

Reproductive Patterns of Two Sympatric Ranid Frogs, *Rana latouchii* and *R. sauteri*, with Comments on Anuran Breeding Seasons in Taiwan

Wen-San Huang^{1*}, Yu-Shan Cheng², and Hsin-Yi Tu²

¹ Department of Zoology, National Museum of Natural Science, Taichung, Taiwan 404, R.O.C.

² Department of Biology, Tunghai University, Taichung, Taiwan 407, R.O.C.

(Received August 6, 2002; Accepted November 10, 2004)

Abstract. A physiological constraint hypothesis suggested that anuran breeding season will be similar among closely related species that shares the same environments. We conducted a test of that hypothesis that the seasonal reproductive pattern would be similar between two sympatric congeneric species of ranid frogs, *Rana latouchii* and *R. sauteri*, by examining testicular, fat body, liver and ovary cycles. In total, 196 and 78 of *R. latouchii* and *R. sauteri* adult frogs, respectively, were collected from Central Taiwan during the period from July 1996 to July 1997. Both species were classified as prolonged breeders, from which *R. latouchii* exhibits breeding activity throughout the year, whereas *R. sauteri* is breeding actively from October to November, and thus seemingly are contrasted to the physiological constraint hypothesis. The breeding season is similar among populations of *R. latouchii*, but is different among populations of *R. sauteri* from those conspecific in Taiwan. In both adult females attained larger snout-vent length (SVL), body mass (BM), head length (HL), and head width (HW) than males. Neither *R. latouchii* nor *R. sauteri* showed a significant positive correlation between female ovary mass and female SVL. Also, timing of reproduction in four other anurans from the present study site is also discussed.

Key words: Physiological constraint, Ranidae, reproduction, sympatric, Taiwan.

INTRODUCTION

It is generally considered that 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 sometimes makes comparisons of all species coexisting 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).

Many anuran species in aseasonal tropical environments are able to reproduce throughout the

year (Duellman and Truebs, 1986), whereas in seasonal environments, breeding activity of most anuran is associated with the rainy season both in the temperate zones and the tropics (Huang *et al.*, 1996, 1997). However, long-term studies of the reproductive patterns are still rare in the subtropical regions.

Amphibians are very adequate target animals 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 histories, we first determine the reproductive cycle of the sympatric species, *Rana latouchii* and *R. sauteri*, based on the fat body cycle, spermatogenic cycle, ovarian cycle, and

*Corresponding author. E-mail: wshuang@mail.nmns.edu.tw

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 for two racophorid frogs, *Buergeria japonica* and *B. robusta* (Huang *et al.*, 2001) from the same study site. Third, we compare reproductive cycles of these six anurans with those of conspecific populations in different geographic regions to determine if the influence of natural selection in these six 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 (24° 10'N, 120° 43'E), Taichung City, west-central Taiwan, from July 1996 to July 1997. The elevation of this area is approximately 150 m. 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 from 16.5 to 18.0 °C. Total annual rainfall in the study area was 2235.2 mm, of which 644.6 mm 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).

Collection of tissues and organs. 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,

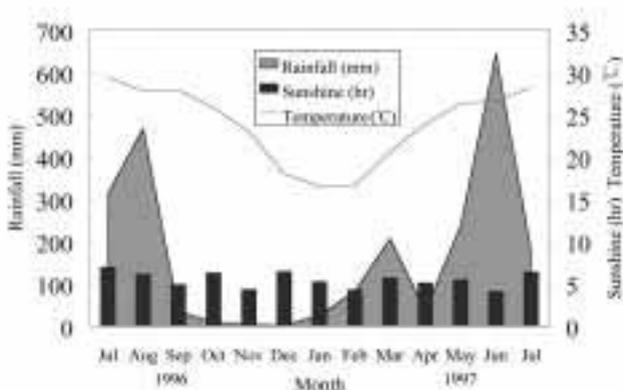


Fig. 1. Annual variations of sunshine duration, temperature, and rainfall during 1996-7 (monthly average) at the Teken area, Taichung, Taiwan. Data are from the Shuijatou Weather Station, Central Weather Bureau, R.O.C.

and then was dissected to remove the liver, fat body, and gonads. All organs were weighed wet to the nearest 0.01 g. Stage of maturity for males was assessed by spermatogenic activity. In males the 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. In some days, we just made observations of behavior without collecting frogs.

