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Geographical Differentiation in a Japanese Stream-Breeding Frog, *Buergeria buergeri*, Elucidated by Morphometric Analyses and Crossing Experiments

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ABSTRACT—Hiroshima and Aomori populations of *Buergeria buergeri* (hereinafter abbreviated as HIROSHIMA and AOMORI, respectively) were morphologically differentiated in both sexually matured frogs and tadpoles. The mean snout-vent lengths of females were 67.4 mm in HIROSHIMA and 50.4 mm in AOMORI, and those of males were 42.9 mm in HIROSHIMA and 37.2 mm in AOMORI. The mean body weights of females of HIROSHIMA and AOMORI just after spawning were 18.0 g and 7.2 g, and those of males of HIROSHIMA and AOMORI were 4.9 g and 3.8 g, respectively. AOMORI tadpoles were rather stocky and their appearance seemed to be more adaptive to lentic water than HIROSHIMA tadpoles. The lower lip of the tadpoles at stage XIII consisted of 2 continuous and 1 broken teeth rows in HIROSHIMA, whereas 3 continuous and 1 broken ones in AOMORI. The growth rate of the embryos was higher in AOMORI than in HIROSHIMA. The embryos of AOMORI were more tolerant to high temperature and less tolerant to low temperature than those of HIROSHIMA. Hybrids between these two populations showed considerably reduced viability in either combination of reciprocal crosses.

INTRODUCTION

There are some definite morphological variations among anuran species widely distributed in the Japanese Archipelago, which stretches from the northeast to southwest along the eastern edge of continental Asia. Such variations are ranked as subspecies and local races in the *Rana porosa* group (Moriya, 1954; Kawamura, 1962) and as subspecies in the *Rana tagoi* group (Okada, 1966; Maeda and Matsui, 1989) and the *Bufo japonicus* group (Okada, 1966; Matsui, 1984; Maeda and Matsui, 1989). Moreover, crossing experiments and electrophoretic analyses revealed substantial differences between *Hyla japonica* populations (Kawamura *et al*., 1990; Nishioka *et al*., 1990) and between *Rana japonica* populations (Sumida, 1981, 1996; Sumida and Nishioka, 1994) despite the absence of great differences in the morphological characters among these populations.

Buergeria buergeri is native to Japan and widely distributed in Honshu, Shikoku and Kyushu (Okada, 1931; Nakamura and Ueno, 1963; Maeda and Matsui, 1989). Although its geographical variation has not yet been investigated, we have observed that *B. buergeri* in Aomori in northern Honshu is considerably smaller than that in Hiroshima in southwestern Honshu. We therefore compared the morphological and physiological characters and conducted crossing experiments between Hiroshima and Aomori populations of *B. buergeri* in order to elucidate the degree of difference.

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MATERIAL AND METHODS

Buergeria buergeri was collected during the breeding seasons of 1995 and 1996. Sexually matured frogs were collected from two branches of Ohta River in Hiroshima Prefecture, that is, Takayama River (34°32´N, 132°23´E, 120 m alt.) and Minochi River (34°28´N, 132°14´E, 290 m alt.). These frogs were treated as HIROSHIMA. Frogs from Aomori (AOMORI) were collected from a branch of Iwaki River in Aomori Prefecture, that is, Aseishi River (40°30´N, 140°45´E, 350 m alt.).

Morphometry

Thirty eight females and 68 males of HIROSHIMA and 27 females and 28 males of AOMORI were examined. The following measurements of matured frogs were undertaken: (1) snout-vent length (SVL), (2) head length (HL), (3) head width (HW), (4) snout length (SL), (5) eye diameter (ED), (6) tympanum-eye distance (T-ED), (7) tympanum diameter (TD), (8) internarial distance (IND), (9) interorbital distance (IOD), (10) forelimb length (FLIMB), (11) hindlimb length (HLIMB), (12) thigh length (THIGH), (13) tibia length (TIBIA), (14) foot length (FOOT), and (15) inner metatarsal tubercle length (IMTL) (Fig. 1). Measurements were taken to 0.1 mm using slide calipers under ether anesthesia.

Of the frogs collected in 1996, 11 gravid females and 22 males of HIROSHIMA and 31 gravid females and 12 males of AOMORI were weighed. Further, of these females, 8 of HIROSHIMA and 17 of AOMORI were weighed again immediately after all their eggs were pressed out.

