

## Mating Calls of the Pond Frog Species Distributed in the Far East and their Artificial Hybrids

By

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### ABSTRACT

Mating calls of *Rana nigromaculata*, two races of *Rana brevipoda brevipoda* and *Rana brevipoda porosa* distributed in Japan, *Rana plancyi chosenica* in Korea, *R. nigromaculata* and *Rana plancyi plancyi* in China and *Rana plancyi fukienensis* in Formosa were recorded under the controlled condition in the laboratory and their structure was analyzed. The Typical race of *R. b. brevipoda* from Okayama and Miyoshi had long continuous calls which consisted of repetitive fine pulses (continuous call). The calls of Japanese and Chinese *R. nigromaculata*, the Nagoya race of *R. b. brevipoda* from Maibara, *R. brevipoda porosa* and *R. plancyi fukienensis* consisted of 3-15 distinct notes (multi-note call). These calls were more or less specialized in structure not only interspecifically but also intraspecifically. The calls of the Nagoya race of *R. b. brevipoda* from Maibara were intermediate between *R. nigromaculata* from Hiroshima and the Typical race of *R. b. brevipoda* from Okayama and resembled the calls of the reciprocal hybrids between the two. The calls of *R. p. plancyi* and *R. plancyi chosenica* consisted of two components which were distinctly different in structure and were mutually joined with each other (two-component call).

Fifteen kinds of inter- and intraspecific hybrids containing seven kinds of reciprocal combinations among the foregoing pond frog species which had different call structure were produced by artificial fertilization methods, and the mating calls of their males were analyzed. The male reciprocal hybrids between the frog species having multi-note call and continuous call or two-component call uttered multi-note calls without fail. The male reciprocal hybrids between the Typical race of *R. b. brevipoda* having continuous calls and *R. R. plancyi chosenica* having two-component calls also uttered multi-note calls. These calls, however, consisted of two phases. The first phase was evidently inherited from the first component of the call of *R. plancyi chosenica*.

Though the reciprocal hybrids must have the same genome constitution, 22 (47.8%) of the total 46 acoustic parameters obtained from the calls of seven kinds of reciprocal hybrids were significantly different between the reciprocal combinations. Of the total 21 acoustic parameters which were significantly different between the parental species, four (19%) were specific to these hybrids, and 15 (71.4%) were intermediate between the parental species, while the maternal effects were more or less emphasized in those parameters. Moreover, there were some other maternal characters which were emphasized in the calls of the reciprocal hybrids.

## INTRODUCTION

Among the pond frogs distributed in the Far East, there are *Rana nigromaculata*, *Rana brevipoda brevipoda* and *Rana brevipoda porosa* in Japan, *R. nigromaculata* and *Rana plancyi chosenica* in Korea, *R. nigromaculata* and *Rana plancyi plancyi* in China and *Rana plancyi fukienensis* in Formosa (KAWAMURA and NISHIOKA, 1979). The relationships among these frog species on the basis of karyotypes (NISHIOKA, 1972; NISHIOKA, OKUMOTO and RYUZAKI, 1987) and genetic distances (NISHIOKA and SUMIDA, 1992) calculated from gene frequencies at 28 loci controlling the allozymes and blood proteins have been clearly demonstrated. Moreover, the postmating reproductive isolating mechanisms among these frog species have been elucidated by extensive artificial hybridization experiments (MORIYA, 1960; KAWAMURA, NISHIOKA and KURAMOTO, 1972; KAWAMURA and NISHIOKA, 1973, 1975, 1977, 1979; KURAMOTO, 1983), but little is known about their premating reproductive isolating mechanisms.

As the mating call structure of anurans is generally species-specific, it has been regarded as an important taxonomic character (BLAIR, 1958; LITTLEJOHN and OLDHAM, 1968; STRAUGHAN, 1973; KURAMOTO, 1977, 1980; SCHNEIDER, SINSCH and NEVO, 1992), and its difference between closely related sympatric species can act as an important premating reproductive isolating barrier (SCHJØTZ, 1973; STRAUGHAN, 1973; NEVO and CAPRANICA, 1985). Among the pond frogs distributed in the Far East, the mating calls of only Japanese *R. nigromaculata*, *R. b. brevipoda* and *R. b. porosa* have been reported by KURAMOTO (1977).

In anurans, natural hybridization between sympatric closely related species has often occurred, such as *R. nigromaculata* and *R. brevipoda porosa* (KAWAMURA and NISHIOKA, 1977). Hybridization is a phenomenon intimately connected with the concept of species and speciation (KAWAMURA and NISHIOKA, 1973, 1975, 1977, 1979; OLIVERIRA, PAILLETTE, ROSA and CRESPO, 1991). For that reason, many studies on hybrid mating calls have been made in America and Europe, (BLAIR, 1956; MECHAM, 1960; GERHARDT, 1974; SCHNEIDER and EICHELBERG, 1974; LITTLEJOHN and WATSON, 1976; KRUSE, 1980; GERHARDT, GUTTMAN and KARLIN, 1980; SCHLEFER, ROMANO, GUTTMAN and RUTH, 1986; SULLIVAN and LAMB, 1988; SCHLYTER, HOGLUND and STROMBERG, 1991; OLIVEIRA, PAILLETTE, ROSA and CRESPO, 1991), but there has been no report on the mating calls of hybrids among the pond frogs distributed in the Far East.

The studies on hybrid calls mentioned above have been mainly made on natural hybrids. These studies were always faced with some problems, such as individual diversities of the materials, discrimination of F<sub>1</sub> hybrids from the backcrosses, and the environmental conditions which influence the call structure. Besides, there is an insoluble problem, that is, which species is maternal or paternal between the parental species of the hybrids?

In this study, the mating calls of three species, three subspecies and three local races among the pond frogs distributed in Japan, Korea, China and Formosa were recorded under controlled conditions in the laboratory, and their structure was

mutually compared. Furthermore, 15 kinds of inter- and intraspecific hybrids among the above pond frogs which had different call structure were produced by artificial insemination, and their mating calls were sonographically analyzed in order to elucidate the heredity of the mating calls.

The preliminary findings of this study have been reported previously (1987).

## MATERIALS AND METHODS

The following species, subspecies and local races which had been preserved from generation to generation in the laboratory or collected from the fields were used in this study.

1. *Rana brevipoda brevipoda* ITO, the sixth generation offspring of wild frogs collected from Konko, Okayama Prefecture, Japan. These frogs belong to the Typical race of *R. b. brevipoda* (MORIYA, 1954 and 1960; KAWAMURA and NISHIOKA, 1977) and are referred to in this report as *bre O*.
2. *Rana brevipoda brevipoda* ITO, the wild frogs collected from Miyoshi, Hiroshima Prefecture, Japan. These frogs belong to the Typical race of *R. b. brevipoda* and are referred to in this report as *bre Miy(W)*.
3. *Rana brevipoda brevipoda* ITO, the wild frogs collected from Maibara, Shiga Prefecture, Japan. These frogs belong to the Nagoya race of *R. b. brevipoda* (MORIYA, 1954 and 1960; NISHIOKA, SUMIDA and OHTANI, 1992) and are referred to in this report as *bre M(W)*.
4. *Rana brevipoda porosa* (COPE), the second generation offspring of wild frogs collected from Ohmiya, Saitama Prefecture, Japan. These frogs are referred to in this report as *por S*.
5. *Rana nigromaculata* HALLOWELL, the third generation offspring of wild frogs collected from Hiroshima, Japan, and the wild frogs whose calls were recorded in the breeding site in Hiroshima. These frogs are referred to in this report as *nig H* and *nig H(W)*, respectively.
6. *Rana nigromaculata* HALLOWELL, the first and second generation offspring of wild frogs collected from Beijing, China, by Dr. Cih-Ye CHANG in 1979 and presented to Dr. T. KAWAMURA and Dr. M. NISHIOKA. These frogs are referred to in this report as *nig B*.
7. *Rana plancyi fukienensis* POPE, the fourth generation offspring of wild frogs collected from Chiayi, Formosa, by Mr. S. LIM in 1976 and sent to Dr. T. KAWAMURA and Dr. M. NISHIOKA. These frogs are referred to in this report as *fuk F*.
8. *Rana plancyi chosenica* OKADA, the fourth generation offspring of the frogs collected from Suwon, Korea, by Dr. H. KIM in 1975 and sent to Dr. T. KAWAMURA and Dr. M. NISHIOKA. These frogs are referred to in this report as *chos K*.
9. *Rana plancyi plancyi* LATASTE, the second generation offspring of wild frogs collected from Beijing, China, by Dr. Cih-Ye CHANG in 1979 and presented to Dr. T. KAWAMURA and Dr. M. NISHIOKA. These frogs are referred to in

this report as *plan B*.

When the hybrids were produced, ovulation was accelerated by injecting *Rana catesbeiana* pituitaries, and all the matings were made by the artificial fertilization method. The tadpoles fed on boiled spinach. Metamorphosed frogs fed on two-spotted cricket, *Gryllus bimaculatus* DE GEER (NISHIOKA and MATSUURA, 1977). All the hybrids and controls attained sexual maturity in two years after metamorphosis.

Before the recording, the males were injected with pituitary suspension of *Rana catesbeiana* (half of a pituitary gland per male) and kept in a sufficiently damp container at  $24 \pm 1^\circ\text{C}$  in the dark. They began to call without fail 3–6 hours thereafter and continued calling for about 12 hours (UEDA, 1993). The container used was 65 cm  $\times$  43 cm  $\times$  15 cm in size. In order to absorb the echo, artificial lawn was placed on each side of the container and fine gravel was spread on the bottom about six cm in depth. Five males were placed in the container which was covered with a wire-gauze. In order to ascertain if the call emitted following the pituitary stimulation is an actual mating call, on June 19, 1984 the mating calls from ten males of wild *R. nigromaculata* were recorded in the breeding site, located in the suburbs of Hiroshima, at an air temperature of  $23.0^\circ\text{C}$ .

Tape recording was made with Sony TCM 500 EV recorder. Analyses of the calls were made with Digital Sona-graph 7800 (Kay). Standard (2.56 sec) sonagrams with 45 Hz or 300 Hz band-filter were used for all calls. Each call was analyzed for the following acoustic parameters: call duration, number of notes per call, note rate, note length, note interval, pulse rate, and spectrum of frequency.

## OBSERVATION

### *I. Mating calls of the pond frogs distributed in the Far East*

Mating calls of the pond frogs distributed in the Far East used in this study were divided into three call types on the basis of structure. As a matter of convenience, in this paper these call types were named as (1) continuous call, (2) multi-note call and (3) two-component call. The continuous call was composed of a series of repetitive pulses which were never divided into pulse groups (notes). Mating calls of this type were uttered by the males of the Typical race of *R. b. brevipoda* from Okayama and Miyoshi. The multi-note call was composed of 3–15 notes which consisted of some pulses. Mating calls of this type were uttered by the males of the Nagoya race of *R. b. brevipoda* from Maibara, *R. nigromaculata* from Hiroshima and Beijing, *R. brevipoda porosa* and *R. plancyi fukienensis*. The two-component call was composed of two acoustic components which were distinctly different in structure. Mating calls of this type were uttered by the males of *R. p. plancyi* and *R. plancyi chosonica*.

TABLE 1  
Acoustic parameters of the mating calls belonging to the continuous call in the Typical races  
of *Rana brevipoda brevipoda* from Okayama and Miyoshi

Kind	Body length (mm)	No. of calls	Call duration (sec)	Pulse rate (pulses/sec)	Dominant frequency (kHz)
<i>bre</i> O, ♀ No. 1 × <i>bre</i> O, ♂ No. 1	55.0 (53~56)	20	1.07 ± 0.02 (0.85~1.35)	151.0 ± 4.8 (124~187)	2.03 ± 0.01 (1.82~2.25)
<i>bre</i> Miy, (W)	50.3 (48~54)	20	0.92 ± 0.01 (0.77~1.18)	189.7 ± 5.9 (148~240)	2.18 ± 0.04 (1.87~2.38)

### 1. Continuous call

#### a. Mating calls of the Typical race of *Rana brevipoda brevipoda*

##### i) *R. b. brevipoda* from Okayama

*R. b. brevipoda* from Okayama used in this study was the sixth generation offspring preserved in the laboratory. It was produced in 1983 as a control mating, *bre* O, ♀ No. 1 × *bre* O, ♂ No. 1, for the hybridizing experiments between this race and each of *R. nigromaculata* from Hiroshima, *R. plancyi chosenica* and *R. plancyi fukienensis*. Mating calls from five males of this control, whose mean body length was 55.0 mm, were recorded at a temperature of 24.5°C on 21 May 1985, and 20 calls were analyzed.

The mating calls of this offspring were long continuous calls (Fig. 1a). These calls lasted for 0.85~1.35 sec, 1.07 sec on the average, and consisted of repetitive fine pulses whose number varied from 114 to 236 with a mean of 162. The pulse rate varied from 124 pulses/sec to 187 pulses/sec with a mean of 151 pulses/sec (Table 1). Each pulse had comparatively clear harmonics. Of these harmonics, the effective ones were restricted within a narrow frequency range between 0.20 kHz and 3.50 kHz (Fig. 1a). Some harmonics often overlapped or were mutually joined. The first harmonic ranging between 0.20 kHz and 1.20 kHz included a fundamental frequency without modulation. The mean fundamental frequency was 0.75 kHz (Fig. 1b). The dominant frequency was included in the third or fourth harmonic. While it ranged around 1.70 kHz at the beginning of the call, it gradually heightened and attained the maximum by the end of the first quarter of the call. The power spectrum analyses showed that the spectral peak (dominant frequency) was around 2.03 kHz (Fig. 1b). Such dominant frequency of high range was retained until the end of calls. It was characteristic of the calls of this offspring that the amplitude modulation was very weak throughout the call.

It was noteworthy that nine of the 20 calls analyzed had short parts with different structure from the main calls at the beginning of the calls (Fig. 1a). These parts which lasted for only about 65 msec ranged between 0.20 kHz and 2.50 kHz, and their energy intensity was very weak. Pulses constituting the parts were too numerous to count on the normal sonagrams.

