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Reproductive Patterns of Two Sympatric Rhacophorid Frogs, *Buergeria japonica* and *B. robusta*, with Comments on Anuran Breeding Seasons in Taiwan

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ABSTRACT—Present study tested a hypothesis that the seasonal reproductive pattern would be similar between two sympatric congeneric species of frogs, *Buergeria japonica* and *B. robusta*, by examining, fat body, liver and gonadal cycle. In total, 132 and 148 adult frogs, respectively, were collected from Taichung, Central Taiwan during July 1996 and July 1997. Both species were classified as prolonged breeders, breeding from March to August. This breeding season differs from those in conspecific populations from other localities. In both species, adult females attained larger snout-vent length (SVL), body mass (BM), head length (HL), and head width (HW) than males. The smaller size of male in these species is inconsistent with a previous energetic constraint hypothesis that smaller males are caused by the costs of advertising, maintaining territories, and lower food intake in prolonged breeding species. Neither *B. japonica* nor *B. robusta* showed a significant positive correlation between female ovary mass and female SVL, indicating that fecundity selection does not directly affect female body size. Also, timing of reproduction in three other anurans from the present study site is also discussed.

INTRODUCTION

It is generally assumed that the natural selection acts to maintain reproductive traits that are adaptive in a particular environment. Such local adaptations are supposedly a primary cause of similarities in reproductive pattern among closely related species that share the same environment (Duellman and Trueb, 1986). However, community complexity constrains the comparisons of all species coexist in a given place difficult. Much of our current knowledge of the reproduction within a community has been inferred from studies of autecologies of single species or of small subsets of the whole community in a given place (e.g., Huang *et al.*, 1996, 1997).

Amphibians are good animal model for reproductive studies, because they are poikilothermic and are greatly affected by physiological constraints (proximate factors) such as temperature, rainfall, and day light length or affected by phylogenetic constraints (ultimate factors), or a mixture of these factors (Zug, 1993). In order to test the hypothesis that natural selection alone can produce similar reproductive patterns in anurans irrespective of their phylogenetic history, we first determined the reproductive cycle of the sympatric species, *Buergeria japonica* and *B. robusta* based on the fat body cycle, spermatogenic cycle, ovarian cycle, and male mating calls as well as amplexus behavior in experimental field observations. Second, we compare the results with previously published data for two bufonid species, *Bufo bankorensis* and *B. melanostictus* (Huang *et al.*, 1996, 1997), and an unpublished data for a ranid *Rana sauteri* from the same study site. Lastly, we compare reproductive cycles of these five anurans with those of conspecific populations in different geographic regions to determine if the influence of natural selection in these five species of anurans has resulted in local adaptations to a particular type of environment.

MATERIALS AND METHODS

Study site

The study was conducted along the Honken River in Teken (Latitude 24°10'N, Longitude 120°43'E), Taichung City, central Taiwan, from July 1996 to July 1997. The elevation of this area is approximately 150 m above sea level. The mean air temperature was highest from July to September, ranging from 27.8 to 29.4°C, and was lowest from December to February ranging from 16.5 to18.0°C. Total rainfall in the study area was 2235.2 mm, of which 644.6 mm

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occurred during the rainy season in early summer. The longest daily sunshine duration occurred in July 1996 and the shortest in June 1997 (Fig. 1). Environmental data are from the Shuijatou Weather Station, Central Weather Bureau, ROC.

Animal descriptions and collection of tissues and organs

There are three species of *Buergeria* are distributed in China. Among these, *B. japonica*, and *B. robusta* are widely distributed in the lowland of Taiwan (Zhao and Adler, 1993). They are sympatric distrbution in Teken, Taichung, and thus, provide a good model to study the variations in reproductive patterns in closed relative species.

Frogs were hand collected each month, but in some months during the torpid period, samples were small or lacking due to the difficulty in finding frogs. Each specimen was weighed to the nearest 0.01 g, measured for snout-vent length (SVL), head length (HL) and head width (HW) to the nearest 0.1 mm, and then was dissected to remove the liver, fat body, and gonads and associated organs. All organs were weighed wet to the nearest 0.01 g. Stage of maturity for males was assessed by spermatogenic activity. In males appearance of sperm bundles and/or free sperm in the seminiferous tubules in a given specimen was interpreted as indicative of maturity. Females were classified as mature if their oviducts were convoluted.

Saidapur's (1983) system as follows: (1) seminiferous tubules involuted but filled with spermatogonia only; (2) primary spermatocytes appearing; (3) secondary spermatocytes and early spermatids abundant; (4) spermatids transforming into a few spermatozoa; (5) spermatids and spermatozoa abundant; (6) spermatozoa abundant; and (7) spermatozoa abundant, but spermatids and spermatocytes greatly reduced. Sperm bundles were counted from 20 random sections of each testis. The abundance of sperm in the seminiferous tubules was quantified by dividing the area of free sperm in the seminiferous tubule lumen by the total area of the seminiferous lumen. Voucher specimens were deposited in the National Museum of Natural Science, Taichung, Taiwan.