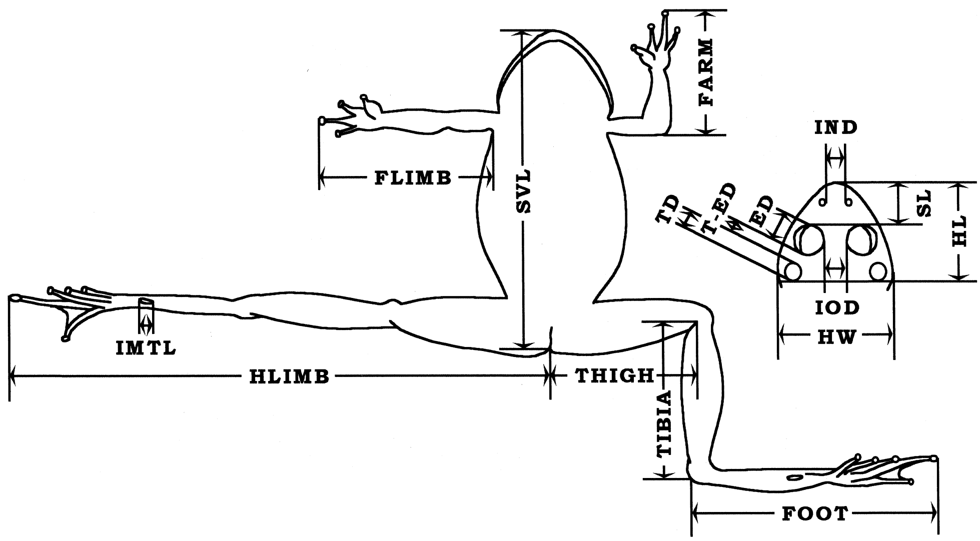

Fig. 1. Frog measurements. See text for abbreviations.

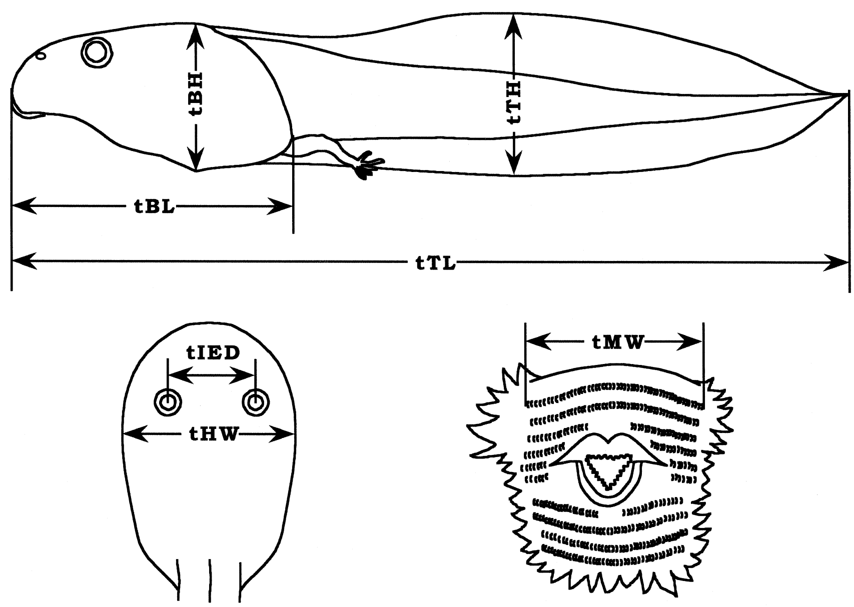

Fig. 2. Tadpole measurements. See text for abbreviations.

Eggs were fixed in 10% formalin solution immediately after they were pressed out and the diameter was measured on 50 eggs per female with an ocular micrometer.

Tadpoles of two populations (n = 30 from HIROSHIMA♀ No. I ~ III; n = 30 from AOMORI♀ No. I ~ III) and their reciprocal interpopulational hybrids (n = 20 from HIROSHIMA♀ No. I, III × AOMORI♂ No. II; n = 20 from AOMORI♀ No. I, III × HIROSHIMA♂ No. I) produced by an artificial fertilization method were measured when they reached stage XIII (Taylor and Kollros, 1946). The following measurements of each tadpole were made under 0.1% MS222 anesthesia: (1) total length (tTL), (2) body length (tBL), (3) body height (tBH), (4) head width (tHW), (5) intereye distance (tIED), (6) mouth width (tMW), and (7) tail height (tTH) (Fig. 2).