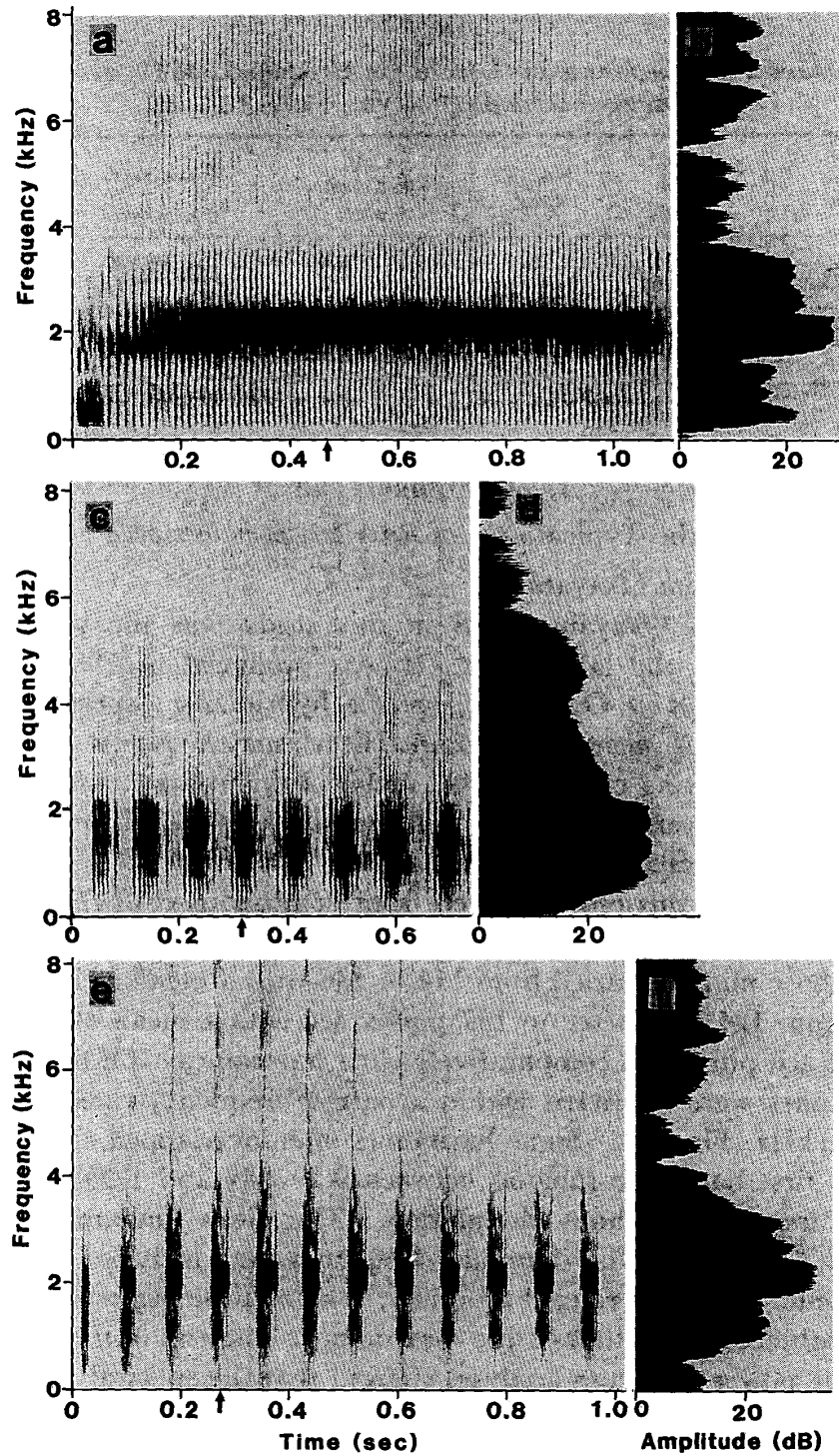


Fig. 1. Sonograms and amplitude spectrograms of the mating calls of *Rana brevipoda brevipoda* and *Rana brevipoda porosa*.

- a. The Typical race of *R. b. brevipoda* from Okayama.
- b. An amplitude spectrogram at the point indicated by arrow in a.
- c. The Nagoya race of *R. b. brevipoda* from Maibara.
- d. An amplitude spectrogram at the point indicated by arrow in c.
- e. *R. brevipoda porosa* from Saitama.
- f. An amplitude spectrogram at the point indicated by arrow in e.

ii) *R. b. brevipoda* from Miyoshi

Five males of *R. b. brevipoda* collected from Miyoshi whose mean body length was 55.0 mm, were used. Their mating calls were recorded at a temperature of 25.0°C on 10 July 1986. Twenty calls were analyzed.

The mating calls of this local race were very similar to those of *R. b. brevipoda* from Okayama. These calls lasted for 0.77~1.18 sec, 0.92 sec on the average, and were composed of repetitive fine pulses whose number varied from 138 to 214 with a mean of 173.9. Pulse rate of the calls varied from 148 pulses/sec to 240 pulses/sec with a mean of 189.7 pulses/sec (Table 1). Each pulse had comparatively clear harmonics. The first harmonic ranging between 480 Hz and 1.5 kHz included a fundamental frequency ranging around 1.06 kHz. Dominant frequencies of the calls were included in the third or fourth harmonic, and their mean value was 2.18 kHz.

## 2. Multi-note call

All species and subspecies which had multi-note call uttered two kinds of calls. The first was that a single call was intermittently uttered, and the other was that two to six calls were successively uttered with short intervals. In this paper, the former is named as a single-call and the latter as 2-, 3-, 4-, 5- and 6-successive calls. The single-calls were overwhelmingly uttered by all the used male frogs.

a. Mating calls of the Nagoya race of *Rana brevipoda brevipoda* from Maibara

Five males of *R. b. brevipoda* collected from Maibara and having a mean body length of 47.0 mm were used. Their mating calls were recorded in the laboratory at a temperature of 24.0°C on 20 May 1986. Twenty single calls were analyzed. The results of these analyses are presented in Table 2.

Though this race was very similar to the *R. b. brevipoda* from Okayama in external characters, these males uttered only multi-note calls (Fig. 1c), differing from the latter. These calls lasted for 0.43~0.76 sec, 0.61 msec on the average, and consisted of 6~9 notes, 7.1 notes on the average. The mean note rate was 11.8 notes/sec. The note duration varied from 49 msec to 84 msec with a mean of 64.3 msec. The note interval varied from two msec to 34 msec with a mean of 23.1 msec. The pulses constituting the notes showed rather similar structure to those of the calls of *R. b. brevipoda* from Okayama. The mean pulse rate of the notes was 142.6 pulses/sec. Each note had some unclear harmonics. The dominant frequency ranged from 1.25 kHz to 1.82 kHz with a mean of 1.48 kHz (Fig. 1d).

The call structure of this race was very similar to that of the reciprocal hybrid males between *R. nigromaculata* from Hiroshima and *R. b. brevipoda* from Okayama in every acoustic parameters as described later.

b. Mating calls of *Rana brevipoda porosa* from Saitama

*R. brevipoda porosa* from Saitama used was produced in 1985 by a crossing between a female and a male first generation offspring whose parents had been

TABLE 2  
Acoustic parameters of the mating calls belonging to multi-note call in the Nagoya race of  
*Rana brevipoda brevipoda* from Maibara, *R. brevipoda porosa* from Saitama, *R. nigromaculata*  
from Hiroshima and Beijing and *R. plancyi fukiensis* from Formosa

Kind	Body length (mm)	No. of calls	Call duration (sec)	No. of notes (notes/call)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Pulse rate (pulses/sec)	Dominant frequency (kHz)
<i>bre</i> M, (W)	47.0 (45~50)	20*	0.61±0.03 (0.43~0.76)	7.1±0.2 (6~9)	11.8±0.2 (11~13)	64.3±2.5 (49~84)	23.3±1.9 (2~34)	142.6±5.9 (109~194)	1.48±0.04 (1.25~1.82)
<i>por</i> S, ♀ No. 1 × <i>por</i> S, ♂ No. 1	60.0 (58~63)	20*	0.88±0.02 (0.63~1.23)	11.0±0.2 (9~15)	12.6±0.1 (11~14)	28.4±0.9 (11~44)	56.9±1.3 (27~87)	227.1±3.5 (179~253)	2.12±0.02 (1.75~2.33)
		15**	0.57±0.03 (0.44~0.71)	7.8±0.2 (6~10)	13.2±0.2 (12~15)	28.5±1.3 (21~34)	52.6±0.9 (47~59)	219.7±2.1 (199~225)	2.12±0.03 (1.67~2.38)
		15***	0.51±0.02 (0.48~0.64)	7.4±0.3 (6~9)	14.7±0.3 (13~16)	26.0±1.0 (21~30)	50.3±1.8 (26~59)	205.7±3.8 (189~223)	2.15±0.02 (1.80~2.38)
<i>nig</i> H, (W)	54.5 (53~56)	20*	0.53±0.01 (0.45~0.62)	6.0±0.1 (5~8)	11.0±0.1 (10~12)	42.1±1.2 (25~61)	55.5±1.0 (40~62)	146.4±2.3 (108~163)	1.63±0.03 (1.43~1.80)
<i>nig</i> H, ♀ No. 1 × <i>nig</i> H, ♂ No. 1	60.0 (58~62)	25*	0.56±0.01 (0.48~0.69)	6.6±0.1 (5~8)	11.8±0.1 (11~13)	40.3±1.2 (15~47)	51.8±1.4 (38~77)	148.8±3.2 (106~169)	1.57±0.02 (1.36~2.00)
		20*	0.55±0.02 (0.42~0.70)	6.5±0.2 (5~8)	11.8±0.2 (10~13)	40.5±1.4 (12~50)	50.6±1.4 (27~74)	149.2±4.6 (110~172)	1.35±0.01 (1.00~1.97)
<i>nig</i> H, ♀ No. 2 × <i>nig</i> H, ♂ No. 2	62.6 (59~67)	20*	0.55±0.02 (0.42~0.70)	6.5±0.2 (5~8)	11.8±0.2 (10~13)	40.5±1.4 (12~50)	50.6±1.4 (27~74)	149.2±4.6 (110~172)	1.35±0.01 (1.00~1.97)
		15**	0.51±0.03 (0.33~0.60)	6.2±0.1 (5~8)	12.3±0.2 (10~15)	39.1±1.3 (18~47)	48.1±1.3 (36~68)	147.8±6.1 (114~180)	1.34±0.02 (1.00~1.88)
		15***	0.38±0.02 (0.28~0.46)	5.1±0.3 (4~7)	13.4±0.2 (10~16)	38.6±1.8 (11~53)	42.8±1.4 (35~62)	151.9±5.6 (104~182)	1.32±0.02 (1.00~1.79)
<i>nig</i> B, ♀ No. 1 × <i>nig</i> B, ♂ No. 1	69.4 (65~73)	20*	0.85±0.02 (0.72~0.96)	11.4±0.1 (10~13)	13.4±0.1 (12~15)	30.8±1.2 (12~42)	50.7±1.2 (31~60)	135.4±2.9 (105~150)	1.82±0.02 (1.45~2.00)
		15**	0.76±0.04 (0.64~0.90)	10.4±0.2 (8~13)	13.7±0.2 (12~16)	30.5±1.8 (15~40)	48.5±2.0 (34~66)	141.0±6.8 (115~200)	1.80±0.02 (1.50~2.00)
		15***	0.51±0.03 (0.35~0.66)	7.7±0.1 (5~11)	14.9±0.2 (12~17)	29.3±1.8 (12~40)	42.0±1.5 (23~60)	149.7±5.0 (114~200)	1.76±0.03 (1.50~2.00)
<i>nig</i> B, ♀ No. 2 × <i>nig</i> B, ♂ No. 2	54.6 (52~59)	20*	0.84±0.01 (0.71~0.99)	11.0±0.1 (9~13)	13.1±0.1 (11~15)	31.1±1.5 (23~47)	52.6±1.0 (42~62)	142.0±2.6 (124~169)	2.01±0.02 (1.50~2.25)
<i>fuk</i> F, ♀ No. 1 × <i>fuk</i> F, ♂ No. 1	61.8 (59~63)	20*	0.25±0.01 (0.19~0.32)	5.6±0.1 (5~7)	22.8±0.1 (21~26)	14.5±0.4 (12~17)	37.4±1.0 (26~50)	————	0.98±0.02 (0.82~1.29)
		15**	0.22±0.01 (0.18~0.28)	5.5±0.1 (5~6)	24.0±0.3 (21~29)	15.5±0.6 (12~21)	31.6±1.2 (26~48)	————	1.00±0.04 (0.85~1.25)
		15***	0.19±0.01 (0.14~0.25)	4.5±0.1 (3~5)	23.3±0.2 (22~27)	15.7±0.8 (12~19)	34.0±1.0 (27~50)	————	0.99±0.03 (0.80~1.25)

\* Single-call. \*\* First call of 2-successive call. \*\*\* Second call of 2-successive call.

collected from the field. Mating calls from the five males whose mean body length was 60.0 mm were recorded at a temperature of 24.5°C on 13 July 1987, and 20 single-calls and 15 2-successive calls were analyzed. The results of these analyses are presented in Table 2.

The call structure of this subspecies was distinctly different from that of each race of the foregoing basic species (Fig. 1e,f; Tables 1 and 2). It showed the most advanced note structure, that is, every note had compact appearances and was separated from adjacent notes with about twice longer distance than the note duration (Fig. 1e). The single call of this subspecies lasted for 0.63~1.23 sec, 0.88 sec on the average, and consisted of 9~15 notes, 11.0 notes on the average. The mean note rate was 12.6 notes/sec. The note duration varied from 11 msec to 44 msec with a mean of 28.4 msec. The note interval varied from 27 msec to 87 msec with a mean of 56.9 msec. The mean pulse rate of the notes was 227.1



pulses/sec. Dominant frequency of the note ranged from 1.75 kHz to 2.33 kHz with a mean of 2.12 kHz (Fig. 1f).

On the other hand, the first and second calls of the 2-successive calls, lasted for 0.57 sec and 0.51 sec, on the average, respectively, and consisted of about 8 notes and 7 notes, respectively. The mean note rate of the first and second calls was 13.2 notes/sec and 14.7 notes/sec, respectively. The mean note interval of the first and second calls was 52.6 msec and 50.3 msec, respectively. The note duration, pulse rate and dominant frequency of the 2-successive calls were not significantly different from those of the single calls of the same males (Table 2). The 2-successive calls generally had shorter call duration, somewhat shorter note interval and higher note rate than those of the single calls in the same males, though the differences were not so large between the first and second calls.

c. Mating calls of *Rana nigromaculata* from Hiroshima

i) Wild frogs

Mating calls from 10 males of wild frogs of *R. nigromaculata*, whose mean body length was 54.5 mm, were recorded in a breeding site, located in the suburbs of Hiroshima, at an air temperature of 23.0°C on 19 June 1984. As only single-calls were recorded in the tape, 20 of them were analyzed. The results of these analyses are presented in Table 2.

These calls lasted for 0.45~0.62 sec, 0.53 sec on the average, and consisted of 5~8 notes, 6.0 notes on the average. The mean note rate was 11.0 notes/sec. The note duration varied from 25 msec to 61 msec with a mean of 42.1 msec. The note interval varied from 40 msec to 62 msec with a mean of 55.5 msec. The first note had the lowest energy intensity and the shortest duration. Those of the subsequent notes, however, became higher and longer with time, attained the maximum level at almost the mid point of the call and became lower and shorter toward the end of the call (Fig. 2a). The notes which were of full energy intensity covered a wide frequency spectrum up to 8 kHz and had unclear harmonic bands. The power spectrum analyses showed that the spectral peak (dominant frequency) was around 1.63 kHz. Each note was composed of 2~7 pulses. The mean pulse rate was 146.4 pulses/sec.

ii) The third generation offspring preserved in the laboratory

This offspring were produced in 1983 and 1984 as control matings, *nig* H, ♀ No. 1 × *nig* H, ♂ No. 1 and *nig* H, ♀ No. 2 × *nig* H, ♂ No. 2, respectively, for the hybridizing experiments between this species and each of *R. nigromaculata* from Beijing, *R. b. brevipoda* from Okayama and *R. plancyi chosonica* and between this species and *R. b. brevipoda* from Okayama, respectively. Mating calls from five males of the former controls and five males of the latter ones, whose mean body length was 60.0 mm and 62.6 mm, respectively, were recorded at a temperature of 25.0°C on 20 and 21 June 1986, respectively. Twenty-five single-calls of the former males and 20 single-calls and 15 2-successive calls of the latter males were analyzed. The results of these analyses are presented in Table 2.

