Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. 12: 177–189 (1993)

Mating calls of autotriploid and autotetraploid males in *Hyla japonica*

By

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ABSTRACT

The calls of artificial autotriploid and autotetraploid males as well as diploid males of *Hyla japonica* were recorded at 24±0.5°C in the laboratory and their structure was sonagraphically analyzed. Note duration, note interval and pulse rate were slightly affected with increasing ploidy, while call duration, note rate and spectrum of frequency were not affected. The mean note duration of the calls of diploid, autotriploid and autotetraploid males was 79 msec, 64 msec, and 59 msec, respectively. The mean note intervals of solo and duet calls were 162 msec and 205 msec, 175 msec and 220 msec, and 183 msec and 231 msec in the diploid, autotriploid and autotetraploid males, respectively. The mean pulse rate of the diploid, autotriploid, and autotetraploid calls was 256 pulses/sec, 226 pulses/sec, and 204 pulses/sec, respectively. All the acoustic parameters, including ploidy correlated parameters, varied at a wide range which overlapped to a large extent with those of other ploidies.

INTRODUCTION

From about 20 years ago, many polyploid species or populations have been discovered in various areas of the world (KAWAMURA, 1984). Among these, the American common treefrog, *Hyla versicolor,* is a well-known tetraploid species and the cryptic species, *H. chrysoscelis,* is a diploid one (WASSERMAN, 1970; BOGART and WASSERMAN, 1972; RALIN, 1977; DANZMANN and BOGART, 1982). The morphological characters of these two species are exactly similar to each other, but *H. chrysoscelis* has a mating call whose pulse rate is nearly twice higher than that of *H. versicolor* (BLAIR, 1958; JOHNSON, 1959; RALIN, 1968; PIERCE and RALIN, 1972; BOGART and WASSERMAN 1972; RALIN, 1977; JASLOW and VOGT, 1977; LITTLE, MONROE and WILEY, 1989). LITTLEJOHN, FOUQUETTE and JOHNSON (1960) have reported that the females of both species can choose the males of their own call type.

Some discussion on the origin of tetraploidy of *H. versicolor* has been made (BOGART and WASSERMAN, 1972; RALIN, 1977; RALIN and SELANDER, 1979; RALIN, ROMANO and KILPATRICK, 1983). However, it may be extremely difficult to elucidate the true genome-constitution of the natural polyploid species (BOGART and WASSERMAN, 1972) and also to determine their ancestral diploid species.

In the present paper, the differences in mating call structure among diploid, autotriploids and autotetraploids in Japanese *Hyla* were elucidated and compared with those between the diploids and tetraploids in the American *Hyla*.

Preliminary findings of this study have been reported by UEDA (1986).

MATERIALS AND METHODS

Autotriploid and autotetraploid *Hyla japonica* males used in this study were artificially produced by NISHIOKA and UEDA (1983, Tables 14 and 15). In 1979, they obtained 110 autotriploid tadpoles by suppressing extrusion of the second polar body by refrigerating fertilized 610 eggs from three females and a male. Of the autotriploid tadpoles, 94 metamorphosed and 76 attained sexual maturity in the following year. In 1980, they found 115 autotetraploid tadpoles in the offspring between three autotriploid females and three control diploid males. Of the autotetraploid tadpoles, 89 metamorphosed and 64 attained sexual maturity. Ten autotriploid males used for recording the mating calls were F_2 offspring between two F_1 autotetraploid females and the two diploid males. Five and five tetraploid males used were F_2 and F_3 offspring, respectively, produced by brother and sister matings. Eleven diploid males used were F_1 offspring between two diploid females and males which were of the control series for producing the autotriploid and autotetraploids.

Before the recording, the males were injected with pituitary suspension of *Rana catesbeiana* (a half of a pituitary gland per male) and kept in a sufficiently damp container at 24±0.5°C in the dark. They began to call without fail 3~6 hours

TABLE 1

Diploid, autotriploid and autotetraploid males of *Hyla japonica* whose mating calls were analyzed

Ploidy	Origin of males	No. of males	Age (years)	Body length (mm)	Temperature (°C)
2n	Field-caught	10	?	29.4 (28.0~31.0)	
	Recorded in the field				20.5
	Recorded in the laboratory				24.0±0.5
2n	2n 80♀, No. 4×2n 80♂, No. 4	6	3	35.8 (35.0~37.0)	24.0±0.5
	2n 80♀, No. 5×2n 80♂, No. 4	5	3	35.0 (34.0~36.0)	24.0±0.5
3n	4n 81♀, No. 1×2n 80♂, No. 4	5	3	39.2 (38.0~41.0)	24.0±0.5
	4n 81♀, No. 2×2n 80♂, No. 5	5	3	35.8 (35.0~37.0)	24.0±0.5
4n	4n 81♀, No. 1×4n 81♂, No. 1	5	3	41.0 (40.5~42.0)	24.0±0.5
	4n 82♀, No. 1×4n 82♂, No. 1	5	1	33.2 (29.5~34.0)	24.0±0.5

( ), Range

thereafter. The container used was 65 cm×43 cm×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. One to 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 23, 1986, the mating calls of 10 wild males were recorded in the breeding field, located in the suburbs of Hiroshima, at an air temperature of 20.5°C. On the following day, these males were treated using the same methods as mentioned above and their calls were recorded in the laboratory. The origin, number, age and body length of the used males and air temperature at the recording times are presented in Table 1.

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 six different acoustic parameters: call duration, note rate (notes/sec), note duration, note interval, pulse rate (pulses/sec), and spectrum of frequency.

