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## MORPHOLOGICAL VARIATION IN TWO CRYPTIC FORMS OF THE COMMON SPADEFOOT TOAD (*Pelobates fuscus*) FROM EASTERN EUROPE

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**Keywords:** *Pelobates fuscus*, Anura, cryptic speciation, morphometrics, coloration.

### INTRODUCTION

According to the nuclear DNA content measured by means of flow cytometry, two forms of *Pelobates fuscus* in eastern Europe were recognized: the “western” form with smaller genome size and the “eastern” form with larger genome size (Barabanov et al., 1998; Borkin et al., 2001a, 2001b, 2003a, 2003b). The existence of the two forms was confirmed by allozyme analysis (Borkin et al., 2001a, 2003b; Khalturin et al., 2003). Apart from biochemical characters, we studied morphological variation in the western and the eastern forms of *P. fuscus*.

### MATERIAL AND METHODS

Three hundred and nineteen adult specimens (178 males and 141 females) from 68 localities in Russia (47), Ukraine (14), Belarus' (4), Moldova (2), and Latvia (1) were used in the study. 228 specimens were allocated to the western or the eastern form by DNA flow cytometry (Borkin et al., 2001b). The remaining spadefoot toads were assigned to either forms on the basis of their localities. Samples from the same physical geographical regions were united together (Fig. 1).

Thirteen standard morphometric measurements (Terentjev and Chernov, 1949; Terentjev, 1950) were taken on each specimen's right side using a digital caliper (to the nearest 0.1 mm). Nineteen ratios expressed various body proportions were calculated and analyzed.

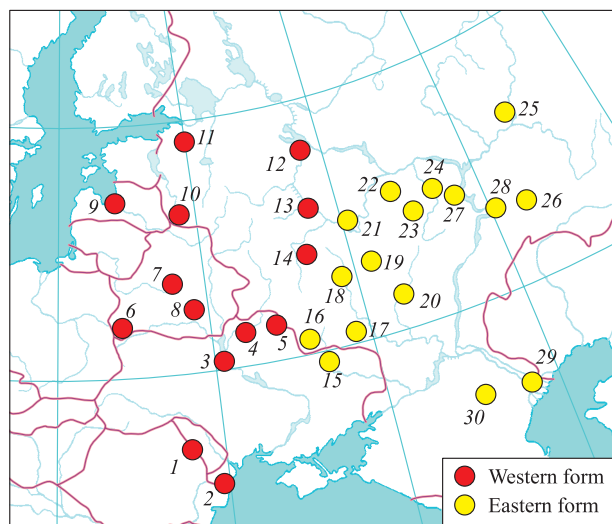
Dorsal coloration patterns of 307 specimens of *P. fuscus* were examined (we failed to identify coloration patterns in others specimens because of inadequate fixation). Frequencies of various kinds of five obvious elements of dorsal patterns were analyzed. These were the light medial stripe, light lateral stripes, the connection between medial

and lateral stripes, the dark stripe between eyes, and its connection with dark dorsal zones (Fig. 2).

Canonical discriminant analysis and standard statistical parameters (mean, SD, min – max, Kolmogorov – Smirnov's test) were used to treat obtained data.

### RESULTS

**Linear parameters.** 294 specimens grouping in 35 samples were studied. Minimum sample size contained three specimens. Canonical discriminant analysis was applied (Fig. 3). Samples of the western and the eastern types formed two groups in terms of external morphology.