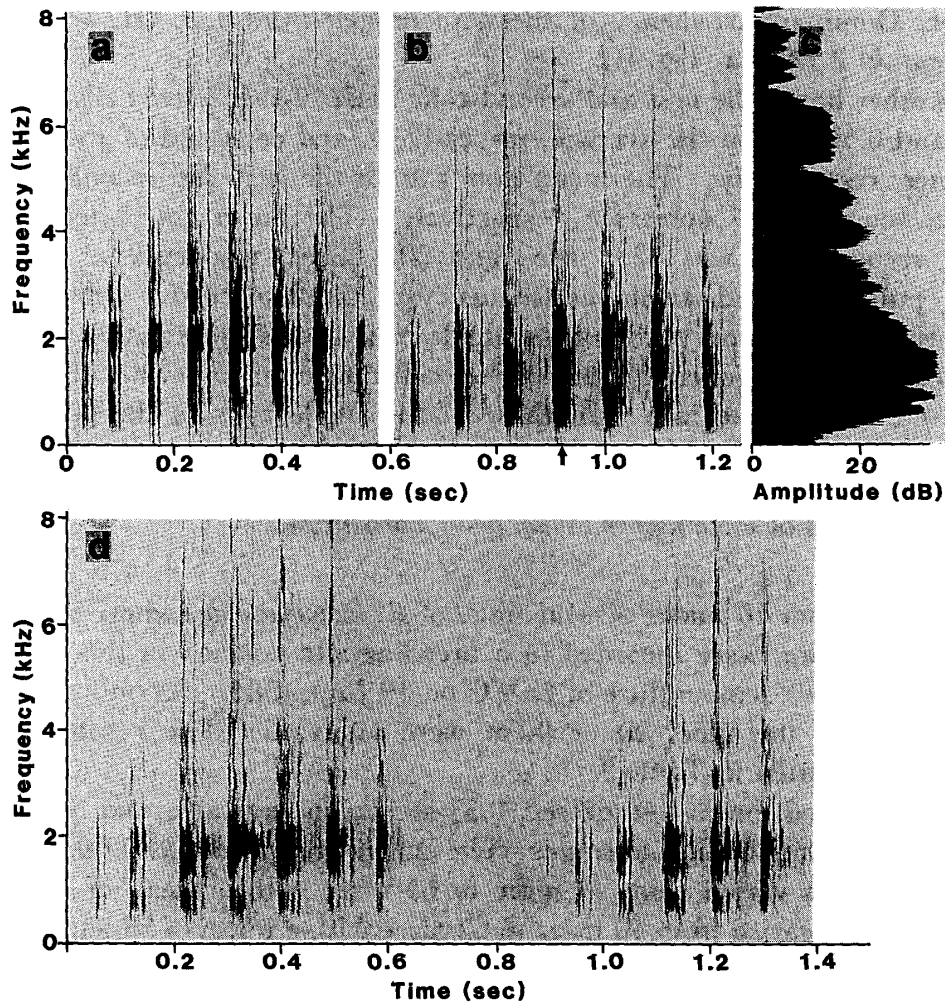


Fig. 2. Sonograms and an amplitude spectrogram of the mating calls of *Rana nigromaculata* from Hiroshima.

- a. A single-call of a male of the wild frog whose call was recorded in the field.
- b. A single-call of a male of the third generation offspring preserved in the laboratory.
- c. An amplitude spectrogram at the point indicated by arrow in b.
- d. A 2-successive call of the same male as b.

The single-calls from the males of these two control series were almost the same in structure to those of the foregoing wild frog males (Fig. 2a, b). Between the two control series, there were no significant differences in any acoustic parameters except dominant frequency. The call durations of the total 10 males of the two series varied from 0.42 sec to 0.70 sec with a mean of 0.56 sec. These calls were composed of 5~8 notes, 6.6 notes on the average, and their mean note rate was 11.8 notes/sec. The note duration varied from 12 msec to 50 msec with a mean of 40.4 msec. The note interval varied from 27 msec to 77 msec with a mean of 51.3 msec. The notes were composed of 2~10 pulses and the mean pulse rate was 149.0 pulses/sec. The mean dominant frequency of the first mating, *nig* H, ♀ No. 1 × *nig* H, ♂ No. 1, was 1.57 kHz (Fig. 2c), and that of the second mating, *nig* H, ♀ No. 2 × *nig* H, ♂ No. 2, was 1.35 kHz.

There were no significant differences in the acoustic parameters of the single-calls except the dominant frequency among the males of the two control series and the wild frogs. The larger males had a lower dominant frequency (Table 2).

The first and second calls of the 2-successive calls (Fig. 2d) recorded from the five males of a control mating, *nig* H, ♀ No 2 × *nig* H, ♂ No. 2, lasted for 0.51 sec and 0.38 sec, on the average, respectively, and were composed of about 6 and 5 notes, respectively. The mean note rate of the first and second calls was 12.3 notes/sec and 13.4 notes/sec, respectively. The mean note duration of the first and second calls was 39.1 msec and 38.6 msec, respectively. The mean note interval of the first and second calls was 48.1 msec and 42.8 msec, respectively. The note structure was not so different between the single-call and 2-successive call in the same males (Fig. 2b, d). These results showed that 2-successive calls had shorter call duration, shorter note interval and higher note rate than those of the single-calls of the same males. Such tendency was emphasized in the second

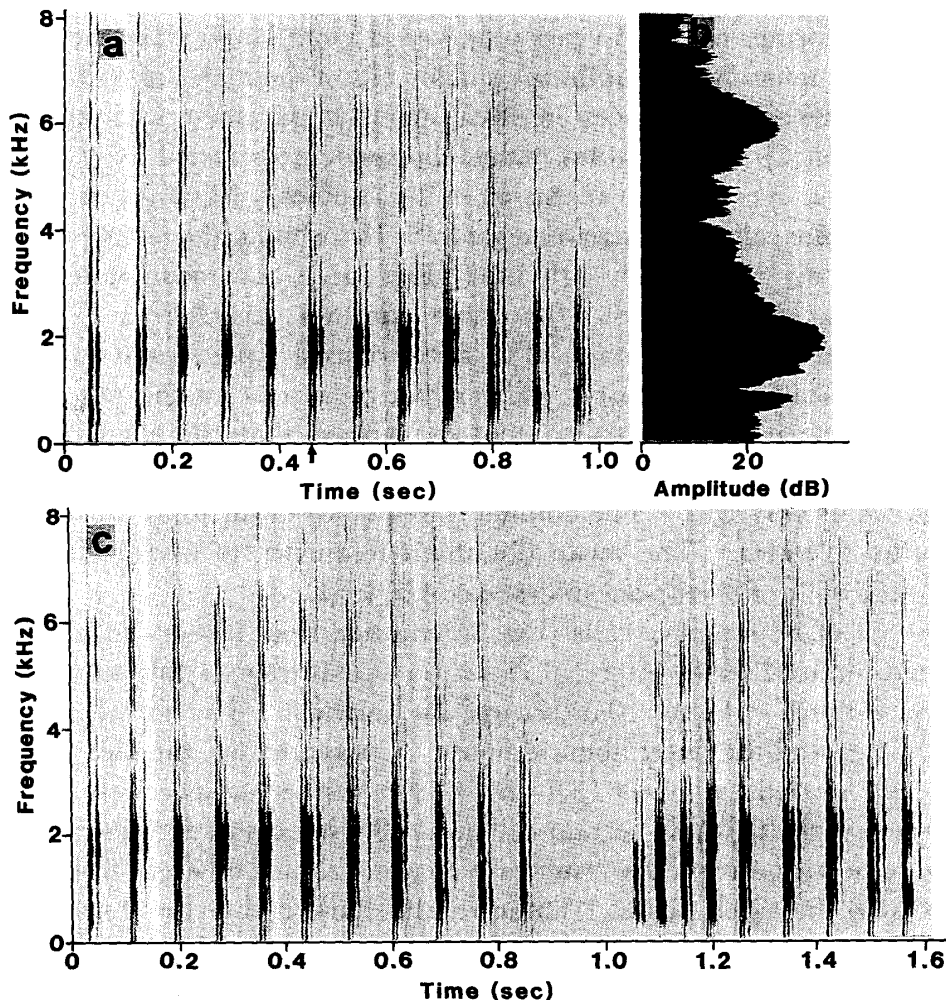


Fig. 3. Sonograms and an amplitude spectrogram of the mating calls of *Rana nigromaculata* from Beijing.

- a. A single-call of a male.
- b. An amplitude spectrogram at the point indicated by arrow in a.
- c. A 2-successive call of the same male as a.

calls of the 2-successive calls (Table 2), differing from the case of *R. brevipoda porosa* from Saitama.

d. Mating calls of *Rana nigromaculata* from Beijing

*R. nigromaculata* from Beijing produced in 1980 by a mating, *nig* B, ♀ No. 1 × *nig* B, ♂ No. 1, was the first generation offspring of one pair of wild frogs collected from Beijing. The second generation offspring was produced in 1983 as the control mating, *nig* B, ♀ No. 2 × *nig* B, ♂ No. 2, for the hybridizing experiments between this species and each of *R. nigromaculata* from Hiroshima and *R. plancyi chosonica*. These first and second generation offspring were used. Mating calls from five males of the former and five males of the latter, whose mean body length was 69.4 mm and 54.6 mm, respectively, were recorded at a temperature of 24.5°C on 6 and 15 June 1986, respectively. Twenty single-calls and 15 2-successive calls of the former and 20 single-calls of the latter were analyzed. The results of these analyses are presented in Table 2.

Though the former males (6 years old) were 15 mm larger than the latter males (3 years old) in mean body length, the single-calls of the males of both matings did not show significant differences in their acoustic parameters except dominant frequency (Table 2). In the total males, the single-calls lasted for 0.71~0.99 sec, 0.85 msec on the average, and consisted of 9~13 notes, 11.2 notes on the average. Differing from the calls of *R. nigromaculata* from Hiroshima, the energy intensities of the notes scarcely modulated with time (Fig. 3a). The mean note rate of the single-calls was 13.3 notes/sec. The note duration varied from 12 msec to 47 msec with a mean of 31.0 msec. The note interval varied from 31 msec to 62 msec with a mean of 51.7 msec. The mean pulse rate of the notes was 138.7 pulses/sec. The power spectrum analyses showed that the energy intensity of each note covered a wide frequency spectrum up to 8 kHz and had unclear harmonic bands (Fig. 3b). The dominant frequency of the total calls ranged from 1.45 kHz to 2.25 kHz. The mean dominant frequency of 6-year-old males was 1.82 kHz and that of 3-year-old males was 2.01 kHz.

In the case of 2-successive calls (Fig. 3c) emitted from 6-year-old males the first calls lasted for 0.64 sec~0.90 sec, 0.76 sec on the average, while the second calls lasted for 0.35 sec~0.66 sec, 0.51 sec on the average. The former consisted of about 10 notes and the latter about 8 notes. Consequently, the mean note rate of the first and second calls was 13.7 and 14.9 notes/sec, respectively. The mean note interval of the first and second calls was 48.5 msec and 42.0 msec, respectively. The note structure of the 2-successive calls was not different from that of the single-calls in the same males. These results showed that the 2-successive calls had generally shorter call duration, shorter note interval and higher note rate than those of the single-calls in the same males. Such tendency strongly appeared in the second calls of the 2-successive calls like those of *R. nigromaculata* from Hiroshima.

It was interesting that the call structure of this frogs was more similar to that of the *R. brevipoda porosa* from Saitama than that of the *R. nigromaculata* from

Hiroshima. Between the *R. nigromaculata* from Beijing and Hiroshima, there were significant differences in call duration, number of notes per call, note duration, note rate and dominant frequency (Table 2; Fig. 8). As the former had longer call, shorter note and higher dominant frequency than the latter, the human ears could easily distinguish which males of the two populations had been calling.

e. Mating calls of *Rana plancyi fukienensis* from Formosa

*R. plancyi fukienensis* used in this study was the fourth generation offspring preserved in the laboratory. It was produced in 1983 as the control mating, *fuk* F, ♀ No. 1 × *fuk* F, ♂ No. 1, for the hybridizing experiments between this species and each of *R. plancyi chosenica* and *R. b. brevipoda* from Okayama. Mating calls from five males of the control *R. plancyi fukienensis*, whose mean body length was 61.8 mm, were recorded at a temperature of 24.0°C on 30 May 1986. Though this subspecies had 2-~6-successive calls, over the third calls of 3-, 4-, 5- and 6-successive calls showed almost same call structure as the second call of the 2-successive calls, and therefore 20 single-calls and 15 2-successive calls were analyzed. The results of these analyses are presented in Table 2.

The duration of the single-calls varied from 0.19 sec to 0.32 sec with a mean of 0.25 sec. These calls were composed of 5~7 notes, 5.6 notes on the average, and the mean note rate was 22.8 notes/sec. The note duration varied from 12 msec to 17 msec with a mean of 14.5 msec. The note interval varied from 26 msec to 50 msec with a mean of 37.4 msec. The pulses which composed the notes were too numerous to count in the normal sonograms. The notes were covered a wide frequency spectrum up to 8 kHz and had unclear harmonic bands. These power spectrum analyses showed that amplitudes somewhat rapidly elevated with increase in frequency to 0.98 kHz, which was the dominant frequency, and gradually

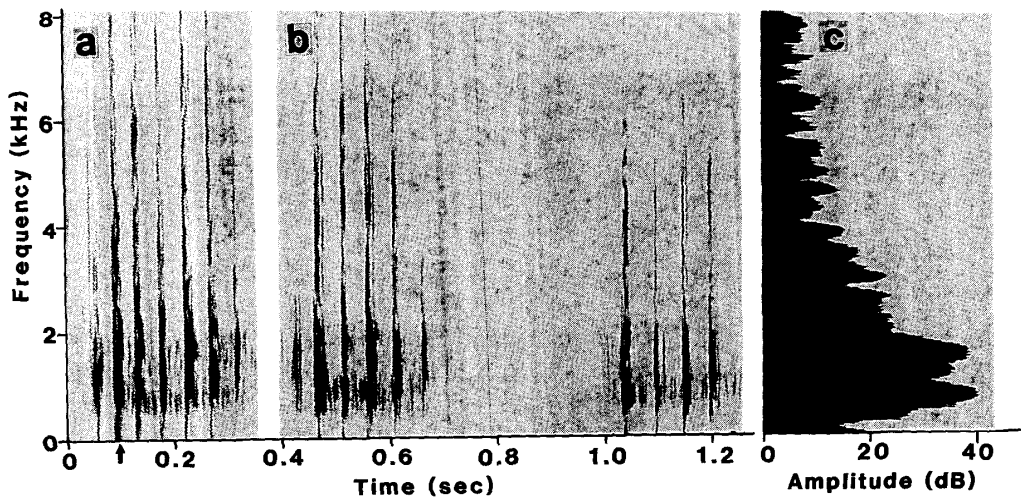


Fig. 4. Sonograms and an amplitude spectrogram of the mating calls of *Rana plancyi fukienensis* from Formosa.

- a. A single-call of a male.
- b. A 2-successive call of the same male as a.
- c. An amplitude spectrogram at the point indicated by arrow in a.

dropped toward 8 kHz (Fig. 4c).

The first and second calls of the 2-successive calls lasted for 0.22 sec and 0.19 sec, on the average, respectively, and were composed of about 5.5 and 4.5 notes, respectively. Subsequently, the mean note rate of the first and second calls was 24.0 notes/sec and 23.3 notes/sec, respectively. The mean note interval of the first and second calls was 31.6 msec and 34.0 msec, respectively. The note structure of the 2-successive calls was not different from that of the single calls in the same males (Fig. 4a, b). The 2-successive calls generally had a shorter call duration, shorter note interval and higher note rate than those of the single-calls of the same males like *R. nigromaculata* and *R. b. porosa*, though these differences in this subspecies were small in all parameters.