Histological examination. The right testis from each adult male was fixed in Bouin's solution, embedded in paraffin, serially sectioned at 7 μ m, and stained with hematoxylin and eosin. Spermatogenic activity was assessed qualitatively by 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 predominant and 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 (CAT# 2909, 2974, 2977, 2979, 2981, 2988, 2991, 2993, 3019, 3022, 3040, 3042, 3049, 3050-1, 3053, 3070, 3073, 3076 of *R. latouchii*; CAT# 2978, 2980, 2982, 3017, 3021, 3041, 3048, 3080 of *R. sauteri*).

Statistical analysis. Analysis of variance (ANOVA) was used to examine sexual differences of SVL and monthly variation in 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, *Rana latouchii* was breeding active throughout the year, whereas *R. sauteri* was breeding active only from October to November. In total, 196 specimens (38 females, and 158 males) of *R. latouchii* and 78 specimens (19 females and 59 males) of *R. sauteri* were collected during 13 months (Table 1). Field observations in the present study showed that amplexus behaviors and male mating vocalization occurred almost year-round except the former was absent in February and April in *R. latouchii* (Table 1). Amplexus behaviors and male mating vocalization were occurred between October and

November in *R. sauteri* (Table 1). Such results indicated that the two sympatric frogs exhibited different breeding seasons in our study site.

Histological observations of testes. Histological examinations of *R. latouchii* and *R. sauteri* testes revealed that the spermatogenic activities were invariably at stage six throughout all monthly samples. The mean numbers of sperm bundles were greatest in June (n = 18) and smallest in March (n = 7.2) in *R. latouchii* (Fig. 2A), whereas sperm bundles were most numerous in October and November (12.5 and 10.5, respectively) and fewest in February (n = 0) in *R. sauteri* (Fig. 3A). Seminiferous tubules were between 70% and 95% full of free sperm in year-round in *R. latouchii* (Fig. 2B), and from 85 to 90% full in October and November, 1997, but had no sperm in July 1996 and February 1997 in *R. sauteri* (Fig. 3B), respectively. Seminiferous tubule diameter did not

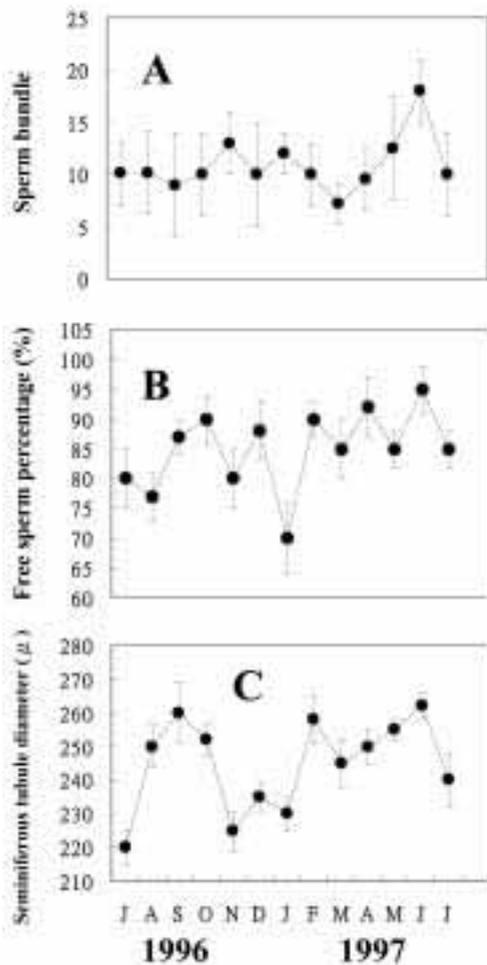


Fig. 2. Monthly changes of the sperm bundles (A), percentage of the free sperm in seminiferous lumen (B), and the seminiferous tubule diameters (C) of *Rana latouchii*. The data are expressed as mean ±SD, and the sample sizes are indicated in Table 1.