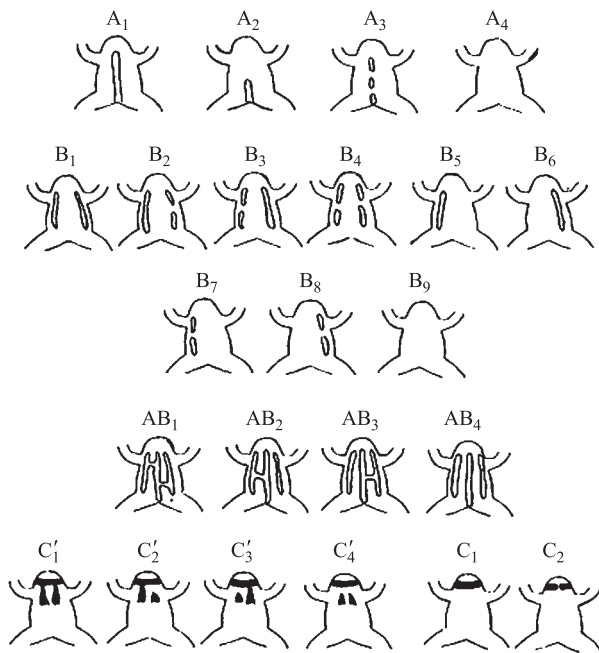


**Fig. 1.** Locality numbers of samples of *Pelobates fuscus*: 1, Moldova; 2, Odessa Oblast'; 3, Kiev Oblast'; 4, Chernigov Oblast'; 5, Sumy Oblast'; 6, Brest Oblast'; 7, Minsk Oblast'; 8, Gomel' Oblast'; 9, Latvia; 10, Pskov Oblast'; 11, Leningradskaya Oblast'; 12, Yaroslavl' Oblast'; 13, Moscow Oblast'; 14, Tula Oblast'; 15, Kharkov Oblast'; 16, Belgorod Oblast'; 17, Voronezh Oblast'; 18, Lipetsk Oblast'; 19, Tambov Oblast'; 20, Saratov Oblast'; 21, Ryazan Oblast'; 22, Nizhegorodskaya Oblast'; 23, Mordovia; 24, Chuvashia; 25, Udmurtia; 26, Bashkortostan; 27, Ul'yanovsk Oblast'; 28, Samara Oblast'; 29, Astrakhan Oblast'; 30, Kalmykia.

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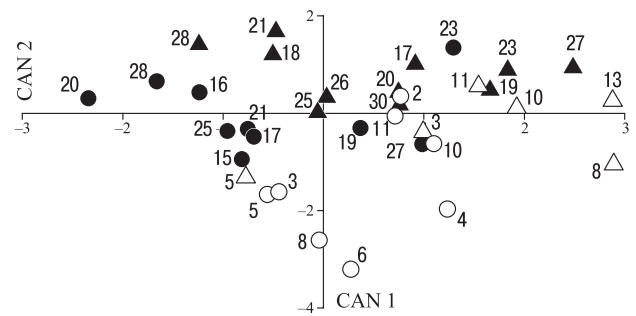
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**Fig. 2.** Variants of 5 elements of the dorsal pattern of *Pelobates fuscus*: A, light medial stripe; B, light lateral stripes; AB, connection between A and B; C, dark stripe between eyes; C', connection between C and dark dorsal zones.

Small overlapping was associated with specimens of the western type from Odessa Oblast' (males), Leningradskaya Oblast' (both sexes), Kiev Oblast' (females), and with specimens of the eastern type from Ul'yanovsk Oblast' (males), Tambov Oblast' (both sexes), and Kalmykia (females). Totally the overlapping included 21% of all specimens under the study. Thus, the majority of samples belonged to either form of *P. fuscus* were separated.

**Ratios.** The comparison of the western and the eastern forms showed significant differences between means of seven ratios for males and females, respectively, Lt.c./Sp.n., Sp.oc./Sp.n., Sp.p./Sp.n. in particular (Table 1). These three most important ratios included the linear parameter Sp.n. (the distance between nostrils). According



**Fig. 3.** Plot of populations centroids for males and females of two cryptic forms of *Pelobates fuscus* in the space of first and second canonical axes (CAN). Sample numbers the same as in Fig. 1. Males of western form males of eastern form; females of western form females of eastern form. ○, Males of western type; △, females of western type; ●, males of eastern type; ▲, females of eastern type.

to Tables 2 – 4, the western form as a whole was characterized by greater distance between nostrils. However, the ranges of three ratios in two forms of *P. fuscus* markedly overlapped. Moreover, significant differences between populations within each form were observed. Moreover, sometimes, differences between two samples of the same type (for instance, between males from Saratov and Ul'yanovsk Oblast's) can reach the level of differences between total samples of these forms.

**Sexual dimorphism.** Among 20 characters, only three ratios (L./L.c., L./D.r.o., and D.r.o./Sp.n.) showed significant differences between males and females of the western form of *P. fuscus* (Table 5). The sexual dimorphism seems to be more expressed in the eastern form because significant differences were found in eight characters.