Crossing experiments

In 1995, interpopulational crossing experiments were conducted using 5 females and 3 males of HIROSHIMA and 3 females and 2 males of AOMORI by an artificial fertilization method. Ovulation of these females spontaneously occurred or was induced by injection of *Rana catesbeiana* pituitaries. Both the hybrid and control embryos from the eggs of AOMORI♀ No. I ~ III and HIROSHIMA♀ No. I ~ III were kept at room temperature of approximately 20°C throughout the embryonic period and at 21 ± 2°C throughout the tadpole period. To elucidate the effect of low temperature on embryonic development, both the hybrid and control embryos from HIROSHIMA♀ No. IV and V were divided into two groups. One group was reared at the same temperature as the embryos from HIROSHIMA♀ No. I ~ III, and the other group was kept at 15°C in a thermostatic chamber throughout the embryonic period.

In 1996, to ascertain the effects of temperature on viability and growth rate of each population and each type of hybrids, hybrid and control embryos were obtained by using 3 females (No. VI ~ VIII) and 2 males (No. IV and V) of HIROSHIMA and 3 females (No. IV ~ VI) and 2 males (No. III and IV) of AOMORI. These hybrid and control eggs from each female were divided into 5 groups. Each group was reared at a temperature of 12°C, 16°C, 20°C, 23°C and 26°C in thermostatic chambers, respectively, from 20 min after fertilization until stage 25, when tadpoles began to feed.

Statistics

Mann-Whitney U test was used to make interpopulational and intersexual comparisons in each morphometric character. The significant level was set at 0.05. In order to determine the overall morphological variation using all characters, discriminant analysis (DISCRIM) was performed using SAS Version 6 (SAS, 1988) at Information Processing Center, Hiroshima University.

RESULTS

Morphometric characters of sexually matured frogs and eggs

Table 1 shows morphometric data of mature frogs. The ranges of SVL in the females of the two populations did not overlap, whereas those in the males partly overlapped. The mean SVL of females of HIROSHIMA and AOMORI were 67.4 mm and 50.4 mm, and those of males of HIROSHIMA and AOMORI were 42.9 mm and 37.2 mm, respectively. All 15 characters showed significant differences between sexes and between populations (HIROSHIMA♀ vs AOMORI♀, HIROSHIMA♂ vs AOMORI♂, HIROSHIMA♀ vs HIROSHIMA ♂, AOMORI♀ vs AOMORI♂).

DISCRIM for raw values of all 15 characters erred to classify only one (1.5%) HIROSHIMA male. All other individuals were correctly classified into respective home groups. When ratios to SVL were used instead of raw values for DISCRIM, 92.6% of HIROSHIMA males, 100% of HIROSHIMA females, 100% of AOMORI males and 96.3% of AOMORI females were correctly classified (Table 2).

HIROSHIMA females were 2.36 times heavier than AOMORI females. The rate slightly increased to 2.49 just after their eggs were completely pressed out (Table 3). On the other hand, the mean weight of HIROSHIMA males was only 1.31 times heavier than that of AOMORI males.

There were significant differences in clutch sizes and egg diameters between the two populations (Table 3). HIROSHIMA females laid about 1.6 times more eggs than AOMORI fe-

Table 1. Measurements for the Hiroshima and Aomori populations of *Buergeria buergeri* (in mm; mean ± SD followed by range)

