, and the remainder showed a heterozygous band, BE. Alleles *b* and *e* were 0.100 and 0.900 in frequency, respectively. All the 15 frogs belonging to the giant-type population from Amami Isl. showed a homozygous band, EE, produced by allele *e* (Table 8; Fig. 10).

It was found that allele *b* is most abundant in the dwarf-type populations, while allele *e* is most abundant in the giant-type and middle-type populations (Table 8; Fig. 10).

21. SOD locus

Six phenotypes produced by five alleles, *a*, *b*, *c*, *d* and *e*, were observed at the SOD locus in the 92 frogs belonging to the six populations. All the 30 frogs belonging to the giant-type populations from Iriomote, Ishigaki and Amami Isls. showed a homozygous band, BB, produced by allele *b*. All the three frogs belonging to the dwarf-type population from Iriomote Isl. and the five frogs belonging to the middle-type population from Okinawa Isl. showed homozygous bands, DD, produced by allele *d*, and CC produced by allele *c*, respectively. Of the 54 frogs belonging to the dwarf-type population from Ishigaki Isl., eight and 26 showed homozygous bands, AA and EE, respectively, and 20 showed a heterozygous band, AE. Alleles *a* and *e* were 0.333 and 0.667 in frequency, respectively (Table 8; Fig. 10).

22. Ab locus

Five phenotypes produced by four alleles, *a*, *b*, *c* and *d*, were observed at the Ab locus in the 92 frogs belonging to the six populations. All the 15 frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, BB, produced by allele *b*. All the 15 frogs belonging to the giant-type population from Amami Isl. showed a homozygous band, AA, produced by allele *a*, all the three frogs belonging to the dwarf-type population from Iriomote Isl. showed a homozygous band, DD, produced by allele *d*, and all the 54 frogs belonging to the dwarf-type population from Ishigaki Isl. showed a homozygous band, CC, produced by allele *c*. Of the five frogs belonging to the middle-type population from Okinawa Isl., 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 (Table 8; Fig. 10).

23. Hb–I locus

Two phenotypes produced by two alleles, *a* and *b*, were observed at the Hb–I locus in the 92 frogs belonging to the six populations. All the seven, eight, five and 15 frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls., middle-type population from Okinawa Isl. and giant-type population from Amami Isl., respectively, showed a homozygous band, AA, produced by allele *a*. All the three and 54 frogs belonging to the dwarf-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, BB, produced by allele *b* (Table 8; Fig. 10).

24. Prot–C locus

Five phenotypes produced by four alleles, *a*, *b*, *c* and *d*, were observed at the Prot–C locus in the 92 frogs belonging to the six populations. All the 15 frogs belonging to the giant-type populations from Iriomote and Ishigaki Isls. showed a homozygous band, AA, produced by allele *a*, and the 57 frogs belonging to the dwarf-type populations from the same islands showed a homozygous band, CC, produced by allele *c*. The five frogs belonging to the middle-type population from Okinawa Isl. showed a homozygous band, BB, produced by allele *b*. Of the 15 frogs belonging to the giant-type population from Amami Isl., 13 and one showed homozygous bands, BB and DD, respectively, and the remainder showed a heterozygous band, BD. Alleles *b* and *d* were 0.900 and 0.100 in frequency, respectively (Table 8; Fig. 10).

V. Genetic variation and genetic distance

1. Genetic variations among the six populations

Three genetic parameters, mean number of alleles per locus, mean proportion of heterozygous loci per individual and mean proportion of polymorphic loci per population, were estimated in order to show the genetic variations among the six populations, including three giant-type, two dwarf-type and one middle-type, of *Rana narina*.

Mean number of alleles at each locus was examined in 30 loci controlling enzymes and blood proteins extracted from the 92 frogs belonging to the six populations of *Rana narina* (Table 9). The results showed that the mean numbers of alleles were 1.27~1.53, 1.37 on the average. The largest 1.53 was found in the giant-type population from Amami Isl., while the smallest 1.27 was in the dwarf-type population from Iriomote Isl.

