Bonn. zool. Beitr.	Jg. 37	H. 1	S. 49—67	Bonn, März 1986

Comparative bioacoustic studies in the Yellow-bellied Toad, *Bombina variegata* (L.), and relationships of European and Asian species and subspecies of the genus *Bombina* (Anura, Amphibia)

by

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Introduction

Recent studies have shown that the anuran mating calls have specific biological significance. They serve to attract females ready to mate (Gerhardt 1978, 1983; Martof 1961; Martof & Thompson 1958; Littlejohn 1961; Littlejohn & Michaud 1959; Littlejohn & Watson 1974, 1976; Schneider 1982), or to mark the territory of the calling male (Brzoska 1982; Emlen 1968; McDiarmid & Adler 1974; Wiewandt 1969). The mating calls of a given species can also have both functions (Narins & Capranica 1978; Brzoska et al. 1982; Schneider et al. 1984).

Because the mating calls of a species are always highly characteristic, they are a useful criterion by which to evaluate inter- and intraspecific relationships. Indeed, they are often better than morphological characteristics for this purpose. But mating calls, too, exhibit a degree of variability, for they are affected in various respects by temperature and to some extent by the size of the calling individual.

The tree frog *Hyla arborea* offers a prime example of the value of the mating call in reexamining systematic relationships (Schneider 1977). Very recently, analysis of the mating call of the Near Eastern tree frog and of the females' behavioral responses to these calls has provided a basis for elevating this tree frog to the status of a species, *Hyla savignyi* (Schneider et al. 1984).

Within the family Discoglossidae, the fire- and yellow-bellied toads *Bombina bombina* and *Bombina variegata* are conspicuous in the European fauna. Although Neubaur (1949) regarded the mating calls of the two species as identical, they do exhibit species-specific differences, as demonstrated by Lörcher (1969) in his extensive comparative study. Hybrids of these fire- and yellow-bellied toads raised in the laboratory by Schneider & Eichelberg (1974) had intermediate mating calls. Another species of fire-bellied toad, *Bombina orientalis*, is native to the Far East; its mating call is very similar to that of the Central European yellow-bellied toad (Akef & Schneider 1985).

The fire-bellied toad *Bombina bombina* is regarded as a uniform species over its entire large range of distribution. *B. variegata*, however, is known to exist in several subspecies, of which three inhabit the Balkan region: in the northern part *B. v. variegata*, in Dalmatia *B. v. kolombatovici*, and in the southern Balkan countries *B. v. scabra* (Mertens & Wermuth 1960). To extend the information available about the Balkan yellow-bellied toads, we have examined the mating calls of animals from the ranges occupied by these three subspecies. To evaluate the systematic relationships of these animals, their calls were then compared with the mating calls of *B. bombina* from the Neusiedler See region of Austria and *B. v. variegata* from southwestern Germany, as well as those of a laboratory population of hybrids of these two species and of the Far Eastern *B. orientalis*.

Material and Methods

Bombina variegata (L.), yellow-bellied toads, were collected in the summer of 1981, three males and two females at Plitvice, Yugoslavia, representing *B. v. variegata*, and another three males and two females in the Cetina river valley near Omiš, Yugoslavia, at two different localities, both in the area of *B. v. kolombatovici*. The toads from the two places near Omiš were studied separately as samples 1 and 2. In addition, one male was collected on the peninsula Chalkidiki, Greece. According to the collection site it is *B. v. scabra*.

All tape recordings of the mating calls evoked from the hormonally injected males (1000 I.U. serum-gonadotropin; Seragon®) were made in the laboratory, using a Beyer M 101 N microphone and a Nagra III tape recorder. The recorded calls were analyzed by means of an oscilloscope (Tektronix 502 A), spectrum analyzer (Nicolet UA-500 A), storage oscilloscope (Tektronix 564 A), and a Toennies Recordine camera. The sonagrams were prepared with a Kay Electric sonagraph 7029 A. The statistical data were analyzed by means of the IBM 7090 computer of the Regionales Rechenzentrum der Universität Bonn.

Results

I. Calling and calls of *B. variegata*-subspecies in southeastern Europe

1. Calling behavior

Reproduction of toads from Yugoslavia occurred periodically in peaks of several months, May, June and July. In the laboratory, hormonal injection (1—3 times) of males from Omiš induced calling activity within 2—3 days after injection. The males called day and night, and the calling activity sometimes extended over 60 days. Hormonal injection (2—3 times) of toads collected at Plitvice also induced normal calling activity but after a longer latency of 7—10 days from injection. Moreover, the calls that the toads from Plitvice emitted during the first 5—7 days showed abnormalities in call rate and amplitude. In general, yellow-bellied toads did not respond to the hormone as promptly as *B. bombina* and *B. orientalis*, which call within 12—24 hours after injection.

The yellow-bellied toads from all places adopt a characteristic posture while calling, with most of the upper part of the body out of water and the hindlegs slightly or widely spread (Fig. 1). The calls are produced during the passage

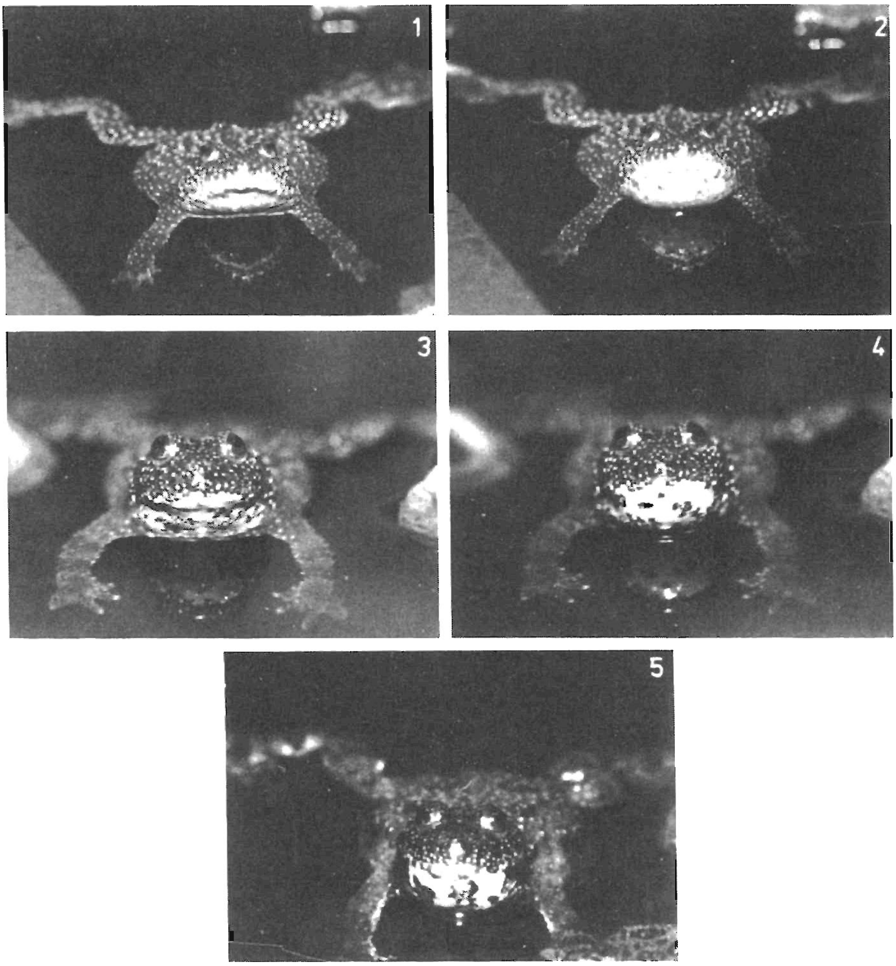

Fig. 1. The calling behavior of male *B. variegata* from Omiš, sample 1 (1,2) and sample 2 (3,4) shown just at the end of call (1,3) and during interval (2,4); 5 calling male from Plitvice during an interval.

of air from the buccal cavity into the lungs, which expand further in the process. The males call in a restricted area for several hours and especially after sunset. Few calls were heard in the morning and during the day. The calling male changes its calling site only when there are disturbances in the water produced by other toads or after disturbances from outside.

When there were two calling males in the pool (45 cm x 25 cm x 6.5 cm) of the experimental terrarium the calling was never antiphonal; instead, the toads defended their territories. But when the two calling males were in different terraria they called in alteration. When calls came from a tape recorder one male established antiphonal calling with the played-back calls.

2. Territorial behavior of males

The behavior of the resident calling males in the pool depended upon the intruding male. If the latter remained motionless and submerged in the water with only its head above the surface, no interaction occurred. However, if either an intruder or the resident or both of them approached the other, and the distance between them decreased to less than about 3 cm, the call rate at first decreased, followed by an increase in the intensity. If the intruder did not stop calling, one male jumped at or upon the other toad. The male below reacted with kicking movements of the hindlegs and by producing release calls until the upper male was turned over on its back. At this moment contact was broken. This fighting lasted about 10—20 seconds.

Another kind of fighting behavior consisted of attacking from the side. A calling male was suddenly attacked by another male and eventually overturned. Fighting sometimes continued under water. After this sudden attack the inferior male either floated again in the area of the figthing or swam some distance under water, usually to a corner of the aquarium, and floated there again. This male remained silent and motionless with just the head above the water surface.

Females showed no defence mechanisms to site-specific territoriality, except during amplexus.

3. The mating call

All yellow-bellied toads studied produce five different types of call, the normal mating call, the modified mating call, the clasping call, and the release call of the first and second order. For testing the relationships within the genus *Bombina* the mating call was chosen. Calls of the two males from Plitvice, three males from Omiš and one male from Chalkidiki were recorded.

The mating call is composed of a fundamental frequency and several harmonics. From the beginning of a call the amplitude increases more or less steadily until a maximum is reached. This may be maintained for some time as a plateau, after which the amplitude decreases again till the end of call (Fig. 2). The mating call is affected by both water temperature and body size. For example, with individuals from Omiš, sample 1, the calls made by a large toad of 48.5 mm have a duration of 312 ms at 18 °C (Fig. 2a) and of 216 ms at 26 °C (Fig. 2b), whereas in the calls of a small toad with a length of 44 mm the duration is 268 ms at 18 °C (Fig. 2c) and 174 ms at 26 °C water temperature (Fig. 2d). In the calls of a 48.5-mm-long male the fundamental frequency is 496.8 Hz at 18 °C and 537 Hz at 26 °C (Fig. 3).

The effect of water temperature and body length upon mating calls was determined by means of the F-test. All regressions derived for the data are described by linear equations. The data obtained from the recordings are given in Tables I—V.

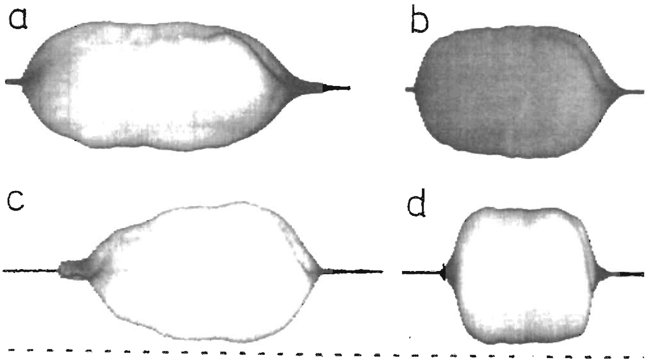

Fig. 2. *B. variegata* from Omiš, sample 1: Oscillograms of mating calls of a 48.5 mm long male at 18 °C (a) and at 26 °C (b), and of a 44 mm long male at 18 °C (c) and at 26 °C (d). Time marks 50 Hz.

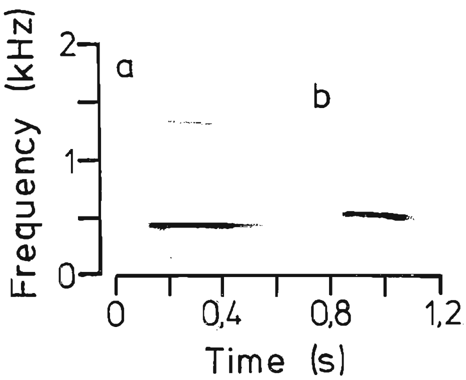

Fig. 3. Sonagram of a mating call of a male from Omiš, sample 2, 48.5 mm in length at a water temperature of 18 °C (3a) and 26 °C (3b).

a. Call rate

The call rate is significantly related to temperature and is unaffected by body size. The correlation is positive and linear (Fig. 4). For example, for males from Omiš, sample 1 and 2, from Plitvice and from Chalkidiki, rise of temperature from 18 ° C to 28 ° C is followed by acceleration of call rate from 60.7 to 112.9 calls per minute (i.e. a factor of 1.86), 67 to 132.3 calls per minute (1.98 x), 77.7 to 127.1 calls per minute (1.64 x), and 70.7 to 114.6 calls per minute (1.64 x), respectively.

Table I. The effect of body length upon call duration in the *B. variegata* studied.

Origin	Snout-vent length (mm)	Call duration at 22 °C (ms)
1. Yugoslavia		
B. variegata from Omiš, sample 1	44.0	215.0
	48.5	236.4
B. variegata from Omiš, sample 2	50.0	206.4
B. variegata from Plitvice	45.5	234.4
	48.5	268.0
2. Greece		
B. variegata from Chalkidiki	38.0	202.8

Table II. The effect of body length on fundamental frequency in the *B. variegata* studied.

Origin	Snout-vent length (mm)	Call frequency at 22 °C (Hz)
1. Yugoslavia		
B. variegata from Omiš, sample 1	44.0	607.6
	48.5	510.8
B. variegata from Omiš, sample 2	50.0	541.7
B. variegata from Plitvice	45.5	622.5
	48.5	599.6
2. Greece		
B. variegata from Chalkidiki	38.0	572.3

Table III. The effect of temperature upon call rate in *Bombina*

	Calls per minute	
	19 °C	26 °C
B. orientalis	61.8	105.9
B. bombina	24.7	36.8
B. variegata from Omiš, sample 1	65.9	102.4
B. variegata from Omiš, sample 2	73.5	119.2
B. variegata from Plitvice	82.6	117.2
B. variegata from Chalkidiki	75.1	105.9
B. v. variegata from Tübingen	79.2	119.8
Hybrid *B. bombina* ♀ x *B. v. variegata* ♂	45.3	66.7

Table IV. The effect of temperature and body size upon call duration in the *Bombina* studied.

Origin	Snout-vent length (mm)	Call duration (ms) 19° C	26° C
B. orientalis	43.0—45.0	154.5	113.8
	47.0	184.3	120.9
B. bombina	40.0	270.2	201.0
	48.0	311.8	218.9
B. variegata from Omiš, sample 1	44.0	234.8	176.7
	48.5	269.7	192.1
B. variegata from Omiš, sample 2	50.0	240.7	160.7
B. variegata from Plitvice	45.5	269.1	188.2
	48.5	300.9	224.2
B. variegata from Chalkidiki	38.0	228.0	169.1
B. v. variegata from Tübingen	31.0	200.6	139.8
	45.0	205.2	160.5
	47.0	235.6	171.1
Hybrid *B. bombina* ♀ x *B. v. variegata* ♂	28.0—32.0	211.5	159.4

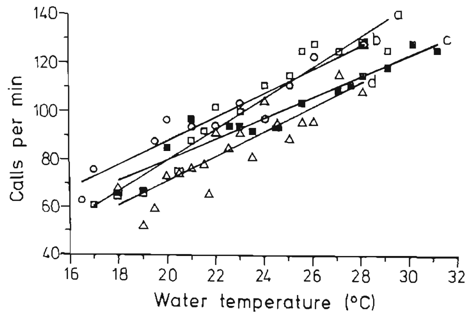

Fig. 4. The effect of temperature upon call rate: a Omiš, sample 2 <□>; b Plitvice <○>; c Chalkidiki <■>; d Omiš, sample 1 <△>.

Statistical comparison between the regression lines of *B. variegata* from Omiš, sample 2, and from Plitvice shows no significant difference ($P > 0.05$), but there is a significant difference in call rate between toads collected at Omiš, sample 1, and that collected on Chalkidiki ($P < 0.05$).

Furthermore, covariance analysis of the regression lines of toads from Omiš, sample 1 and sample 2, exhibited very significant differences at the 0.01 level.

b. Call duration

Generally, call duration decreased in all samples as water temperature increased. In order to make valid comparisons of duration of mating calls, these calls are at first corrected to a common temperature (Table I).

There is a highly significant difference between the regression lines of the toads of equal size collected near Omiš, (48.5 mm) and Plitvice (48.5 mm) at the P = 0.001 level, but there is no significant difference (P >0.05) in call duration of the largest toad from Omiš (48.5 mm) as compared with the smaller one from Plitivice (45.5 mm). Comparison of regression lines of the toads from Omiš measuring 44 mm and 50 mm demonstrates highly significant differences (P <0.001), while the regression of the 44-mm toad from Omiš differs significantly, at the 0.05 level, from that of the Chalkidiki toad (38 mm). Moreover, highly significant differences were found between the regression lines of the toad from Chalkidiki (38 mm) and the 50-mm toad from Omiš.

The effect of body length is also important in the analysis of intraspecific variations in mating call. For *B. variegata* from Plitvice and Omiš, sample 1, the relationship between call duration and snout-vent length is highly significant and positively correlated (Fig. 5; Table V). Statistical evaluation of regressions of the Plitvice sample shows a highly significant difference between the two toads (P <0.001). In addition comparison between the regressions of the toads from Omiš, sample 1, reveals very significant differences at P = 0.001 level.

The statistical analysis shows that the mating call of the toad from Plitvice is significantly longer than of the other *B. variegata* with the same body length.

c. Call frequency

The effect of temperature upon frequency is statistically very significant; the

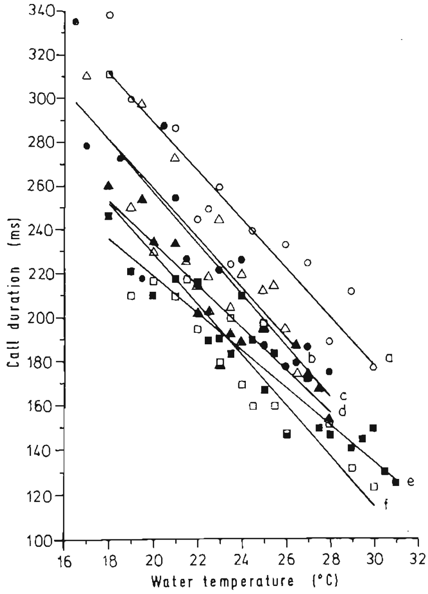

Fig. 5. The effect of temperature and body length upon call duration a Plitvice, 48.5 mm <○>; b Omiš, sample 1, 48.5 mm <△>; c Plitvice, 45.5 mm <●>;d Omiš, sample 1, 44 mm <▲>; e Chalkidiki, 38 mm <■>; f Omiš, sample 2, 50 mm <□>.

two are positively correlated in both the sample from Yugoslavia and that from Greece (Fig. 6).

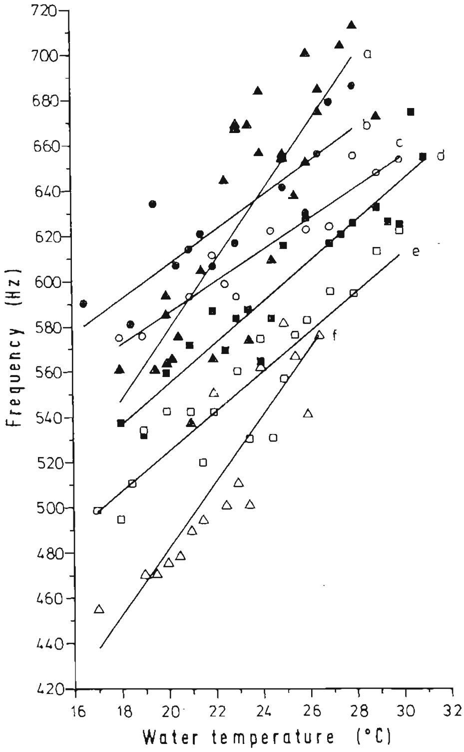

Fig. 6. The effect of temperature and body length upon fundamental frequency: a Omiš, sample 1, 44 mm <▲>; b Plitvice, 45.5 mm <●>; c Plitvice, 48.5 mm <○>; d Chalkidiki, 38 mm <■>; e Omiš, sample 2, 50 mm <□>; f Omiš, sample 1, 48.5 mm <△>

The mating call of the *B. variegata* studied follows the general rule of decreasing frequency with increasing body length. For example, in the Omiš individuals the increase in snout-vent length from 44 mm to 48.5 mm results in a highly significant decrease of frequency. Highly significant correlation was also found for the individuals from Plitvice. It is noteworthy that the frequency of males from Plitvice is statistically higher than that of the other studied *B. variegata* (Table II and V).

Males from Omiš, sample 1, produce calls which form regression lines that intersect the lines of the other toads (Fig. 6). The frequency increases faster with increasing water temperature than in the other *B. variegata.* Moreover, the regression line for the male from Omiš, sample 2, runs in a different direction from that of sample 1, and is more or less parallel to that of the males from Plitvice and Chalkidiki.

Males from Omiš, sample 1, emit calls the frequencies of which follow the general rule that increasing body length will be accompanied by decreasing fundamental frequency. But the male from Omiš, sample 2, body length 50 mm, emits calls of higher frequency than that produced by the male of about the same body length (48.5 mm) from Omiš, sample 1. There is a significant difference in the regressions of the two males from the same area of Omiš, but of different body length (48.5 mm and 50 mm) at 0.01 level (Table V).
The harmonics are also affected by water temperature and body length (Fig. 10). At 18 °C *B. variegata* from Omiš sample 1, body length 44 mm, emits calls having first and second harmonics at 1094.3 and 1650.9 Hz, respectively. On the other hand the male 48.5 mm in length produces calls with frequencies of 925.1 and 1391.8 Hz for the first and second harmonics. Furthermore, at 26 °C, the male 44 mm in length emits calls with first and second harmonic at 1322.1 Hz and 1975.5 Hz, while the male 48.5 mm in length produces calls with 1122.7 and 1683.6 Hz for the first and second harmonics.

The greatest intraspecific variation with respect to the parameters of the mating call is found in the Omiš samples. Variation consists primarily of differences in call duration and in frequency. Although the male from Omiš, sample 2, is large (50 mm), it produces calls with frequencies higher than those of the 48.5-mm-long male of sample 1, (length 48.5 mm) and also shorter duration than that produced by a male with 44 mm body length.

II. Comparison between mating-call parameters of European and Asian *Bombina*

The analysis of the three Balkan subspecies of *B. variegata* provides a highly differentiated picture, indicating considerable intraspecific variability. It is therefore of interest to compare these results with what has been found in the other representatives of the genus *Bombina* so far investigated. Among them are *B. v. variegata* from the vicinity of Tübingen in southwestern Germany and *B. bombina* from the region of the Neusiedler See in Austria, both studied by Lörcher (1969). The regression lines for call duration and fundamental frequency of *B. v. variegata* and for the call duration of *B. bombina* are based on Lörcher's original data, and were calculated from his mean values. In addition, the comparison includes data on hybrids of *B. bombina* ♀ x *B. v. variegata* ♂ (Schneider & Eichelberg 1974) as well as recently obtained data on *B. orientalis* (Akef & Schneider 1985). The mating calls of *B. orientalis* included in the latter analysis proved not to be uniform and were divided into Groups A and B; the comparison here is with Group B. The results of this extended comparison are illustrated in Figures 7—10, and the statistical data are presented in Table 5.

Table V. The results of the statistical analyses. Significance is indicated by: P = 0.05*, P = 0.01**, P = 0.001***. SVL: Snout-vent length (mm). (a) Equations according to Lörcher (1969) and (b) to Schneider & Eichelberg (1974); (c) calculations based on the original data of Lörcher (1969).

Variable (y)	N	Regression equation	Test for linearity x	x^2
1. Calls per minute				
B. orientalis				
SVL: 43.0—47.0	159	$y = -57.67 + 6.29x$	1427.9***	
B. bombina[a]	243	$y = -8.14 + 1.73x$		
B. v. variegata[a]	174	$y = -31.04 + 5.8x$		
Hybrids[b]				
SVL: 28.0—32.0	75	$y = -12.85 + 3.06x$	176.2***	
B. variegata Omiš, sample 1				
SVL: 44.0—48.5	39	$y = -33.07 + 5.21x$	87.4***	
B. variegata Omiš, sample 2				
SVL: 50.0	15	$y = -50.58 + 6.53x$	168.5***	
B. variegata Plitvice				
SVL: 45.5—48.5	11	$y = -11.23 + 4.94x$	90.3***	
B. variegata Chalkidiki				
SVL: 38.0	15	$y = -8.29 + 4.39x$	159.6***	
2. Call duration				
B. orientalis				
SVL: 43.0—45.0	104	$y = 369.6 - 15.35x + 0.212x^2$	692.1***	34.8***
47.0	66	$y = 648.3 - 35.65x + 0.591x^2$	246.4***	42.1***
B. bombina				
SVL: 40.0[b]	8	$y = 877.82 - 48.13x + 0.850x^2$	33.3***	16.3***
48.0[c]	9	$y = 727.7 - 28.20x + 0.332x^2$	257.3***	30.1***
B. variegata				
SVL: 31.0[b]	6	$y = 365.73 - 8.69x$	13.2*	
45.0[c]	8	$y = 673.6 - 38.01x + 0.703x^2$	114.6***	22.2***
47.0[c]	5	$y = 747.5 - 39.9x + 0.682x^2$	36.5***	14.6***
Hybrids[b]				
SVL: 28.0—32.0	69	$y = 352.86 - 7.44x$	143.2***	
B. variegata Omiš, sample 1				
SVL: 44.0	14	$y = 425.8 - 9.58x$	74.7***	
48.5	15	$y = 480.2 - 11.08x$	36.7***	
B. variegata Omiš, sample 2				
SVL: 50.0	16	$y = 458.1 - 11.44x$	48.8***	
B. variegata Plitvice				
SVL: 45.5	15	$y = 488.5 - 11.55x$	50.6***	
48.5	13	$y = 508.9 - 10.95x$	73.7***	
B. variegata Chalkidiki				
SVL: 38.0	21	$y = 388.0 - 8.42x$	123.4***	
3. Fundamental frequency				
B. orientalis				
SVL: 43.0	34	$y = 531.0 + 6.04x$	131.1***	
45.0	55	$y = 492.3 + 6.83x$	294.2***	
47.0	62	$y = 493.7 + 4.35x$	81.0***	
B. bombina[a]				
SVL: 40.0	104	$y = 377.0 + 7.1x$		
42.0	141	$y = 382.2 + 6.1x$		
48.0	56	$y = 384.0 + 4.0x$		
B. v. variegata[c]				
SVL: 40.0	6	$y = 386.9 + 8.6x$	71.3***	
42.0	8	$y = 431.6 + 5.41x$	269.4***	
45.0	8	$y = 345.5 + 8.77x$	109.2***	
Hybrids[b]				
SVL: 28.0—32.0	58	$y = 373.68 + 10.19x$	49.1***	

Table V. (continued)

Variable (y)	N	Regression equation	Test for linearity x	x^2
B. variegata Omiš, sample 1				
SVL: 44.0	28	y=271.7+15.27x	57.9***	
48.5	16	y=189.2+14.62x	55.4***	
B. variegata Omiš, sample 2				
SVL: 50.0	19	y=349.4+ 8.74x	90.8***	
B. variegata Plitvice				
SVL: 45.5	13	y=454.6+ 7.63x	33.3***	
48.5	12	y=446.0+ 6.98x	113.0***	
B. variegata Chalkidiki				
SVL: 38.0	20	y=374.5+ 8.99x	135.1***	
4. Harmonics				
B. orientalis				
SVL: 43.0				
First harmonic	39	y=1112.3+10.27x	80.4***	
Second harmonic	40	y=1654.4+15.64x	76.8***	
SVL: 47.0				
First harmonic	50	y= 972.2+10.09x	63.8***	
Second harmonic	51	y=1439.5+15.35x	66.0***	
B. variegata Omiš, sample 1				
SVL: 44.0				
First harmonic	30	y=475.8+32.55x	47.0***	
Second harmonic	31	y=769.9+46.37x	48.2***	
SVL: 48.5				
First harmonic	15	y=388.5+28.24x	19.4***	
Second harmonicd	15	y=599.9+41.68x	19.0***	

1. Call rate

As shown in Table III and Fig. 7, an increase of water temperature from 19 °C to 26 °C accelerates the call rate of the various toads studied. The regression lines of *B. variegata* lie above that of *B. bombina*; i.e. the former species give calls at a much faster rate. The regression of *B. orientalis* runs near those of *B. variegata* complex and far from that of *B. bombina.*

According to Fisher's transformation, there is no significant difference between the call rates of individuals from Plitvice and of *B. v. variegata* from Tübingen. Statistically, there is no significant difference between the regression lines of *B. orientalis* and of *B. v. variegata* from Omiš, sample 1. Moreover, the regression line of the hybrids of *B. bombina* ♀ x *B. v. variegata* ♂ is located between those of all *B. variegata* and *B. orientalis* and *B. bombina.*

2. Call duration

Call duration of all toads varies inversely with temperature. The relation may either be linear or nonlinear. Furthermore, comparison of calls produced by males different in size establishes the size-dependence of call duration in all *Bombina* studied. Table IV and Fig. 8 illustrate that *B. orientalis* has the briefest calls of all toads studies, while the calls of *B. bombina* are considerably longer

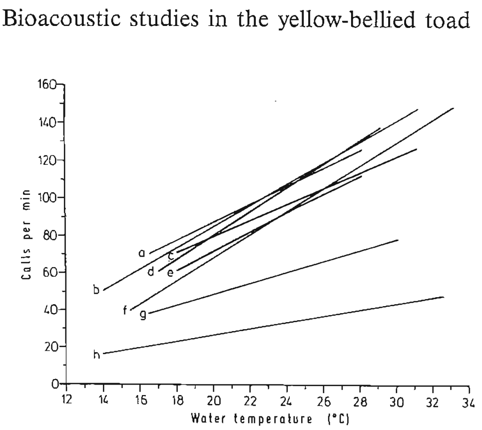

Fig. 7. The effect of temperature upon call rate in *B. variegata* from: a Plitvice; b Tübingen; c Chalkidiki; d Omiš, sample 2; e Omiš, sample 1; f *B. orientalis*; g hybrids *B. bombina* ♂ x *B. v. variegata* ♂; h *B. bombina*.

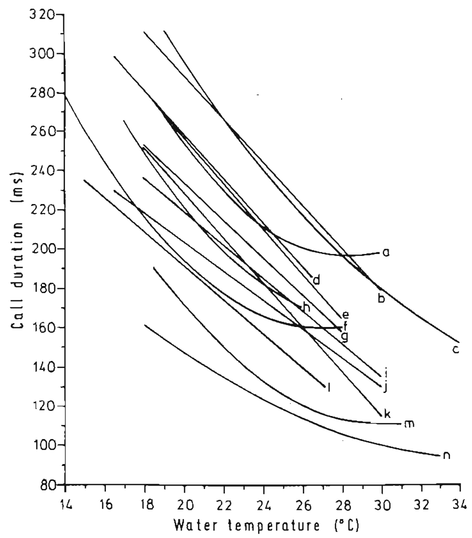

Fig. 8. The effect of temperature and body length upon call duration: a *B. bombina* (40 mm); b *B. v. variegata* from Plitvice (48.5 mm); c *B. bombina* (48 mm); *B. variegata* from d Omiš, sample 1 (48.5 mm); e Plitvice (45.5 mm); f Tübingen (45 mm); g Omiš, sample 1 (44 mm); h Tübingen (47 mm); i Chalkidiki (38 mm). j hybrids *B. bombina* ♂ x *B. v. variegata* ♂ (28—32 mm). *B. variegata* from k Omiš, sample 2 (50 mm) and l Tübingen (31 mm). *B. orientalis* m (47 mm) and n (43—45 mm).

than those of the other toads. *B. bombina*, *B. orientalis* and *B. variegata* from Tübingen emit calls that have a logarithmic relation to temperature (i.e. nonlinear), while the *B. variegata* investigated have call durations that vary linearly with temperature. Moreover, the regression line of hybrids lies between those of *B. v. variegata* and *B. bombina*. Generally, the regression lines of the *B. variegata* studied are located between the curves of *B. orientalis* and *B. bombina*.

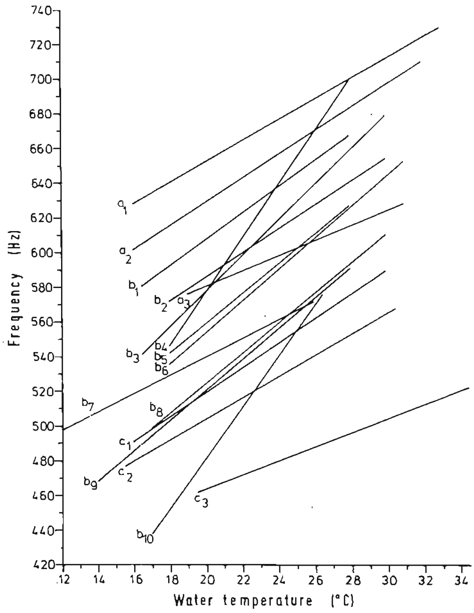

Fig. 9. The effect of temperature and body length upon fundamental frequency. *B. orientalis:* a_1 (43 mm), a_2 (45 mm), a_3 (47 mm); *B. variegata* from Plitvice: b_1 (45.5 mm), b_2 (48.5 mm); hybrids *B. bombina* ♀ x *B. v. variegata* ♂: b_3 (28—32 mm); *B. variegata* from: b_4 Omiš, sample 1 (44 mm), b_5 Tübingen (40 mm), b_6 Chalkidiki (38 mm), b_7 Tübingen (42 mm), b_8 Omiš, sample 2 (50 mm), b_9 Tübingen (45 mm), b_{10} Omiš, sample 1 (48.5 mm); *B. bombina:* c_1 (40 mm), c_2 (42 mm), c_3 (48 mm).

3. Call frequency

The calls of the various toads studied are strongly differentiated interspecifically with respect to the fundamental frequency and its harmonics.

In the calls of some *Bombina* the harmonics are strongly expressed, in others certain harmonics are damped and others are emphasized. Moreover, the yellow-bellied toads and *B. bombina* show modulated frequencies, while *B. orientalis* shows a slightly modulated frequency.

In all toads the fundamental frequency varies inversely with the body length, as illustrated in Table V and Fig. 9. It is obvious that male *B. orientalis* emit call frequencies which are significantly higher than those of the other toads studied.

The regression line of the hybrids (length 28—32 mm) is located between the lines of *B. variegata* from Plitvice (length 45.5 mm) above it and that of *B. bombina* (length 40 mm) below it; the latter species has a remarkably low call fre-

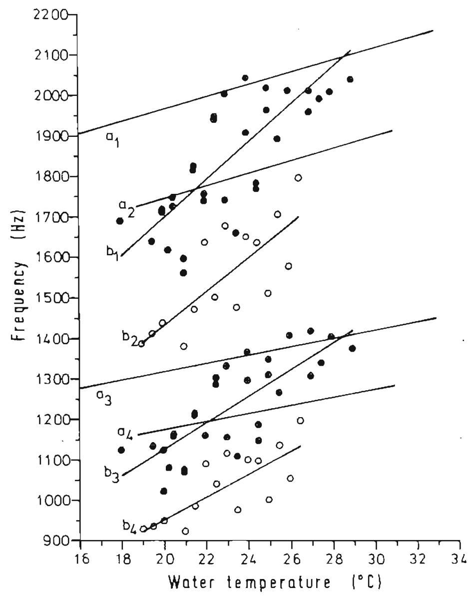

Fig. 10. The effect of temperature and body length upon harmonics. *B. orientalis:* a_1 and a_3 second and first harmonics of a 43 mm long male, a_2 and a_4 of a 47 mm long male. *B. variegata* from Omiš, sample 1: b_1 and b_3 <●> second and first harmonics of a 44 mm long male, b_2 and b_4 <○> of a 48.5 mm long male.

quency for its size. Within the *B. variegata* complex, the positions of the regression lines on the ordinate do not reflect a close correlation with body size, for those of the toads from Plitvice are the highest although the animals are of intermediate to large size. The line for the animals from Chalkidiki almost coincides with that for the Tübingen toad nearest its own size (2 mm larger) Fig. 9, Table V).

The calls of *B. orientalis* and *B. variegata* often have three emphasized harmonics. In *B. orientalis* and *B. variegata* from Omiš, sample 1, the first and second harmonics are always highly significantly correlated with water temperature and body size (Table V).

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The first and second harmonics of *B. variegata* from Omiš, sample 1, run steeply through the regression lines of *B. orientalis* (Fig. 10). Moreover, the harmonics of *B. orientalis* are always higher than those of *B. variegata* from Omiš. *B. orientalis* and *B. variegata* from Omiš differ principally in that at a temperature of 22 ° C the former (body length 43 mm) has the first harmonic at 1338.2 Hz and the second at 1998.5 Hz, while in the toad from Omiš (body length 44 mm) the first and second harmonics, respectively, were 1191.9 Hz and 1790 Hz.

In general, the regression lines of the frequencies of *B. variegata* are located between *B. orientalis* and *B. bombina.*

Discussion

The first question to be considered is whether data obtained from one or a few individuals provide an adequate basis for conclusions about the mating calls of a population. Lörcher (1969) measured the temperature dependence of various parameters of the mating calls of *B. bombina* and *B. v. variegata* both for samples comprising large numbers of animals and for individual males differing in size. Heinzmann (1970) studied another member of the family Discoglossidae, *Alytes o. obstetricans*, and compared the calls of single individuals with the data for a large number of animals. Both of these publications show that data from individual animals are representative of the calls of a population. Because the calls are highly dependent on animal size, it is actually essential to compare the data from single males in order to draw precise conclusions. Therefore the results obtained here for the Balkan *B. variegata* do support further inference.

The general conclusion from this study is that the European and Asian discoglossid species considered are related in unexpected ways. Although the Asian *B. orientalis* differs from *B. variegata* in coloration, having a red rather than yellow belly, the two are very similar in other respects. In contrast, *B. bombina* bears no detectable relationship to either *B. orientalis* or *B. variegata.*

The situation is most readily analyzed in the case of call repetition rate, for this parameter is not affected by the size of the animal. *B. bombina* calls at the lowest rate of all the toads in this study, and the call rate increases only moderately with rising temperature. The lines for the *B. variegata* complex lie within a small range, which also includes the regression line of *B. orientalis.* Akef & Schneider (1985) had previously found a close relation between the mating calls of *B. orientalis* and those of *B. v. variegata*, and this resemblance is even more clearly revealed by the distribution of regression lines within the *B. variegata* complex. The regression lines for call rate of males from Omiš, sample 1, are the lowest within the *B. variegata* group and do not differ significantly from that of *B. orientalis.* With respect to call duration, the regression curves of the *B. variegata* complex are distributed between those of *B. bombina*, which has the longest call duration, and *B. orientalis*, with the shortest calls.

The yellow-bellied toads of Plitvice and Tübingen, southwestern Germany, which belong to the same subspecies, *B. v. variegata*, call at the same repetition rates but differ in two important respects: *B. v. variegata* from Plitvice have much higher frequencies and longer call duration than *B. v. variegata* of the same length from Tübingen.

The species of *Bombina* studied varied most widely with respect to the frequency of the calls. Even a small increase in body length results in a significant decrease in call frequency. For example, an increase in length of individuals of *B. orientalis* and *B. v. variegata* by 2 mm is accompanied by highly significant changes.

Another difference with regard to frequency is particularly noteworthy because it sets off one of the Omiš samples from all the other toads in this study. That is, the frequency-vs.-temperature regression lines for the *B. variegata* in sample 1 from Omiš, for both the fundamental and the harmonics, rise much more steeply than do those of the other *B. variegata* (including sample 2 from Omiš) as well as those of *B. orientalis*, *B. bombina* and the hybrids of *B. bombina* ♀ × *B. v. variegata* ♂. Evidently the animals in the region of Omiš are highly differentiated, for the distance between the sites at which the samples were collected is only about 5 km. It is likely that the samples also differ in other characteristics.

The call of the yellow-bellied toad from Chalkidiki is characterized by low frequency, closer to that of the geographically more distant *B. v. variegata* from Tübingen than to that of the nearer *B. v. variegata* from Plitvice. However, the toads from Chalkidiki and Tübingen show differences in call rate and call duration.

The statistical comparisons of *B. variegata* from Omiš and *B. v. variegata* from Tübingen reveal great differences in the mating call parameters.

A c k n o w l e d g e m e n t s. M.A.S. Akef is grateful to the General Department for Mission, Ministry of Higher Education, Arab Republic of Egypt, for financial support. All authors thank Mrs. U. Dung and Mrs. B. Zarbock for skilled technical assistance.

Summary

1. The mating calls of *Bombina variegata* from Plitvice (*B. v. variegata*) and two localities in the Cetina river valley near Omiš (*B. v. kolombatovici*), Yugoslavia, and from Chalkidiki, Greece, (*B. v. scabra*) were analyzed with respect to call rate, call duration, fundamental frequency and harmonics. — 2. Call repetition rate and call frequency show a positive linear correlation with water temperature, whereas call duration is negatively, linearely correlated with temperature. — 3. Call duration and frequency are related to the length of the males. Small males have calls of shorter duration and higher frequencies than large males. Repetition rate of call is not related to size. — 4. Comparison of mating calls of these three subspecies and of other species, *B. bombina*, *B. orientalis* and hybrids of *B. bombina* ♀ × *B. v. variegata* ♂, reveals intra- and interspecific differences. — 5. Although *B. orientalis* is a red-bellied toad the mating call is close to that of *B. variegata* complex. — 6. The parameters of the mating call of *B. bombina* suggest no relationship to either *B. variegata* complex or to *B. orientalis*. — 7. The differences within the *B. variegata* complex are very differentiated. *B. v. variegata* from Plitvice and Southwest Germany have identical call rates, but different call durations and frequencies. *B. v. scabra* from Chalkidiki has calls of low frequency. *B. v. kolombatovici* from Dalmatia show differences not only to other subspecies but also within their own range.

Zusammenfassung

1. Bei *B. variegata* der Unterarten *B. v. variegata* aus dem Gebiet von Plitwitz, *B. v. kolombatovici* von zwei Fundorten im Cetina-Tal bei Omiš, Jugoslawien, und *B. v. scabra* aus Chalkidike, Griechenland, wurden die Paarungsrufe im Hinblick auf Wiederholungsrate, Dauer, Grundfrequenz und Obertöne analysiert. — 2. Wiederholungsrate und Tonhöhe der Rufe sind mit der Wassertemperatur positiv linear korreliert, die Rufdauer zeigt dagegen eine negative lineare Korrelation. — 3. Rufdauer und Tonhöhe sind von der Körpergröße der Männchen abhängig. Kleine Männchen geben Rufe mit kürzerer Dauer und höherer Frequenz ab als große. Die Wiederholungsrate der Rufe ändert sich nicht mit der Tiergröße. — 4. Der Vergleich der Paarungsrufe dieser drei Unterarten mit denen von *B. orientalis*, *B. bombina* und den Hybriden von *B. bombina* ♀ x *B. v. variegata* ♀ läßt intra- und interspezifische Unterschiede erkennen. — 5. Obgleich *B. orientalis* auf Grund der Färbung auf der Bauchseite eine Rotbauchunke ist, steht diese Art durch die Paarungsruf-Parameter dem *B. variegata* Komplex sehr nahe. — 6. Die Parameter des Paarungsrufes von *B. bombina* lassen keine enge Beziehung zu den Paarungsrufen des *B. variegata*-Komplexes und *B. orientalis* erkennen. — 7. Innerhalb des *B. variegata*-Komplexes sind die Unterschiede sehr differenziert. *B. v. variegata* aus dem Gebiet von Plitwitz und Südwestdeutschland haben identische Wiederholungsraten, jedoch sind Rufdauer und Tonhöhe verschieden. Bei *B. v. scabra* aus Chalkidike ist die Ruffrequenz niedrig. *B. v. kolombatovici* aus Dalmatien zeigt Unterschiede nicht nur im Vergleich zu den Paarungsrufen der anderen Unterarten, sondern auch innerhalb des eigenen Verbreitungsgebietes sind die Paarungsrufe nicht einheitlich.

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