OBSERVATION

A call of a single male (solo call) is the most useful for sonagraphical analysis, but *H. japonica* mostly call in chorus in the breeding fields. It is conceivable that the call structure of a solo call is different from that of a chorus. Detailed observations of the chorus formation in this species showed the following facts. When the first male of a group of males standing adjacent to each other began to call, and the others followed him, each note of the second male was generally inserted at almost the midpoint of the note interval of the first male by widening

TABLE 2

Acoustic parameters of the solo calls in diploid, autotriploid and autotetraploid males of *Hyla japonica*

Ploidy	Origin of males	No. of calls	Call duration (sec)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Pulse rate (pulses/sec)	Frequency (kHz)	
								Fundamental	Dominant
2n	Field-caught	20	15.5±1.1 (9.4~34.1)	4.5±0.1 (4.1~5.0)	81.8±1.0 (64.6~106.6)	155.8±2.1 (121.6~194.3)	258.7±2.3 (201.9~327.9)	1.51±0.02 (1.43~1.54)	3.10±0.07 (2.82~3.30)
2n	2n 80♀, No. 4 ×2n 80♂, No. 4	20	11.1±1.4 (5.6~18.2)	4.5±0.1 (4.0~4.7)	77.0±1.5 (63.7~106.7)	162.2±2.8 (128.6~225.1)	252.3±2.9 (205.1~326.0)	1.27±0.03 (1.19~1.39)	2.53±0.05 (2.38~2.69)
	2n 80♀, No. 5 ×2n 80♂, No. 4	20	13.0±1.8 (8.2~31.0)	4.5±0.1 (4.2~4.6)	76.7±1.2 (68.5~107.5)	168.0±1.3 (129.8~196.7)	254.6±2.8 (216.1~357.3)	1.34±0.03 (1.16~1.35)	2.63±0.04 (2.33~2.69)
3n	4n 81♀, No. 1 ×2n 80♂, No. 4	18	11.1±1.5 (5.0~18.5)	4.6±0.1 (4.4~5.1)	60.6±1.0 (49.9~77.4)	176.8±1.9 (148.7~230.6)	226.1±1.5 (192.7~258.7)	1.16±0.01 (1.13~1.24)	2.33±0.04 (2.12~2.49)
	4n 81♀, No. 2 ×2n 80♂, No. 5	20	11.9±0.7 (7.2~21.0)	4.5±0.1 (4.1~4.9)	68.0±0.6 (50.4~86.4)	173.7±2.3 (146.3~221.0)	223.5±1.9 (158.6~263.1)	1.23±0.02 (1.15~1.34)	2.54±0.04 (2.42~2.72)
4n	4n 81♀, No. 1 ×4n 81♂, No. 1	20	18.3±1.5 (10.1~29.4)	4.6±0.1 (4.3~5.1)	57.9±0.5 (43.9~70.4)	186.7±1.8 (152.4~236.9)	198.2±1.1 (158.7~224.8)	1.13±0.03 (1.93~1.28)	2.25±0.05 (2.02~2.54)
	4n 82♀, No. 1 ×4n 82♂, No. 1	17	7.0±1.9 (5.6~20.0)	4.5±0.1 (4.2~4.8)	61.5±0.2 (49.3~72.6)	178.8±1.2 (153.0~224.5)	215.1±2.0 (152.3~228.2)	1.41±0.03 (1.40~1.52)	2.86±0.05 (2.63~2.97)

TABLE 3

Acoustic parameters of the duet or chorus calls in diploid, autotriploid and autotetraploid males of *Hyla japonica*

Ploidy	Origin of males	No. of calls	Call duration (sec)	Note rate (notes/sec)	Note duration (msec)	Note interval (msec)	Pulse rate (pulses/sec)	Frequency (kHz)	
								Fundamental	Dominant
2n	Field-caught Recorded in the field	20 C	14.3±2.2 (9.0~31.0)	3.6±0.1 (3.1~4.1)	92.3±1.4 (69.2~129.4)	214.4±3.4 (158.5~285.2)	175.8±1.7 (139.1~218.2)	1.49±0.03 (1.38~1.64)	3.07±0.05 (2.65~3.14)
	Recorded in the laboratory	14 D	16.8±1.9 (9.0~28.5)	3.9±0.1 (3.6~4.1)	82.4±1.2 (66.1~103.4)	202.3±3.6 (195.1~237.8)	254.5±3.7 (203.1~316.5)	1.52±0.05 (1.40~1.55)	3.10±0.07 (2.96~3.25)
2n	2n 80♀, No. 4 ×2n 80♂, No. 4	12 D	14.8±2.5 (7.6~22.6)	3.8±0.1 (3.5~4.0)	75.7±1.6 (61.2~107.2)	208.2±4.2 (183.0~242.1)	264.4±3.8 (219.3~322.8)	1.30±0.04 (1.19~1.36)	2.57±0.05 (2.35~2.74)
3n	4n 81♀, No. 1 ×2n 80♂, No. 4	10 D	16.5±1.4 (8.0~25.5)	4.0±0.1 (3.8~4.1)	62.6±1.0 (47.8~75.5)	219.7±1.5 (192.9~253.4)	230.1±2.0 (191.0~259.3)	1.17±0.03 (1.08~1.21)	2.30±0.04 (2.08~2.45)
4n	4n 81♀, No. 1 ×4n 81♂, No. 1	10 D	19.0±1.7 (13.5~30.0)	3.9±0.1 (3.7~4.1)	57.8±0.7 (42.1~72.6)	231.3±2.2 (198.3~278.9)	198.6±1.8 (145.2~231.0)	1.15±0.03 (1.02~1.24)	2.32±0.07 (2.23~2.72)

C, Chorus call D, Duet call

the width of the note interval (Fig. 1b). When the third and fourth males joined in the duet, their notes generally overlapped on the notes of either the first or second male. Roughly speaking, a chorus of this species corresponded to a troll of two kinds of notes. Then both solo and duet were analyzed in diploid, autotriploid, and autotetraploid males. While the solo calls were analyzed in two mating groups, the duet calls were analyzed in one mating group. The results are presented in Tables 2 and 3.

The basic call structure of the wild males recorded with pituitary stimulation in the laboratory was not different from that of the same males recorded in the field (Tables 2 and 3).

