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Topography of Morphological Abnormalities and Mate Selection in the Toads *Bufo bufo* L., 1758 and *B. viridis* Laur., 1768

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Abstract—Collection material (the Museum of the Institute of Plant and Animal Ecology of the Ural Branch of the Russian Academy of Sciences) on the common and green toads collected by L.Ya. Toporkova in 1976–1977 in the Middle and Southern Urals was analyzed. In addition to species-specific characteristics of mate selection, differences in the frequency and the pattern of topographic confinement were found. They were determined by different mating models and differences in the degree of ecological flexibility of the species studied.

Key words: morphological abnormalities, topographic confinement, amphibians, mating pairs.

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Individuals with morphological deviations regularly occur in amphibian populations. The causes of the malformations are various: parasites, biotopic isolation, developmental deviations, abnormal regeneration, environmental pollution, appearance of genetically isolated populations as a result of fragmentation of natural biotopes, changes in the genetic structure of populations because of a decrease in their sizes, etc.

The ranges of variation in different morphological structures, including its extreme forms (referred to as abnormalities), are different. The prevalence of morphological deviations is usually higher in males (Szekely and Nemes, 2003; Gridi-Papp and Gridi-Papp, 2005); anatomical deviations of fore legs are rarer than those of hind legs in anurans (Dubois, 1979; Helgen et al., 2000). The spectrum of these deviations is genetically determined as a species-specific reaction norm (it is narrower in some species and wider in others), which reflects the phylogenetic position and ecological flexibility of the species. Forms with higher individual variability are also more flexible in the course of evolution; they more readily respond to environmental changes (Schmalhausen, 1983).

The following types of abnormalities are found in common and green toads (*Bufo bufo* L., 1758 and *B. viridis* Laur., 1768): ectrodactyly, oligodactyly, syndactyly, clinodactyly, brachymelia, taumelia, polymelia, ectromelia, retinal depigmentation (Engelmann and Obst, 1976), albinism, monorenia, and synrenia (Flindt, 1985; Rostand, 1958). In most anurans, females are larger than males. This phenomenon is explained by the main population role of females, namely reproduction: a large female produces more eggs. Sexual maturation of amphibians usually correlates with attainment of a certain body size. The male body size is not directly related to fecundity, which is reflected in the sex-related differences in body size.

Mate selection is important for successful fertilization (Licht, 1976). Large males are believed to have advantage in mating, because this phenomenon is based on territorial behavior (Davies and Halliday, 1977). A large male better protects its territory and occupies a more suitable site for spawning; i.e., it has a better chance than a small one. These are, in brief, theoretical speculations on sexual selection in anurans. However, factual data indicate that this often is not the case (Davies and Halliday, 1979).

According to another hypothesis, the advantage of large males consists in a longer calling, which, in turn, is related to the amount of energy reserves (Halliday and Verrel, 1986), which is not always correlated with body size. Ishchenko (1999) has demonstrated that small, slowly growing male moor frogs have a longer life span and, hence, can participate in reproduction more times and eventually have a larger offspring, which is the biological sense of reproduction. It is also assumed (Kondrashev, 1981) that the average and extreme sizes of females are not included in the region of maximum preference of males of either common or green toad. Thus, to fully understand the assortative mating and sexual selection, it is necessary to know much about the physiological characteristics of the animals and all stages of their life cycle.

It is known that the mating period of the common frog is brief and its mating behavior is extremely active. Small males lose competition with large ones; i.e., assortative mating takes place. In the Caucasian toad *B. verrucosissimus circassicus* Orlova et Tuniyev, 1989, the male and female body lengths in amplexus are negatively correlated: the larger the female, the smaller the male. In the green toad, mating is also assortative. It is believed (Reading, 2001) that selective mating, together with sex-related differences in the age of sexual maturation, favors a decrease in the inbreeding rate. Two main mating strategies have been described: an active choice of a female by competing males and an active choice of a male by a female. However, observations have not confirmed that only males choose the mate (Arntzen, 1999). Large males are more successful in competition for females. The main criteria in choosing the mate are the frequency of vocalization and the pitch height and duration of calling, which are positively correlated with the male body size (Kuzmin, 1999).

The purpose of this study was to compare the spectra of visually observable morphological abnormalities in the common and green toads, determine the animal body lengths by means of a vernier caliper with a scale division of 0.1 mm, and estimate the patterns of mate selection in the two species.

