PHENOTYPIC SELECTION IN COMMON TOAD *(BUFO BUFO)*

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Abstract — One of the most important problems for evolutionary biologists is to investigate the patterns and strength of phenotypic selection acting on quantitative traits in natural populations. Measurement of selection is complicated by the presence of correlations between characters; selection on a particular trait produces not only a direct effect, but indirect effects as well. Despite the growing body of phenotypic selection studies in a variety of taxa, studies on amphibians are still sparse. The aim of this study was to estimate patterns and strength of selection acting on a set of correlated characters in a natural population of *Bufo bufo* from the vicinity of Belgrade, Serbia. Morphological traits (body length, fore- and hind leg length) were measured, while fitness was assayed as fecundity and gonad weight for females and males, respectively. The regression approach was used to estimate selection differentials and gradients. Selection patterns differed between the sexes – linear selection differentials showed significant total directional selection for body size in females, but not in males. In males, differentials were significant for both fore- and hind leg lengths. Sample size did not permit identification of significant nonlinear (quadratic) selection.

Key words: Phenotypic selection, correlated characters, selection differentials, selection gradients, body size, *Bufo bufo,* Serbia

INTRODUCTION

One of the most important problems for evolutionary biologists is to investigate the patterns and estimate the strength of phenotypic selection acting on quantitative traits in natural populations. The process of natural selection occurring within generations is distinguished from the evolutionary consequences of that process, which appear across generations (L a n d e and A r n o l d, 1983; F a i r b a i r n and R e e v e, 2001). Selection can be measured from the observed changes in distribution of phenotypic characters within a generation. This is complicated by the fact that natural selection acts on numerous traits simultaneously. Any given trait is correlated with many others and these correlations influence the patterns observed. Selection on a given trait produces a direct effect (on the distribution of that trait) and indirect effects as well (on the distribution of correlated characters). One of the questions that researchers have to answer is the relative importance of indirect effects compared to direct ones.

Commonly used univariate methods of measuring selection could not distinguish direct from indirect selection. Thus, multivariate methods were developed, based on the linear and quadratic regressions of relative fitness on morphological or other traits, to analyze selection acting on multiple, correlated traits, and to disentangle direct from indirect effects (L a n d e, 1979; L a n d e and A r n o l d, 1983; A r n o l d and W a d e, 1984a, 1984b; P h i l l i p s and A r n o l d, 1989).

These methods permit calculation of linear as well as nonlinear (quadratic) selection differentials and gradients (L a n d e and A r n o l d, 1983). Selection differentials estimate the total strength of selection, including both direct effects on a given trait and indirect effects through other, phenotypically correlated traits. Selection gradients estimate the direct effects of selection on a trait, excluding

the residual effects of other traits. Linear selection gradients and differentials estimate the strength of directional selection, while nonlinear (quadratic) estimate the strength of stabilizing/disruptive selection. The linear selection differential describes changes in the trait mean; the nonlinear selection differential describes changes in trait variance.

In years following the development of these models, our knowledge about the strength of phenotypic selection in natural populations has increased substantially. Thus, K i n g s o l v e r et al. (2001) present a synthetic review of phenotypic selection studies on more than 60 species of invertebrates, vertebrates, and plants; F a i r b a i r n and R e e v e (2001) give an overview of 188 studies performed on 184 species. K i n g s o l v e r et al. (2001) analyzed a heterogeneous set of studies in order to show how different biological and methodological features (taxon, trait, sample size, etc.) influence our estimates of the strength of selection and draw general conclusions about selection patterns in nature.

Despite this ever growing body of information about phenotypic selection in a variety of taxa, studies on amphibians are still very sparse. Among anurans, the few analyzed species include *Rana catesbeiana* (A r n o l d and W a d e, 1984b); *Bufo bufo* (H ö g l u n d and S ä t e r b e r g, 1989); *Rana lessonae* and *R. esculenta* (A l t w e g g and R e y e r, 2003). *Bufo bufo* is a widespread and well-studied anuran species; various aspects of its biology have attracted much research interest (H e m e l a a r, 1988; R e a d i n g, 1991, 2007; K u h n, 1994; S c r i b n e r et al., 2001; C v e t k o v i ć et al., 2005; B r e d e and B e e b e e, 2006), yet so far only H ö g l u n d and S ä t e r b e r g (1989) have investigated the problem of multivariate selection in this species.

Accordingly, the aim of this study was to investigate the pattern and quantify the strength of phenotypic selection acting on a set of correlated morphological characters in a natural population of *B. bufo* from the vicinity of Belgrade, Serbia.