When the proportion of heterozygous loci per individual was examined at the 30 loci in the six populations, the highest, 11.9%, was found in the giant-type population from Amami Isl., followed by 10.5% which was found in the middle-type population from Okinawa Isl. In contrast, it was lower in the other four populations, that is, the giant-type population from Iriomote Isl., the

TABLE 9
Genetic variabilities at 30 loci in six populations of *Rana narina*

Population	Sample size	Mean number of alleles per locus	Mean proportion of heterozygous loci per individual (%)	Mean proportion of polymorphic loci per population (%)
Iriomote Giant-type	7	1.33	7.9 (10.0)	26.7
〃 Dwarf-type	3	1.27	7.4 (8.9)	20.0
Ishigaki Giant-type	8	1.30	6.3 (6.7)	30.0
〃 Dwarf-type	54	1.37	5.2 (4.4)	30.0
Okinawa Middle-type	5	1.40	10.5 (12.0)	30.0
Amami Giant-type	15	1.53	11.9 (10.7)	33.3
Average	15.3	1.37	8.2 (8.8)	28.3

Parentheses show an expected value.

dwarf-type population from Iriomote Isl., the giant-type population from Ishigaki Isl. and the dwarf-type population from Ishigaki Isl. The mean proportions of heterozygous loci in these four populations were 5.2~7.9%, 6.7% on the average.

When the proportions of polymorphic loci containing multiple alleles at the rate of more than 1% were estimated in the six populations, they were 26.7~33.3%, 28.3% on the average. The highest, 33.3%, was found in the giant-type population from Amami Isl. and the lowest, 20.0%, was in the dwarf-type population from Iriomote Isl. The giant-type and dwarf-type populations from Ishigaki Isl. and the middle-type population from Okinawa Isl. were 30.0% in mean proportion of polymorphic loci, while the giant-type population from Iriomote Isl. was 26.7% in mean proportion of polymorphic loci (Table 9).

2. Genetic distance and dendrogram

The genetic identity (I) and the genetic distance (D) among the six populations were estimated on the basis of gene frequencies at the 30 loci in the 92 frogs of *Rana narina* (Nei, 1975). The results showed that the genetic distance between the giant-type populations from Iriomote and Ishigaki Isls. was the smallest, being 0.012, followed by that between the dwarf-type populations from Iriomote and Ishigaki Isls. being 0.137. The genetic distances between the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl., between the middle-type population and the giant-type population from Ishigaki Isl., and between the middle-type population and the giant-type population from Iriomote Isl. were 0.232, 0.456 and 0.471, respectively. The genetic distances between the giant-type population from Amami Isl. and the giant-type populations from Iriomote and Ishigaki Isls. were 0.462.

The genetic distances between the giant-type or middle-type populations and the dwarf-type populations were remarkably larger than those between the giant-type or middle-type populations and the giant-type populations. The genetic distances between the giant-type and dwarf-type populations from

Iriomote Isl., and between the giant-type and dwarf-type populations from Ishigaki Isl. were 0.714 and 0.809, respectively. Those between the giant-type population from Iriomote Isl. and the dwarf-type population from Ishigaki Isl. and between the giant-type population from Ishigaki Isl. and the dwarf-type population from Iriomote Isl. were 0.754 and 0.759, respectively. The genetic distances between the middle-type population from Okinawa Isl. and the two dwarf-type populations from Iriomote and Ishigaki Isls. were 0.809 and 0.865, respectively. Those between the giant-type population from Amami Isl. and the two dwarf-type populations from Iriomote and Ishigaki Isls. were the largest, being 1.061 and 1.079, respectively (Table 10).