The mating calls of the males of *R. plancyi fukienensis* were remarkably characteristic. This is because these calls showed the shortest call duration and note duration, the highest note rate and pulse rate, and the lowest dominant frequency among all the species and subspecies which had the multi-note calls (Table 2).

TABLE 3  
Acoustic parameters of the mating calls belonging to two-component call in *Rana plancyi plancyi* from Beijing and *R. plancyi chosonica* from Korea

Kind	Body length (mm)	No. of calls	Call duration (sec)	First component		Second component		
				Duration (sec)	Dominant frequency (kHz)	Duration (sec)	Pulse rate (pulses/sec)	Dominant frequency (kHz)
<i>plan</i> B, ♀ No. 1 × <i>plan</i> B, ♂ No. 1	41.8 (40~45)	35	0.53 ± 0.01 (0.40~0.76)	0.33 ± 0.01 (0.26~0.55)	1.53 ± 0.05 (1.18~1.84)	0.20 ± 0.02 (0.13~0.35)	173.4 ± 3.9 (130~200)	1.84 ± 0.03 (1.25~2.28)
<i>chos</i> K, ♀ No. 1 × <i>chos</i> K, ♂ No. 1	42.6 (41~45)	20	0.31 ± 0.01 (0.23~0.39)	0.11 ± 0.01 (0.04~0.18)	1.56 ± 0.09 (1.22~2.00)	0.21 ± 0.01 (0.13~0.26)	178.3 ± 2.6 (160~188)	1.46 ± 0.02 (1.12~1.75)

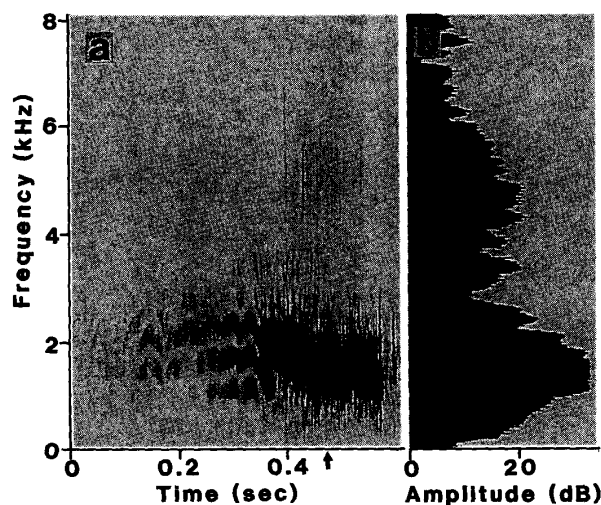


Fig. 5. A sonagram (a) and an amplitude spectrogram (b) of a mating calls of *Rana plancyi plancyi* from Beijing.

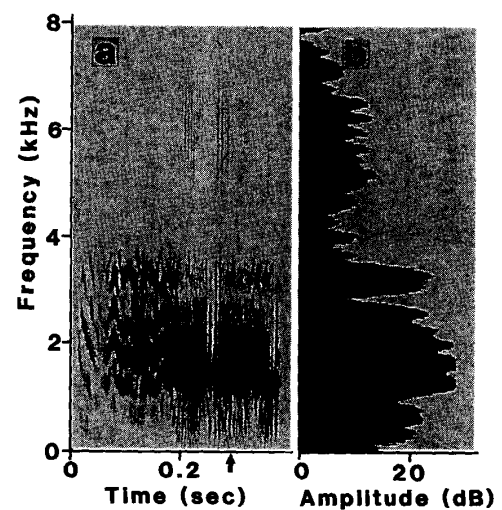


Fig. 6. A sonagram (a) and an amplitude spectrogram (b) of a mating calls of *Rana plancyi chosonica* from Korea.

### 3. Two-component call

#### a. Mating calls of *Rana plancyi plancyi* from Beijing

*R. p. plancyi* from Beijing used in this study was the second generation offspring preserved in the laboratory. It was produced in 1983 by a mating, *plan* B, ♀ No. 1 × *plan* B, ♂ No. 1. Mating calls from five males of the offspring, whose mean body length was 41.8 mm, were recorded at a temperature of 25.0°C on 4 June 1986, and 35 calls were analyzed. The results of these analyses are presented in Table 3.

The males of this basic species uttered very interesting mating calls, because their calls were composed of two components which were arranged without intervals, and these components were distinctly different in structure (Fig. 5). The first component lasted for 0.26 sec~0.55 sec, 0.33 sec on the average, and had 3~6 clear harmonic bands which showed wavelike frequency modulations. As the pulses which constituted the first component were too numerous to count in the normal sonagrams, these harmonic bands appeared homogeneous (Fig. 5a). The second, third and fourth harmonic bands always showed stable amplitude, but the others varied. The second harmonic bands generally contained a dominant frequency which ranged from 1.18 kHz to 1.84 kHz with a mean of 1.53 kHz.

On the other hand, the second component lasted for 0.13~0.35 sec, 0.20 sec on the average. These components consisted of repetitive fine pulses each of which scarcely varied in structure (Fig. 5a). Therefore, they somewhat resembled the calls of *R. b. brevipoda* from Okayama, though the latter were considerably longer in duration. In the sonagrams of some second components, the darker lines of the pulse were sparsely distributed around the center of them. The number of pulses of the second components varied from 21 to 49 with a mean of 34.7. The mean pulse rate was 173.4 pulses/sec. Each pulse had unclear harmonics. The dominant frequency varied from 1.25 kHz to 2.28 kHz with a mean of 1.84 kHz (Fig. 5b).

#### b. Mating calls of *Rana plancyi chosenica* from Korea

*R. plancyi chosenica* used in this study was the fourth generation offspring preserved in the laboratory. It was produced in 1983 as the control mating, *chos* K, ♀ No. 1 × *chos* K, ♂ No. 1, for the hybridizing experiments between this subspecies and each of *R. nigromaculata* from Hiroshima and Beijing, *R. b. brevipoda* from Okayama and *R. plancyi fukienensis*. Mating calls from five males of the controls, whose mean body length was 42.6 mm, were recorded at a temperature of 24.0°C on 26 May 1986, and 20 calls were analyzed. The results of these analyses are presented in Table 3.

The males of this subspecies had two-component calls like *R. p. plancyi* (Fig. 6a). The first components lasted for 0.04 sec~0.18 sec, 0.11 sec on the average, and had 4~6 clear harmonic bands whose structure was very similar to that of *R. p. plancyi*. The second harmonic band generally contained a dominant frequency which ranged from 1.22 kHz to 2.00 kHz with a mean of 1.56 kHz. On the other hand,



the second components, which were very similar to those of the calls of *R. p. plancyi* males in structure, also, lasted for 0.13~0.26 sec, 0.21 sec on the average. The number of pulses of the second components varied from 23 to 47 with a mean of 37.6. The mean pulse rate was 178.3 pulses/sec. Each pulse had unclear harmonics. The dominant frequency varied from 1.12 kHz to 1.75 kHz with a mean of 1.46 kHz (Fig. 6b).

The mating call of *R. plancyi chosenica* was quite similar to that of *R. p. plancyi* in structure. There were only quantitative differences in two acoustic parameters between them. The first components of the latter lasted about three times longer than those of the former, and the second components of the latter showed higher dominant frequency than that of the former (Table 3).

## II. Mating calls of the hybrid males

### 1. Reciprocal hybrids between Hiroshima and Beijing populations of *Rana nigromaculata*

The reciprocal interpopulational hybrids between *R. nigromaculata* from Hiroshima and Beijing were produced in 1983 by the crossings, *nig* H, ♀ No. 1 × *nig* B, ♂ No. 2 and *nig* B, ♀ No. 2 × *nig* H, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 65.1 mm and 67.8 mm, respectively, were recorded at a temperature of 25.0°C on 7 and 11 July 1987, respectively. Though both the reciprocal hybrids had the single-calls and 2-successive calls, 20 single-calls of each reciprocal hybrid were analyzed.

The interpopulational reciprocal hybrids between *R. nigromaculata* from Hiroshima and Beijing were extremely similar to each other in external characters, but their calls sounded somewhat different (Fig. 7a, b). Between the parental offsprings, there were significant differences in call duration, number of notes per call, note rate, note duration and dominant frequency of the mating calls (Fig. 8). On the other hand, there were moderate differences in those acoustic parameters between the reciprocal hybrids (Table 4). While the mean values of call dura-

TABLE 4  
Mating calls of the intra- and interspecific reciprocal hybrids between the pond frogs having multi-note calls with different acoustic parameters

Kind	Body length (mm)	No. of calls	Call duration (sec)	No. of notes (notes/call)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Pulse rate (pulses/sec)	Dominant frequency (kHz)
<i>nig</i> H, ♀ No. 1 × <i>nig</i> B, ♂ No. 2	65.1 (60~68)	20	0.63±0.02 (0.44~0.75)	7.7±0.2 (6~9)	12.4±0.2 (11~15)	38.2±1.7 (10~47)	50.8±1.1 (25~61)	147.7±4.7 (80~204)	1.66±0.03 (1.02~2.04)
<i>nig</i> B, ♀ No. 2 × <i>nig</i> H, ♂ No. 1	67.8 (64~71)	20	0.70±0.02 (0.52~0.83)	9.0±0.2 (7~11)	12.8±0.3 (11~15)	32.3±2.5 (17~56)	51.1±1.2 (27~59)	142.6±4.9 (70~234)	1.88±0.03 (1.24~2.14)
<i>nig</i> H, ♀ No. 1 × <i>fuk</i> F, ♂ No. 1	60.6 (58~63)	20	0.44±0.02 (0.25~0.59)	8.9±0.3 (6~13)	22.3±0.2 (20~25)	27.6±1.1 (18~36)	25.7±1.3 (15~42)	274.3±3.8 (159~397)	1.27±0.05 (0.73~1.85)
<i>fuk</i> F, ♀ No. 1 × <i>nig</i> H, ♂ No. 1	61.3 (59~65)	20	0.31±0.02 (0.20~0.51)	7.9±0.2 (6~11)	24.5±0.2 (22~27)	17.4±1.0 (11~33)	23.9±1.5 (13~50)	————	0.95±0.05 (0.56~1.74)



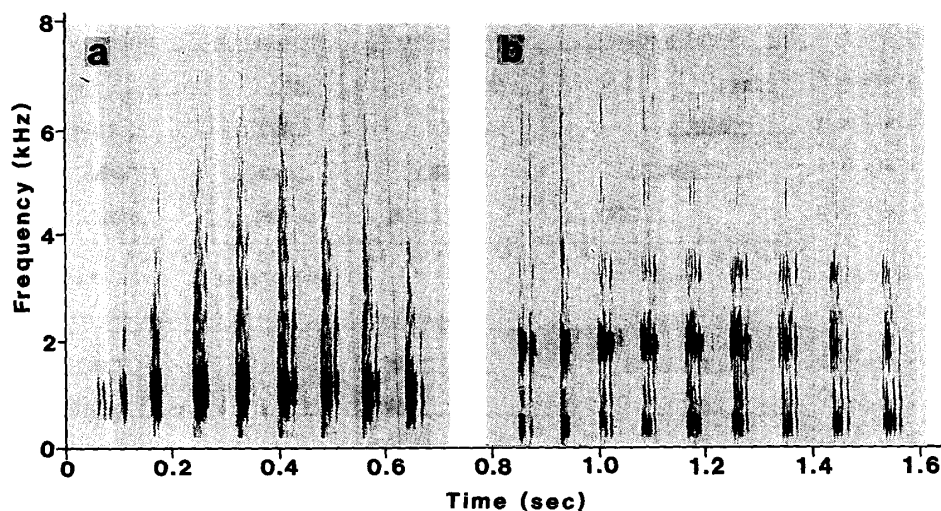


Fig. 7. Sonograms of the mating calls of the reciprocal intraspecific hybrids between *Rana nigromaculata* from Hiroshima and Beijing.

- a. Hybrid between a female from Hiroshima and a male from Beijing.
- b. Hybrid between a female from Beijing and a male from Hiroshima.

tions, numbers of notes per call, note rates, note durations, note intervals, pulse rates and dominant frequencies of the mating calls of the hybrids, *nig* H, ♀ No. 1 × *nig* B, ♂ No. 2, was 0.63 sec, and 7.7, 12.4 notes/sec, 38.2 msec, 50.8 msec, 147.7 pulses/sec and 1.66 kHz, respectively, those of the reciprocal hybrids, *nig* B, ♀ No. 2 × *nig* H, ♂ No. 1, were 0.70 sec, 9.0, 12.8 notes/sec, 32.3 msec, 51.1 msec, 142.6 pulses/sec and 1.88 kHz, respectively. The energy intensities and energy distributions over the frequency range of each note in the hybrids, *nig* H, ♀ No. 1 × *nig* B, ♂ No. 2, modulated with time like the calls of Hiroshima *nigromaculata* (Fig. 7a), while those of the reciprocal hybrid, *nig* B, ♀ No. 2 × *nig* H, ♂ No. 1, did not modulate like the calls of Beijing *nigromaculata* (Fig. 7b).

Between the mating calls of these interpopulational reciprocal hybrids, there were significant differences in the call duration, number of the notes per call and dominant frequency and some differences in the note duration and pulse rate. All these parameters were intermediate between those of the parental populations, while their values approached the means of the parental populations or were intermediate the means of the parental populations and those of the maternal populations (Table 4; Fig. 8).

## 2. Reciprocal hybrids between *Rana nigromaculata* from Hiroshima and *Rana plancyi fukiensis* from Formosa

Reciprocal interspecific hybrids between *R. nigromaculata* from Hiroshima and *R. plancyi fukiensis* from Formosa were produced in 1983 by the crossings, *nig* H, ♀ No. 1 × *fuk* F, ♂ No. 1 and *fuk* F, ♀ No. 1 × *nig* H, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 60.6 mm and 61.3 mm, respectively, were recorded at a temperature of 25.0°C on 18 and 20 July 1992, respectively. Twenty single-calls of each reciprocal hybrid were analyzed.