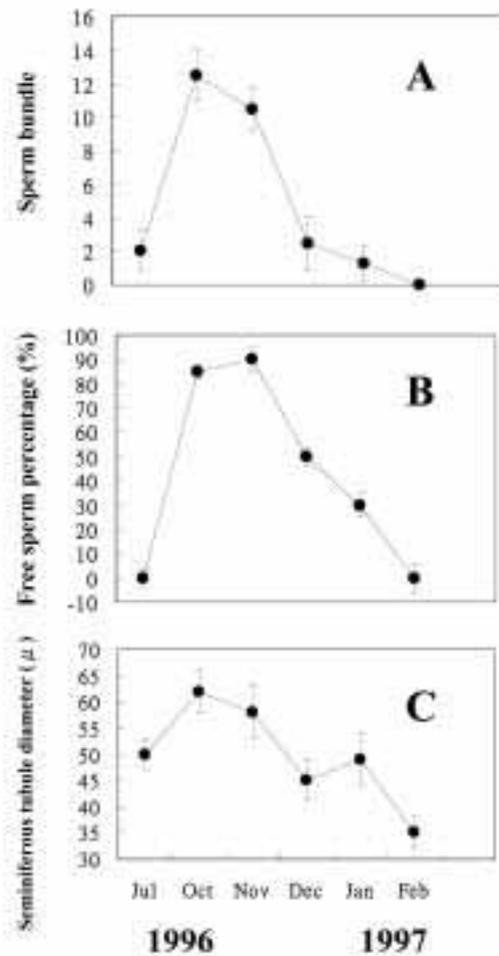


Fig. 3. Monthly changes of the sperm bundles (A), percentage of the free sperm in seminiferous lumen (B), and the seminiferous tubule diameters (C) of *Rana sauteri*. The data are expressed as means ±SD, and the sample sizes are indicated in Table 1.

Table 1. Numbers of amplexus (NA) and vocalization (V) behaviors observed in *Rana latouchii* and *R. sauteri* in an annual reproductive cycle at Tekon areas; - and + indicate absence or presence of vocalization in field observations. MN: Male sample.

Species	1996						1997						
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
<i>R. latouchii</i>													
NA	2	2	3	1	1	2	1	0	2	0	1	1	0
V	+	+	+	+	+	+	+	+	+	+	+	+	+
MN	12	26	13	2	8	12	16	18	27	6	11	2	4
FN	2	3	3	8	3	8	2	0	5	0	2	2	0
<i>R. sauteri</i>													
NA	0	0	0	6	3	0	0	0	0	0	0	0	0
V	-	-	-	+	+	-	-	-	-	-	-	-	-
MN	0	0	0	25	23	4	3	2	0	0	0	0	2
FN	0	0	0	6	3	5	3	0	2	0	0	0	0

vary significantly among the months in *R. latouchii* ($P > 0.05$; Fig. 2C; ANOVA), but showed significantly fluctuation in *R. sauteri* with high diameter in October and November ($P < 0.05$; ANOVA; Fig. 3C).

Annual changes in LM, FM, TM, and OM.

Males. In *R. latouchii*, male LM showed significant monthly variation ($F_{13,144} = 1.93$, $p = 0.04$; Fig. 4A), with the highest peak in June and the lowest value in July, whereas LM showed no significant variation in *R. sauteri* ($F_{6,52} = 1.64$, $P = 0.17$; Fig. 5A). LM showed no significant correlation with male FM or any climatic variables considered in *R. latouchii* (Table 2). In contrast, male LM in *R. sauteri* showed a positive correlation with temperature ($r = 0.85$, $P < 0.05$; Table 2). Male FM showed significant monthly variation in both frogs ($F_{13,144} = 10.3$, $P < 0.0001$; Fig. 4B in *R. latouchii*; $F_{6,52} = 2.1$, $P < 0.05$; Fig. 5B in *R. sauteri*). There was a significant positive correlation between fat body and temperature in *R. latouchii* ($r = 0.75$, $P < 0.05$; Table 2), but showed no significantly different between fat body and any climatic variables in *R. sauteri* (Table 2). Male *R. latouchii* TM showed a significant monthly variation ($F_{13,144} = 2.37$, $P = 0.008$; Fig. 4C), with the highest peak in October (Fig. 4C), but this was not seen in *R. sauteri* ($F_{6,52} = 1.43$, $P = 0.22$; Fig. 5C). TM showed a significant negative correlation with temperature in *R. sauteri* ($r = -0.88$, $P < 0.05$; Table 2).