1. Call duration

A call was formed by repetitive reciprocal movement between the lungs and vocal sac of the air that had been once inhaled in the respiratory organs. The notes were uttered whenever the air inhaled in the lungs was exhaled to the vocal sac.

The durations of both solo and duet calls varied at a wide range, but they were not influenced by increasing ploidy (Tables 2 and 3; Fig. 4). The solo calls lasted for 5.0~34.1 sec, 12.7 sec on the average, in all males used, while the duet calls lasted for 7.6~30.0 sec, 16.7 sec on the average. The differences in the call duration between duet calls and solo calls were statistically significant in each ploidy (Fig. 4). The results showed that duet calls were slightly longer than solo calls.

During the calling time, 21~158 notes, 57 notes on the average, continued at a regular interval in solo calls, while 29~121 notes, 65 notes on the average, continued in duet calls.

2. Note rate (number of notes per second)

The mean number of notes which appeared in a standard sonagram (2.56 sec) of solo calls was 11.5, 11.6, and 11.7 in the diploid, autotriploid, and autotetraploid males, respectively (Fig. 2), while that of duet calls was 9.8, 10.0, and 9.9 in the diploid, autotriploid, and autotetraploid males, respectively (Fig. 1). The results showed that there were no significant differences in the number of notes of both solo calls and duet calls among the three kinds of ploidies and that the duet call had a smaller number of notes than the solo call in each ploidy. Consequently, among the three kinds of ploidies, there were no significant differences in the note rates of both solo calls and duet calls at the same temperature, while between solo calls and duet calls, the note rate of the latter was lower than that of the former in each ploidy (Fig. 4). In all males used, the note rates of solo calls were 4.0~5.1, 4.5 on the average, and those of duet calls were 3.1~4.1, 3.9 on the average (Tables 2 and 3).

A comparison between the chorus calls of the wild males at 20.5°C and the duet calls of the same males at 24±0.5°C indicated that the note rate fairly increased with increasing temperature (Tables 1 and 3).

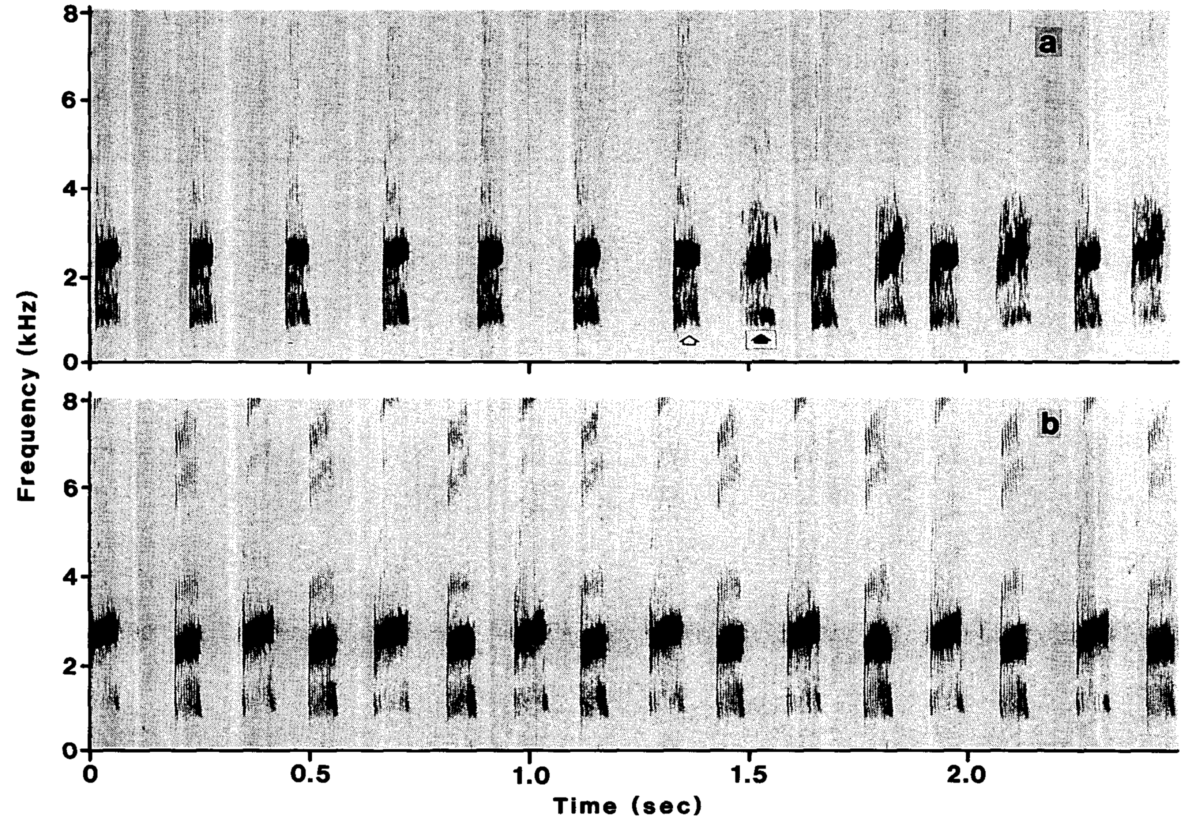

Fig. 1. Sonagrams of two duets recorded at 24±0.5°C.

a. The duet formation in autotetraploid males. The note interval of a preceding male were widened by insertion of the notes (solid arrow) of a succeeding male. Hollow arrow shows a note of a preceding male.

b. Two duet calls emitted by two autotetraploid males. The notes of a succeeding male were usually situated at almost the midpoints of the note intervals of a preceding male.