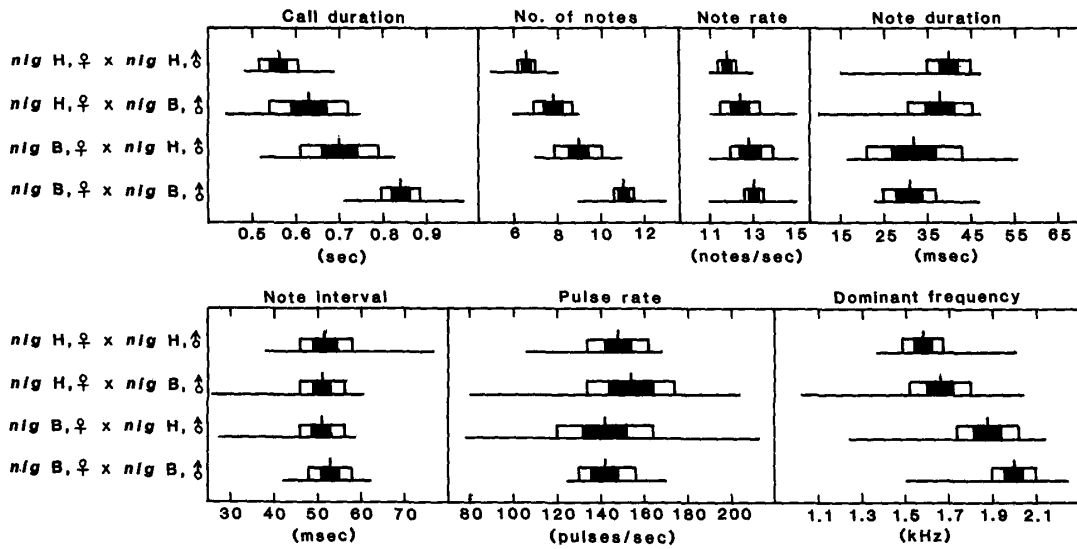


Fig. 8. Comparison of the seven kinds of acoustic parameters among the mating calls of the reciprocal intraspecific hybrids between *Rana nigromaculata* from Hiroshima and Beijing and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

Though the males of both *R. nigromaculata* and *R. p. fukiensis* had multi-note calls, there were significant differences in all analyzed parameters between these two species (Table 2). The males of each reciprocal hybrid had multi-note calls (Fig. 9a, b). While the mean values of the call durations, numbers of notes per call, note rates, note durations, note intervals, pulse rate and dominant frequencies of the calls of the hybrids, *nig* H, ♀ No. 1 × *fuk* F, ♂ No. 1, were 0.44 sec, 8.9, 22.3 notes/sec, 27.6 msec, 25.7 msec, 274.3 pulses/sec and 1.27 kHz, respectively, those of the reciprocal hybrid, *fuk* F, ♀ No. 1 × *nig* H, ♂ No. 1, were 0.31 sec, 7.9, 24.5

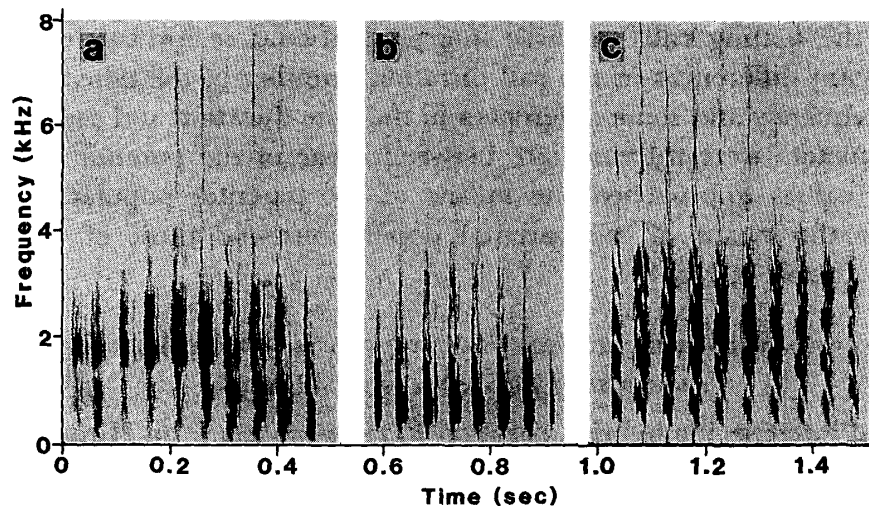


Fig. 9. Sonograms of the mating calls of the hybrids among *Rana nigromaculata* from Hiroshima, *R. plancyi fukiensis* from Formosa and the Typical race of *Rana brevipoda brevipoda* from Okayama.

- Hybrid between a female *R. nigromaculata* and a male *R. plancyi fukiensis*.
- Hybrid between a female *R. plancyi fukiensis* and a male *R. nigromaculata*.
- Hybrid between a female *R. b. brevipoda* and a male *R. plancyi fukiensis*.

notes/sec, 17.4 msec, 23.9 msec and 0.95 kHz, respectively (Table 4). The pulses which constituted the note in the latter were too numerous to count in the normal sonagrams that the pulse rate could not be calculated (Fig. 9).

There were significant differences in call duration, note rate, note duration, and dominant frequency between the mating calls of these reciprocal hybrids. The number of notes per call, note rate and note interval of the reciprocal hybrid were specific to these hybrids. On the other hand, call durations, note durations and dominant frequencies of each reciprocal hybrid were intermediate between the parental species, while the maternal effects seemed to be emphasized in those parameters (Table 4; Fig. 10).

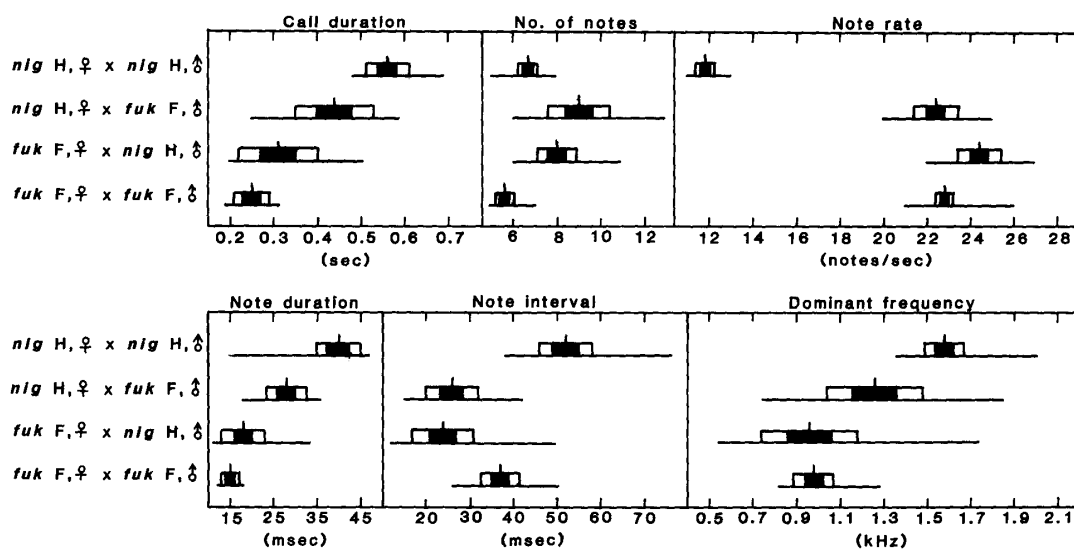


Fig. 10. Comparison of the six kinds of acoustic parameters among the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Hiroshima and *R. plancyi fukienensis* from Formosa and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

### 3. Reciprocal hybrids between *Rana nigromaculata* from Hiroshima and the Typical race of *Rana brevipoda brevipoda* from Okayama

Two series of reciprocal interspecific hybrids between *R. nigromaculata* from Hiroshima and *R. b. brevipoda* from Okayama were produced in 1983 and 1984 by the crossings, *nig* H, ♀ No. 1 × *bre* O, ♂ No. 1 and *bre* O, ♀ No. 1 × *nig* H, ♂ No. 1, and by the crossings, *nig* H, ♀ No. 2 × *bre* O, ♂ No. 2 and *bre* O, ♀ No. 2 × *nig* H, ♂ No. 2, respectively. Mating calls from five males of each combination were recorded at a temperature of  $24.0 \pm 1^\circ\text{C}$  on 16~20 May 1986. While the male hybrids, *nig* H, ♀ × *bre* O, ♂, produced in 1983 and 1984 were 65.0 mm and 60.8 mm in mean body length, respectively, those of the reciprocal combination, *bre* O, ♀ × *nig* H, ♂, were 63.8 mm and 55.4 mm, respectively. Twenty single-calls of each hybrid produced by the four crossings were analyzed.

While the male of *R. b. brevipoda* from Okayama had continuous call, the male of *R. nigromaculata* from Hiroshima had multi-note call. The reciprocal hybrid males between these two species had only multi-note calls (Fig. 11a, b). The note

TABLE 5  
Mating calls of interspecific hybrids between pond frog species having multi-note calls  
and continuous calls. These hybrids had multi-note calls

Kind	Body length (mm)	No. of calls	Call duration (sec)	No. of notes (notes/call)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Pulse rate (pulses/sec)	Dominant frequency (kHz)
<i>nig</i> H, ♀ No. 1 × <i>bre</i> O, ♂ No. 1	65.0 (62~69)	20	0.68±0.03 (0.55~0.76)	9.3±0.2 (8~12)	13.7±0.1 (12~15)	49.0±1.5 (15~70)	29.6±2.0 (15~42)	118.6±2.0 (104~147)	1.35±0.03 (1.00~1.74)
<i>nig</i> H, ♀ No. 2 × <i>bre</i> O, ♂ No. 2	60.8 (58~62)	20	0.70±0.03 (0.67~0.85)	9.4±0.2 (8~11)	13.1±0.1 (12~14)	46.4±1.8 (15~63)	33.9±2.3 (15~65)	125.1±2.5 (101~133)	1.62±0.04 (1.00~1.95)
<i>bre</i> O, ♀ No. 1 × <i>nig</i> H, ♂ No. 1	63.8 (61~67)	20	0.85±0.04 (0.62~1.25)	10.6±0.3 (6~15)	12.5±0.2 (11~14)	56.7±1.0 (24~72)	29.6±1.0 (12~49)	147.9±5.5 (107~179)	1.70±0.03 (1.45~2.00)
<i>bre</i> O, ♀ No. 2 × <i>nig</i> H, ♂ No. 2	55.4 (53~58)	20	0.82±0.04 (0.57~0.93)	10.8±0.3 (8~13)	13.1±0.3 (12~16)	57.2±1.8 (17~68)	27.6±1.6 (12~40)	156.4±5.9 (115~210)	1.83±0.03 (1.00~2.20)
<i>bre</i> O, ♀ No. 1 × <i>fuk</i> F, ♂ No. 1	59.6 (57~62)	18	0.44±0.03 (0.26~0.70)	9.8±0.3 (6~12)	22.4±0.2 (21~24)	27.2±1.7 (11~64)	19.9±1.2 (7~35)	—————	1.45±0.03 (1.09~1.75)

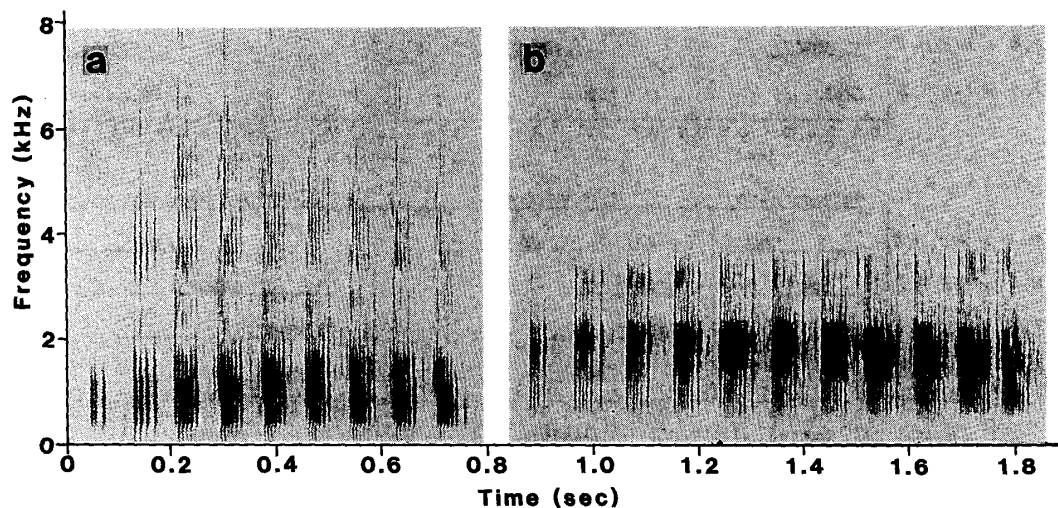


Fig. 11. Sonograms of the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Hiroshima and the Typical race of *R. b. brevipoda* from Okayama.

- Hybrid between a female *R. nigromaculata* and a male *R. b. brevipoda*.
- Hybrid between a female *R. b. brevipoda* and a male *R. nigromaculata*.

structure of the mating calls of each reciprocal hybrid was fairly different from that of the Hiroshima *nigromaculata*. The pulse-lines appeared in the sonograms of the hybrid calls were more clear and uniform throughout the calls than those of the Hiroshima *nigromaculata*, and they arranged more regularly than those of the latter in each note. Thus, they appeared to rather resembled those of Okayama *brevipoda*. Such tendency was more emphasized in the hybrids, *bre* O, ♀ × *nig* H, ♂ (Fig. 11b). The energy distribution in each note throughout the calls were different between the reciprocal hybrids. The energy intensity of the notes in the hybrids, *nig* H, ♀ Nos. 1, 2 × *bre* O, ♂ Nos. 1, 2, modulated with time like that of the Hiroshima *nigromaculata*, while that of the reciprocal hybrids, *bre* O, ♀ Nos. 1, 2 × *nig* H, ♂ Nos. 1, 2, scarcely modulated like that of the Okayama *brevipoda*. The frequency distribution in the pulses of each reciprocal hybrid was also more similar to those of the maternal species. Though the body lengths of each hybrid

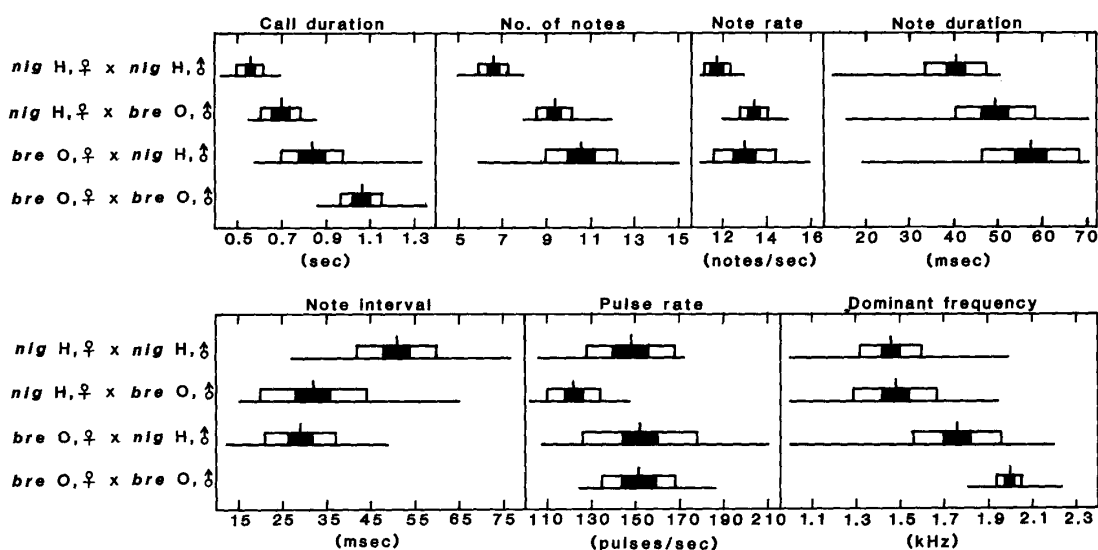


Fig. 12. Comparison of the seven kinds of acoustic parameters among the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Hiroshima and the Typical race of *R. b. brevipoda* from Okayama and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

were fairly different between the two experimental series, and there were some differences in some parameters, the differences were not significant except the dominant frequency. The mean values of the call durations, numbers of notes per call, note rates, note durations, note intervals, pulse rates and dominant frequencies of the hybrids, *nig* H, ♀ Nos. 1, 2 × *bre* O, ♂ Nos. 1, 2, were 0.69 sec, 9.4, 13.4 notes/sec, 47.7 msec, 31.8 msec, 121.9 pulses/sec and 1.49 kHz, respectively, while those of the reciprocal hybrids, *bre* O, ♀ Nos. 1, 2 × *nig* H, ♂ Nos. 1, 2, were 0.84 sec, 10.7, 12.8 notes/sec, 57.0 msec, 28.6 msec, 152.2 pulses/sec and 1.77 kHz, respectively (Table 5).