Character	HIROSHIMA♂ n = 68	AOMORI♂ n = 28	HIROSHIMA♀ n = 38	AOMORI♀ n = 27
SVL	42.9 ± 1.6 39.0 ~ 46.4	37.2 ± 1.5 33.8 ~ 39.5	67.4 ± 4.3 59.0 ~ 75.9	50.4 ± 2.8 45.1 ~ 57.0
HL	12.5 ± 0.6 11.0 ~ 13.6	10.7 ± 0.6 9.5 ~ 11.9	18.4 ± 0.9 16.3 ~ 20.2	13.6 ± 0.9 11.9 ~ 15.9
HW	14.6 ± 0.5 13.6 ~ 16.0	12.3 ± 0.6 11.0 ~ 13.8	23.2 ± 1.3 21.0 ~ 25.3	16.7 ± 1.1 14.6 ~ 19.0
SL	5.4 ± 0.4 4.7 ~ 6.2	4.9 ± 0.4 3.9 ~ 5.8	8.0 ± 0.8 6.7 ~ 10.2	6.3 ± 0.5 5.5 ~ 7.8
ED	4.5 ± 0.5 3.2 ~ 5.8	3.5 ± 0.3 3.1 ~ 4.2	5.7 ± 0.4 4.8 ~ 6.8	4.3 ± 0.4 3.8 ~ 5.2
T-ED	1.2 ± 0.2 0.8 ~ 1.8	1.0 ± 0.2 0.7 ~ 1.4	2.0 ± 0.4 1.2 ~ 3.0	1.5 ± 0.4 0.7 ~ 2.5
TD	1.7 ± 0.3 1.1 ~ 2.6	1.3 ± 0.2 0.9 ~ 1.7	2.7 ± 0.4 2.0 ~ 3.5	2.3 ± 0.6 1.8 ~ 4.7
IND	3.4 ± 0.3 2.6 ~ 4.2	3.1 ± 0.3 2.3 ~ 3.6	5.1 ± 0.4 4.3 ~ 5.8	4.0 ± 0.6 2.5 ~ 4.9
IOD	5.2 ± 0.5 3.7 ~ 6.2	4.6 ± 0.2 4.2 ~ 5.0	7.8 ± 0.8 6.5 ~ 9.6	5.8 ± 0.7 4.8 ~ 8.0
FLIMB	27.9 ± 1.9 23.7 ~ 32.8	24.0 ± 1.7 18.8 ~ 26.4	44.0 ± 2.4 40.3 ~ 49.7	33.6 ± 2.8 27.8 ~ 39.3
HLIMB	71.5 ± 3.0 64.8 ~ 77.4	62.4 ± 2.4 58.6 ~ 66.5	106.9 ± 5.0 97.8 ~ 119.5	83.3 ± 4.2 75.0 ~ 89.8
THIGH	19.6 ± 1.0 17.4 ~ 21.9	16.6 ± 0.9 14.8 ~ 18.8	30.2 ± 1.6 27.0 ~ 33.2	22.5 ± 1.6 19.2 ~ 25.8
TIBIA	20.9 ± 1.3 18.0 ~ 23.7	18.2 ± 0.9 16.8 ~ 19.9	31.5 ± 1.9 26.3 ~ 35.0	23.9 ± 1.4 21.2 ~ 26.5
FOOT	31.1 ± 1.6 28.2 ~ 34.5	26.8 ± 1.5 22.0 ~ 29.6	46.7 ± 3.3 40.1 ~ 53.2	36.1 ± 3.9 23.5 ~ 39.5
IMTL	2.0 ± 0.2 1.4 ~ 2.7	1.7 ± 0.2 1.3 ~ 2.2	3.0 ± 0.3 2.1 ~ 3.6	2.3 ± 0.3 1.8 ~ 2.8

males, and the egg diameter of the former was about 1.2 times larger than that of the latter. Eggs laid by AOMORI females were always more blackish in both animal and vegetal hemispheres than those laid by HIROSHIMA females.

Interpopulational hybrids

No difference could be detected with respect to the rate of normally cleaved eggs (97.1 ~ 100%) between interpopulational hybrid eggs and control eggs (Table 4). The development of hybrid embryos, however, was delayed from the tail-bud stage, and a large number of them became abnormal tadpoles with undeveloped gills or lacking gills. Moreover, most of the larvae died of edema and underdevelopment before feeding.

The fate of the controls and hybrids reared at 20 ± 2°C is summarized as follows (Table 4): Of the 429 control eggs from HIROSHIMA♀ No. I ~ V, 99.5% normally cleaved, 78.1% hatched and 65.5% metamorphosed, whereas 99.1% of 587 HIROSHIMA eggs inseminated with the sperms of AOMORI males normally cleaved, 79.9% hatched, only 19.4% began to feed and 13.8% metamorphosed. Of the total 314 control eggs from AOMORI♀ No. I ~ III, 99.4% normally cleaved, 79.9% hatched, 66.9% began to feed, and 57.0% metamorphosed, whereas 99.3% of 446 AOMORI eggs inseminated with the sperms of HIROSHIMA males normally cleaved, 74.0% hatched, only 46.0% began to feed and 32.3% metamorphosed.