Females. Female LM in *R. latouchii* showed significant monthly variation ($F_{10,27} = 4.5$, $P = 0.001$; Fig. 6A), with the highest peak in August and was significantly positively correlated with rainfall ($r = 0.78$, $P < 0.05$; Table 2). Likewise, LM showed significant monthly variation in *R. sauteri* ($F_{5,13} = 6.9$, $P < 0.05$; Fig. 7A), and showed a negative correlation with FM ($r = -0.91$, $P < 0.05$; Table 2). In both frogs, female FM showed significant monthly variations ($F_{10,27} = 3.85$, $P = 0.003$, Fig. 6B in *R. latouchii*; $F_{5,13} = 278.0$, $P < 0.0001$, Fig. 7B in *R. sauteri*). FM in *R. latouchii* increased to a highest in July, and decreased to a lowest in November (Fig. 6B). FM in *R. sauteri* increased to a highest peak in March and followed to decrease a lowest in October and November (Fig. 7B). There were no correlation between this parameter and any climatic variables in both frogs (Table 2). Left OM showed significant monthly variation in *R. latouchii* ($F_{10,27} = 2.97$, $P = 0.02$; Fig. 6C), but such a pattern of dynamic was not seen in *R. sauteri* ($F_{5,13} = 1.7$, $P = 0.24$; Fig. 7C). There was no any correlation between this parameter and any climatic variables (Table 2). Likewise, there was no correlation between OM and female SVLs in both frogs ($r = 0.05$, $P = 0.82$ in *R. latouchii*; $r = 0.07$, $P = 0.79$ in *R. sauteri*; Table 2).

Sexual size dimorphism.-Mean adult SVLs of males and females were 44.3 and 55.1 mm in *R. latouchii*, and were 42.1 and 52.4 mm in *R. sauteri*, respectively, and differed significantly

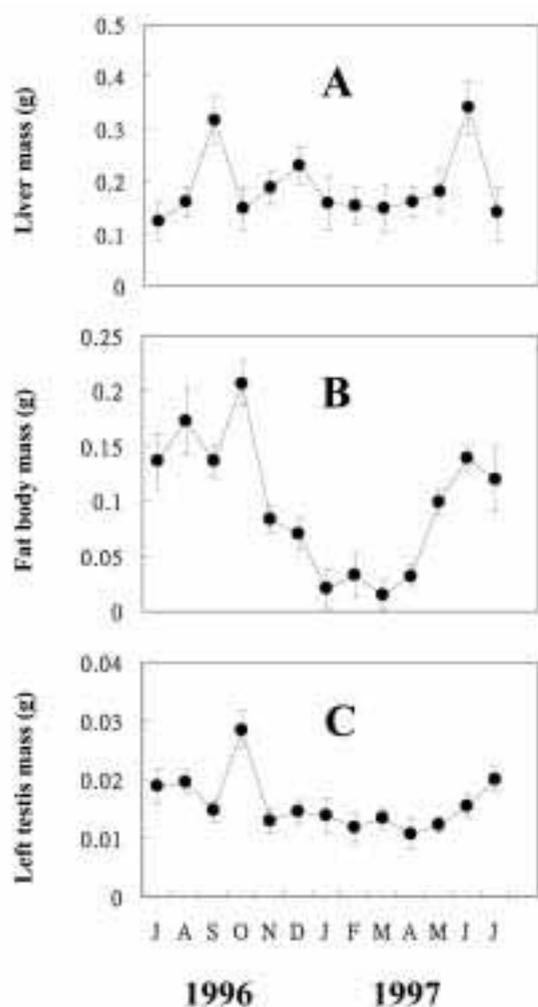


Fig. 4. Monthly changes of the liver mass (A), fat body mass (B), and the left testis mass (C) of male *Rana latouchii*. The data are expressed as means \pm SD, and the sample sizes are indicated in Table 1.

($F_{1,194} = 469.5$; $P < 0.0001$ in *R. latouchii*; $F_{1,76} = 75.6$, $P < 0.0001$ in *R. sauteri*; Table 3). Likewise, female BM, HL, and HW in both species were significantly greater than those of adult males ($F_{2,193} = 596.3$, $P < 0.0001$ for BM; $F_{2,193} = 177.1$, $P < 0.0001$ for HL; $F_{2,193} = 345.2$, $P < 0.0001$ for HW in *R. latouchii*; $F_{2,75} = 65.2$, $P < 0.0001$ for BM; $F_{2,75} = 41.1$, $P < 0.0001$ for HL; $F_{2,75} = 65.5$, $P < 0.0001$ for HW in *R. sauteri*; ANCOVA; Table 3).