Between the mating calls of these reciprocal hybrids, there were significant differences in call duration, number of notes per call, note duration, pulse rate and dominant frequency. Call duration and dominant frequency of each reciprocal hybrid were intermediate between those of the parental species, while the maternal effects were more or less emphasized in those parameters (Table 5; Fig. 12). Moreover, the maternal characters of the energy and frequency distribution patterns of the calls of each species were predominately transmitted to each reciprocal hybrids. Therefore, the structure of the mating calls of each reciprocal hybrid rather resembled that of the maternal species, disregarding the note-structured calls of the hybrids, *bre* O, ♀ × *nig* H, ♂ (Fig. 11a, b).

#### 4. Hybrids between a female of *Rana brevipoda brevipoda* from Okayama and a male of *Rana plancyi fukienensis* from Formosa

Interspecific hybrids between a female of *R. b. brevipoda* from Okayama and a male of *R. plancyi fukienensis* from Formosa were produced in 1983 by the crossing, *bre* O, ♀ No. 1 × *fuk* F, ♂ No. 1. Mating calls from the five male hybrids, whose mean body length was 59.6 mm, were recorded at a temperature of 25.0°C on 28 June 1986. Eighteen single-calls of the hybrid males were analyzed.

While the male of *R. b. brevipoda* from Okayama had typical continuous calls and the male of *R. plancyi fukiensis* from Formosa had multi-note calls, the hybrids between these two species had only multi-note calls (Fig. 9c). The call duration of this hybrids varied from 0.26 sec to 0.70 sec with a mean of 0.44 sec. So far the calling times, about 10 notes whose mean duration was 27.2 msec, were repeated at intervals of about 20 msec (Table 5). These notes showed such specific structure which those of the parental species never showed. They had five to seven comparatively clear harmonics with strong frequency modulation (Fig. 9c). In these harmonics, three of the lower frequency level, ranging from 0.5 to 2.5 kHz, showed high energy intensity and the second or third harmonic contained dominant frequency ranging around 1.45 kHz (Table 5). The call duration and dominant frequency of the hybrids were intermediate between those of the parental species (Fig. 13).

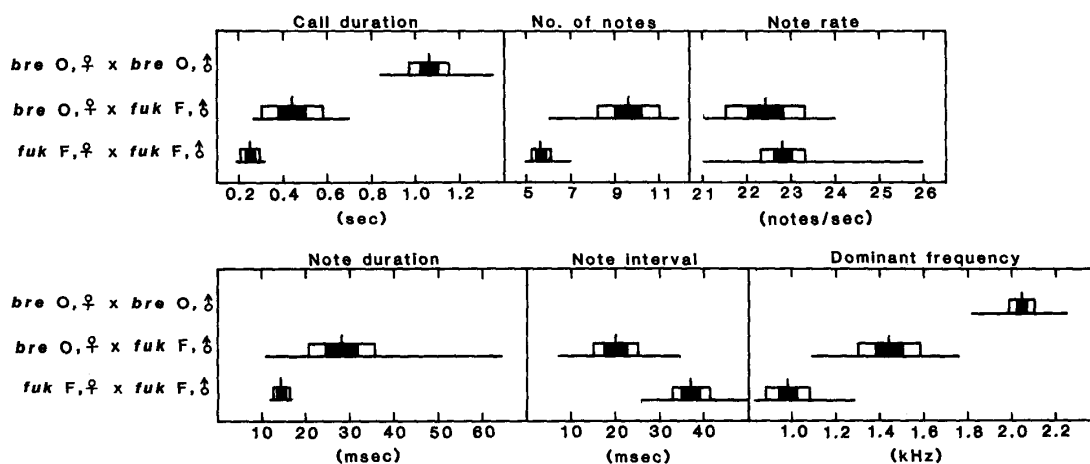


Fig. 13. Comparison of the six kinds of acoustic parameters among the mating calls of the hybrids between a female of *Rana brevipoda brevipoda* from Okayama and a male of *Rana plancyi fukiensis* from Formosa and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

##### 5. Reciprocal hybrids between *Rana nigromaculata* from Hiroshima and *Rana plancyi chosonica* from Korea

Reciprocal interspecific hybrids between *R. nigromaculata* from Hiroshima and *R. plancyi chosonica* from Korea were produced in 1983 by the crossings, *nig* H, ♀ No. 1 × *chos* K, ♂ No. 1 and *chos* K, ♀ No. 1 × *nig* H, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 49.3 mm and 50.8 mm, respectively, were recorded at a temperature of 25.0°C on 9 and 10 June 1986, respectively. Twenty single-calls of each reciprocal hybrid were analyzed.

Though the males of *R. plancyi chosonica* from Korea had two-component calls, the reciprocal hybrids between this subspecies and *R. nigromaculata* from Hiroshima had only multi-note calls (Fig. 14a, b). The note structure of these hybrids was greatly different from that of the Hiroshima *nigromaculata*. While the mean values of the call durations, numbers of the notes per call, note rate, note durations, note intervals and dominant frequencies of the mating calls of the hybrids, *nig* H, ♀ No.

TABLE 6

Mating calls of two kinds of the interspecific reciprocal hybrids between pond frog species having multi-note calls and two-component calls. These hybrids had multi-note calls

Kind	Body length (mm)	No. of calls	Call duration (sec)	No. of notes (notes/call)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Dominant frequency (kHz)
<i>nig</i> H, ♀ No. 1 × <i>chos</i> K, ♂ No. 1	49.3 (47~52)	20	0.46±0.02 (0.31~0.60)	8.8±0.4 (6~12)	19.2±0.2 (18~21)	30.4±1.8 (8~42)	25.5±1.1 (18~35)	1.26±0.03 (0.85~1.57)
<i>chos</i> K, ♀ No. 1 × <i>nig</i> H, ♂ No. 1	50.8 (48~55)	20	0.39±0.02 (0.28~0.54)	9.0±0.4 (6~13)	23.3±0.3 (18~26)	18.3±1.0 (8~31)	30.6±1.3 (19~43)	1.35±0.05 (0.86~2.04)
<i>nig</i> B, ♀ No. 2 × <i>chos</i> K, ♂ No. 1	51.2 (48~54)	20	0.65±0.03 (0.46~0.91)	16.0±0.6 (11~19)	25.2±0.2 (23~27)	17.5±1.4 (7~42)	25.4±1.0 (7~41)	1.53±0.05 (1.07~2.00)
<i>chos</i> K, ♀ No. 1 × <i>nig</i> B, ♂ No. 2	52.4 (48~58)	20	0.55±0.02 (0.36~0.71)	13.3±0.2 (9~16)	25.0±0.2 (23~28)	17.4±0.8 (5~25)	23.3±0.9 (13~36)	1.33±0.05 (0.62~1.87)

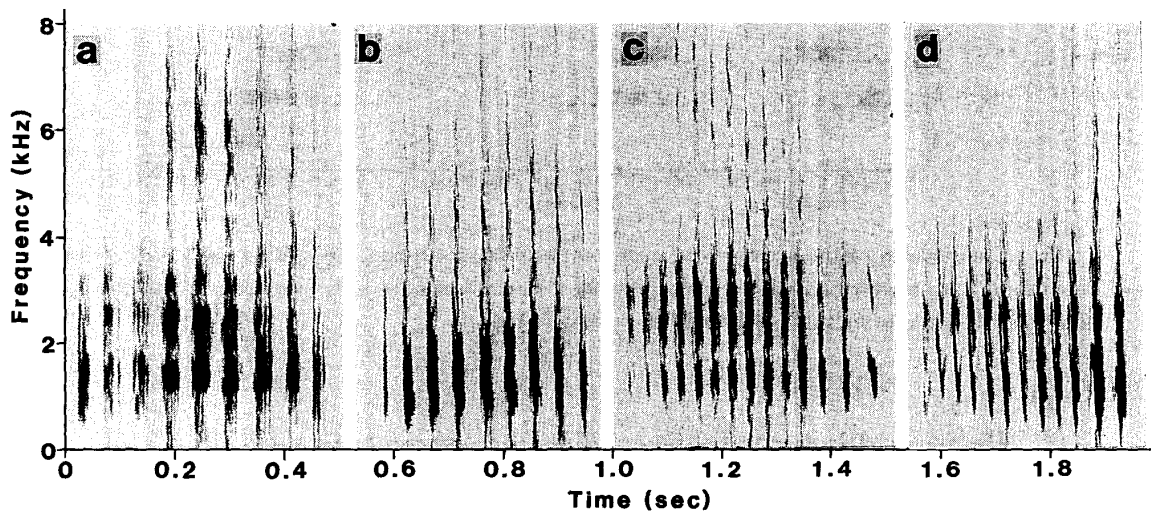


Fig. 14. Sonograms of the mating calls of the reciprocal hybrids between *Rana plancyi chosenuca* from Korea and *Rana nigromaculata* from Hiroshima or *Rana plancyi fukienensis* from Formosa.

- Hybrid between a female *R. nigromaculata* and a male *R. plancyi chosenuca*.
- Hybrid between a female *R. plancyi chosenuca* and a male *R. nigromaculata*.
- Hybrid between a female *R. plancyi fukienensis* and a male *R. plancyi chosenuca*.
- Hybrid between a female *R. plancyi chosenuca* and a male *R. plancyi fukienensis*.

1 × *chos* K, ♂ No. 1, were 0.46 sec, 8.8, 19.2 notes/sec, 30.4 msec, 25.5 msec and 1.26 kHz, respectively, those of the reciprocal hybrids, *chos* K, ♀ No. 1 × *nig* H, ♂ No. 1, were 0.39 sec, 9.0, 23.3 note/sec, 18.3 msec, 30.6 msec and 1.35 kHz, respectively (Table 6). The mean note duration and note interval of the reciprocal hybrids were reduced to under 61% and 55% of those of the Hiroshima *nigromaculata*, respectively. The mean note rate of each reciprocal hybrids was nearly twice as high as that of the Hiroshima *nigromaculata*. The mean number of notes per call of each reciprocal hybrid was over 1.3 times larger than that of the Hiroshima *nigromaculata*. The mean dominant frequency of each reciprocal hybrids was lower than that of the parental species.

Between the mating calls of these reciprocal hybrids, there were significant differences in note rate, note duration and note interval. In the parameters which were significantly different between the parental species, dominant frequencies of

the reciprocal hybrids were lower than those of both parental species, but call durations were intermediate between those of the parental species (Table 6; Fig 15).

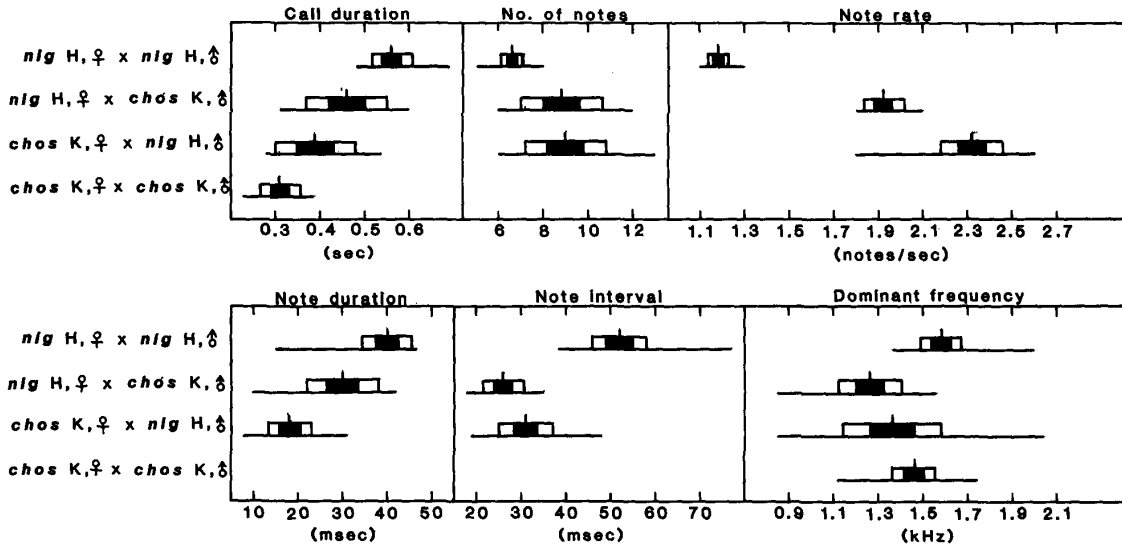


Fig. 15. Comparison of the six kinds of acoustic parameters among the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Hiroshima and *Rana plancyi chosonica* from Korea and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

#### 6. Reciprocal hybrids between *Rana nigromaculata* from Beijing and *Rana plancyi chosonica* from Korea

Reciprocal interspecific hybrids between *R. nigromaculata* from Beijing and *R. plancyi chosonica* from Korea were produced in 1983 by the crossings, *nig* B, ♂ No. 1 × *chos* K, ♂ No. 1 and *chos* K, ♀ No. 1 × *nig* B, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 51.2 mm and 52.4 mm, respectively, were recorded at a temperature of 25.0°C on 18 and 19 July 1986, respectively. Twenty single-calls of each reciprocal hybrid were analyzed.

Both the reciprocal hybrids had only multi-note calls like the hybrids between *R. nigromaculata* from Hiroshima and *R. plancyi chosonica* from Korea (Fig. 16a, b). While the mean values of call durations, numbers of notes per call, note rates, note durations, note intervals, and dominant frequencies of the hybrids, *nig* B, ♀ No. 1 × *chos* K, ♂ No. 1, were 0.65 sec, 16.0, 25.2 notes/sec, 17.5 msec, 25.4 msec, and 1.53 kHz, respectively, those of the reciprocal hybrids, *chos* K, ♀ No. 1 × *nig* B, ♂ No. 1, were 0.55 sec, 13.3, 25.0 notes/sec, 17.4 msec, 23.3 msec, and 1.33 kHz, respectively (Table 6).

There were significant differences in the call duration, number of notes per call and dominant frequency between the reciprocal hybrids. Call duration of each reciprocal hybrid was intermediate between those of the parental species (Table 6; Fig. 17).



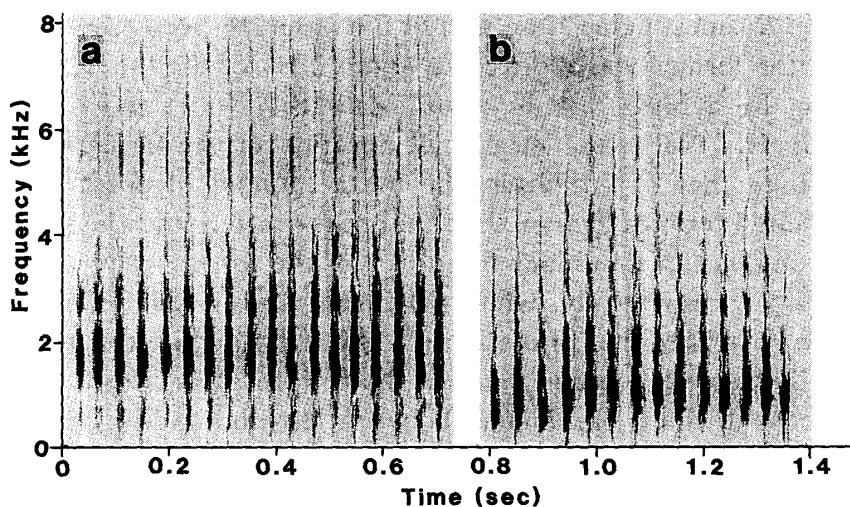


Fig. 16. Sonograms of the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Beijing and *Rana plancyi chosonica* from Korea.