On the other hand, in hybrids from HIROSHIMA♀ No. IV and V kept at a temperature of 15°C throughout the embryonic stage, the rate of feeding tadpoles increased by 34.3% in comparison with those (13.1%) reared at 20 ± 2°C. In the controls, however, the rate of feeding tadpoles was almost the same in low (94.9%) and high (91.1%) temperature conditions (Table 4).

Figures 3 and 4 show the effects of temperature on viability and growth rate of the hybrid and control embryos, respectively, plotting the mean values of each hybrid type and the control derived from HIROSHIMA♀ No. VI ~ VIII and AOMORI ♀ No. IV ~ VI. These results showed that the optimum tem-

Table 2. Results of discriminant analyses of the Hiroshima (Hr) and Aomori (Am) populations of *Buergeria buergeri*, using raw dimensions and ratios to SVL

	Actual group	n	Predicted group	
			Hr♂	Am♂
Raw data	Hr♂	68	67 (98.5%)	1 (1.5%)
	Am♂	28	0 (0%)	28 (100%)
Ratio	Hr♂	68	63 (92.6%)	5 (7.4%)
	Am♂	28	0 (0%)	28 (100%)

	Actual group	n	Predicted group	
			Hr♀	Am♀
Raw data	Hr♀	38	38 (100%)	0 (0%)
	Am♀	27	0 (0%)	27 (100%)
Ratio	Hr♀	38	38 (100%)	0 (0%)
	Am♀	27	1 (3.7%)	26 (96.3%)

Table 3. Comparisons of 9 characters between the Hiroshima and Aomori populations of *Buergeria buergeri* (mean followed by range in parentheses)

Character	HIROSHIMA	AOMORI
Mature frog		
Male body weight (g)	4.9 (3.7 ~ 5.8) n = 22	3.8 (3.2 ~ 5.0) n = 12
Female body weight before spawning (g)	25.5 (19.8 ~ 30.2) n = 11	10.8 (7.2 ~ 18.1) n = 31
Female body weight after spawning (g)	18.0 (16.4 ~ 19.8) n = 8	7.2 (5.8 ~ 9.3) n = 17
Clutch size	475 (213 ~ 659) n = 10	298 (177 ~ 490) n = 15
Egg diameter (mm)	2.64 (2.30 ~ 2.83) 400 from 8 clutches	2.24 (2.03 ~ 2.43) 400 from 8 clutches
Length and width of testis (mm)	6.1 × 2.4 n = 10	5.0 × 2.0 n = 10
Embryo and tadpole		
Age at the day when feeding began	10.0 3 clutches	9.0 3 clutches
Age at the day of landing (22 ± 2°C)	32.1 (29 ~ 38) 195 tadpoles from 3 clutches	31.4 (28 ~ 54) 179 tadpoles from 3 clutches
Dental formula	2:4 + 4 / 2:1 + 1 50 tadpoles from 5 clutches	2:4 + 4 / 3:1 + 1 50 tadpoles from 5 clutches

Table 4. Results of crossing experiments between the Hiroshima (Hr) and Aomori (Am) populations of *Buergeria buergeri* reared at 20 ± 2°C and 15°C throughout the embryonic period

	Parents		No. of eggs	Cleaved eggs	Tail-bud embryos	Hatched larvae	Feeding tadpoles	Froglets
	Female	Male		%	%	%	%	%
20 ± 2°C	Hr I	Hr II	111	100	81.1	78.4	77.5	52.3
		Am II	137	100	97.1	83.9	49.6	36.5
	Hr II	Hr II	121	100	77.7	65.3	65.3	62.8
		Am II	137	97.1	62.0	20.4	0	0
	Hr III	Hr II	98	98.0	85.7	73.5	69.4	62.2
		Am II	176	99.4	81.2	59.7	15.9	9.1
	Hr IV	Hr III	51	100	100	98.0	98.0	84.3
		Am III	67	100	95.5	49.3	23.9	20.9
	Hr V	Hr III	48	100	97.9	97.9	91.7	89.6
		Am III	70	100	97.1	72.9	2.9	1.4
	Am I	Am I	101	100	86.1	86.1	83.2	74.3
		Hr I	110	99.1	88.2	86.4	70.0	55.5
	Am II	Am I	96	100	82.3	72.9	35.4	29.2
		Hr I	72	100	66.7	50.0	2.8	0
	Am III	Am I	117	98.3	81.2	80.3	78.6	65.0
		Hr I	264	99.2	89.8	75.4	47.7	31.4
15°C	Hr IV	Hr III	183	97.3	96.2	90.7	90.7	61.2
		Am III	150	100	97.3	74.0	63.3	52.7
	Hr V	Hr III	167	99.4	98.2	92.2	91.6	67.7
		Am III	175	100	97.7	76.6	33.7	5.1