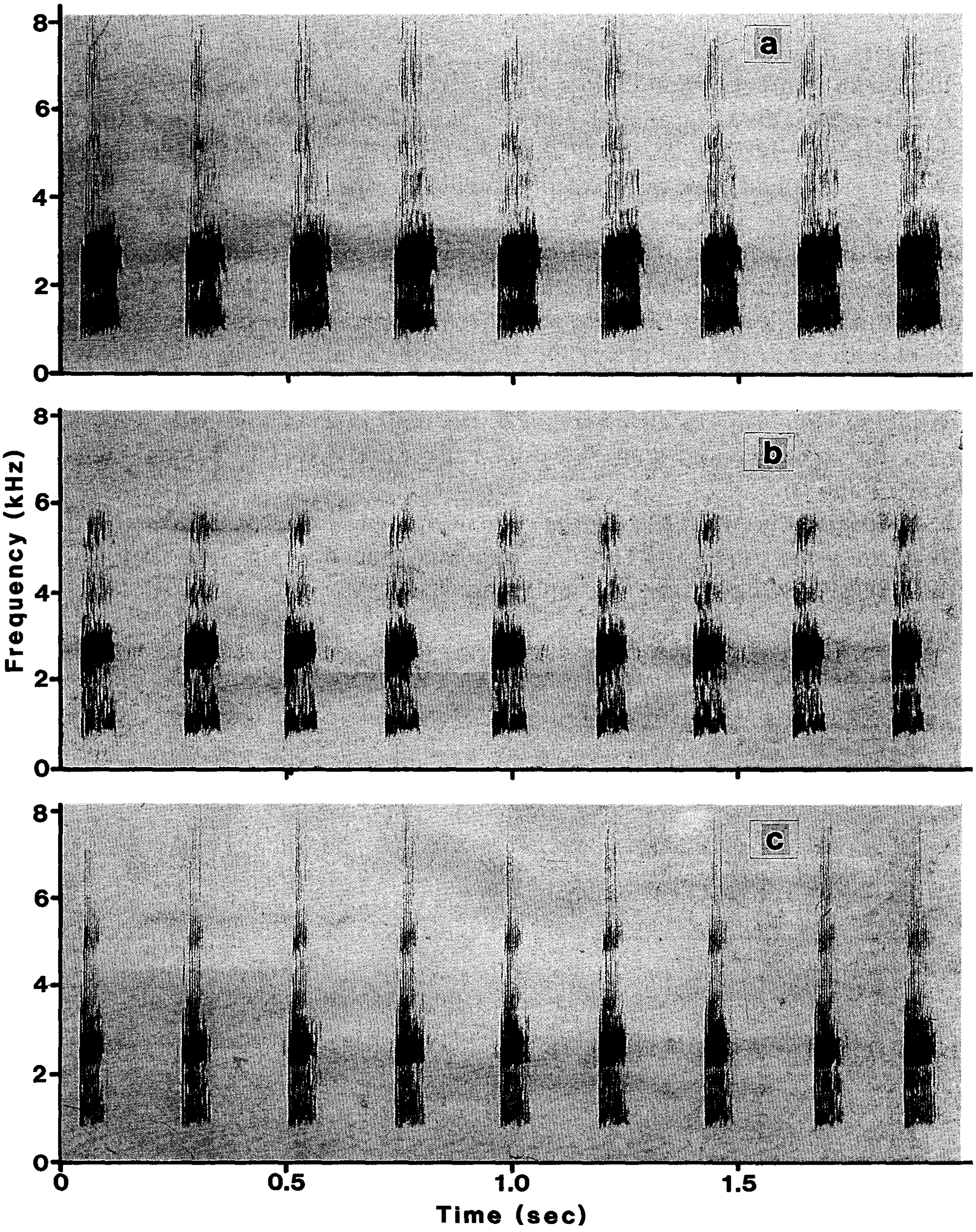

Fig. 2. Sonagrams of the mating calls at 24±0.5°C in diploid (a), autotriploid (b), and autotetraploid (c) males.

3. Note duration

The note durations of solo calls were not significantly different from those of duet calls in every ploidies (Fig. 4). The mean note duration calculated from solo calls and duet calls was 78.7 msec, 64.1 msec, and 59.2 msec in diploid, autotriploid, and autotetraploid males, respectively (Tables 2 and 3; Fig. 3). These differences in mean note duration among the three kinds of ploidies were statistically significant. Consequently, it may be safe to say that the higher ploid males tend to emit a shorter note in both solo and duet calls at the same temperature (Fig. 3).

A comparison between the chorus calls of the wild males at 20.5°C and the duet calls of the same males at 24±0.5°C indicated that the note duration became shorter with increasing temperature (Tables 1 and 3). When the note duration was compared between the two mating groups whose body lengths were different, the larger males had a shorter note duration in every ploidies (Tables 1 and 2).

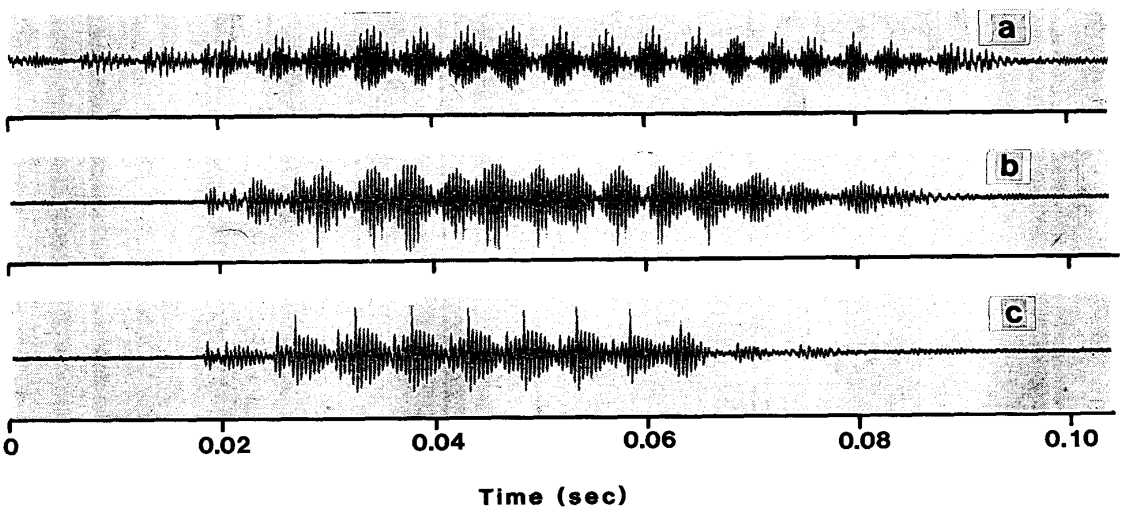

Fig. 3. Waveforms of the single notes at 24±0.5°C in diploid (a), autotriploid (b), and autotetraploid (c) males.