MATERIAL AND METHODS

Adult common toads (*B. bufo*) were collected during the breeding period in 2003. The study site was a pond near the village of Zuce (44º 41' N, 20º 33' E, altitude of 240 m a.s.l.), situated in an agricultural area near Belgrade and surrounded by remnants of deciduous sclerophylous woods.

The traits included in the analysis were: body size (measured as snout-vent length, SVL), fore leg length, and hind leg length [the original number of traits examined was reduced to three to improve statistical power (F a i r b a i r n and R e e v e, 2001)]. Body size was chosen as the phenotypic trait most commonly analyzed in studies of this kind; it typically correlates with fitness, e.g., with increase in survival or fecundity (J a n z e n et al., 2007; review in H o n e and B e n t o n, 2005). Measures were taken with dial calipers to 0.1 mm precision. Fitness was assayed as fecundity (i.e., the number of eggs) for females, while gonad weight was used as the indicator of fitness for males. The total number of eggs per female was determined using image analysis software (Image Tool, v. 3.0, UTHSC SA). Weights were measured with an electronic balance to 0.001 g precision. The individual age data used in analysis of age-size relationships were obtained from T o m a š e v i ć et al. (in press); age was assessed by the skeletochronological method (details in C v e t k o v i ć et al., 2005). Total sample size was 58 individuals (25 males and 33 females).

Statistical methods

To quantify phenotypic selection, we applied statistical models based on regression of relative fitness on standardized morphological traits. Relative fitness is defined with reference to mean fitness; thus, individual fitness was divided by mean fitness in the population. Morphological variables were standardized to have normal distribution with a mean of zero and a standard deviation of one; these standardized values are also termed "z-scores" (Q u i n n and K e o u g h, 2002).

Standardized linear selection differentials (S') and gradients (β'), as well as nonlinear (quadratic) selection differentials (C') and gradients (γ') were calculated according to L a n d e and A r n o l d (1983), employing linear and quadratic regression

models. To allow for calculation of quadratic gradients, sample size had to exceed the number of coefficients to be estimated:

$$N > n + n(n+1)/2$$

where N is sample size and n is the number of analyzed traits (L a n d e and A r n o l d, 1983; F a i r b a i r n and R e e v e, 2001). Analyses were performed using the Statistica v.6 statistical package (Statsoft Inc., USA).

RESULTS

Bufo bufo is known for high levels of sexual size dimorphism (SSD); males and females were therefore analyzed separately (L a n d e and A r n o l d, 1983). Correlations between the analyzed morphological characters were positive and highly significant, ranging from 0.618 to 0.786 in males, and from 0.778 to 0.795 in females (p<0.001 in all cases). Standardized linear (directional) selection differentials (S') and gradients (β') for the analyzed traits in B. bufo females and males are given in Table 1.

Table 1. Linear (directional) standardized selection differentials (S') and gradients (β') for analyzed traits in *B. bufo* females and males (SVL – snout-vent length, FLL and HLL – fore and hind leg length, respectively); p<0.05, **p<0.01.

	S'	p	β'	p
females				
SVL	**0.204***±0.077	0.014	0.223±0.143	0.131
FLL	**0.165***±0.073	0.032	0.096±0.125	0.450
HLL	0.121±0.076	0.124	-0.115±0.130	0.384
males				
SVL	0.081±0.058	0.178	-0. 057±0.072	0.437
FLL	**0.157****±0.051	0.006	0.133±0.091	0.161
HLL	**0.149****±0.052	0.009	0.079±0.086	0.368

Linear selection differentials pointed to significant total directional selection for body size in females, but not in males. Females also showed significant positive selection for fore leg length, while in males differentials were significant for both fore and hind leg lengths. However, there is a marked contrast between selection differentials and

gradients – none of the directional gradients was significant.

Standardized nonlinear (quadratic, stabilizing/disruptive) selection differentials and gradients for the analyzed morphological traits in *B. bufo* females and males are given in Table 2. None of the calculated differentials and gradients (i.e., indicators of total and direct stabilizing/disruptive selection) was significant, which is a result commonly obtained in studies of this type.

Table 2. Nonlinear (quadratic, stabilizing/disruptive) standardized selection differentials (C') and gradients (γ') for analyzed traits in *B. bufo* females and males (SVL – snout-vent length, FLL and HLL – fore and hind leg length, respectively).