I ~ V, individual number of the parents.

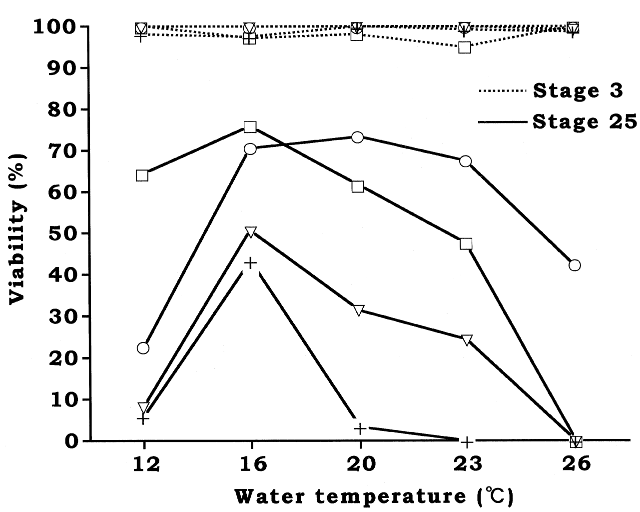

Fig. 3. Effects of water temperature on the viability. Viability at stage 3 (first cleavage; broken lines) and stage 25 (beginning to feed; solid lines) in the Hiroshima (□) and Aomori (○) populations of *Buergeria buergeri* and the reciprocal hybrids (HIROSHIMA♀ × AOMORI♂, +; AOMORI♀ × HIROSHIMA♂, ▽).

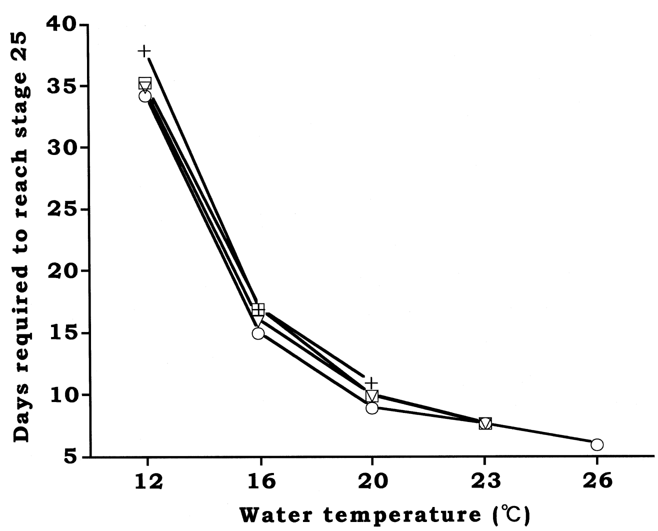

Fig. 4. Effects of water temperature on growth rate. Days for developing to stage 25 (beginning to feed) in the Hiroshima (□) and Aomori (○) populations of *Buergeria buergeri* and the reciprocal hybrids (HIROSHIMA♀ × AOMORI♂, +; AOMORI♀ × HIROSHIMA♂, ▽).

perature for embryonic growth for AOMORI was higher than that for HIROSHIMA and that the embryos of AOMORI could tolerate higher temperature than those of HIROSHIMA. The control embryos of HIROSHIMA showed the best viability at 16°C (Fig. 3), and their viability decreased under higher temperature with all of them dying by stage 17 at 26°C. On the other hand, 42.6% of the control embryos of AOMORI developed to normal feeding tadpoles at the same temperature of 26°C. The controls of AOMORI showed the best viability at temperature of 20°C. However, only 22.7% could become normal feeding tadpoles by rearing at temperature of 12°C, although 64.1% of the controls of HIROSHIMA did. Like HIROSHIMA, the interpopulational hybrids from each reciprocal cross had the best viability at temperature of 16°C, but the viability was considerably lower than the controls.