**Coloration pattern.** Significant differences in the frequency of seven variants (especially light lateral stripes and dark stripe between eyes) were found between two cryptic forms of *P. fuscus*. Frequencies of various kinds of light lateral stripes and dark stripe between eyes are demonstrated in Table 6. The largest differences were revealed in variants B<sub>1</sub>, B<sub>4</sub> as well as in two variants of C. However,

**TABLE 1.** The Significant Level of Differences in Morphometric Characters Between Two Forms of *Pelobates fuscus*

Sex	L.	L./T.	L./D.p.	L./L.c.	L./D.r.o.	F./T.	T./D.p.	D.p./C.i.	L.c./Lt.c.	L.c./D.r.o.
Males	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.	n.s.	n.s.	0.001
Females	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.	n.s.	n.s.	0.01
Sex	L.c./L.o.	Lt.c./Sp.oc.	Lt.c./Sp.p.	Lt.c./Sp.n.	Sp.oc./Sp.p.	Sp.oc./Sp.n.	Sp.oc./D.r.o.	Lt.p./Sp.p.	Sp.p./Sp.n.	D.r.o./Sp.n.
Males	n.s.	0.01	n.s.	0.001	n.s.	0.001	0.001	n.s.	0.001	n.s.
Females	n.s.	n.s.	n.s.	0.001	n.s.	0.001	0.01	n.s.	0.001	0.01

**Note.** n.s., non significant,  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ .

**TABLE 2.** The Ratio Lt.c./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
<b>The “Western” Type</b>								
Sumy	8	4.48	0.33	4.11 – 5.10	7	4.44	0.21	4.17 – 4.74
Gomel’	6	4.60	0.25	4.17 – 4.90	—	—	—	—
Pskov	6	4.10	0.25	3.71 – 4.38	11	4.13	0.25	3.61 – 4.43
<b>Total</b>	44	4.42	0.39	3.27 – 5.29	40	4.31	0.42	3.61 – 6.15
<b>The “Eastern” Type</b>								
Voronezh	19	4.68	0.21	4.36 – 5.29	9	4.51	0.21	4.33 – 5.33
Tambov	19	4.48	0.23	3.98 – 4.97	13	4.51	0.43	3.32 – 5.11
Saratov	15	4.97	0.49	3.72 – 5.75	—	—	—	—
Ryazan	11	4.67	0.26	4.28 – 5.13	—	—	—	—
Mordovia	—	—	—	—	13	4.49	0.17	4.22 – 4.83
Udmurtia	19	4.86	0.33	4.21 – 5.48	16	4.70	0.23	4.42 – 5.09
Ul’yanovsk	7	4.43	0.46	3.45 – 4.76	—	—	—	¾
Samara	27	4.81	0.37	4.24 – 6.11	16	4.75	0.03	4.39 – 5.00
Kalmykya	—	—	—	—	9	4.82	0.30	4.26 – 5.12
<b>Total</b>	134	4.71	0.36	3.45 – 6.11	111	4.69	0.33	3.32 – 5.76

**TABLE 3.** The Ratio Sp.oc./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
<b>The “Western” Type</b>								
Sumy	8	1.71	0.13	1.54 – 1.90	7	1.80	0.14	1.54 – 2.00
Gomel’	6	1.67	0.08	1.59 – 1.77	—	—	—	—
Pskov	6	1.65	0.10	1.51 – 1.79	11	1.68	0.11	1.48 – 1.89
<b>Total</b>	44	1.69	0.15	1.29 – 2.08	40	1.69	0.16	1.41 – 2.15
<b>The “Eastern” Type</b>								
Voronezh	19	1.73	0.13	1.47 – 2.03	9	1.71	0.16	1.36 – 1.88
Tambov	19	1.78	0.16	1.51 – 2.10	13	1.75	0.20	1.35 – 2.06
Saratov	15	2.14	0.24	1.59 – 2.50	—	—	—	—
Ryazan	11	1.92	0.24	1.53 – 2.16	—	—	—	—
Mordovia	—	—	—	—	13	1.81	0.15	1.53 – 2.03
Udmurtia	19	1.90	0.21	1.49 – 2.17	16	1.86	0.15	1.58 – 2.07
Ul’yanovsk	7	1.70	0.16	1.46 – 1.94	—	—	—	—
Samara	27	1.99	0.15	1.76 – 2.35	16	2.06	0.02	1.82 – 2.30
Kalmykya	—	—	—	—	9	1.83	0.15	1.60 – 2.00
<b>Total</b>	134	1.88	0.23	1.30 – 2.50	111	1.86	0.19	1.35 – 2.30