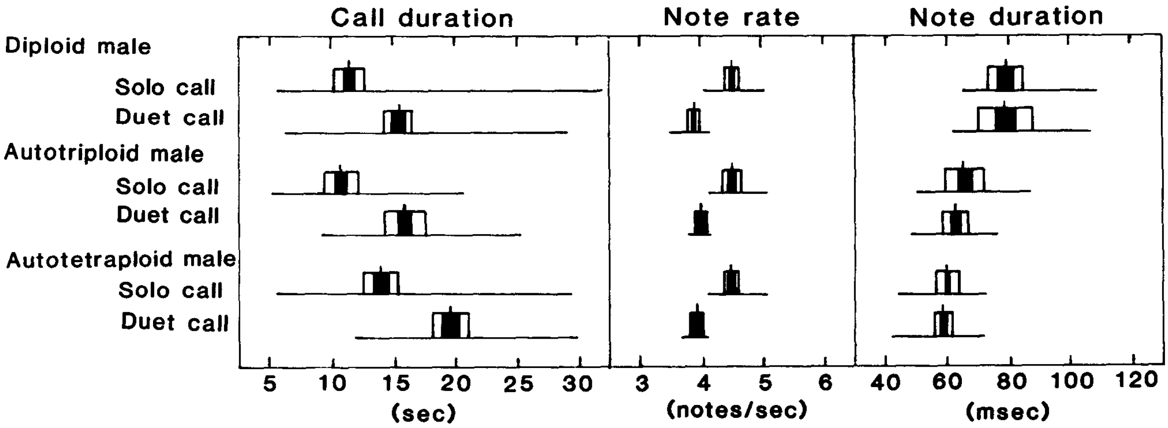

Fig. 4. Comparison of the call durations, note rates, and note durations among diploid, autotriploid, and autotetraploid males. Each recording was made at 24±0.5°C. The bar graphs follow the method of Hubbs and Hubbs (1953).

4. Note interval

Note interval was essentially a different parameter between solo and duet calls. While the mean note interval of solo calls in diploid, autotriploid, and autotetraploid males at 24±0.5°C was 162.0 msec, 175.2 msec and 183.1 msec, respectively, that of duet calls in diploid, autotriploid, and autotetraploid males was 205.0 msec, 219.7 msec, and 231.3 msec, respectively (Tables 2 and 3). These results showed that the mean note interval of duet calls was longer than that of solo calls by about 1.3 times in each ploidy.

It was very interesting that in a duet formed when the succeeding male joined in a solo call which had been emitting by the preceding male, the latter lengthened his note intervals just after the first note of the former (Fig. 1a). It was also interesting that the succeeding male began to emit the first note at a regular interval to the note of the preceding male. When two males began to call at the same or almost same time, the first three or more notes were in confusion, but immediately thereafter, the notes of one male staggered at a regular interval to those of the other male and then a normal duet was formed.

The differences in mean note intervals of both solo and duet calls among the three kinds of males in ploidy were statistically significant (Fig. 5). Such an increase of note interval in the higher ploid males seemed to be mainly due to the decrease in note duration of the higher ploid males as mentioned previously.

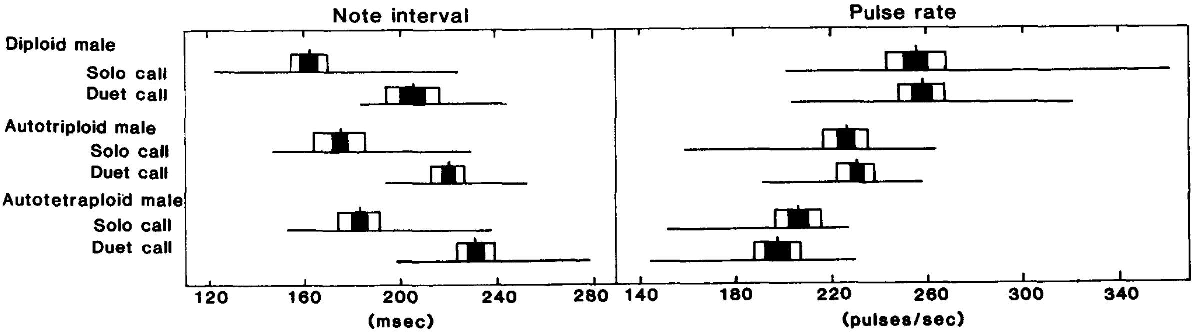

Fig. 5. Comparison of the note intervals and pulse rates among diploid, autotriploid, and autotetraploid males. Each recording was made at 24±0.5°C. The bar graphs follow the method of Hubbs and Hubbs (1953).

A comparison between the chorus calls of the wild males at 20.5°C and the duet calls of the same males at 24±0.5°C indicated that the note interval became shorter with increasing temperature (Tables 1 and 3). When the note interval was compared between the two mating groups whose body length was different, the larger males showed the somewhat longer note interval than the smaller males in each ploidy (Tables 1 and 2).

5. Pulse rate (number of pulses per second)

Each note emitted by the diploid, autotriploid, and autotetraploid males at 24±0.5°C was composed of about 20, 16, and 13 pulses, respectively. The mean pulse rate of solo calls was not significantly different from that of duet calls in each ploidy (Fig. 5). The mean pulse rate calculated from both solo and duet calls was 256.4, 225.9, and 204.4 in diploid, autotriploid, and autotetraploid males, respectively (Tables 2 and 3). These differences in mean pulse rate among the ploidies were statistically significant. The higher ploid males tended to have somewhat lower pulse rates, though the ranges of the pulse rates of every ploid males were too wide to overlap with each other.

The pulse rate of chorus call of the wild males at 20.5°C varied from 139.1 to 218.2 with a mean of 175.8, while those of duet calls of the same males at 24±0.5°C varied from 203.1 to 316.5 with a mean of 254.5 (Table 3). These results showed that the pulse rate was considerably increased with increasing temperature. One-year-old autotetraploid males (4n 82♀,No. 1×4n 82♂,No. 1) which were the smallest in body length among the laboratory raised males including the diploids and the autotriploids had a mean pulse rate of 215.1, while three-year-old autotetraploid males (4n 81♀,No. 1×4n 81♂,No. 1) which were the largest in body length among them had a mean pulse rate of 198.2 (Table 2). In diploid and autotriploid males, no effect of body length could be found.

6. Frequency

Each note of both solo and duet calls had six harmonic bands up to 8 kHz. The first band contained a fundamental frequency ranging from 1.02 kHz to 1.64 kHz and the second band contained a dominant frequency ranging from 2.02 kHz to 3.30 kHz (Figs. 2 and 6). The other bands were generally weak in energy distribution so that two of them often disappeared in the sonagrams. Both fundamental and dominant frequencies were not influenced with increasing ploidy and air temperature (Tables 1, 2 and 3) but were greatly influenced by body length of the males (Fig. 7). The larger males had a lower frequency in both fundamental and dominant frequencies.

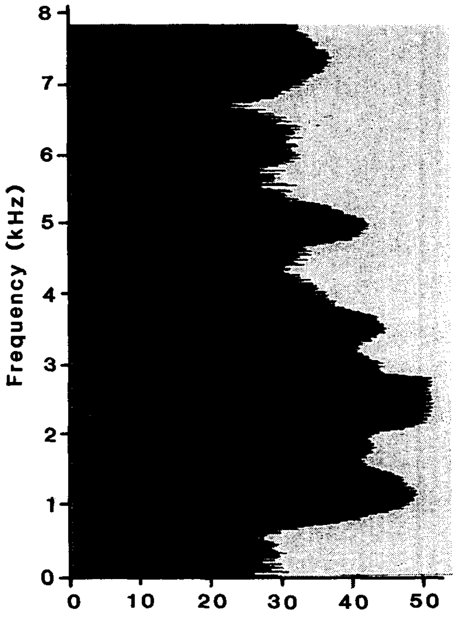

Fig. 6. A spectrographic section of the energy distribution in the middle of a note of an autotetraploid male.

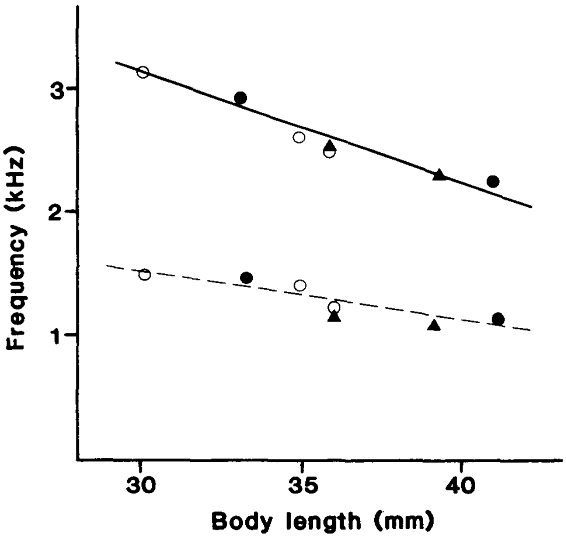

Fig. 7. Effect of male body lengths upon fundamental (dashed line) and dominant (straight line) frequencies in diploid (hollow circles), autotriploid (solid triangles), and autotetraploid (solid circles) males. Each recording was made at 24±0.5°C.

DISCUSSION

Artificial autotriploid anurans have been sexually matured in *Rana nigromaculata* (Kawamura, 1941, 1951; Nishioka, 1971; Kawamura, Nishioka and Okumoto, 1983), in *R. brevipoda* (Nishioka, 1971; Kawamura, Nishioka and Okumoto, 1983), in *Bombina orientalis* (Ueda, 1980), in *Hyla arborea japonica* (=*Hyla japonica*) (Nishioka and Ueda, 1983), in *Bufo japonicus miyakonis* (Nishioka and Ueda, 1988) and in *Rana rugosa* (Kashiwagi, 1993). On the other hand, artificial autotetraploid anurans have been sexually matured in *Xenopus laevis* (Gurdon, 1959), in *R. japonica* (Kawamura, Nishioka and Myorei, 1963), in *R. nigromaculata* (Kawamura and Nishioka, 1963, 1983), in *H. arborea japonica* (Nishioka and Ueda, 1983), and in *B. japonicus miyakonis* (Nishioka and Ueda, 1988). These autopolyploid anurans have been studied for viability, morphology, sex, and reproductive capacity, but not for mating calls. In the present study, the mating calls of artificial autotriploid and autotetraploid males and the control diploid males of *H. japonica,* together with wild males collected from the field, were sonagraphically analyzed. Every analyzed calls were recorded at 24±0.5°C in the laboratory except for the chorus calls of wild males which were recorded at 20.5°C in the breeding site. As the autotriploid, autotetraploid and the control diploid males used were F_1, F_2 or F_3 offspring produced by brother and sister matings, they should be comparatively uniform in genes.

H. japonica males usually call in chorus throughout the active breeding season. The sonagrams of the duet calls showed that each note of the succeeding call was situated almost at the midpoint of note interval of the preceding call. When solo calls were compared with duet calls or chorus calls, it became evident that the note intervals of the latter were always longer than those of the former, though no difference in note structure could be found between them.