DISCUSSION

Reproductive cycle. Field observations in the present study showed that amplexus behaviors and male mating vocalization occurred throughout the year in *Rana latouchii* and those behaviors occurred between October and November in *R.*

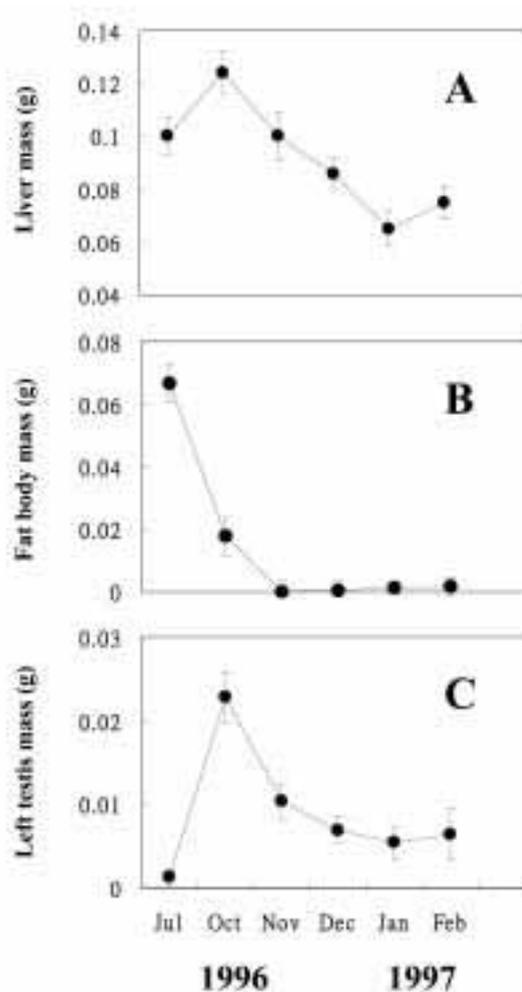


Fig. 5. Monthly changes of the liver mass (A), fat body mass (B), and the left testis mass (C) of male *Rana sauteri*. The data are expressed as means \pm SD, and the sample sizes are indicated in Table 1.

sauteri. Sperm bundles in the seminiferous tubules and free sperm in the lumen were observed every month in the former species, and thus, *R. latouchii* exhibits a year-round reproductive pattern, whereas those two spermatogenic variables are observed only in October and November in *R. sauteri*. Thus, *R. sauteri* exhibits a two months breeding period.

Sympatric populations of the two ranid species exhibited different reproductive cycles in sympatry which is seemingly contrasted to the physiological constraint hypothesis that local adaptations are a primary cause of reproduction similarities among individuals within a community that share the same environment. Likewise, two other closely related toads, *Bufo bankorensis* and *B. melanostictus*, also inhabiting the same environment at the same locality had

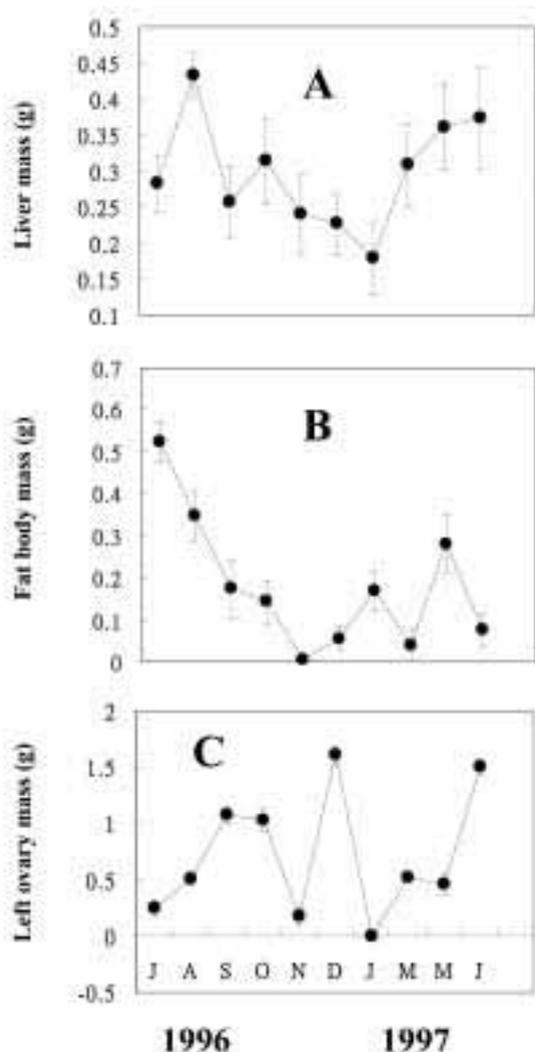


Fig. 6. Monthly changes of the liver mass (A), fat body mass (B), and the left ovary mass (C) of female *Rana latouchii*. The data are expressed as means \pm SD, and the sample sizes are indicated in Table 1.

definitively different reproductive cycles (Huang *et al.*, 1996, 1997; Table 4). 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 breeding seasons are more clearly cut than that of present study, and this is also contrasted to the physiological hypothesis that closely related species develop similar reproductive patterns under the same environments.