**TABLE 4.** The Ratio Sp.p./Sp.n. of Various Samples of Two Forms of *Pelobates fuscus*.

Samples	Males				Females			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
<b>The “Western” Type</b>								
Sumy	8	1.40	0.09	1.28 – 1.55	7	1.37	0.19	1.19 – 1.55
Gomel’	6	1.27	0.06	1.21 – 1.35	—	—	—	—
Pskov	6	1.33	0.13	1.16 – 1.55	11	1.30	0.13	1.06 – 1.47
<b>Total</b>	44	1.33	0.15	1.02 – 1.94	16	1.30	0.14	1.05 – 1.70
<b>The “Eastern” Type</b>								
Voronezh	19	1.48	0.11	1.33 – 1.76	9	1.50	0.09	1.38 – 1.65
Tambov	19	1.31	0.13	1.14 – 1.60	13	1.35	0.14	1.00 – 1.57
Saratov	15	1.53	0.20	1.18 – 1.85	—	—	—	—
Ryazan	11	1.40	0.13	1.18 – 1.61	—	—	—	—
Mordovia	—	—	—	—	13	1.28	0.13	1.02 – 1.48
Udmurtia	19	1.45	0.16	1.16 – 1.76	16	1.37	0.12	1.07 – 1.54
Ul’yanovsk	7	1.27	0.17	0.93 – 1.47	—	—	—	—
Samara	27	1.54	0.14	1.18 – 1.78	16	1.56	0.02	1.40 – 1.93
Kalmykya	—	—	—	—	9	1.46	0.12	1.31 – 1.75
<b>Total</b>	134	1.43	0.17	0.93 – 1.85	111	1.41	0.16	1.00 – 1.93

**TABLE 5.** The significant level of differences in morphometric characters between males and females in two forms of *Pelobates fuscus*

<i>P. fuscus</i> form	L.	L./T.	L./D.p.	L./L.c.	L./D.r.o.	F./T.	T./D.p.	D.p./C.i.	L.c./Lt.c.	L.c./D.r.o.
“Western”	n.s.	n.s.	n.s.	0.05	0.01	n.s.	n.s.	n.s.	n.s.	n.s.
“Eastern”	0.001	0.01	0.001	0.001	0.001	n.s.	0.01	0.001	0.05	n.s.

<i>P. fuscus</i> form	L.c./L.o.	Lt.c./Sp.oc.	Lt.c./Sp.p.	Lt.c./Sp.n.	Sp.oc./Sp.p.	Sp.oc./Sp.n.	Sp.oc./D.r.o.	Lt.p./Sp.p.	Sp.p./Sp.n.	D.r.o./Sp.n.
“Western”	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01
“Eastern”	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

**Note.** n.s., non significant;  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ .

**TABLE 6.** Frequencies (per cent) of various kinds of some elements of the dorsal pattern in two forms of *Pelobates fuscus*

<i>P. fuscus</i> form	Light lateral stripes (B)							Dark stripe between eyes (C)	
	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>	B <sub>7</sub>	B <sub>8</sub>	B <sub>9</sub>	C <sub>1</sub>	C <sub>2</sub>
“Western”	29.4	2.9	1.5	35.3	0	0	14.7	26.2	73.8
“Eastern”	7.0	2.6	1.7	81.6	0.4	0.4	6.1	66.1	33.9

almost all variants of all elements were observed in the both forms of *P. fuscus*.

## CONCLUSION

To summarize, some significant differences between averaged values of morphological characters of two forms of *P. fuscus* were revealed. However, diagnostic characters, which could allow to make reliable identification of each specimen, were not found. This fact confirms that these forms could be recognized cryptic. Such a morphological stasis of *P. fuscus* may be explained by the effect of stabilizing selection which maintains the optimum phenotype as a result of the adaptation to burrowing mode of life of this anuran species (Borkin et al., 2003a; Khalturin et al., 2003).

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