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DIAGNOSTIC TRAITS IN THE MORPHOLOGY OF GREEN FROGS (*Rana esculenta* COMPLEX) IN THE MIDDLE DNEPR BASIN

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INTRODUCTION

To solve problems of diagnostics specimens belonging to the green frogs of the *Rana esculenta* complex (*Rana ridibunda* Pall., 1771; *R. lessonae* Camerano, 1882; and their hybrid *R. esculenta* Linnaeus, 1758) on the basis of morphological traits has become possible only after successful using of the genetic markers (Mezhzherin and Morozov-Leonov, 1992). The morphological analysis of hybrid populations of green frogs was carried out, among other regions, in Poland, Hungary, France, Germany, Latvia, Russia, and Ukraine.

Morphological features are known to be subject to significant geographical variability. They depend on ploidy of hybrids and often are not fully reliable in mixed populations. Studies of diagnostic traits that allow taking into account the genetic variability and habitat choice of the pop-

ulations are of particular interest. These traits also may be the most reliable ones when distinguishing representatives of the *Rana esculenta* complex. The aim of the present study was to detect the most effective diagnostic indices using the ANOVA-MANOVA, discriminant analyses.

MATERIAL AND METHODS

Investigations were carried out in the Middle Dnepr basin (Fig. 1) from 1992 — 2002 on the basis of series of specimens that were genetically identified using electrophoresis. A total of 854 specimens of the *Rana esculenta* complex from 52 localities were analyzed. Sixteen standard morphometric indices (Terentiev, 1950; Berger, 1968, 1973; Nekrasova and Morozov-Leonov, 2001) were used (see Fig. 2): head — Lt.c./S.n., L./D.r.o., L./Sp.oc.,

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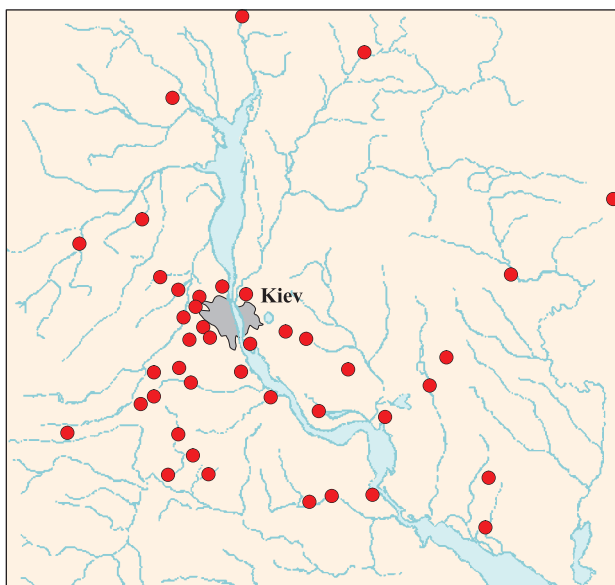


Fig. 1. Study area: the Middle Dnepr Basin.

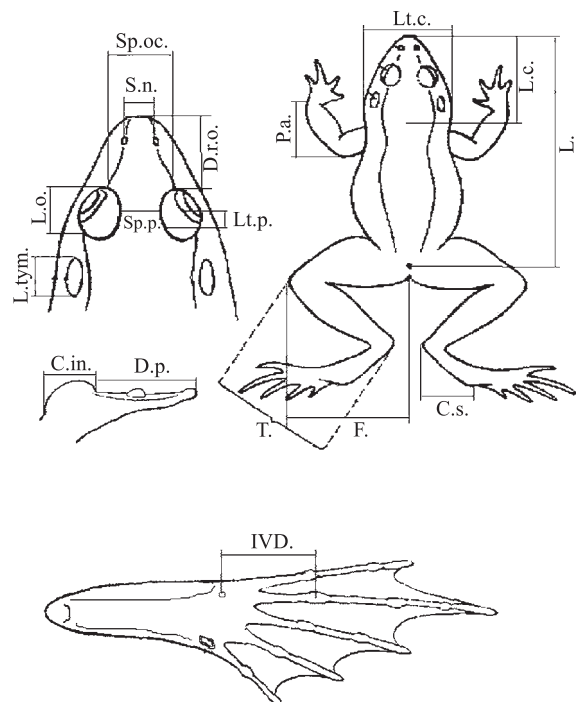


Fig. 2. Standard morphometric measurements as used in this study.

L./L.o., L./Lt.c., L./Sp.p., L./L.tym., L./S.n.; limbs — L./T., T./IVD., L./C.s., D.p./C.in., T./C.in., F./T., T./C.s., and index of Tarashchuk (1985): $I_x = T./C.in. \times D.p./C.in. \times T./C.s.$ Several coloration traits (see Nekrasova, 2002) were also recorded: (1) general body color, (2) number and size of the dorsal spots, (3) presence of dorsal-medial stripe, (4) number of stripes on the limbs (tibia, femur); (5) pigmentation of the ventral side (Figs. 2 and 3). The obtained morphometric ratios were used in standard statistical analysis using the discriminant, cluster and factor analysis modules of Statistica for Windows v. 5.0 (StatSoft, Inc., 1984 – 1995, USA).