MATERIALS AND METHODS

We used materials from the herpetological collection of the Institute of Plant and Animal Ecology of the Ural Branch of the Russian Academy of Sciences: the 1976 collection of L.Ya. Toporkova (collector, O.I. Shilova) (a sample of 183 common toads (*B. bufo* L.) from the Kuzino village, Sverdlovsk oblast) and the 1977 collection of L.Ya. Toporkova (collector, L.A. Kobzeva) (a sample of 171 adult and 123 young-of-the-year green toads (*B. viridis* Laur.) from the vicinities of a heat power plant and the central power plant and the Severnyi Plyazh beach in the city of Magnitogorsk, Chelyabinsk oblast. The adult animals were captured during the spawning period.

Regression analysis was performed using the Statistica for Windows 6.0 and StatGraph 2.1 program packages. The overlap of the spectra of abnormalities was calculated using modified Morisita's index (Morisita, 1959).

RESULTS AND DISCUSSION

Studies on the samples from spawner groups of the two toad species, *B. bufo* and *B. viridis*, showed the presence of four types of abnormalities: ectrodactyly, brachymelia, clinodactyly, and syndactyly. Thus, we found only the "background abnormalities" typical of these species (Kovalenko, 2000) that do not preclude survival or reproduction of the animals.

Among the 183 common toads examined, 55 (30.05%) had abnormalities. The prevalence rates of abnormalities in males and females were 33.6 and 21.15%, respectively; i.e., the difference between males and females in the total frequency of abnormalities was nonsignificant ($\chi^2 = 2.74$; $p > 0.05$). In general, most abnormalities were found in hind legs (abnormalities of the fore and hind legs accounted for 43.6 and 60% of the total number of cases, respectively). In males, abnormalities of the hind and fore legs accounted for 68.2 and 36.4% of their total number, respectively. Therefore, the prevalence rates of abnormalities of the fore and hind legs in males and females considerably differed from each other ($\chi^2 = 5.24$; $p < 0.05$). Note that abnormalities of fore and hind legs were found in six and one female copulants, respectively, and two and nine male copulants, respectively.

Since the overlap of the abnormality spectra of male and female common toads was 97.7% ($C = 0.977$), we are reporting the summary proportions of individual abnormalities among the total number of abnormalities in all common toads. The proportions of ectrodactyly, syndactyly, clinodactyly, and brachymelia were 85.9, 1.8, 10.5, and 1.8%, respectively.

Abnormalities were found in 23 out of 294 examined adult green toads (13.45%). As in common toads, we found four types of abnormalities: ectrodactyly, brachymelia, clinodactyly, and syndactyly.

Most abnormalities were found in both fore and hind legs, the abnormalities of the fore legs being slightly more frequent (52.2% versus 47.8% in the hind legs). Only two of the abnormal animals were female, which was 8.7% of the total number of abnormal adult animals. In the females, abnormalities were found only in the hind legs. Among copulants, there were two females with abnormalities of the hind legs, one male with clinodactyly of a fore leg and one male with ectrodactyly of a hind leg.

Twelve out of 21 abnormal males had abnormalities of the fore legs; nine males, those of the hind legs. Thus, the ratio of the proportions of abnormalities of the fore and hind legs of male green toads were 57.1 and 42.9%, respectively. Young-of-the-year and adult toads significantly differed in the total prevalence rate of malformations ($\chi^2 = 12.86$; $p < 0.001$); males and females also significantly differed in this respect ($\chi^2 = 16.83$; $p < 0.001$).

The overlap of the abnormality spectra in male and female green toads was 98.3% ($C = 0.983$), which was almost the same as in *B. bufo*; therefore, we are reporting the summary proportions of individual abnormalities among the total number of abnormalities in all the animals. The proportions of ectrodactyly, syndactyly, clinodactyly, and brachymelia were 52, 4, 40, and 4%, respectively. In addition, cases of combined abnormalities were found in males of both species: two cases in common toads (double ectrodactyly) and one case in a green toad (brachymelia and ectrodactyly).