The sonagraphical analyses of the mating calls in diploid, autotriploid and autotetraploid males showed that the note duration, note interval and pulse rate were actually affected with increasing ploidy, while the call duration, note rate and spectrum of frequency were not affected. The mean note duration calculated from solo and duet calls was 79 msec, 64 msec, and 59 msec in diploid, autotriploid, and autotetraploid males, respectively. While the mean note interval of solo calls was 162 msec, 175 msec, and 183 msec in diploid, autotriploid, and autotetraploid males, respectively, and that of duet calls was 205 msec, 220 msec and 231 msec in diploid, autotriploid, and autotetraploid males, respectively. It was found that the mean note interval of duet calls was always longer than that of solo calls by about 1.3 times in each ploidy. Consequently, the mean note rate of duet calls was reduced to about 86% of that of solo calls. The mean pulse rate calculated from solo and duet calls was 256, 226, and 204 in diploid, autotriploid, and autotetraploid males, respectively.

It was noteworthy that every acoustic parameters, including ploidy correlated parameters, varied at a wide range, which overlapped to a large extent with those of other ploidies. In this species, the ploidy correlated acoustic parameters became apparent only when the mean values were statistically calculated. Fouquette (1960, 1975) have suggested that acoustic parameters of high variability which overlapped in range were not useful in discrimination. It seemed likely that *H. japonica* female could not discriminate their own ploid male with the mating calls.

The present author (unpublished) also analyzed the mating calls of artificial autotriploid and autotetraploid males together with diploid males in *Rana nigromaculata.* In this species, call duration, note duration, and pulse rate tended to decrease with increasing ploidy, though these parameters varied with a wide range which overlapped to a large extent with those of other ploidies, as found in *H. japonica.* On the other hand, note rate, note interval, and spectrum of frequency were not significantly different among the three kinds of ploidies. The effects of increasing ploidy on note rate, note duration, pulse rate and spectrum of frequency were common to *H. japonica* and *R. nigromaculata,* but those on note interval and call duration were characteristic in species. These differences between the two species seemed to be caused by the different processes of call formation. When the air inhaled in the lungs was once exhaled to vocal sacs, a call which was composed of five to eight notes was formed in *R. nigromaculata,* but only one note was uttered in *H. japonica.* In the latter, a call was formed by repetitive reciprocal movement of air between the lungs and vocal sac by the same times as the note number.

Diploid-tetraploid cryptic species, *H. chrysoscelis* ($2n=24$) and *H. versicolor* ($4n=48$) (Wasserman, 1970; Bogart and Wasserman, 1972; Marion and Bogart, 1978), distributed widely in the eastern half of the United States were morphologically indistinguishable (Johnson, 1966; Bogart and Wasserman, 1972). The males of the two species had mating calls that differed markedly in pulse rate (Blair, 1958; Johnson, 1966; Ralin, 1968; Brown and Brown, 1972; Piece and Ralin, 1972; Ralin, 1977; Little, Monroe and Wiley, 1989). Ralin (1977) who compared the mating calls of the two species distributed in Texas, Oklahoma, Mississippi, North Carolina, and New York observed that the mean pulse rate of *H. chrysoscelis* was higher than that of *H. versicolor* by 1.8 times at 21°C. The pulse rate ratio of *H. chrysoscelis* per *H. versicolor* distributed in Wisconsin was 2.3 at 23°C (Jaslow and Vogt, 1977), 2.2 at 22°C in Illinois (Brown and Brown, 1972) and 2.3 at 22°C in West Virginia (Little, Monroe and Wiley, 1989). Differing from *H. japonica,* the ranges of the pulse rates of the two species did not overlap in all populations when the calls are recorded at the same temperature (Ralin, 1977; Jaslow and Vogt, 1977). All the other acoustic parameters of the two species, however, overlapped to a large extent (Ralin, 1968, 1977). Bogart and Wasserman (1972) compared the mating calls of diploid and tetraploid populations in *Odntophyrnus americanus* and showed that the pulse rate of tetraploid males was reduced. However, diploid per tetraploid pulse rate ratio was unknown. In the present study, diploid per autotetraploid pulse rate ratio was only 1.26. In *Rana nigromaculata,* the mean pulse rate at 25°C was 156, 136, and 129 in diploid, triploid, and tetraploid males, respectively (unpublished). Consequently, diploid per tetraploid pulse rate ratio was 1.21.

Why are there differences in pulse rate ratio of diploid per tetraploid between *H. versicolor* complex and *H. japonica* or *R. nigromaculata*? Here, we must resolve the problem whether *H. versicolor* is autotetraploid or allotetraploid. Based on the results from albumin immunological experiments, MAXSON, PEPPER and MAXSON (1977) stated that *H. versicolor* arose from the hybrid between the eastern and western *H. chrysoscelis* which had specialized to distinct species. Therefore, *H. versicolor* may be an allotetraploid. Allotetraploidy of *H. versicolor* seems to be supported by the observations by RALIN (1968) that the males of *H. versicolor* and *H. chrysoscelis* have different calling positions and food habits. However, RALIN, ROMANO and KILPATRICK (1983) compared allele frequencies of 12 enzyme loci of *H. versicolor* and *H. chrysoscelis* and proposed that *H. versicolor* had a single origin from the central populations of *H. chrysoscelis* during the Sangamon interglacial period. They insisted on an assumption of autotetraploidy of *Hyla versicolor*.

UEDA (unpublished) analyzed the mating calls of four kinds of amphidiploid (allotetraploid) males which are composed of two genomes of *R. nigromaculata* and two genomes of *R. brevipoda,* two genomes of *R. brevipoda* and two genomes of *R. plancy fukienensis,* two genomes of *R. plancy chosenics* and two genomes of *R. brevipoda,* and two genomes of *R. ridibunda* and two genomes of *R. lessonae.* The acoustic parameters of these amphidiploid males were intermediate between the two original diploid species.