The control embryos of AOMORI tended to show a higher growth rate than those of HIROSHIMA and the hybrid embryos were always slower in growth than the maternal controls when reared at 12 ~ 20°C (Fig. 4).

Morphology of tadpoles

Table 5 shows morphometric data for tadpoles at stage XIII. Total length of the tadpoles did not significantly differ between HIROSHIMA and AOMORI controls, although the length significantly differed between hybrids and the two controls and between the hybrids from two reciprocal crosses. Moreover, differences in all relative lengths (tBL/tTL, tHW/tBL, tIED/tBL, tMW/tBL, tBH/tBL, tTH/tTL) between the hybrids and the maternal controls were significant. In short, AOMORI tadpoles had rather stocky trunk with small mouth and short tail with wide fin in comparison with HIROSHIMA tadpoles, and most characters of both hybrid tadpoles were between parental tadpoles except tTL and tBL (Table 5).

DISCRIM using relative lengths as input variables correctly classified 100% of HIROSHIMA, 80% of AOMORI, 80% of HIROSHIMA♀ × AOMORI♂ and 95% of AOMORI♀ × HIROSHIMA♂ into respective home groups (Table 6).

Another difference between the tadpoles of the two populations was seen in the arrangement of teeth rows on the lower lip of their mouth. All the tadpoles from three females of HIROSHIMA had 2 continuous and 1 broken teeth rows on the lower lip (Hosoi *et al.*, 1996), whereas AOMORI tadpoles from three females had 3 continuous and 1 broken teeth rows. The upper lip, however, had the same arrangement of teeth rows, 2 continuous and 4 broken ones in both populations.

In the interpopulational hybrids, HIROSHIMA♀ ×

Table 5. Measurements of the hybrid tadpoles at stage XIII in reciprocal crosses between the Hiroshima (Hr) and Aomori (Am) populations of *Buergeria buergeri* and the controls (upper 2 rows, mean ± SD followed by range; lower 6 rows, median followed by range)

Kind	Hr♀ I~III × Hr♂ II	Am♀ I~III × Am♂ I	Hr♀ I, III × Am♂ II	Am♀ I, III × Hr♂ I
	n = 30	n = 30	n = 20	n = 20
tTL	40.5 ± 1.9	40.1 ± 2.3	37.4 ± 4.6	33.1 ± 5.3
(mm)	37.0 ~ 43.3	36.0 ~ 45.5	25.6 ~ 44.1	25.6 ~ 43.4
tBL	12.3 ± 0.8	13.6 ± 0.9	12.1 ± 1.1	12.1 ± 1.2
(mm)	10.6 ~ 13.4	12.0 ~ 15.5	9.8 ~ 14.1	9.8 ~ 14.6
tBL/tTL	0.304	0.340	0.325	0.366
	0.270 ~ 0.322	0.311 ~ 0.356	0.286 ~ 0.387	0.314 ~ 0.422
tBH/tBL	0.450	0.504	0.535	0.534
	0.400 ~ 0.529	0.436 ~ 0.583	0.434 ~ 0.602	0.433 ~ 0.611
tHW/tBL	0.607	0.633	0.654	0.692
	0.546 ~ 0.667	0.569 ~ 0.702	0.556 ~ 0.723	0.623 ~ 0.771
tED/tBL	0.342	0.324	0.332	0.364
	0.320 ~ 0.366	0.292 ~ 0.355	0.297 ~ 0.361	0.274 ~ 0.396
tMW/tBL	0.249	0.220	0.236	0.256
	0.223 ~ 0.299	0.179 ~ 0.267	0.208 ~ 0.265	0.192 ~ 0.297
tTH/tTL	0.155	0.172	0.177	0.211
	0.132 ~ 0.172	0.149 ~ 0.214	0.162 ~ 0.219	0.161 ~ 0.269

I ~ III, individual number of the parents.