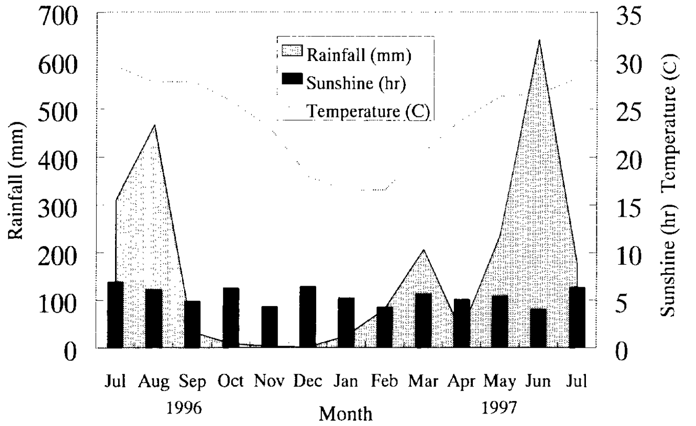

Fig. 1. Annual variations of sunshine duration, temperature, and rainfall during 1996–97 (monthly average) at the Teken area, Taichung, Taiwan (latitude 24°10'N, longitude 120°43'E). [Data source: Shuijatou Weather Station, Central Weather Bureau, Taiwan, ROC]

Statistical analysis

Analysis of variance (ANOVA) was used to examine sexual differences of SVL and seminiferous tubule diameter. The body mass (BM), head length (HL), head width (HW), and monthly variation of each sex were assessed by ANCOVA using log SVL as the covariate (SAS, 1994). Regression analysis was performed to evaluate the correlation of environmental variables with masses of liver (LM), fat body (FM), left testis (TM), and ovary (OM) mass, as well as with seminiferous tubule diameter (STD). In the analyses, LM, FM, TM, OM, and STD for each monthly sample were treated as dependent variables, whereas the climatic factors (i.e., monthly air temperature, photoperiod, and rainfall) were treated as independent variables. A probability of 0.05 or less was regarded as indicative of statistical significance.

RESULTS

Reproductive behaviors

In this study, both *Buergeria japonica* and *B. robusta* were active from March to August. In total, 132 specimens (26 females, and 106 males) of *B. japonica* and 148 specimens (12 females and 136 males) of *B. robusta* were collected. Field observations in the present study showed that amplexus behaviors and mating vocalization occurred between March and August in both frogs (Table 1), indicating that the breeding periods of these two closely related frogs are similar.

Histological observations of testis

Histological examinations of *B. japonica* and *B. robusta* testes revealed that the spermatogenic activities were invariably at stage six throughout all monthly samples (Fig. 2). The numbers of sperm bundles were greatest in February and smallest in April in *B. japonica* (Fig. 3A) whereas sperm bundles were greatest in August and smallest in November in *B. robusta* (Fig. 4A). Seminiferous tubules were full of free sperm in February, and half full in June 1997 in *B. japonica* (Fig. 3B), and from 70% full in October to 95% full in July 1997 in *B. robusta* (Fig. 4B). Seminiferous tubule diameter did not significantly vary among the monthly samples for both

Table 1. Numbers of amplexus (NA) and vocalization (V) behaviors observed during the annual reproductive cycle of *Buergeria japonica* and *B. robusta* at Teken areas; – and + indicate none or the existence of vocalization in field observations. MN: male sample sizes

	1997							1996					
Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Jul	Aug	Sep	Oct	Nov	Dec
B. japonica													
NA	0	0	2	3	4	1	2	2	0	0	0	0	0
V	–	–	+	+	+	+	+	+	–	–	–	–	–
MN	0	4	22	37	14	4	5	10	7	3	0	0	0
B. robusta													
NA	0	0	1	0	1	1	1	0	0	0	0	0	0
V	–	–	+	+	+	+	+	+	–	–	–	–	–
MN	0	0	16	15	13	24	27	7	26	3	1	2	2

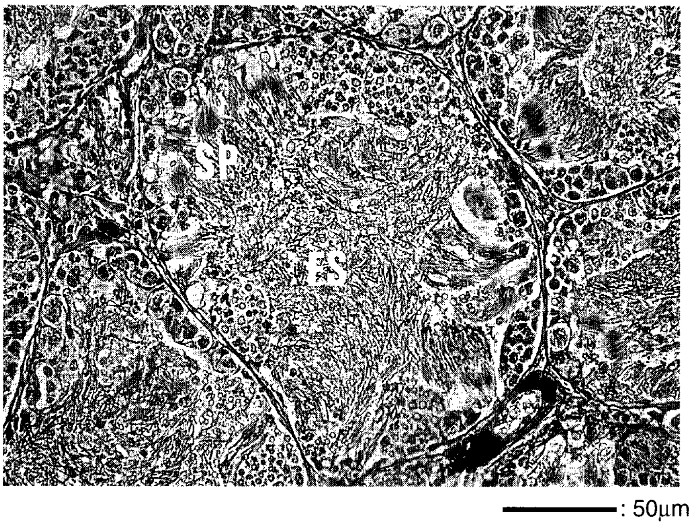

Fig. 2. Numberous sperm bundles and free sperm were present during breeding season of *Buergeria japonica*. FS: free sperm in seminiferous tubule; SP: sperm bundle.