Species of the same genus may have homologous genes and exhibit the same variability (Vavilov, 1967). Therefore, it is conceivable that the characteristics of deviant forms in species of the same genus may be similar, which is confirmed by our data. Calculation of the

Table 1. Numbers of pairs formed by *B. bufo* from different size classes

Female size class, mm	Male size class, mm			
	60–65	65–70	70–75	75–80
75–80	1	4	2	0
80–85	5	3	2	0
85–90	0	3	4	0
90–95	2	0	0	2
95–100	1	0	0	0

Table 2. Numbers of pairs formed by *B. viridis* from different size classes

Female size class, mm	Male size class, mm				
	50–55	55–60	60–65	65–70	70–75
50–55	0	1	0	0	0
55–60	0	1	1	0	0
60–65	1	2	2	0	0
65–70	0	0	0	1	1
70–75	0	0	1	0	1

overlap of the abnormality spectra in *B. bufo* and *B. viridis* showed their close similarity ($C = 0.828$).

At the same time, we found considerable interspecific differences in the total prevalence rates of abnormalities between the common and green toads. Probably, this was related to a higher ecological flexibility of *B. viridis*, which is less susceptible to adverse effects of the environment promoting morphological deviations. Although the captured animals lived in the vicinities of industrial objects (a heat power plant and the central power plant), the frequency of abnormalities in this species was only about one-third of that in the common toad population from a relatively unpolluted biotope near the Kuzino village. These results agree with published data on the state of green toad populations in industrially altered areas. Estimation of the state of green toad populations in anthropogenic landscapes (Chikin et al., 1997) based on morphological criteria showed that it was relatively stable. The frequency of abnormalities among young-of-the-year (2.4%) fell within the range reported earlier (0.28–3.21%) (Flindt, 1985). For comparison, the frequencies of polydactyly and polymelia in young-of-the-year common toads may sometimes be as high as 51.6 and 16.67%, respectively (Rostand, 1958).

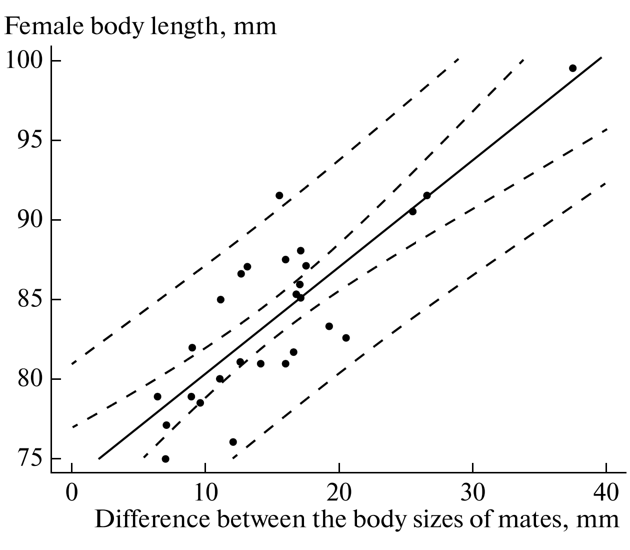

Fig. 1. Relationship between the female body size and the difference between the body sizes of mates in the common toad.

To analyze the data on mate selection, we grouped common and green toads into size classes (Table 1). Male common toads were divided into four classes and female toads, into five classes. It is known that male common toads are always smaller than female ones (Grosse and Hempel, 2004). Most males fell within size classes 1 (from 60 to 65 mm) and 2 (from 65 to 70 mm); most females, within class 2 (from 80 to 85 mm) and, slightly fewer, classes 1 (from 75 to 80 mm) and 3 (from 85 to 90 mm). The smallest males (size class 1) formed pairs with females from all size classes except class 3, and their sizes in all pairs were smaller than those of females, the mean difference being 15.3 ± 6.69 (6.3–37.5, $n = 28$)). Males from classes 2 and 3 formed pairs with females from classes 1, 2, and 3. The largest males formed pairs with females from size class 4. Probably, small males intercept females approaching a water body, as is the case with *Rana temporaria* (Marzona and Giacoma, 1999).

Regression analysis (the linear model) did not reveal any significant relationship between the body sizes of mates in *B. bufo* couples. However, we found a highly significant correlation ($R = 0.82$; $F = 52.65$; $p \ll 0.00001$) between the female body length and the difference between the female and male body lengths (Fig. 1). A significant negative linear relationship was found between the male body length and the difference between the female and male body lengths ($R = -0.58$; $F = 12.91$; $p = 0.0013$).