TABLE 10

Genetic identity (I) and genetic distance (D) among six populations of *Rana narina*

Population	Iriomote Isl.		Ishigaki Isl.		Okinawa Isl.	Amami Isl.
	Giant-type	Dwarf-type	Giant-type	Dwarf-type	Middle-type	Giant-type
Iriomote Giant-type	—	0.490	0.988	0.470	0.624	0.630
〃 Dwarf-type	0.714	—	0.468	0.872	0.445	0.346
Ishigaki Giant-type	0.012	0.759	—	0.445	0.634	0.630
〃 Dwarf-type	0.754	0.137	0.809	—	0.421	0.340
Okinawa Middle-type	0.471	0.809	0.456	0.865	—	0.793
Amami Giant-type	0.462	1.061	0.462	1.079	0.232	—

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

As the genetic distances among the six populations of *Rana narina* show the degree of genetic differentiation of this species, a dendrogram can be drawn by using the genetic distances. Although there are several methods, a dendrogram was drawn by the unweighted pair-group arithmetic average (UPGMA) clustering method (SNEATH and SOKAL, 1973; NEI, 1975), as this method is most commonly used (Fig. 11).

As the dendrogram in Fig. 11 indicated, the dwarf-type populations seem to have branched off from the giant-type populations in a very ancient period,

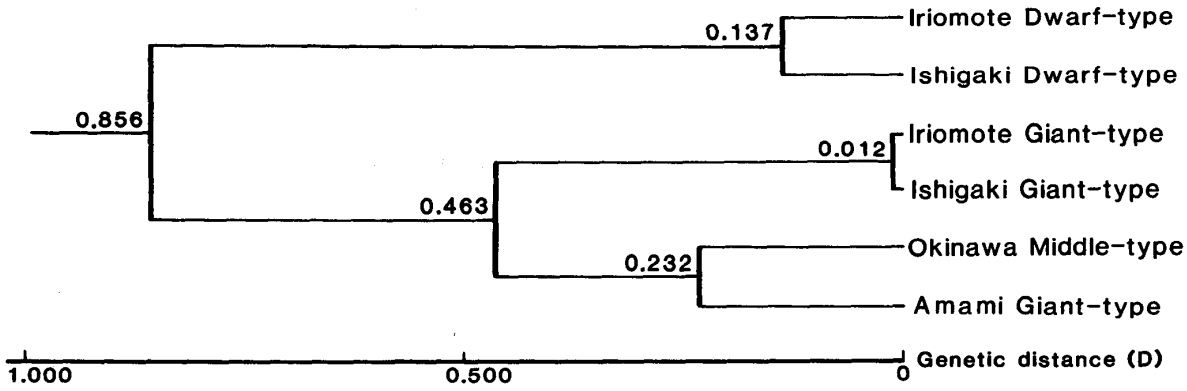

Fig. 11. Dendrogram for the six populations of *Rana narina*.

probably more than million years ago. From the giant-type populations, the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. seem to have branched off thereafter. As the genetic distance between the dwarf-type populations from Iriomote and Ishigaki Isls. is somewhat larger than that between the giant-type populations from these two islands, it is probable that the dwarf-type populations were the former occupants of these islands and later the giant-type populations had entered there. It is also probable that the differentiation between the middle-type population from Okinawa Isl. and the giant-type population from Amami Isl. occurred earlier than those between the two dwarf-type or giant-type populations from Iriomote and Ishigaki Isls.

DISCUSSION

It was found that the dwarf-type and giant-type populations of *Rana narina* are sympatrically distributed in each of two small islands, Ishigaki and Iriomote, situated in the southwestern end of Japan. These two islands are about 221 and 284 square kilometers in area, respectively, and are about 15 kilometers apart from each other. The dwarf-type and giant-type populations distributed in Iriomote Isl. are nearly the same as those distributed in Ishigaki Isl., respectively, in various respects. The frogs of the dwarf-type population are smaller in body size and lay fewer eggs as compared with those of the giant-type population. While the dwarf-type and giant-type populations of Ishigaki Isl. are not isolated from the dwarf-type and giant-type populations of Iriomote Isl., respectively, by reproductively isolating mechanisms, the dwarf-type populations of the two islands are completely isolated from the giant-type populations of the two islands and Amami Isl. by gametic isolation or hybrid inviability by the gastrula stage.