	C'	p	γ'	p
females				
SVL	0.025±0.080	0.760	0.010±0.140	0.964
FLL	-0.065±0.056	0.259	-0.117±0.086	0.187
HLL	0.065±0.061	0.297	0.129±0.113	0.267
males				
SVL	-0.005±0. 055	0.924	-0.023±0.057	0.692
FLL	0.036±0.043	0.414	-0.010±0.050	0.849
HLL	0.050±0.053	0.362	0.013±0.060	0.844

One major point that needs to be kept in mind in various studies considering body size in amphibians is that individual age may have important implications. Inspection of the age-size distribution revealed no significant relationship between age and size in either sex and no significant differences between females and males. Figure 1 shows the regressions of body size (measured as snout-vent length) on age in female and male common toads (Y=99.133–0.481X, p= 0.71 and Y=71.736–0.256X, p= 0.65 in females and males, respectively). H ö g l u n d and S ä t e r b e r g (1989) also tested the age-size relationship in a study concerning selection on correlated morphological characters in *B. bufo*. They found nonsignificant correlations between age and body size in males, but significant ones in females. Other morphological traits showed low and nonsignificant correlations with age in both sexes, and the same result was obtained in our study.
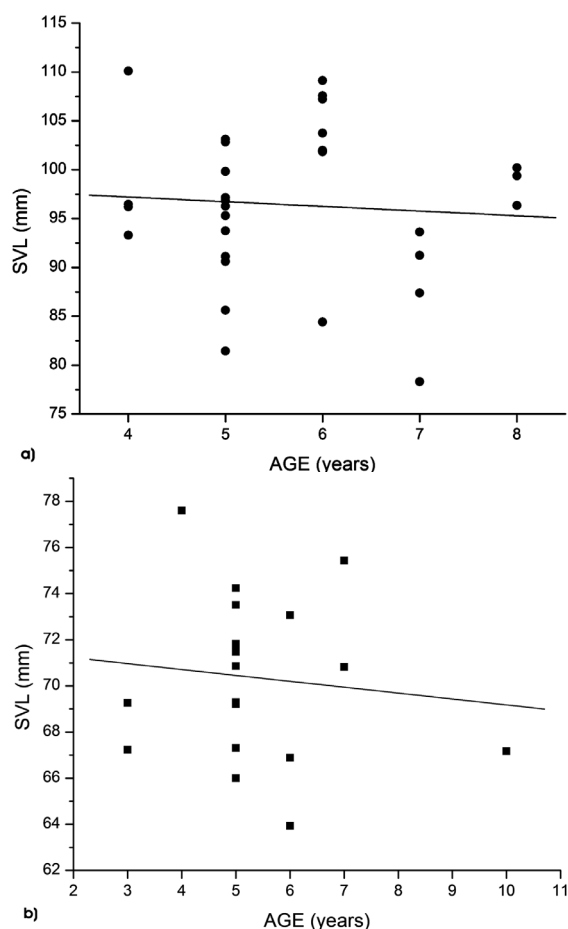
Fig. 1. – Linear regression of body length (SVL) on age in female (a) and male (b) common toads.
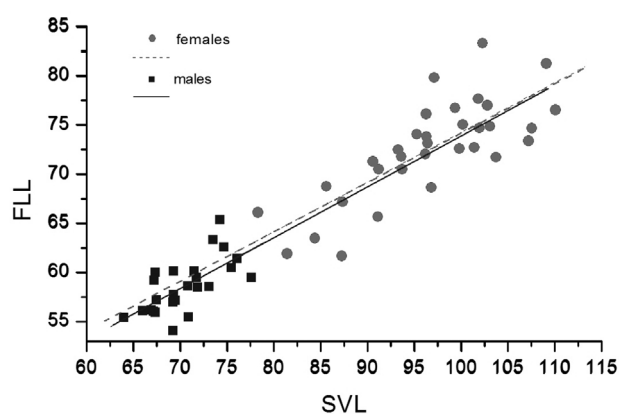

Fig. 2. – Linear regression of forelimb length (FLL) on body length (SVL) in female and male common toads.

We also examined whether there were significant intersexual differences in the relationship between leg lengths and total body length in females and males. Figure 2 shows the regressions of total fore leg length (FLL) on body size in females and males (Y = 23.704 + 0.505 X, p < 0.001 and Y = 21.714 + 0.524 X, p< 0.001 in females and males, respectively). The difference between regression slopes was not significant. The results for total hind leg length (HLL) are not presented graphically, for the conclusions were essentially the same (Y=37.608+0.997X, p< 0.001 and Y=42.547+0.932X, p=0.001 in females and males, respectively; nonsignificant difference between regression slopes). H ö g l u n d and S ä t e r b e r g (1989) also examined the relationship between fore leg length and total body length in females and males and found significant intersexual differences, males having proportionally longer fore legs than females.

DISCUSSION

Selection patterns differed between the sexes in our analysis. Linear selection differentials showed significant total directional selection for body size in females, but not in males. There was also significant selection for longer fore legs in females, while in males differentials were significant for both fore and hind leg lengths.