For green toads (Table 2), in contrast to common toads, we obtained the same intervals of the size classes and their numbers in males and females (interval 1, 50–55; interval 2, 55–60; interval 3, 60–65; interval 4, 65–70; and interval 5, 70–75 mm).In addition, the mates in pairs did not differ much in body length. There were pairs with almost equal body lengths of males and females and with larger males. The mean difference between the body lengths of the mates was 1.6 ± 5.29 mm (5.0–12.5 mm, $n = 12$).

Practically all males of the first three size classes (with one exception) formed pairs with females from

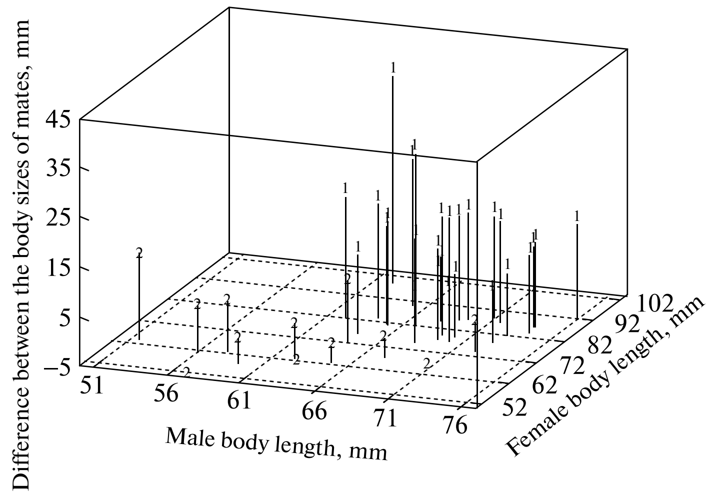

Fig. 2. Relationship between the body sizes of mates in the (1) common and (2) green toads.

the respective classes, whereas all males from the two classes with the largest size mated with females from the last two classes. Regression analysis showed a positive linear relationship ($R = 0.61$) between the male and female body lengths in pairs of green toads ($F = 6.049$; $p = 0.0337$), which was not the case with common toads (Fig. 2).

Thus, the species with a distinct sexual dimorphism exhibited assortative mating, with small males (size class 1, from 60 to 65 mm) being advantaged in forming pairs; they mated with females from all size classes. Interestingly, an experiment using a system of fences and traps for studying intra- and interpopulation relationships of *B. bufo* under terrestrial conditions (Arntzen, 1999) demonstrated that large males formed pairs, while small ones are left without mates. Gentilli et al. (2001) noted contradictions in interpreting sexual selection for morphometric traits in *B. bufo* populations.

In our case, the consistent pattern of mate selection in common toads is that the difference between the body lengths of mates in pairs and the female body length increase as the male body length decreases. Apparently, small males are very active and form pairs irrespective of the female size, thereby disturbing the common rule of selecting mates that are more similar in size. Probably, this is favored by formation pairs on land (Marzona and Giacoma, 1999). According to Arntzen (1999), 30–35% of spawning females form pairs on land, which is an important peculiarity of the reproduction biology of *B. bufo* that should be borne in mind.

The green toad has hardly any sexual dimorphism with respect to the body size. According to available data, toads of similar sizes form pairs more or less randomly, with the differences between the mates' body sizes being not unidirectional (in 50% of cases, males are larger than females).

The higher prevalence of abnormalities in males of both species is not surprising: the variability of the main parameters is always greater in females, which is related to reproduction, their major population function.

Evidently, the smaller variability of the structure of the fore legs compared to the hind legs in male common frogs, representative s of a species with a distinct sexual dimorphism, is related to their important functional role: they are used in amplexus. Some authors (Lee and Corrales, 2002) note that selection affects the musculoskeletal system of the fore legs, with which a male holds a female. This is why abnormalities of the hind legs in male *B.bufo* are more frequent, whereas abnormalities of the fore legs are more prevalent in females.

Thus, the two species of toads exhibit both similarities and dissimilarities with respect to occurrence of morphological abnormalities. Evidently, the similarities are determined by the taxonomic closeness of the species belonging to the same genus, and the dissimilarities are related to species specificity.

The common toad is characterized by considerable, functionally determined differences in the topographic confinement of abnormalities in males and females. This species exhibits assortative mating, which is poorly expressed in *B. viridis*. We think that the observed differences are related to the distinct sexual dimorphism of *B. bufo*. The probable biological sense of this phenomenon is increasing the genetic heterogeneity of populations (maintaining a certain level of heterozygosity).

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