The results of electrophoretic analyses of 19 enzymes extracted from the skeletal muscles and livers and three kinds of blood proteins showed that the giant-type and dwarf-type populations of Iriomote Isl. are 0.012 and 0.137 in genetic distance, respectively, from those of Ishigaki Isl. The giant-type populations of the two islands are 0.714 and 0.809 in genetic distance from the dwarf-type populations of the same islands, while they are 0.754 and 0.759 in genetic distance from the dwarf-type populations of the other islands. These numerical values seem to show that the giant-type and dwarf-type populations from Iriomote or Ishigaki Isl. are biochemically differentiated from each other to a large extent. On the basis of the foregoing findings about the morphological differences in body and eggs, the existence of complete isolating mechanisms in reproduction and the biochemical differences in enzymes and blood proteins, the giant-type and dwarf-type populations seem to be two different species distributed sympatrically, although they resemble each other in appearance.

The sympatric distribution of two allied brown-frog species has been reported by KAWAMURA and NISHIOKA (1973). *Rana tsushimensis* (2n=26) and *Rana dybowskii* (2n=24) are distributed in Tsushima Isl., while *Rana amurensis coreana* (2n=26) and *Rana dybowskii* (2n=24) are found in Korea. *Rana japonica* (2n=26)

and *Rana ornativentris* (2n=24) are sympatrically distributed in many districts of Honshu, Shikoku and Kyushu of Japan. The two species distributed in the same district and having different chromosome numbers are completely isolated by hybrid sterility. Although mature hybrids are produced by crossings, they are all males and completely sterile. Two European brown-frog species, *Rana temporaria* (2n=26) and *Rana arvalis* (2n=24), seem to be sympatric in many districts. Such a sympatric distribution of two allied species is also found in several other anurans distributed in Japan. *Rhacophorus arboreus* and *Rh. schlegelii* are sympatric in many hills. *Rana nigromaculata* and *Rana brevipoda* are also sympatric in the contact areas of these two species. In this case, natural hybrids are rarely discovered, owing to incompleteness of reproductively isolating mechanisms.

The middle-type population of Okinawa Isl. and the giant-type population of Amami Isl. are 0.471 and 0.462 in genetic distance from the giant-type population of Iriomote Isl., respectively, while they are 0.809 and 1.061 in genetic distance from the dwarf-type population of Iriomote Isl. Nearly the same numerical values are obtained between the middle-type population of Okinawa Isl. or the giant-type population of Amami Isl. and the two populations of Ishigaki Isl. Iriomote Isl. and Ishigaki Isl. are about 380 kilometers apart from Okinawa Isl. and 620 kilometers apart from Amami Isl. Okinawa Isl. and Amami Isl. are about 1185 and 709 square kilometers in area and about 180 kilometers apart from each other. The frogs of the middle-type population are intermediate in body size and egg number between those of the dwarf-type and giant-type populations. The genetic distance between the giant-type population of Amami Isl. and the middle-type population of Okinawa Isl. is 0.232. This numerical value seems to show that the middle-type population of Okinawa Isl. and the giant-type population of Amami Isl. are fairly close to each other in relation. The genetic distances between these two populations and the two dwarf-type populations of Iriomote and Ishigaki Isls. seem to be far remoter than those between the former two populations and the giant-type populations of Iriomote and Ishigaki Isls. The results of crossing experiments between the middle-type population of Okinawa Isl. or the giant-type population of Amami Isl. and the dwarf-type or giant-type population of Iriomote or Ishigaki Isl. seem to support the results of electrophoretic analyses. While there is a complete gametic isolation or hybrid inviability by the early gastrula stage between the giant-type population of Amami Isl. or the middle-type population of Okinawa Isl. and the dwarf-type population of Iriomote or Ishigaki Isl., the hybrids between the former two populations and the giant-type population of Iriomote or Ishigaki Isl. are viable to some extent. A few normally cleaved eggs became normally feeding tadpoles. A crossing between a female belonging to the giant-type population of Iriomote Isl. and a male belonging to the giant-type population of Amami Isl. produced a few metamorphosed frogs.