- a. Hybrid between a female *R. nigromaculata* from Beijing and a male *R. plancyi chosonica*.
- b. Hybrid between a female *R. plancyi chosonica* and a male *R. nigromaculata* from Beijing.

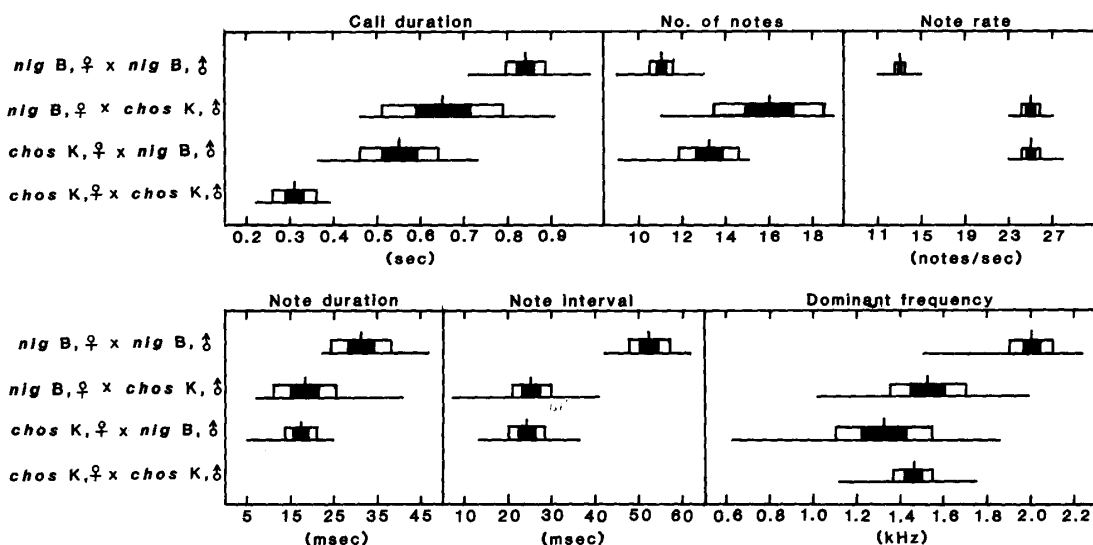


Fig. 17. Comparison of the six kinds of acoustic parameters among the mating calls of the reciprocal hybrids between *Rana nigromaculata* from Beijing and *Rana plancyi chosonica* from Korea and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

Thus, the mating calls of these reciprocal hybrid males were generally similar to those of the reciprocal hybrid males between *R. nigromaculata* from Hiroshima and *R. plancyi chosonica* from Korea, but some differences in call structure were found (Table 6). Comparing the mating calls of the hybrids, *nig B*, ♀ No. 1 × *chos K*, ♂ No. 1, with those of the hybrids, *nig H*, ♀ No. 1 × *chos K*, ♂ No. 1, the mean call duration of the former was about 1.4 times longer than that of the latter, the mean number of notes per call of the former was about two times numerous than that of the latter, and the mean note rate and dominant frequency of the former was

about 1.3 and 1.2 times higher than that of the latter, respectively, while the mean note duration the former was reduced to about 58% of the latter. On the other hand, between the hybrids, *chos* K, ♀ No. 1 × *nig* B, ♂ No. 1, and the hybrids, *chos* K, ♀ No. 1 × *nig* H, ♂ No. 1, the mean call duration of the former was about 1.4 times longer than that of the latter, the mean number of notes per call of the former was about 1.5 times greater than that of the latter, and the mean note rate, note duration and mean dominant frequency of the former were almost equal to those of the latter, respectively, while the mean note interval of the former was reduced to about 76% of that of the latter. All these differences in parameters of the mating calls between the two kinds of the reciprocal hybrids were mainly due to the differences in those parameters between Hiroshima and Beijing *nigromaculata*.

#### 7. Reciprocal hybrids between *Rana plancyi fukienensis* from Formosa and *R. plancyi chosonica* from Korea

Reciprocal intraspecific hybrids between *R. plancyi fukienensis* from Formosa and *R. plancyi chosonica* from Korea were produced in 1985 by the crossings, *fuk* F, ♀ No. 1 × *chos* K, ♂ No. 1 and *chos* K, ♀ No. 1 × *fuk* F, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 46.8 mm and 45.4 mm, respectively, were recorded at a temperature of 25.0°C on 18 and 20 July 1987, respectively. Twenty calls of the former crossing and 18 single-calls of the latter were analyzed, respectively.

TABLE 7

Mating calls of the inter- and intraspecific reciprocal hybrids between pond frog species having continuous calls or multi-note call and two-component calls. These hybrids had multi-note calls consisting of two phases excluding one combination, a female of *R. plancyi fukienensis* and a male of *R. plancyi chosonica*

Kind	Body length (mm)	No. of calls	Call duration (sec)	Duration of first phase (sec)	No. of notes (notes/call)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Dominant frequency (kHz)
<i>fuk</i> F, ♀ No. 1 × <i>chos</i> K, ♂ No. 1	46.8 (46~48)	20	0.48 ± 0.01 (0.40~0.55)	—	15.9 ± 0.5 (11~19)	32.7 ± 0.3 (27~35)	17.2 ± 0.8 (11~34)	14.0 ± 0.5 (10~21)	1.24 ± 0.04 (1.00~2.14)
<i>chos</i> K, ♀ No. 1 × <i>fuk</i> F, ♂ No. 1	45.4 (44~48)	18	0.33 ± 0.01 (0.25~0.44)	0.15 ± 0.01 (0.05~0.22)	11.0 ± 0.5 (8~16)	33.3 ± 0.1 (31~36)	17.6 ± 0.7 (11~25)	13.8 ± 1.5 (0~25)	1.46 ± 0.03 (1.17~2.10)
<i>bre</i> O, ♀ No. 1 × <i>chos</i> K, ♂ No. 1	48.8 (46~52)	20	0.90 ± 0.03 (0.63~1.16)	0.15 ± 0.01 (0.08~0.20)	23.6 ± 0.3 (21~28)	26.4 ± 0.1 (24~28)	22.5 ± 1.0 (14~28)	16.0 ± 1.1 (6~24)	1.90 ± 0.04 (1.35~2.17)
<i>chos</i> K, ♀ No. 1 × <i>bre</i> O, ♂ No. 1	47.5 (46~51)	20	0.59 ± 0.04 (0.29~0.90)	0.19 ± 0.01 (0.13~0.24)	15.2 ± 0.4 (11~21)	25.9 ± 0.2 (24~31)	25.0 ± 1.1 (18~35)	14.5 ± 0.6 (5~19)	1.48 ± 0.02 (1.00~2.04)

The males of both reciprocal hybrids between these two subspecies had only multi-note calls (Fig. 14c, d) like the males of *R. plancyi fukienensis*, though the males of *R. plancyi chosonica* had two-component calls. The calls of the hybrids, *fuk* F, ♀ No. 1 × *chos* K, ♂ No. 1, were composed of about 16 notes and lasted for about 0.48 sec (Table 7). Every note had unclear harmonics without frequency modulation and did not differentiate into two groups (Fig. 14c). The mean dominant frequency of these notes was 1.24 kHz. On the other hand, the mating calls of the reciprocal hybrid, *chos* K, ♀ No. 1 × *fuk* F, ♂ No. 1 lasted for about 0.33 sec and

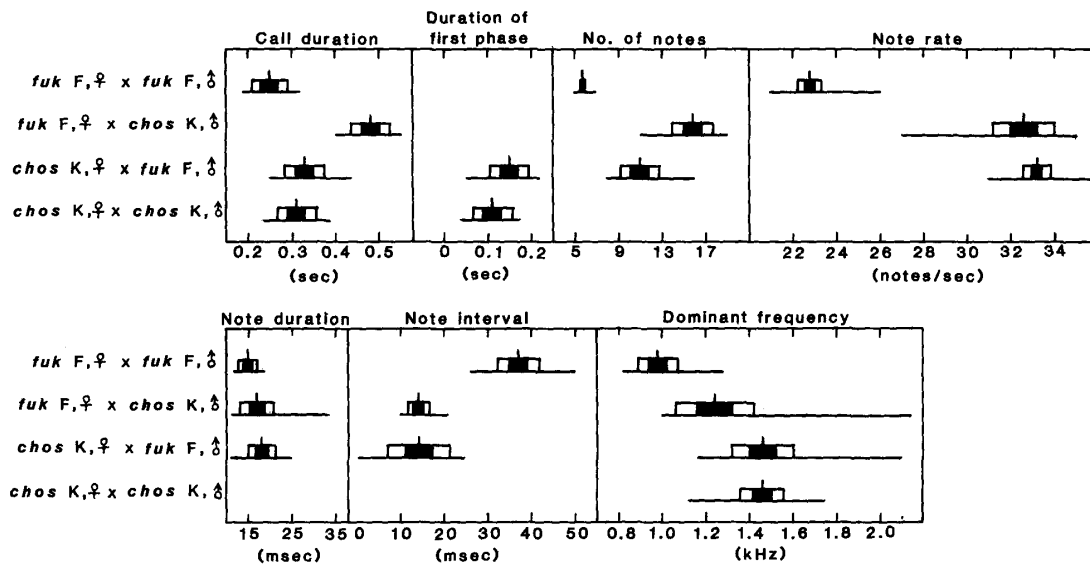


Fig. 18. Comparison of the seven kinds of acoustic parameters among the mating calls of the reciprocal hybrids between *Rana plancyi fukienensis* from Formosa and *Rana plancyi chosonica* from Korea and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

could be divided into two phases (Fig. 14d). The first phase was composed of 2~9 notes which had clearer harmonics with marked frequency modulation and somewhat shorter duration than the notes which constituted the second phase. The mean duration of the first phase was 0.15 sec. The mean number of the notes per call was 11.0 and the mean dominant frequency was 1.46 kHz (Table 7).

Though the call durations of all the other hybrids were always intermediate between those of the parental species, only the reciprocal hybrids between *R. plancyi fukienensis* from Formosa and *R. plancyi chosonica* from Korea had longer call durations than both parental subspecies. The call duration, duration of the first phase, number of the notes per call and dominant frequency were significantly different between the reciprocal hybrids. Of these parameters, dominant frequencies of the reciprocal hybrids were intermediate between those of the parental subspecies, while the maternal effect was emphasized in that parameter (Table 7; Fig. 18).

#### 8. Reciprocal hybrids between *Rana brevipoda brevipoda* from Okayama and *Rana plancyi chosonica* from Korea

Reciprocal interspecific hybrids between *R. b. brevipoda* from Okayama and *R. plancyi chosonica* from Korea were produced in 1983 by the crossings, *bre* O, ♀ No. 1 × *chos* K, ♂ No. 1 and *chos* K, ♀ No. 1 × *bre* O, ♂ No. 1. Mating calls from five males of the former crossing and five males of the latter crossing, whose mean body length was 48.8 mm and 47.5 mm, respectively, were recorded at a temperature of 24.5°C on 19 and 21 June 1986, respectively. Twenty single-calls of each reciprocal hybrid were analyzed.

It was very interesting that though neither males of *R. b. brevipoda* from Okayama and *R. plancyi chosonica* from Korea had multi-note calls, the reciprocal

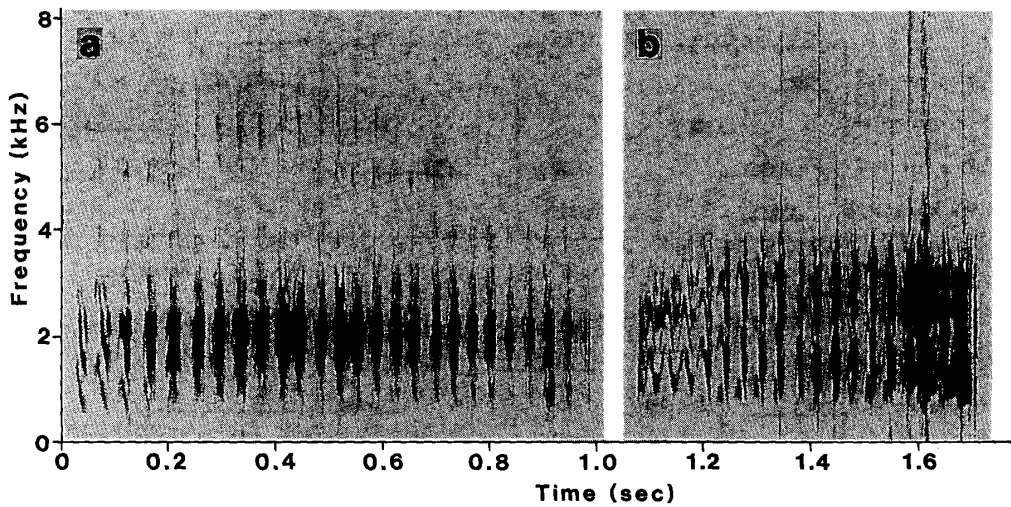


Fig. 19. Sonograms of the mating calls of the reciprocal hybrids between the Typical race of *Rana brevipoda brevipoda* from Okayama and *Rana plancyi chosonica* from Korea.

- Hybrid between a female *R. b. brevipoda* and a male *R. plancyi chosonica*.
- Hybrid between a female *R. plancyi chosonica* and a male *R. b. brevipoda*.

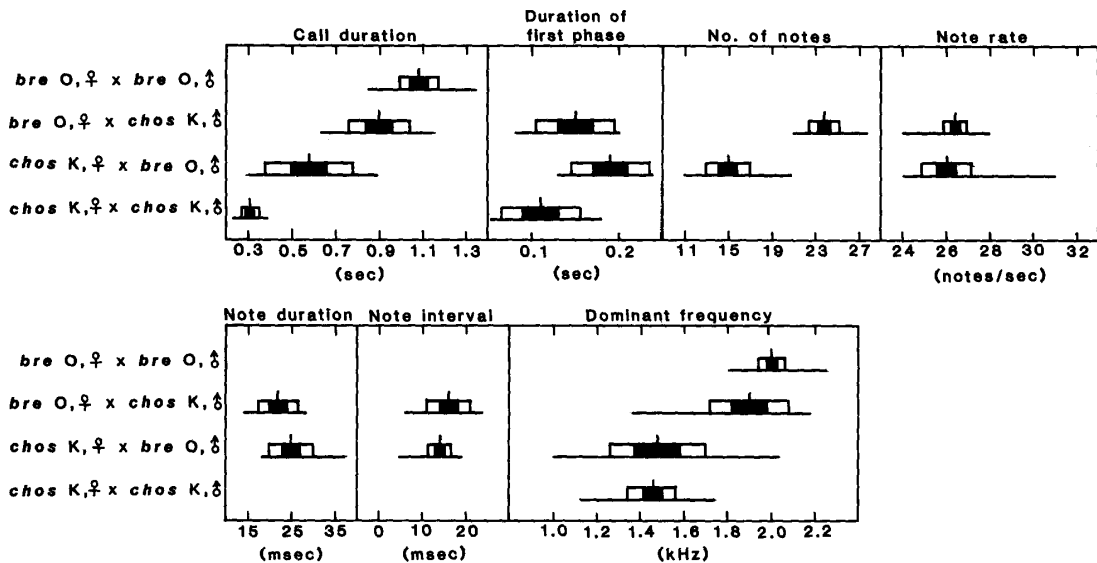


Fig. 20. Comparison of the seven kinds of acoustic parameters among the mating calls of the reciprocal hybrids between the Typical race of *Rana brevipoda brevipoda* from Okayama and *R. plancyi chosonica* from Korea and the controls. The bar graphs follow the method of HUBBS and HUBBS (1953).

hybrids between these two offsprings had only multi-note calls which were composed of two phases (Fig. 19a, b). The first phase consisted of 2~7 notes which had clear harmonics with strong frequency modulation in each reciprocal hybrid, while the second phase consisted of many notes without frequency modulations.