Table 6. Results of discriminant analyses of tadpoles at stage XIII in the Hiroshima (Hr) and Aomori (Am) populations of *Buergeria buergeri*, using relative dimensions

Actual group	n	Predicted group			
		Hr♀ × Hr♂	Hr♀ × Am♂	Am♀ × Hr♂	Am♀ × Am♂
Hr♀ × Hr♂	30	30 (100%)	0 (0%)	0 (0%)	0 (0%)
Hr♀ × Am♂	20	0 (0%)	16 (80.0%)	1 (5.0%)	3 (15.0%)
Am♀ × Hr♂	20	0 (0%)	0 (0%)	19 (95.0%)	1 (5.0%)
Am♀ × Am♂	30	2 (6.7%)	2 (6.7%)	2 (6.7%)	24 (80.0%)

AOMORI ♂, 1 (2.5%) of 40 tadpoles examined had teeth rows of the AOMORI type on the lower lip, 18 (45.0%) had the HIROSHIMA type and the remaining 21 (52.5%) had the intermediate type, with diversely shortened outer teeth row. On the other hand, all the 40 hybrid tadpoles of the reciprocal combination had teeth rows of the AOMORI type with a formula of 3:1 + 1, although almost all their outer rows were somewhat shortened.

DISCUSSION

Morphometric analyses of mature frogs indicated that Hiroshima and Aomori populations of *B. buergeri* are morphologically differentiated. HIROSHIMA females are 1.31 times longer than AOMORI females, and HIROSHIMA males are 1.15 times longer than AOMORI males. There are large differences between the two populations not only in body size but also in proportion (Table 2).

To date, numerous kinds of hybridizing experiments among closely related anuran species, subspecies, and/or local populations distributed in Japan and its neighboring countries have been carried out mainly in our laboratory. These results showed that there are interspecific hybrids together with intraspecific ones that are completely viable; that is the *Rana nigromaculata* groups, (Moriya, 1960; Kawamura and Nishioka, 1979; Kuramoto, 1983), the brown frog groups (Kawamura *et al*., 1981; Sumida, 1981, 1996), the *Bufo* groups (Kawamura *et al*., 1980), and the Asian and European *Bombina* (Ueda, 1977), although the fertility is not always complete. If the hybrids show reduced viability, they never have complete fertility. Only the exception from this general trend has been known in *Hyla japonica*. Interpopulational hybrids among the Hiroshima, Tsushima and Korean populations of *Hyla japonica* had somewhat reduced viability, but these male and female hybrids showed low fertility of various degree and some hybrids had complete fertility (Kawamura *et al*., 1990). Interpopulational hybrids of *B. buergeri* in this study rather resembled those of *H. japonica*, though fertility of the former has not yet been ascertained.

Embryos of AOMORI showed a higher growth rate than HIROSHIMA at various temperatures (Fig. 4). Although the mortality rate of interpopulational hybrids of either combination in *B. buergeri* was improved by rearing at the optimum temperature, the improved rates were not high. The mortality rate of hybrids was always considerably higher than that of the respective controls. These results suggest that the reduction of viability observed in these interpopulational hybrids is not only due to the discordance of the growth rate of parental populations but also to other genetic incompatibilities by many other genes controlling development.

Because the estimated mean annual air temperature (Japan Meteorological Agency, 1996) of AOMORI (7.6°C) is lower than that of HIROSHIMA (13.6°C), the activity period of froglets of AOMORI should be much shorter than that of HIROSHIMA. The embryos of AOMORI were more tolerant to high temperature and higher in growth rate than those of HIROSHIMA. Moreover, the shape of AOMORI tadpoles was more adapted to lentic habitat than that of HIROSHIMA tadpoles. These results suggest that AOMORI tadpoles may shorten the tadpole period by spending more time on shoal and in small streams where water temperature readily rises.

All the data from morphometric analyses and physiological comparisons together with crossing experiments mentioned above suggest that the difference between Hiroshima and Aomori populations of *B. buergeri* may have reached a distinct species level. However, it should be pointed out that the present study compared only two populations distributed at both ends of Honshu in Japan. The mean SVL of a population from Boso in middle Honshu is between that of AOMORI and HIROSHIMA (Fukuyama and Kusano, 1989). In order to substantiate this assumption, it is necessary not only to extend such comparisons to as many populations as possible in the country but also to ascertain the fertility of the hybrids between HIROSHIMA and AOMORI which are maintained in our laboratory.

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