ACKNOWLEDGMENTS

The author is especially indebted to Emeritus Professor Toshijiro KAWAMURA and Professor Midori NISHIOKA of Hiroshima University for their encouragement and guidance during the course of this work and for their critical review of the manuscript.

LITERATURE

BLAIR, W. F. 1958. Mating call in the speciation of anuran amphibians. Amer. Natur., **92**: 27–51.

BOGART, J. P. and A. O. WASSERMAN 1972. Diploid-polyploid cryptic pairs: A possible clue to evolution by polyploidization in anuran amphibians. Cytogenetics, **11**: 7–24.

BROWN, L. E. and J. R. BROWN 1972. Mating calls and distributional records of treefrogs of the *Hyla versicolor* complex in Illinois. J. Herpetol., **6**: 233–234.

DANZMANN, R. G. and J. P. BOGART 1982. Gene dosage effects on MDH isozyme expression in diploid, triploid and tetraploid treefrogs of genus *Hyla*. J. Hered., **73**: 277–280.

GURDON, J. B. 1959. Tetraploid frogs. J. Exp. Zool., **141**: 519–544.

FOUQUETTE, M. J. 1960. Isolating mechanisms in three sympatric treefrogs in the Canal Zone. Evolution, **14**: 484–497.

———— 1975. Speciation in chorus frogs I. Reproductive character displacement in the *Pseudacris nigrita* complex. Syst. Zool., **24**: 16–23.

HUBBS, C. L. and C. HUBBS 1953. An improved graphical analysis and comparison of series of samples. Syst. Zool., **2**: 49–56.

JASLOW, A. P. and R. C. VOGT 1977. Identification and distribution of *Hyla versicolor* and *Hyla chrysoscelis* in

Wisconsin. Herpetologica, **33**: 201–205.

Johnson, C. 1959. Genetic incompatibility in the call races of *Hyla versicolor* Le Conte in Texas. Copeia, 1959: 327–335.

——— 1966. Species recognition in the *Hyla versicolor* complex. Texas J. Sci., **18**: 361–364.

Kashiwagi, K. 1993. Production of triploids and their reproductive capacity in *Rana rugosa*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **12**: 23–36.

Kawamura, T. 1941. Triploid frogs developed from fertilized eggs in *Rana nigromaculata*. Proc. Imp. Acad. Tokyo, **17**: 523–526.

——— 1951. The offspring of triploid males of the frog, *Rana nigromaculata*. J. Sci. Hiroshima Univ., Ser. B. Div. 1, **12**: 11–20.

——— 1984. Polyploidy in amphibians. Zoological Science, **1**: 1–15.

Kawamura, T. and M. Nishioka 1963. Autotetraploids and the production of allotetraploids and diploid nucleo-cytoplasmic hybrids in pond frogs. J. Sci. Hiroshima Univ., Ser. B. Div. 1, **21**: 85–106.

——— 1983. Reproductive capacity of male autotetraploid *Rana nigromaculata* and male and female amphidiploids produced from them by mating with female diploid *Rana brevipoda*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **6**: 1–45.

Kawamura, T., M. Nishioka and Y. Myorei 1963. Reproductive capacity of autotetraploid males in brown frogs. J. Sci. Hiroshima Univ., Ser. B. Div. 1, **21**: 15–24.

Kawamura, T., M. Nishioka and H. Okumoto 1983. Production of autotetraploids and amphidiploids from auto- and allotriploids in *Rana nigromaculata* and *Rana brevipoda*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **6**: 47–80.

Little, M. L., B. L. Monroe and J. E. Wiley 1989. The distribution of the *Hyla versicolor* complex in the Northern Appalachian Highlands. J. Herpetol., **23**: 299–303.

Littlejohn, M. J., M. J. Fouquette and C. Johnson 1960. Call discrimination by female frogs of *Hyla versicolor* complex. Copeia, 1960: 47–49.

Maxson, L. R., E. Pepper and R. D. Maxson 1977. Immunological resolution of a diploid-tetraploid species complex of treefrogs. Science, **197**: 1012–1013.

Marion, N. C. and J. P. Bogart 1978. Cytological differentiation of the diploid-tetraploid species pair of North American treefrogs (Amphibian, Anuran, Hylidae). J. Herpetol., **12**: 555–558.

Nishioka, M. 1971. Abnormal combinations of the nucleus and cytoplasm and their effects in amphibians. Symposia Cell. Biol., **22**: 189–203.

Nishioka, M. and H. Ueda 1983. Studies on polyploidy in Japanese treefrogs. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **6**: 207–252.

——— 1988. Reproductive capacity of tetraploids and pentaploids in *Bufo japonicus miyakonis*. Jpn. J. Genet., **63**: 585.

Pierce, J. R. and D. B. Ralin 1972. Vocalization and behavior of the males of three species in the *Hyla versicolor* complex. Herpetologica, **28**: 329–337.

Ralin, D. B. 1968. Ecological and reproductive differentiation in the cryptic species of the *Hyla versicolor* complex (Hylidae). Southwestern Natur., **13**: 283–299.

——— 1977. Evolutionary aspects of mating call variation in a diploid-tetraploid species complex of treefrogs (Anura). Evolution, **31**: 721–736.

Ralin, D. B., M. A. Romano and C. W. Kilpatrick 1983. The tetraploid treefrog *Hyla versicolor*: Evidence for a single origin from the diploid *Hyla chrysoscelis*. Herpetologica, **39**: 212–225.

Ralin, D. B. and R. K. Selander 1979. Evolutionary genetics of diploid-tetraploid species of treefrogs of the genus *Hyla*. Evolution, **33**: 595–608.

Ueda, H. 1980. The sex of triploids and gynogenetic diploids in *Bombina orientalis*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **4**: 185–199.

——— 1986. On the mating calls of triploid and tetraploid males in *Hyla arborea japonica*. (In Japanese). Jpn. J. Genet., **61**: 627.

Wasserman, A. O. 1970. Polyploidy in the common tree toad, *Hyla versicolor* Le Conte. Science, **167**: 385–386.