frogs ($p > 0.05$; ANOVA; Figs. 3C, 4C), but showed a significant positive correlation with rainfall in *B. japonica* ($r = 0.79$, $p < 0.05$; Table 2).

Annual changes in LM, FM, TM, and OM

Males In *B. japonica*, male LM showed significant monthly variation ($F_{9,95} = 4.1$, $p = 0.005$; Fig. 5A), with the highest peak in March and the lowest value in August. In contrast, male LM showed no significant variation in *B. robusta* ($F_{11,123} = 0.67$, $p = 0.77$; Fig. 6A). Male LM showed no significant correlation with FM or any climatic variables considered (Table 2), whereas LM of male *B. robusta* showed a positive correlation with fat body mass. In, both frogs, male FM showed significant monthly variations ($F_{9,95} = 5.02$, $p < 0.0001$; Fig. 5B in *B. japonica*; $F_{11,123} = 19.5$, $p < 0.0001$; Fig. 6B in *B. robusta*). There was a significant positive correlation between this parameter and rainfall in *B. japonica* ($r = 0.73$, $p < 0.05$; Table 2), but such a correlation was not recognized in *B. robusta* (Table 2). In *B. japonica* TM showed a significant monthly variation ($F_{9,95} = 3.04$, $p = 0.006$), with the highest peak in August (Fig. 5C), but this was not seen in *B. robusta* (Fig. 6C). TM showed a significant negative correlation with LM in *B. japonica* ($r = -0.81$, $p < 0.05$; Table 2).

Females Female LM in *B. japonica* showed significant monthly variation ($F_{8,17} = 3.2$, $p = 0.02$; Table 3), with the highest peak in June and was significantly positively correlated with ovary mass ($r = 0.77$, $p < 0.05$; Table 2), but these parameters did not show significant correlation in *B. robusta* (Table 2). In both frogs, female FM showed significant monthly variation ($F_{8,16} = 10.24$, $p < 0.0001$ in *B. japonica*; $F_{8,2} = 53.0$, $p = 0.02$ in *B. robusta*; Table 3), with the highest peak in June. There was a positive correlation between this parameter and rainfall in *B. japonica* ($r = 0.70$, $p < 0.05$; Table 2). OM also showed significant monthly variation ($F_{8,17} = 3.69$, $p = 0.01$; Table 3), with a decrease to the lowest value in November and an increase to the highest value in July 1997 in *B. japonica*, but such a pattern of dynamics were not recognized in *B. robusta* (Table 3).