NISHIOKA, OHTA and SUMIDA (1987) have reported that the genetic distances for seven populations of *Rana tagoi* collected from three mainlands and three small islands of Japan are 0.031~0.335. A dendrogram drawn by the UPGMA

clustering method seemed to indicate that the population from Yaku Isl. (*Rana tagoi yakushimensis)* was differentiated earlier than the other six populations, as this population showed somewhat larger numerical values in genetic distance among the seven populations in addition to the presence of a low degree of hybrid inviability between this population and the Nabara population from Hiroshima Prefecture. NISHIOKA, SUMIDA, OHTA and SUZUKI (1987) have also described that the genetic distances for four populations of *Buergeria japonica* collected from three islands, Amami, Tokara and Okinawa, belonging to the Southwest Islands of Japan are 0.003~0.270. In contrast, the genetic distances between *B. japonica* from the foregoing islands and *B. buergeri* from Hiroshima Prefecture situated in one of the mainlands of Japan are 2.142~2.243. The genetic distance between two subspecies of *Rhacophorus, Rh. v. viridis* and *Rh. v. owstoni,* distributed in different islands is 0.819, which seemed to be at a species level, as compared with 0.301~0.387 between two mainland species, *Rhacophorus schlegelii* and *Rh. arboreus,* which was placed as a valid species from the position of a subspecies by KAWAMURA (1962).

LARSON and HIGHTON (1978) have reported that the genetic distances between the northern and southern populations of *Plethodon dorsalis* are 1.67~2.15, and that such genetic distances are greater than those observed in any species previously studied. According to TILLEY and SCHWERDTFEGER (1981), the genetic distances between the northern and southern populations of *Desmognathus fuscus* distributed in eastern North America are 0.176~0.462. WAKE, MAXSON and WURST (1978) have reported that the genetic distances among five populations of *Hydromantes shastae* are 0.003~0.275, while those among five *Hydromantes* species are 0.121 ~1.836. KALEZIĆ and HEDGECOCK (1979) have calculated the genetic distances between certain Yugoslavian populations of *Triturus vulgaris, T. alpestris, T. cristatus dobrogicus* and *T. c. karelinii.* The mean values between consubspecific, subspecific and specific taxa are 0.031, 0.347 and 0.906, respectively.

In the present study, the genetic distances among the six populations of *Rana narina* were found to be 0.012~1.079. The results of electrophoretic analyses of enzymes and blood proteins in addition to those of crossing experiments suggest that the dwarf-type populations distributed in Iriomote and Ishigaki Islands have differentiated into a species level in these islands during the period of time when they were exclusive occupants and geographically isolated from the other populations. It is very probable that the giant-type populations of Iriomote and Ishigaki Islands were produced in Taiwan before they entered these two islands. It is reported by LUE and CHEN (1982) that a giant-type subspecies named *Rana narina swinhoana* is widely distributed in Taiwan. The dendrogram of Fig. 11 seems to show that the ancestor of *Rana narina* populations was divided into two branches, one of which became the dwarf-type populations in Iriomote and Ishigaki Islands and the other were differentiated into giant-type and middle-type populations which evolved into a subspecies level. A part of the giant-type population entered the two islands as newcomers and became sympatric with the two dwarf-type populations, while the other part of the giant-type population and

the middle-type population occupied Amami Isl. and Okinawa Isl., respectively. The larger value in genetic distance between the dwarf-type populations of the Iriomote and Ishigaki Islands than that between the giant-type populations of these islands seems to show that the dwarf-type populations were the former inhabitants in these islands.

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