The absolute values of linear selection gradient estimates obtained in our study, ranging from 0.06 to 0.22, are in accordance with findings of K i n g s o l v e r et al. (2001) that the magnitude of linear selection is generally rather modest, the median β value for morphological traits being 0.17.

A significant selection differential for body size could be the result of sexual selection or fecundity selection (J o n e s et al., 2005). The selection for larger body observed in this study is consistent with the expected pattern of fecundity selection favoring larger body size in female toads. A strong tendency of larger females to produce more eggs has been documented in numerous amphibian species (G i b b o n s and M c C a r t h y, 1986; H ö g l u n d and R o b e r t s o n, 1987; K u h n, 1994). It is interesting that the same pattern was found in sex-role-reversed species, where fecundity selection favored larger males (J o n e s et al., 2005).

The results for other traits are less clear. Net directional selection for longer legs in males could be associated with the importance of agility in males (H a l l i d a y and V e r r e l l, 1986). However, we did not detect significant intersexual differences in total leg length relative to body length, though in a previous study on this species H ö g l u n d and S ä t e r b e r g (1989) found that males had proportionally longer fore legs than females.

Although significant total directional selection was detected for some of the characters, analysis of directional gradients failed to reveal significant direct effects in either sex. The marked contrast between selection differentials and gradients is not an uncommon result in studies of this type. One possible explanation for such contrast is that the observed change of character mean may be attributable to indirect effects of selection acting on some other, correlated trait. However, in this case it seems more likely that the relatively small sample size resulted in lower power to detect significant selection; it has been noticed that standard errors of estimates can be quite large relative to the magnitude of selection. It should be mentioned, however, that our sample size still falls within the range reported in reviews of previous studies (F a i r b a i r n and R e e v e, 2001; K i n g s o l v e r et al., 2001) – most published phenotypic selection studies had relatively small sample sizes (n < 135), and in some it was very small (10 < n < 20).

With respect to the possibility that the observed pattern may reflect selection on a correlated character, K i n g s o l v e r et al. (2001) also concluded that their results do not indicate that indirect effects frequently mask or reverse direct effects, which suggests that indirect selection is usually weak compared to direct selection.

Sample size in our study was not large enough to permit identification of significant nonlinear (quadratic) selection; however, this result does not eliminate the possibility of stabilizing/disruptive selection acting on the analyzed traits. Again, the absolute values of quadratic selection gradient estimates obtained in our study, ranging from 0.01 to 0.13, are in accordance with conclusions of K i n g s o l v e r et

al. (2001) that the magnitude of quadratic selection is typically small (median $\gamma = 0.10$, 84% of estimates not significantly different from 0).

When drawing more general conclusions about the quadratic selection, it has to be kept in mind that quadratic differentials and gradients are often not estimated (if sample size is modest) or not reported (if values are nonsignificant). There is also a publishing bias, the so-called "file drawer" phenomenon (I y e n g a r and G r e e n h o u s e, 1988; P a l m e r, 1999) – studies with modest sample size reporting nonsignificant selection are not likely to be submitted/accepted.

To conclude: phenotypic selection studies show a strong bias in favor of some taxa (e.g., insects, herbaceous plants); for logistic reasons, studies on amphibians are sparse. One of the main problems is how to obtain adequate sample size and analyze diverse fitness traits without at the same time endangering natural populations. Performed on one of the most widespread and well studied amphibian species in Europe, the present study yielded some intriguing results, especially with respect to intersexual differences in selection patterns, and stresses the need for more studies on phenotypic selection in this group.

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ФЕНОТИПСКА СЕЛЕКЦИЈА КОД *BUFO BUFO*

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Један од најважнијих проблема за еволуционе биологе је процена образаца и интензитета фенотипске селекције која делује на квантитативне особине у природним популацијама. Процену селекције компликује и присуство корелације између особина; селекција која делује на дату особину има не само директан ефекат на ту особину, већ и индиректан на расподелу корелисаних особина. Упркос растућој количини информација о фенотипској селекцији код различитих таксона, студије на водоземцима су још увек врло ретке. Циљ овог рада био је да се процене обрасци и интензитет селекције која је деловала на скуп корелисаних особина у природној популацији *Bufo bufo* из околине Београда. Мерене су морфолошке особине (дужина тела, дужина предњих и задњих екстремитета); фитнес је процењен преко фекундитета код женки, односно тежине гонада код мужјака. Регресиони метод је примењен за процену селекционих диференцијала и градијената. Обрасци селекције су се разликовали међу половима – линеарни селекционих диференцијали указују на значајну укупну дирекциону селекцију за величину тела код женки, али не и код мужјака. Код мужјака, диференцијали су били значајни и за предње и за задње екстремитете. Због величине узорка није било могуће наћи значајну нелинеарну (квадратну) селекцију.