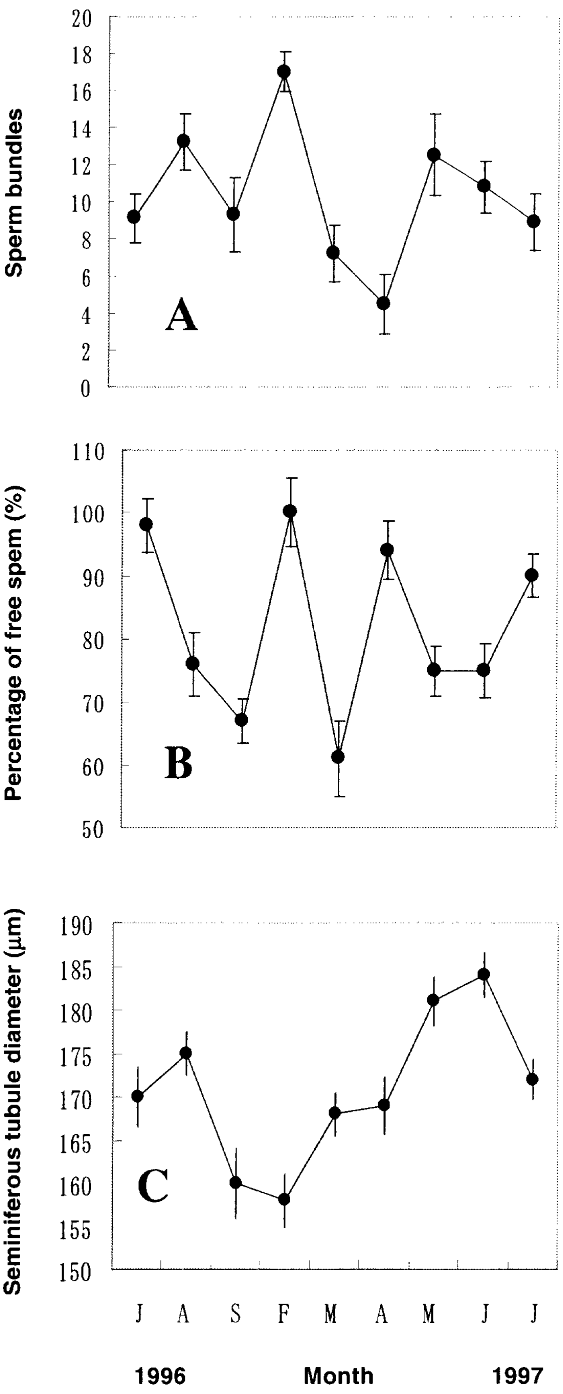

Fig. 3. Monthly changes of the sperm bundles (A), percentage of the free sperm in seminiferous lumen (B), and the seminiferous tubule diameters (C) of *Buergeria japonica*. The data are expressed as means ± SD, and the sample sizes are indicated in Table 1.

Sexual size dimorphism

Mean adult SVLs of females and males were 31.1 and 25.5 mm in *B. japonica*, and were 67.1 and 49.1 mm in *B. robusta*, respectively, and differed significantly ($F_{1,118} = 262.7$; $p < 0.0001$ in *B. japonica*; $F_{1,144} = 539.38$, $p < 0.0001$ in *B. robusta*; Table 4). Likewise, female BM, HL, and HW in both species were significantly greater than those of adult males ($F_{2,116} = 184.2$, $p < 0.0001$ for BM; $F_{2,117} = 37.6$, $p < 0.0001$ for

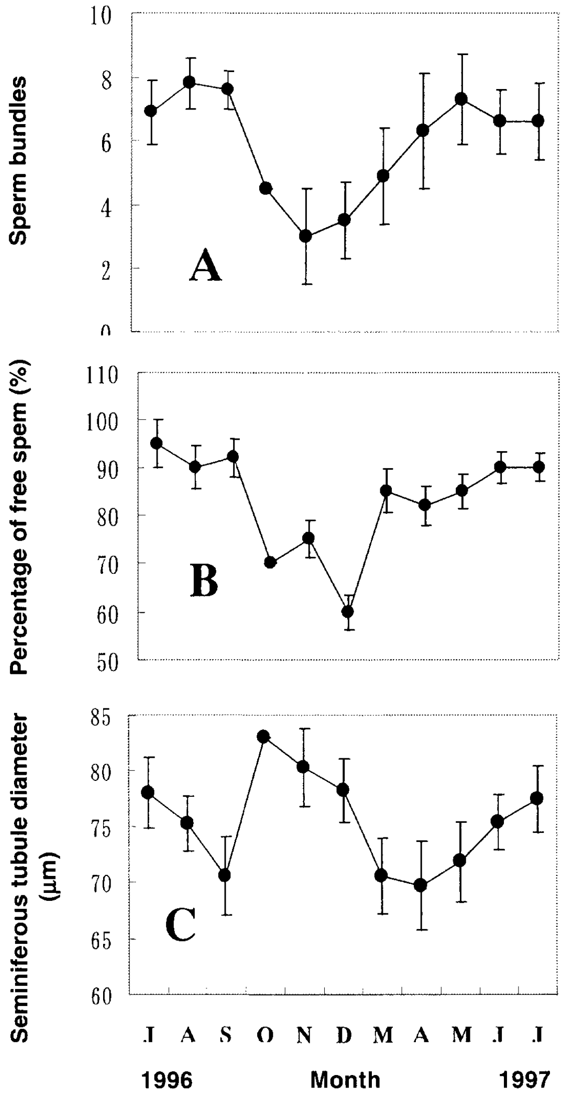

Fig. 4. Monthly changes of the sperm bundles (A), percentage of the free sperm in seminiferous lumen (B), and the seminiferous tubule diameters (C) of *Buergeria robusta.* The data are expressed as means ± SD, and the sample sizes are indicated in Table 1.

HL; $F_{2,117} = 95.5$, $p < 0.0001$ for HW in *B. japonica*; $F_{2,143} = 592.4$, $p < 0.0001$ for BM; $F_{2,143} = 114.9$, $p < 0.0001$ for HL; $F_{2,143} = 380.9$, $p < 0.0001$ for HW in *B. robusta*; ANCOVA; Table 4). Female OM was not significantly correlated with female SVL in *B. japonica* ($r = 0.061$, $p = 0.807$) or *B. robusta* ($r = 0.10$, $p = 0.451$).

DISCUSSION

Reproductive cycle

Field observations in both *Buergeria japonica* and *B. robusta* showed that reproductive behaviors, such as amplexus and mating vocalization, occur only between March and August. During each month of this period, sperm bundles in the seminiferous tubules and free sperm in the lumen were observed. Thus, March to August was considered to be the breeding period of both species.

Sympatric populations of the two rhacophorid exhibited similar reproductive cycles in sympatry which seemly supports the hypothesis that local adaptations are a primary cause of reproduction similarities among individuals within a community that share the same environment, and thus, fits the physiological constraint hypothesis. However, two other closely related toads, *Bufo bankorensis* and *B. melanostictus*, also inhabiting the same environment at the same locality, had definitively different reproductive cycles (Huang *et al.*, 1996, 1997; Table 5). The former species showed breeding activity from August to February of the following year (Huang *et al.*, 1996), and the latter species was active from February to June (Huang *et al.*, 1997). Such results contrast to those of the present study that closely related species develop similar reproductive patterns under the same environments. If reproductive cycles in amphibians are indeed influenced by the local environment independently, then one may expect that closely related species or whole anurans taxa should exhibit

Table 2. Correlation coefficients (r) of male fat body mass (MFM), liver mass (MLM), male left testis mass (MTM), seminiferous tubule diameter (STD), numbers of spermatid cell cysts (SPE), female fat body mass (FFM), liver mass (FLM), and left ovary mass (FOM) as well as rainfall (RF), sunshine duration (SSD), and temperature (TEM), during the annual reproductive cycle of *Buergeria japonica* and *B. robusta*; # represent as *B. japonica* and ## represent as *B. robusta*.

	MFM	MLM	MTM	STD	SPE	FFM	FLM	FOM
MFM#	–	–0.13	0.38	0.69	0.38	–	–	–
MFM##	–	0.95***	0.09	0.59	–	–	–	–
MLM#	–	–	–0.81*	–0.07	–0.26	–	–	–
MLM##	–	–	0.37	0.49	–	–	–	–
MTM#	–	–	–	0.04	0.01	–	–	–
MTM##	–	–	–	0.18	–	–	–	–
DIA#	–	–	–	–	–0.41	–	–	–
FFM#	–	–	–	–	–	–	–0.21	–0.01
FFM##	–	–	–	–	–	–	0.71	0.05
FLM#	–	–	–	–	–	–	–	0.77*
FLM##	–	–	–	–	–	–	–	0.43
RF#	0.73*	–0.20	0.28	0.79*	0.49	0.70*	0.01	0.01
RF##	0.09	0.19	0.13	0.19	0.33	0.52	0.25	0.06
SSD#	–0.17	–0.05	0.23	0.15	0.19	–0.29	0.00	0.43
SSD##	0.17	0.29	0.08	0.00	0.09	0.03	0.14	0.17
TEM#	0.29	–0.27	0.16	0.48	0.87**	0.32	0.03	0.46
TEM##	0. 10	0.01	0.20	0.06	0.77*	0.16	0.02	0.06

Correlation coefficients (r) were analyzed from the monthly means of the parameters.
* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

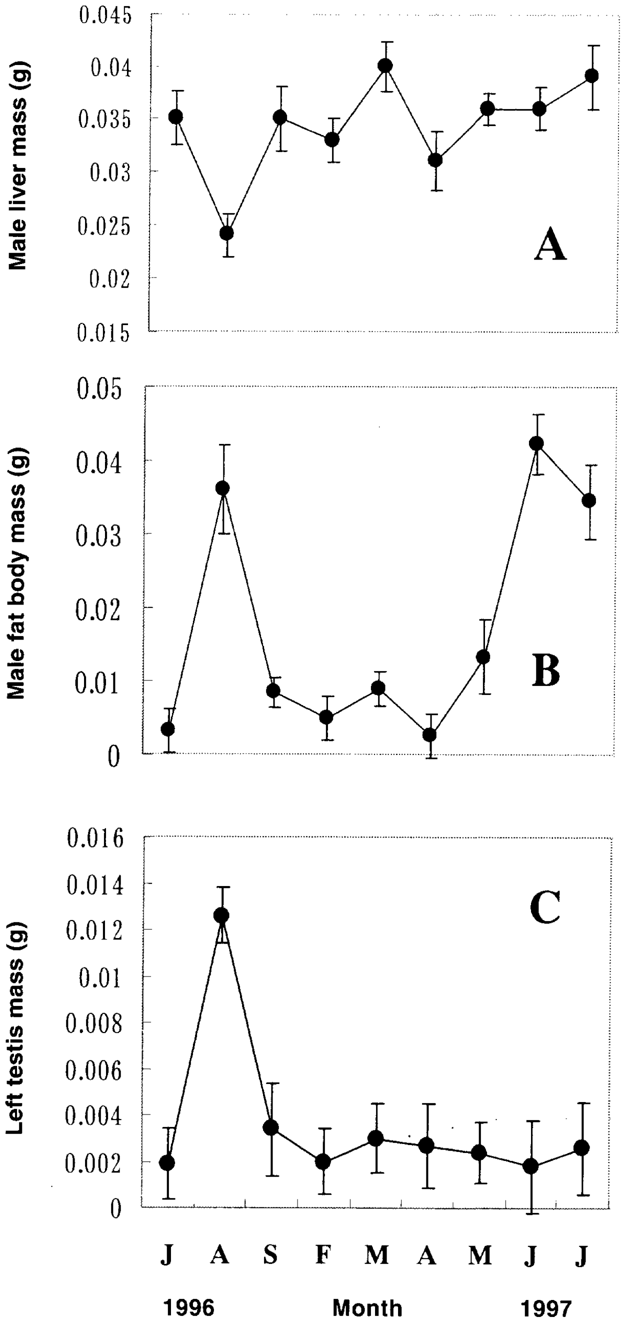

Fig. 5. Monthly changes of the liver mass (A), fat body mass (B), and the left testis mass (C) of *Buergeria japonica*. The data are expressed as means ± SD, and the sample sizes are indicated in Table 1.

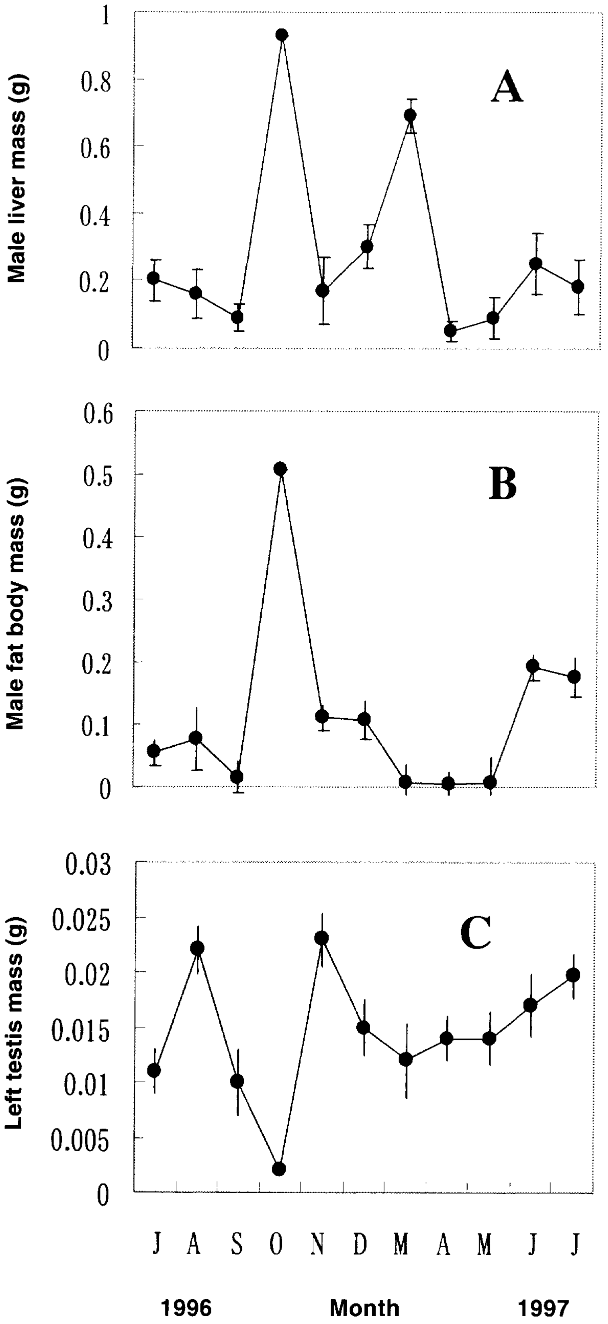

Fig. 6. Monthly changes of the liver mass (A), fat body mass (B), and the left testis mass (C) of *Buergeria robusta*. The data are expressed as means ± SD, and the sample sizes are indicated in Table 1.

the same reproductive patterns in the same habitats, and at least, in the closely related species.

However, such difference may be caused by the variation in evolutionary history of the two toads. For example, *B. bankorensis* previously recognized as *B. gargarizans*, a species widely distributed from Russia, China, Korea, and Japan, is a temperate species. Consequently, its reproduction is adapted to the temperate climate pattern (Zhao and Adler, 1993), whereas *B. melanostictus*, is a tropical species that is distributed in Southern Asia, Sri Lanka, Indonesia, and Borneo (Zhao and Adler, 1993). Likewise, its reproductive cycle is adapted to the tropical climate pattern. Reproductive patterns of present two sympatric toads located in the subtropical climate area in Teken, Central Taiwan are mainly adapted to each own evolutionary history, and thus, exhibit a spring breeder in tropical species, and a winter breeder in temperate species. Therefore, reproductive patterns in sympatric closely related toads are not necessary to the same, but entrained mainly by their local environments.