Although above results inconsistent with the physiological constraint hypotheses, two racophorid frogs, *Buergeria japonica* and *B. robusta*, also inhabiting in the same study site exhibited a similar breeding season from March to August seemingly contrasted to the present study

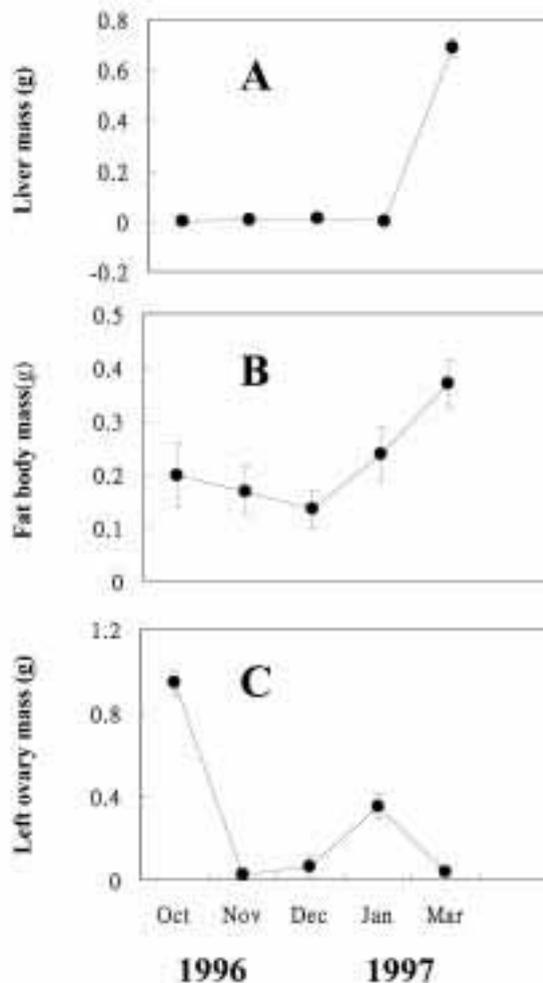


Fig. 7. Monthly changes of the liver mass (A), fat body mass (B), and the left ovary mass (C) of female *Rana sauteri*. The data are expressed as means \pm SD, and the sample sizes are indicated in Table 1.

(Huang *et al.*, 2001; Table 4). If the reproductive cycle in amphibians is indeed influenced by the local environment independent, then one may expect that the closed relative species or whole anurans should exhibit the same reproductive pattern under the same habitats.

Reproductive cycles of allopatric conspecific populations are compared in Table 4, providing evidence that the reproductive cycles are susceptible to influence by local environment in the six sympatric anurans. For example, the reproductive cycle of *B. bankorensis*, distributed in the Teken and Wushe areas, exhibit almost a similar breeding period from August to February, and September to March, respectively, whereas a conspecific population inhabiting in a high elevation above 2000 m has active breeding

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) of *Rana latouchii* and *R. sauteri* as well as rainfall (RF), photoperiod (PHO), and temperature (TEM), during an annual reproductive cycle; # and ## indicate *R. latouchii* and *R. sauteri*, respectively. Correlation coefficients (r) were analyzed from the monthly means of the parameters. *: $p < 0.05$.

	MFM	MLM	MTM	STD	FFM	FLM	FOM
MFM#	-	0.23	0.8	0.41	-	-	-
MFM##	-	0.44	0.26	0.71	-	-	-
MLM#	-	-	0.16	0.66*	-	-	-
MLM##	-	-	0.67	0.84*	-	-	-
MTM#	-	-	-	0.05	-	-	-
MTM##	-	-	-	0.84*	-	-	-
FFM#	-	-	-	-	-	0.31	0.42
FFM##	-	-	-	-	-	-0.91*	0.34
FLM#	-	-	-	-	-	-	0.10
FLM##	-	-	-	-	-	-	0.13
RF#	0.37	0.29	0.07	0.43	0.33	0.78*	0.04
RF##	0.12	0.49	0.27	0.14	0.46	0.52	0.08
PHO#	0.33	0.45	0.56	0.31	0.57	0.04	0.12
PHO##	0.51	0.43	0.15	0.24	0.21	0.02	0.41
TEM#	0.75*	0.23	0.36	0.39	0.60	0.65	0.31
TEM##	0.05	0.85*	0.88*	0.95*	0.03	0.07	0.53