These reciprocal hybrids had considerably different mating calls in structure. The mating calls of the hybrids, *bre* O, ♀ No. 1 × *chos* K, ♂ No. 1, lasted for 0.90 sec on the average, and were composed of about 24 notes whose mean duration was

22.5 msec with mean note interval of 16 msec. Of these calls, the first phase lasted for about 0.15 sec. During that time, 2~5 notes were repeated with distinct note intervals. Each note of the first phase had 3~5 clear harmonics with strong frequency modulation (Table 7; Fig. 19a). The other hand, the mating calls of the reciprocal combination, *chos* K, ♀ No. 1 × *bre* O, ♂ No. 1, lasted for 0.59 sec on the average, and were composed of about 15 notes whose mean duration was 25.0 msec with mean note interval of 14.5 msec. Of these calls, the first phase lasted for about 0.19 sec, and was composed of 3~7 notes, each of which had clear harmonics with strong frequency modulation appearing v-shape or inverted v-shape in the sonagrams (Table 7; Fig. 19b). The note intervals of these phases were so short that the harmonics looked like waves throughout the phases with the harmonics being connected to those of the adjacent notes. Thus, the first phase of this hybrid call resembled the first component of the mating call of *R. p. chosonica* male. It was evident that these reciprocal hybrids had inherited the first phase structure from the first component of the calls of the *R. plancyi chosonica* from Korea offspring, though the maternal effect strongly appeared in this case, too (Fig. 19b).

While the second phases of the mating calls of the hybrids, *bre* O, ♀ No. 1 × *chos* K, ♂ No. 1, lasted for about 0.75 sec, those of the reciprocal combination, *chos* K, ♀ No. 1 × *bre* O, ♂ No. 1, lasted for 0.40 sec. The notes which constituted the second phases of the mating calls of the former hybrids had somewhat clearer harmonics than those of the latter. While the mean dominant frequency of the former hybrids was 1.90 kHz, that of the latter was 1.48 kHz (Table 7). The value of the dominant frequency in each combination was close to that of the maternal species.

The call duration, first phase duration, number of the notes per call, dominant frequency, and structure of the first phase were significantly different between the reciprocal hybrids, and the maternal characters of the call duration, dominant frequency and structure of the first phase were predominantly transmitted to the calls of each reciprocal hybrid (Table 7; Fig. 20).

## DISCUSSION

### 1. Mating calls of the pond frogs distributed in the Far East

Kuramoto (1977) analyzed the mating calls of Japanese pond frogs and stated that the Typical race of *Rana brevipoda brevipoda* from Okayama had long continuous calls, and *R. nigromaculata*, *R. brevipoda porosa* and the Nagoya race of *R. b. brevipoda* had multi-note calls. In this study, it was found that *R. nigromaculata* from Beijing and *R. plancyi fukienensis* from Formosa had multi-note calls, and *R. plancyi chosonica* from Korea and *R. p. plancyi* from Beijing showed a new call type. The mating calls of these two were composed of two components which were distinctly different in structure. Thus, the mating calls of the pond frogs distributed in the Far East could be divided into three types from their call structure: (1) continuous call, (2) multi-note call, and (3) two-component call.

There is a parallelism in the call types between foregoing frog species and the

European water frogs species which belong to the same phyletic line as the former. *R. lessonae* from Firenze, Italy have long continuous calls (UEDA, 1989). The same species from Bonn and Luxembourg, however, have multi-note calls (RADWAN and SCHNEIDER, 1988; UEDA, 1989). *R. ridibunda* (JOERMANN, BARAN and SCHNEIDER, 1988; UEDA, 1989; SCHNEIDER and EGIASARJAN, 1991) and *R. esculenta* (WAHL, 1969; SCHNEIDER, 1990) also have the multi-note calls.

Moreover, the multi-note calls of the Far Eastern frogs should be subdivided into two types: single-call and 2- to 6-successive call, because some acoustic parameters were significantly different between these two types. The call duration, number of notes per call, and note interval of the latter were always reduced, but the note rate was increased. Such a tendency was markedly shown in the succeeding calls of 2- to 6-successive calls. The mean call duration and mean note interval of the succeeding calls of 2-successive calls in Hiroshima and Beijing *R. nigromaculata*, *R. brevipoda porosa*, and *R. plancyi fukienensis* were reduced to 69% and 85%, 60% and 83, 58% and 88%, and 76% and 91% of those of the single-calls, respectively. The mean note rate of those calls, however, increased to 114%, 111%, 117% and 102% of that of the single-calls, respectively.

No postmating reproductive isolating mechanisms between Hiroshima and Beijing *R. nigromaculata* have been confirmed by the hybridization experiments (KAWAMURA and NISHIOKA, unpublished) and the genetic distance between them calculated from 28 loci controlling the blood proteins and allozymes is only 0.158 (NISHIOKA, SUMIDA and OHTANI, 1992). However, these two populations of *R. nigromaculata* had such different mating calls that five of seven acoustic parameters analyzed were significantly different. The mean call duration and number of notes per call of Beijing *R. nigromaculata* was 1.5 and 1.8 times larger than the respective parameters of Hiroshima *R. nigromaculata*, while the mean note duration of the former was 0.8 time shorter than that of the latter. In comparing the mating groups of each population with the same mean body length of 55 mm, the mean dominant frequency of Beijing *R. nigromaculata* was 2.01 kHz, while that of the Hiroshima *R. nigromaculata* was 1.63 kHz. Thus, it is evident that though these two populations of *R. nigromaculata* were closely related from a genetic viewpoint, such as the postmating isolation and genetic distance, their mating calls were markedly specialized.

Formosan *R. plancyi fukienensis* males had multi-note calls differing from its basic species and a subspecies, *R. p. plancyi* and *R. plancyi chosenica*. The latter two had similar call structure. Besides, among the pond frogs species which had multi-note calls, *R. plancyi fukienensis* males showed the shortest call duration, the highest note rate, the narrowest note and the lowest dominant frequency. KAWAMURA and NISHIOKA (1973, 1977, 1979) conducted hybridization experiments among these three subspecies and elucidated that while there are no postmating reproductive isolating mechanisms between *R. p. plancyi* and *R. plancyi chosenica*, there are hybrid sterilities between *R. plancyi fukienensis* and *R. p. plancyi* or *R. plancyi chosenica*. On the basis of these results, KAWAMURA (1985) have proposed that *R. plancyi fukienensis* should be treated as a distinct species. The results of this study

strongly support his proposal from the viewpoint of premating reproductive isolation.

The mating calls of *R. p. plancyi* and *R. plancyi chosenica* were unique. These calls consisted of two components which were distinctly different in structure. While the first component had three to six clear harmonics with wavelike frequency modulation, the second component consisted of fine repetitive pulses without frequency modulation and thus resembled the calls of Typical race of *R. b. brevipoda*. The short curious parts found at the beginning of the calls of the Typical race of *R. b. brevipoda* might correspond to the first components of the calls of the *R. plancyi chosenica*.

The mating calls which are composed of two phases or components have been known in *Eleutherodactylus coqui* (NARINS and CAPRANICA, 1976, 1978; CAPRANICA, 1977), in *Pseudophyrne corroborae* (PENGILLEY, 1971), in *Rhacophorus moltrechti* and *Buergeria japonica* (KURAMOTO, 1986). NARINS and CAPRANICA (1976, 1978) have found that males of *Eleutherodactylus coqui* distributed in Puerto Rico always utter two different notes with short interval. They have suggested that the first note "co" conveys an agonistic message to other males, while the second note "qui" serve to attract females. WELLS (1977) have presented a hypothesis that the elements of the different call types which have separate functions might have been incorporated into a new compound mating calls which convey several messages simultaneously. Playback experiments may elucidate the possible role of each component of the mating calls of *R. p. plancyi* and *R. plancyi chosenica* males.

KURAMOTO (1977) stated that the mating calls of *R. brevipoda porosa* in Niigata and Shizuoka Prefectures and the Nagoya race of *R. b. brevipoda* are intermediate between *R. nigromaculata* and the Typical race of *R. b. brevipoda* from Okayama. In this study, the Nagoya race of *R. b. brevipoda* from Maibara had calls intermediate between *R. nigromaculata* from Hiroshima and the Typical race of *R. b. brevipoda* from Okayama like KURAMOTO's observations, and the call structure of these males resembled that of the reciprocal hybrids between the latter two. The calls of *R. brevipoda porosa* from Saitama, however, did not resemble those of the Nagoya race of *R. b. brevipoda* from Maibara and the hybrids between *R. nigromaculata* from Hiroshima and the Typical race of *R. b. brevipoda* from Okayama but rather resembled those of *R. nigromaculata*.

A similar intraspecific divergence in the call structure is known in the populations of European water frog, *R. lessonae* mentioned above. Italian population has long continuous call like Okayama *R. b. brevipoda* race (UEDA, 1989). The calls of German population (Bonn) are divided into the pulse groups (=note) by inserting special pulses in some pulses, though there are no intervals between the pulse groups (RADWAN and SCHNEIDER, 1988). On the other hand, the population from Luxembourg have multi-note calls with distinct note intervals (UEDA, 1989). Furthermore, SCHNEIDER (1990) have stated that the calls of *R. esculenta* are intermediate between *R. lessonae* and *R. ridibunda*.

KURAMOTO (1977) have surmised that the call structure of *R. nigromaculata* can be derived from that of the Typical race of *R. b. brevipoda*. A sequence of call

structure changes in the local races of *R. b. brevipoda* and *R. lessonae* seems to clearly show that multi-note call may develop from continuous call, which consists of almost homogeneous pulses, by inserting the differentiated pulses or voiceless intervals in some pulses. Thus, it can easily be imagined that the mating call of each pond and water frog species distributed in the Palaearctic region should be differentiated from the call of a common ancestral species. However, their calls were specialized in structure not only interspecifically but also intraspecifically, and the degree of specialization in each species, subspecies or local races was not always parallel with the level of its evolutionary ladder.

LITTLEJOHN (1965) and SCHIØTZ (1973) have stated that both biotic and abiotic components of the environment often affect call structure and its evolution. As all the species treated in this study inhabit the same habitat as the rice paddy, their call divergences seem to be caused by the former components, together with long geographical isolation, and some selective pressure.

## 2. Mating calls of the hybrid males

BLAIR (1956) reported that the males of natural hybrids between *Bufo woodhousei* and *B. americanus* had call structure intermediate between the parental species. There are many other reports that the calls of hybrid are intermediate between the parental species: natural hybrids between *Hyla avivoca* and *H. chrysoscelis* (GERHARDT, 1974), artificial hybrids between *Bombina bombina* ♀ × *B. variegata* ♂ (SCHNEIDER and EICHELBERG, 1974), natural hybrids between *H. arborea* and *H. meridionalis* (OLIBEIRA, PAILLETTE, ROSA and CRESPO, 1991), natural hybrids between *R. pipiens* and *R. blairi* (KRUSE, 1980), and natural hybrids between *B. viridis* and *B. calamita* (SCHLYTER, HOGLUND and STROMBERG, 1991). On the other hand, the natural hybrids between *B. woodhousei* and *B. valliceps* had calls similar to those of *B. valliceps* (BLAIR, 1956) and the natural hybrids between *H. femoralis* and *H. chrysoscelis* had calls similar to those of *H. chrysoscelis* (GERHARDT, 1974). In the case of the natural hybrids between *Geococcyx laevis* and *G. victriana*, these hybrid males had mosaic calls which contained elements characteristic of the parental species (LITTLEJOHN and WATSON, 1976). The natural hybrids between *H. cinerea* and *H. gratiosa* distributed in the southeast of North America are often found, though these species are different in some external characters and ecological habits (MECHAM, 1960; GERHARDT, GUTTMAN and KARLIN, 1980; SCHLEFER, ROMANO, GUTTMAN and RUTH, 1986). MECHAM (1960) stated that mating calls of these natural hybrids were intermediate between the parental species. GERHARDT, GUTTMAN and KARLIN (1980) had treated 12 putative hybrid males and one female and stated that six males showed *gratiosa*-like calls and the other six males showed *cinerea*-like calls. Of the 13 putative hybrids, only two males having *cinerea*-like calls and one female were confirmed as F<sub>1</sub> hybrids by the analyses of their allozymes. The other 10 males were backcrosses. SCHLEFER, ROMANO, GUTTMAN and RUTH (1986) revealed that 38% of the individuals morphologically identified as *H. cinerea* were electrophoretically *H. cinerea* backcrosses. Thus, there are always some problems and confusions in the cases of natural hybrids. Such



problems and confusions may be mainly because that the investigators do not know the parental individuals of the natural hybrids, even though their parental species are known.

The species, subspecies and local races used in this hybridization experiments were preserved from generation to generation in the laboratory. All the hybrids and controls were produced by artificial inseminations and were reared to the adult stage and the recording of their mating calls were made under controlled conditions. Therefore, the sonographical analyses of their calls should permit elucidation of the heredity of the mating calls at acoustic parameter level.

Since the anuran calls are uttered in collaboration with the vocal sacs, vocal chords, lungs, and nervous system controlling the vocalization, the call structure must be determined by many genes (LEROY, 1979). It is conceivable that some acoustic parameters of the mating calls may be controlled by a single gene as LEROY (1979) has surmised. In this study, the inter- and intraspecific hybrid males between the pond frogs whose male had the multi-note call and the continuous call or two-component call, such combinations as *R. nigromaculata* from Hiroshima and *R. b. brevipoda* from Okayama, *R. plancyi fukienensis* and *R. b. brevipoda* from Okayama, *R. nigromaculata* from Hiroshima or Beijing and *R. plancyi chosenica*, and *R. plancyi fukienensis* and *R. plancyi chosenica*, uttered the multi-note call without fail. Similar facts were seen in the reciprocal hybrids among the European water frogs, such combinations as *R. lessonae* from Italy and *R. lessonae* from Luxembourg, and *R. lessonae* from Italy and *R. ridibunda* from Turkey (UEDA, 1989). Thus, it is evident that the note-structure of the mating call is dominant to non note-structure. Ratio of segregation of the note-structured calls and non note-structured calls which will appear in the F<sub>2</sub> offspring or the backcrosses of F<sub>1</sub> hybrids should reveal whether the note structure of mating call is controlled by a single gene or not.

The reciprocal hybrids must have the same genome constitution. It is worthy of special mention that the mating calls of the reciprocal hybrids had many acoustic parameters which were significantly different between the reciprocal combinations. Indeed, 22 (47.8%) of the total 46 acoustic parameters from seven kinds of the reciprocal hybrids were significantly different between the reciprocal combinations. Of the total 21 acoustic parameters which were significantly different between the parental species, four (19%) were specific to the hybrids, and 15 (71.4%) were intermediate between the parental species, while the maternal effects were more or less emphasized in those parameters. Moreover, the maternal effects were emphasized in the other hereditary characters, such as the pulse structure, energy and frequency distribution patterns in the calls of each species and the first component structure of the calls of *R. plancyi chosenica*. As the results, the call structure of the reciprocal hybrids tend to resembled that of the maternal species. These findings suggest that the phenotypic expression of such acoustic parameters and characters should be influenced by some cytoplasmic factors of the eggs. Some other reports that the reciprocal hybrids resemble their maternal species in external and physiological characters have been presented (UEDA, 1977;

KAWAMURA, NISHIOKA and UEDA, 1980).

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