Reproductive cycles of allopatric conspecific populations are compared in Table 5, providing evidence that the reproductive cycles are susceptible to influence by local environment of the five sympatric anurans. For example, the reproductive cycle of *B. bankorensis*, distributed in the Teken and Wushe areas, exhibit a similar breeding period from August to February, and from September to March, respectively, whereas a conspecific population inhabiting a high elevation 2000 m above from sea level has active breeding throughout the year

(Table 5). Likewise, a population of *B. japonica* living near the hot spring in Gukam central Taiwan exhibits reproductive activity throughout the year (Kam, unpublished data) which differs from other localities of present study site (Table 5).

Table 3. Liver mass (LM±SE), fat body mass (FM±SE), and left ovary mass (OM±SE) of adult females of *Buergeria japonica* and *B. robusta* from the Teken area. N = monthly sample sizes; # represent as *B. japonica* and ## represent as *B. robusta*.

		Female		
Months	N	LM (mg)	FM (mg)	OM (mg)
1996				
July#	4	4.4 ± 1.0	2.9 ± 0.5	8.9 ± 1.2
July##	1	0.3	0.0	0.1
Aug#	4	6.9 ± 0.2	0.7 ± 0.2	24 ± 3.1
Sept##	1	36.4	0	22
Oct##	2	52 ± 8	46 ± 4	111 ± 2
Nov#	1	5	0.7	0
Nov##	1	60.3	15.7	5
1997				
Mar#	4	7.5 ± 2.1	1.3 ± 0.4	10.3 ± 2.5
Mar##	1	50.1	0	75.8
May#	9	6.5 ± 1.5	0.3 ± 0.1	27.1 ± 5.5
May##	4	40.8 ± 5	0.4 ± 0.1	296 ± 33
Jun#	1	17.3	6.5	10.9
Jun##	1	80.4	85.3	16.9
Jul#	3	6.8 ± 1.1	4.3 ± 0.5	43.8 ± 3.5
Jul##	1	107	45	198

Although the reproductive cycle is strongly affected by local environments, *Rana sauteri*, a widely distributed species in Taiwan (Yang, 1998), exhibits various breeding periods in different localities (Table 5). However, the breeding period in this species is always limited to two months, and this seems to reflect a phylogenetic constraint that the breeding season is confined to a definite period. Thus, the different patterns of reproductive cycle expression observed in this study from allopatric and sympatric populations are not only affected by local adaptations to environment factors but are also influenced by phylogenetic constraints. Consequently, only by investigating patterns of evolution of reproduction in total sympatry and allopatry can one determine whether or not these species will always exhibit similar reproductive cycles when they share a common environment. We indicate that, at least in one location, two species exhibit similar reproductive cycles and that this pattern cannot be the result of representing a single species reproduction. This suggests that further study of this kind and other closely related sympatric and allopatric species is warranted.

Histological observations

Lofts (1974) and Saidapur (1983) proposed that the spermatogenic cycle of species which have free sperm in the seminiferous tubules is of the continuous type. However, Huang

Table 4. Adult body sizes of two species, *Buergeria japonica* and *B. robusta*, at Teken. SVL, BM, HL, and HW indicate snout-vent length, body mass, head length, and head width, respectively; N = sample sizes.

Species and sex	N	SVL ± SE	BM ± SE	HL ± SE	HW ± SE
Buergeria japonica					
Males	94	25.5 ± 1.8	1.65 ± 0.31	9.76 ± 0.71	9.36 ± 0.67
Females	26	31.1 ± 1.89	3.03 ± 0.76	10.91 ± 0.90	11.04 ± 0.70
Statistical test		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
B. robusta					
Males	135	49.1 ± 2.9	8.0 ± 1.2	18.22 ± 2.0	16.41 ± 1.5
Females	12	67.1 ± 7.2	23.1 ± 4.0	24.08 ± 1.2	23.44 ± 2.2
Statistical test		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Table 5. Breeding periods of five anurans of Taiwan; C indicate wide and central regions in Taiwan; M1 and 2 indicate geographical distribution above sea level of 1000 and 2000 m, respectively; * indicates unpublished data.

Species	Distribution	Breeding period	Reference
Bufo bankorensis	Teken	Aug-Feb	Huang *et al.*, 1996
	Wushe (M1)	Sep-Mar	Huang and Yu*
	C(M2)	year round	Huang and Yu*
B. melanostictus	Teken	Feb-Jun	Huang *et al.*, 1997
	Tunghai Univ.	Feb-Sep	Hsieh, 1993
Buergeria japonica	Teken	Mar-Aug	Present study
	–	Feb-Oct	Yang,1998
B. robusta	Teken	Mar-Aug	Present study
	–	May-Aug	Yang, 1998
R. sauteri	–	Apr-May	Yang, 1998
	Gukam (M1)	Jun-Jul	Kam*
	Teken	Oct-Nov	Huang*

et al. (1997) examined the fluctuations of several reproductive characters (i.e., sperm bundles, free sperm, fat body, and individual collection numbers) of the toad, *Bufo melanostictus*, and found that the numbers of free sperm and sperm bundles were significantly higher during the breeding period than in any other period. They thus recognized that it was inappropriate to determine the nature of the reproductive cycle of toads based solely on the presence of free sperm in the seminiferous tubules. Accordingly, they subdivided the continuous type into fluctuating and constant continuous types and assigned *B. melanostictus* in central Taiwan to the latter.

The numbers of free sperm and sperm bundles in *B. japonica* and *B. robusta* did not fluctuate as clearly as in *B. melanostictus*, but were similar to those of other amphibians inhabiting more tropical areas. In such areas, climatic conditions do not show appreciable fluctuations, and local amphibians often exhibit a constant continuous type of spermatogenesis (Lofts, 1974), in which spermatozoa are produced throughout the year, and testes always contain spermatic cell nests, as well as a complete spectrum of spermatogenic stages (Kao *et al.*, 1993, Huang *et al.*, 1996). In contrast, the spermatogenic activity of species living in seasonal environments tends to be restricted to late spring and summer. In these species, maturation, shedding of gametes, and the secretion of sex steroid hormones are associated with mating and fertilization. Spermatogenesis and oogenesis are initiated more or less simultaneously (Pough *et al.*, 1998).

Changes in testicular mass have also been used to determine the reproductive cycle of amphibians (Rastogi *et al.*, 1986, Huang *et al.*, 1997). The results of the present study reveal significant variations in testicular mass by month in *B. japonica*, but this did not occur in *B. robusta.* Besides, this parameter was not correlated with other parameters presumably more highly associated with the degree of spermatogenesis, such as seminiferous tubule diameter and numbers of spermatids (Table 2) in *B. japonica.* This contrasts with the general pattern, in which intensity of testicular activity is largely reflected by gonadal mass (Huang *et al.*, 1996). Thus, testis mass seems to be an inappropriate index to assess testicular function in *B. japonica* and *B. robusta.*

Relations of changes in fat body and liver mass to the reproductive cycle

Stored lipids may be used during winter dormancy and/or reproduction in anurans (Long, 1987). Body and liver lipids may be reduced through metabolic use during dormancy, vitellogenesis, spermatogenesis, and possibly steroid production during the reproductive period (Kanamadi and Saidapur, 1988, Huang *et al.*, 1996). Significant negative correlation between liver mass and testis mass observed in *B. japonica* suggests that the energy for testicular activity is provided from the liver in this species. Nevertheless, spermatids and the diameter of seminiferous tubules showed no significant correlation with male liver mass. This suggests that the energy may be used for the production of spermatocytes. In contrast, a positive correlation did exist between female liver mass and ovary mass (Table 2). This strongly suggests that the energy used for vitellogenesis in *B. japonica* is not directly from the liver. However, there are no significant correlations between reproductive organs and lipids in *B. robusta* indicating that the energy requirement to produce sperm or ovaries might not come directly from the liver or fat body in this species.

Association of environmental factors with the reproductive cycle

Three major environmental factors have been implicated in the regulation of the amphibian breeding cycle: rainfall, photoperiod, and temperature (Lofts, 1974). Temperature and photoperiod play important roles in the timing of the reproductive cycle (Zug, 1993). For example, rising temperatures and increasing day length stimulate gonadal activity (Huang *et al.*, 1997). Rainfall acts as the most proximate stimulus for the breeding behavior of amphibians in both temperate and tropical zones (Pough *et al.*, 1998). Our present study of the subtropical anuran species, *B. japonica*, and *B. robusta* reveal that spermatid production is induced by high temperature (Table 2).

Sexual size dimorphism

Female amphibians usually attain larger body size than do conspecific males. Among anurans, the body size of the female is larger than that of the male in 90% of species (Shine, 1979). This pattern has been attributed to female fecundity selection and higher mortality in males than in females (Shine, 1979). However, in some species, males are equal in size or slightly larger than females (Duellman and Trueb, 1986). Shine (1979) analyzed 589 anurans and found that large-sized males are significantly correlated with male-male combat and the presence of tusks or spines. However, our data showed no correlation between female ovary mass and female SVL, indicating that the fecundity selection of *B. japonica* and *B. robusta* does not directly affect female body size.

There are several hypotheses suggesting that energetic constraints on reproductive males, caused by the costs of advertising, maintaining territories, and lower food intake, also affect male body size (Woolbright,1983). Prolonged breeding uses up energy for mating behaviors rather than for body size growth. Thus, body size is considerably smaller in males of species that are prolonged breeders when compared to species that are explosive breeders. The breeding period of male *B. japonica* and *B. robusta* lasts from March to August at this locality, and is thus prolonged. The amount of fat bodies found in males captured at vocalizing sites was large (May-August in *B. japonica*; June-July in *B. robusta*; Figs. 5B, 6B) when compared to that of the non-breeding period which is contrasted with an energetic constraint hypothesis.

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