RESULTS AND DISCUSSION

Populations of green frogs in Middle Dnepr basin are characterised by several genetic and morphological peculiarities. The most significant diagnostic indices of the

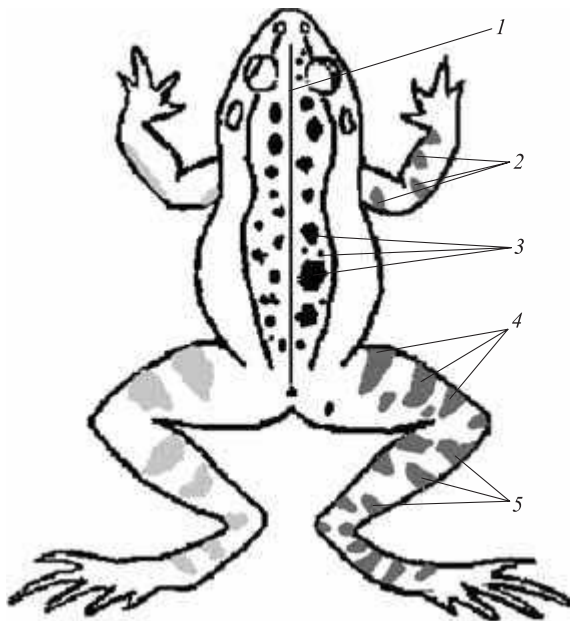


Fig. 3. Coloration traits. Right half, *Rana ridibunda*; left half, *R. lessonae*; 1, dorsal stripe; 2, basic stripes (or spots) on front limb; 3, dorsal spots; 4, main stripes (or spots) on femur; 5, main stripes (or spots) on tibia.

hind legs were determined green frogs genetic forms (I_x , D.p./C.in., T./C.in.). Limits of variability were specified (for most of the individuals): $I_x - R. lessonae < 21 < \text{hybrid} < 32 < R. ridibunda$; D.p./C.in. — $R. lessonae < 1.8 < \text{hybrid} < 2.3 < R. ridibunda$; T./C.in. — $R. lessonae < 6.7 < \text{hybrid} < 8.3 < R. ridibunda$.

Comparison of the results obtained using discriminant and factor analysis shows that it is possible to identify only three basic forms. The more clear results were achieved in distinguishing of parental species (Table 1): *R. ridibunda* (99%) and *R. lessonae* (97%). The hybrids were the least distinguishable (95%). The diagnostics gives the most reliable results when all the selected indices are included into the analysis. Contrary to that, the use of single traits resulted in 10 to 50% identification error. Traditionally used for diagnostics T./C.in. and D.p./C.in. provide the most accurate identification (91 and 88%, respectively).

To further improve the diagnostic resolution, the morphometric indices were compared to the body coloration pattern. The phenetic traits of *Rana esculenta* are largely intermediate between the two paternal species (Fig. 4), although some traits may be closer to one of the paternal species. This effect could be due to dominant-recessive gene interactions, which may influence the coloration pattern. It was shown that the level of the fluctuating asymmetry of dorsal spots in the hybrids exceeds one of the paternal species (Nekrasova, 2002).

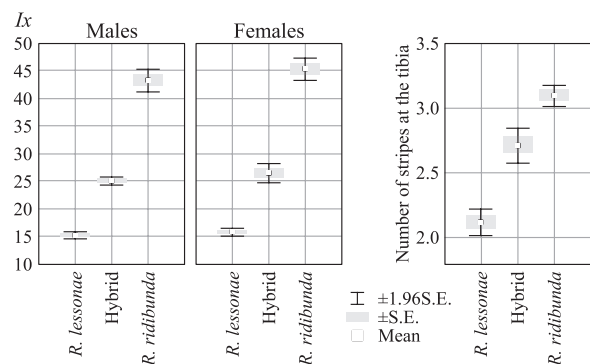


Fig. 4. Mean values (mean), standard error of mean (S.E.) and confidence interval (1.96S.E.).

TABLE 1. Ratio of Diagnosed Individuals of Paternal Species and Their Hybrids According to Different Traits (Terentiev, 1950) Combinations, Calculated by the Medium of Discriminant Analysis

Traits	3 forms, %			All, %
	<i>R. lessonae</i>	hybrid	<i>R. ridibunda</i>	
Indexes (head): L.c./Lt.c., L.c./L.o., Sp.oc./D.r.o., Lt.c./S.n., L./D.r.o., L./Lt.c., L./Sp.p., L./L.tym.	89.6	50.6	87.2	80.0
Indexes (limbs): I_x , F./T., T./IVD., 2IVD./D.p., C.s./2IVD., L./T., T./C.s., T./C.in., D.p./C.in.	96.9	95.2	99.0	98.0
Coloration (1 – 5, see <i>Material and Methods</i>)	88.6	50.0	92.9	80.2

The indexes of the head as well as the coloration traits allow for discriminating parental species at the level of 89 – 93%, while hybrids are only diagnosed at levels around 50%.

The following characteristics were identified to be the most significant for the diagnostics:

- Color traits and body pattern: color of vocal sacs, the location and the number of stripes on the limbs.

- