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## Epicoracoid overlap in fire-bellied toads, *Bombina bombina*, from parents of known morphology

## Valentin G. Borkhvardt, Yegor B. Malashichev

Department of Vertebrate Zoology, St.-Petersburg State University, Universitetskaya nab. 7/9, St.-Petersburg, 199034, Russia e-mail: Yegor@EM1915.spb.edu

Recent studies (Borkhvardt and Ivashintsova, 1994; Borkhvardt and Malashichev, 1997; Greer and Mills, 1997) have supported evidence (Martin, 1972) that the ratio of animals with the left epicoracoid in the dorsal (i.e. the deep, not superficial, when viewed ventrally) position to those with the right epicoracoid in the dorsal position (L.top/R.top ratio, hereafter called also overlap index) is specific for a species or group of species. At the same time, the index in the fire-bellied toad, *Bombina bombina* can vary drastically in the progenies of different parents (Borkhvardt and Malashichev, 1997). In this study we tested the hypothesis that the pattern of the epicoracoid overlap may depend on the type of the pectoral girdle structure in parents. Earlier we proposed a simple and effective non-invasive technique for determining epicoracoid arrangement in living animals by moving the forelimbs of a toad and observing the medial edge of the superficial epicoracoid (Borkhvardt and Malashichev, 1997; see also Malashichev and Nikitina, 2001). Therefore, the question of the heritability of the epicoracoid position could be addressed. We also aimed to determine whether other factors such as mortality of tadpoles may influence the overlap index.

Twenty-four mature *B. bombina* (8 from the Tula region,  $54^{\circ}12'N$ ,  $37^{\circ}36'E$ , and 16 from the Belgorod region,  $50^{\circ}36'N$ ,  $36^{\circ}36'E$  of Russia) were caught in natural habitats in April-May, 1999, during the breeding season, and transported to St.-Petersburg. Animals from the Belgorod region were captured in the vicinities of the same artificial reservoir as those used in Borkhvardt and Malashichev (1997). We combined five pairs (No. 1-5), in which both parents had the left epicoracoid in the dorsal position (L.top pairs) and five pairs (No. 6-10), in

which both parents had the right epicoracoid in the dorsal position (R.top pairs). Two pairs were mixed, with R.top female and L.top male (pair No. 11) or vice versa (pair No. 12). Both parents in each pair came from the same locality. Animals were stimulated for breeding with a single application of mixture of 100 IU chorionic gonadotropin ("Profasi," Serono Inc., Italy) and 1.25  $\mu$ g of a mammalian isoform of gonadotropin releasing hormone ("Surphagone," Bior Pharm. Enterprise, Ukraine). Eggs were incubated and larvae were reared in plastic aquaria of 9 to 40 1 at densities of not more than 15 eggs or larvae/l, at room temperature with scald (dry or fresh) dandelion and nettle leaves as the food for tadpoles. Newly metamorphosing juveniles were sacrificed and

fresh) dandelion and nettle leaves as the food for tadpoles. Newly metamorphosing juveniles were sacrificed and stored in 4% formaldehyde. We formed three groups of approximately equal numbers of juveniles, according to the dates at which they had successively metamorphosed, so that they represented correspondingly the first, the intermediate and the last third of the total number of metamorphosing juveniles. We determined the number of L.top and R.top individuals in each group and calculated L.top/R.top ratio. The epicoracoid position in juveniles was determined under the microscope after removal of the ventral skin. We applied one-sample and two-sample chi-square tests to analyze departures in the distribution of the number of L.top animals and R.top animals from random and differences between samples. The effect of time of metamorphosis on the overlap index was tested using one-way ANOVA. Standard coefficient of correlation was used as well.

Our data (table 1) clearly show that the L.top/R.top ratio in F<sub>1</sub> progeny did not depend on the epicoracoid arrangement of their parents. There was no significant difference in the proportions of both phenotypes between descendants of all L.top versus all R.top pairs ( $\chi^2 = 0.354$ , P > 0.54). Moreover, the maximum value of the L.top/R.top ratio (3.24) was noted in the progeny of a R.top pair (No. 8). As significant difference ( $\chi^2 = 12.18$ , P < 0.001) in distributions of L.top and R.top juveniles between progenies of Tula's and Belgorod's crosses was noted, we compared also the progenies of L.top and R.top pairs separately for Tula (pair No. 1 against pairs No. 6 and No. 7) and Belgorod (pairs No. 2-5 against pairs No. 8-10) broods. Again, the distribution of two phenotypes did not differ significantly between the L.top and R.top broods if either pairs from Tula ( $\chi^2 = 0.4$ , P = 0.52) or Belgorod ( $\chi^2 = 1.31$ , P > 0.25) were considered.

Borkhvardt and Ivashintsova (1995) suggested that the position of epicoracoids was a manifestation of handedness. Malashichev and Nikitina (2001) have found concordance between the structure of the pectoral girdle and fore-limb use in the fire-bellied toad, *B. bombina* — toads with the left (right) epicoracoid in dorsal position preferentially use their left (right) forelimbs to wipe a strip of paper from their snouts. At the moment, the mechanism of the inheritance of handedness is not clear. Bianky et al. (1979), for example, found that the left-, right-handed, and ambidextrous mice appeared in constant quantitative proportions whatever the phenotype of the parents was. These authors failed to increase the percentage of the right- or the left-handed mice after five inbred generations.

In more recent literature (e.g. Corbalis et al., 1996) there is evidence of sex-linked inheritance of handedness in humans. We showed previously (Borkhvardt and Malashichev, 1997) that there is a significant difference in frequency of R.top phenotype between the sexes. Our current results from reciprocal crossings (table 1, pairs No. 11 and 12) show non-significant difference between these two pairs of parents ( $\chi^2 = 2.18$ , P > 0.15). It is noteworthy, however, that the overlap index is greater in the progeny of the pair No. 11 (R.top female) with parents from Tula, while other F<sub>1</sub> Tula progenies show the proportion of L.top animals almost always significantly lower than F<sub>1</sub> Belgorod progeny (table 1). As we have few data, this issue warrants further investigation.

								Par	ents						
Pair No.		L.	top × L.t	do		$\Sigma$		R	$top \times R$	.top		$\Sigma$	$R.top \times L.top$	$L.top \times R.top$	$\Sigma$
	1	7	ю	4	S		9	٢	8	6	10		11	12	
Number of eggs	61	213	370	63	131	838	240	227	256	159	204	1086	320	191	2435
Mortality, %	5	31	34	24	36	30.7	22	32	11	36	55	29.7	47	43	33
Number of toadlets	58	146	245	48	84	581	187	155	229	101	91	763	170	109	1623
L.top	31	100	164	34	63	392	109	89	175	73	57	503	119	67	1081
R.top	27	46	81	14	21	189	78	99	54	28	34	260	51	42	542
L.top/R.top ratio	1.15	2.2	2.0	2.43	3.0	2.07	1.4	1.35	3.24	2.6	1.68	1.93	2.33	1.59	1.99
Ρ	0.6	<0.001	< 0.001	<0.01	< 0.001	< 0.001	< 0.05	0.06	< 0.001	<0.001	< 0.05	< 0.001	< 0.001	< 0.05	<0.001

progenies of parents forming L top  $\times$  L top, R top  $\times$  R top, or reciprocal crossings. Parents in pairs 1, 6, 7, and 11 are from Tula region, the others from Belgorod Table 1. Mortality before metamorphosis and the number of reared fire-bellied toads, Bombina bombina, with the left or right epicoracoids in dorsal position —

Short Notes

One could assume that the position of epicoracoids is simply not inherited, i.e. the overlap index for embryos equals 1. In this case biased ratios in adults could result from biased mortality of embryos and/or larvae, which will develop into R.top animals or postmetamorphic specimens that already have the right epicoracoid in the dorsal position. The data from Borkhvardt and Malashichev (1997) seemed to support this suggestion. They showed that L.top/R.top ratio was slightly greater in adults than in juveniles (1.86 versus 1.46, although difference was not significant,  $\chi^2 = 1.82$ , P > 0.18). However, in the current study the progeny of Belgorod toads showed a greater ratio (2.29) than adults from the same locality (1.86). Although the difference is also not significant ( $\chi^2 = 2.19$ , P > 0.15), it is beyond reason to think that postmetamorphic toads with the right epicoracoid in dorsal position die much more frequently than their L.top siblings. We found no evidence to suggest greater mortality in R.top embryos and larvae; there was no correlation between the mortality percentages (table 1) and the values of the overlap index (table 1) ( $r = -0.0057 \pm 0.3171$ ; t = 0.018; P > 0.9). Similarly, there was no correlation between sample size and the overlap index ( $r = 0.1424 \pm 0.2928$ ; t = 0.455; P > 0.3).

Surprisingly, the ANOVA, in three groups of juveniles, which successively metamorphosed (L.top/R.top ratios are 2.6, 1.97, and 1.52 correspondingly), revealed significant difference in the rates of development between the L.top and R.top larvae (F = 8.66; P < 0.01). Tadpoles that will have the right epicoracoid in the dorsal position develop slower and metamorphose later. The overlap index decreased from 2.6 to 1.97 and 1.52 in the first, second and third groups of toadlets subsequently: i.e., there were more R.top animals among later metamorphosing juveniles. Principally, such a delay in metamorphosis sometimes may increase probability of higher mortality of R.top juveniles during the first wintering, thus, altering to some extent the overlap index for the population.

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## Description of the tadpole of *Scinax perereca* (Anura, Hylidae)

Adriana Pugliese<sup>1</sup>, Rogério P. Bastos<sup>2</sup>

<sup>1</sup> Departamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, 20940-040 Rio de Janeiro, RJ, Brazil

e-mail: pugliese@acd.ufrj.br

<sup>2</sup> Departamento de Biologia Geral, Universidade Federal de Goiás, Cx. Postal 131, 74001-970 Goiânia, GO, Brazil

e-mail: bastos@icb1.ufg.br

The *Scinax ruber* species group is characterized by small to moderate size frogs, vocal sac single, median and subgular, snout not pointed, posterior surfaces of thighs with flash colors, advertisement call with mutipulsed notes, generally egg laying in open areas (Pombal et al., 1995) and tadpoles with high fins. *Scinax perereca* is one of twelve species of *Scinax ruber* group known from southeastern Brazil (Pombal et al., 1995). Among these species, only *S. alter* (B. Lutz), *S. cuspidatus* (A. Lutz), *S. nasicus* (Cope) and *S. perereca* Pombal, Haddad and Kasahara lack tadpoles descriptions. Herein, we describe and illustrate the tadpole of *Scinax perereca* and compare it to known larvae of the *Scinax ruber group* from southeastern Brazil.

The specimens examined were collected in the type locality, Fazenda São Luis, Municipality of Ribeirão Branco  $(24^{\circ}13'S; 48^{\circ}46'W; 800 \text{ m above sea level})$ , São Paulo State, Brazil. Tadpoles of *Scinax perereca* are deposited in MNRJ (Museu Nacional, Rio de Janeiro, Brazil: MNRJ 25440).

Tadpoles were obtained from eggs laid in a plastic bag by an amplectant pair of *Scinax perereca* (Coleção F. B. Haddad, Depto. de Biologia/UNESP, Rio Claro, São Paulo, Brazil: CFBH 2206  $\varphi$ ; 2207 rightarrow). They were raised in an aquarium (measurements:  $25 \times 25 \times 10$  cm) with about 6.0 liters of water and fed fish food and lettuce ad libitum.

The description and measurements are based on tadpoles (n = 16; table 1) in stages 36-37 (Gosner, 1960). Other stages were used to corroborate changes of some structures in development. The dental formula and measurements follow Altig (1970), except interorbital distances which were taken between the inner margins of eyes. All measurements were taken to the nearest 0.1 millimeters using an ocular micrometer in a Zeiss stereomicroscope; total length was measured with calipers. Remarks about spiracle and tail tip and musculature morphology follow Altig and McDiarmid (1999). Drawings were made using a Zeiss stereomicroscope with a camera lucida. Tadpoles were preserved in 5% formalin.