Table 3. Adult body sizes of two species, *Rana latouchii* and *R. sauteri*, 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 \pm SE	BM \pm SE	HL \pm SE	HW \pm SE
<i>Rana latouchii</i>					
Males	158	44.3 \pm 2.8	6.4 \pm 1.3	17.0 \pm 1.7	14.7 \pm 0.7
Females	38	55.1 \pm 1.9	13.0 \pm 0.8	20.3 \pm 1.0	18.1 \pm 1.1
Statistical test	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	
<i>R. sauteri</i>					
Males	59	42.1 \pm 2.3	5.2 \pm 0.7	15.5 \pm 1.1	13.0 \pm 1.5
Females	19	52.4 \pm 3.4	10.6 \pm 1.3	18.5 \pm 1.2	16.1 \pm 2.2
Statistical test		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

throughout the year (Table 4). Likewise, a population of *B. japonica* living near the hot spring in Gukam exhibits reproductive activity throughout the year (Kam, unpubl. data) which differs from results of the present study site (Table 4).

Although the reproductive cycle is strongly affected by local environments discussions above, *Rana sauteri*, a widely distributed species in Taiwan (Yang, 1998), exhibits various breeding periods in different localities (Table 4). However,

the breeding period in this species is always limited to two months, and this seems to reflect a phylogenetic constraint that even the month of breeding season is strongly influenced by the local environments but the breeding periods is still 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.

Table 4. Breeding seasons of six anurans of Taiwan; WD and C indicate wide and central regions in Taiwan; M1 and 2 indicate geographical distribution above sea level of 1,000 and 2,000 m, respectively; * indicates unpublished data.

Species	Locality	Breeding season	Reference
<i>Bufo bankorensis</i>	Teken	Aug-Feb	Huang <i>et al.</i> , 1996
	Wushe (M1)	Sep-Mar	Huang & Yu*
	Meifong (M2)	year-round	Huang & Yu*
<i>B. melanostictus</i>	Teken	Feb-Jun	Huang <i>et al.</i> , 1997
	Tunghai Univ.	Feb-Sep	Hsieh, 1993
<i>Buergeria japonica</i>	Teken	Mar-Aug	Huang <i>et al.</i> 2001
	-	Feb-Oct	Yang, 1998
	Gukam (M1)	Jun-Jul	Kam*
<i>Bue. robusta</i>	Teken	Mar-Aug	Huang <i>et al.</i> 2001
	-	May-Aug	Yang, 1998
<i>Rana latouchii</i>	Teken	year-round	Present study
	-	year-round	Yang, 1998
<i>R. sauteri</i>	-	Apr-May	Yang, 1998
	Teken	Oct-Nov	Present study

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 demonstrate that two species exhibit different reproductive cycles and that this pattern cannot be the result of representing a single species' reproduction. This suggests that further study of these 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 *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 divided 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 *R. latouchii* and *R. sauteri* 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 spermatid cell nests, as well as a complete spectrum of spermatogenic stages (Huang *et al.*, 1996; Kao *et al.*, 1993). In contrast, the spermatogenic activity of species living in seasonal environments tends to be restricted to late spring and summer. In these species, maturation, the 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 (Huang *et al.*, 1997; Rastogi *et al.*, 1986). The results of the present study reveal significant variations in testicular mass by month in *R. latouchii*, but this did not occur in *R. sauteri*. 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 *R. latouchii*. 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 *R. latouchii* and *R. sauteri*.

Relations of changes in fat body, liver mass and environmental factors 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 positive correlation between liver mass and seminiferous tubule diameters observed in *R. latouchii* and *R. sauteri* suggests that the energy for testicular activity is not provided from liver stores in these species. Nevertheless, diameter of seminiferous tubules, testis mass or female ovary mass showed no significant correlation with liver or fat body mass. This suggests that the energy for production gametes may not be directly from the liver or fat body in these species.

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. 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). However, our present study of the subtropical anuran species, *R. sauteri*, revealed that spermatid production and testis mass are inhibited by high temperature (Table 2).

ACKNOWLEDGMENTS

We would like to thank C.H. Chang, J.K. Lee and J.H. Ho for their assistance with field and laboratory work. Financial support was provided by M.M. Su and the National Museum of Natural Science, Taichung, Taiwan, R.O.C..

REFERENCES

- Duellman, W.E. and L. Trueb. 1986. Reproductive strategies. *In* Duellman W.E. and L. Trueb (eds.), *Biology of Amphibians*, pp. 54-55. McGraw-Hill, New York.
- Huang, W.S., J.K. Lee, and C.H. Ho. 2001. Reproductive patterns of two sympatric rhacophorid frogs, *Buergeria japonica* and *B. robusta*, with comments on anuran breeding seasons in Taiwan. *Zoological Science* 18: 63-70.
- Huang, W.S., J.Y. Lin, and Y.L. Yu. 1996. The male reproductive cycle of the toad, *Bufo bankorensis*, in Taiwan. *Zoological Studies* 35: 128-137.
- Huang, W.S., J.Y. Lin, and Y.L. Yu. 1997. Male reproductive cycle of the toad *Bufo melanostictus* in Taiwan. *Zoological Science* 14: 497-503.
- Hsieh, J.N. 1993. The breeding ecology of *Bufo melanostictus*. Master Thesis, Tunghai University, Taichung, Taiwan. (in Chinese)
- Kanamadi, R.D., and S.K. Saidapur. 1988. Effect of fat body ablation on spermatogenesis in the Indian skipper frog *Rana cyanophlyctis* (Schn.). *Zoological Anz* 220: 79-81.
- Kao, Y.H., P.S. Alexander, V.V.C. Yang, and Y.L. Yu. 1993. Annual patterns of testicular development and activity in the Chinese bullfrog (*Rana rugulosa* Wiegmann). *Zoological Science* 10: 337-351.
- Lofts, B. 1974. Reproduction. *In* Lofts B. (ed.), *Physiology of the Amphibian*, Vol. 2. Academic Press, New York.
- Long, D.R. 1987. A comparison of energy substrates and reproductive patterns of two anurans, *Acris crepitans* and *Bufo woodhousei*. *Comparative Biochemical Physiology* 87A: 81-91.
- Pough, F.H., R.M. Andrews, J.E. Cadle, M.L. Crump, A.H. Savitzky, and K.D. Wells. 1998. *Herpetology*. Prentice-Hall, New Jersey.
- Rastogi, R.K., L. Iela, G. Delrio, and J.T. Bagnara. 1986. Reproduction in the Mexican leaf frog, *Pachymedusa dacnicolor*. *General Comparative Endocrinology* 62: 23-35.
- Saidapur, S.K. 1983. Patterns of testicular activity in Indian amphibians. *Indian Review Life Science* 3: 157-184.
- SAS Institute. 1994. SAS/STAT Licensed for National Museum of Natural Science, For Window, SAS Institute, Inc, Cary, North Carolina.
- Yang, Y.J. 1998. A field guide to the frogs and toads of Taiwan. Nature and Ecology Photo Association, Taipei. (in Chinese).
- Zug, G.R. 1993. *Herpetology: an introductory biology of amphibians and reptiles*. Academic Press, California.

同域分佈拉都西氏蛙和梭德氏蛙之生殖週期並兼論臺灣兩 生類生殖週期

黃文山¹ 鄭妤珊² 杜心怡²

¹國立自然科學博物館動物學組

²東海大學生物系

有一生理限制假說認為生活於相同環境下，相近種的兩生類其生殖季應當一致。本研究利用生活於臺中大坑同一環境下同一屬的拉都西氏蛙和梭德氏蛙的生殖週期測試此一生理限制假說；包括青蛙的睪丸、脂肪體、肝臟和卵巢等生殖週期；時間由1996年7月至1997年7月共採集了196隻拉都西氏蛙和78隻梭德氏蛙。兩種蛙都屬長期生殖型，拉都西氏蛙屬全年生殖型而梭德氏蛙生殖季介於10-11月；此一結果似乎違反上述的生理限制假說。分佈於其它地區的拉都西氏蛙也屬全年生殖，但是分佈於其它地區的梭德氏蛙卻與大坑族群不同。兩種的雌蛙其吻肛長（SVL），體重（BM），頭長（HL）和頭寬（HW）皆比雄蛙大。兩種雌蛙的卵巢重量和吻肛長未成正相關。本文亦將其它四種大坑兩生類生殖週期一併納入討論。

關鍵詞：生理限制，生殖，同域分佈，赤蛙科，臺灣。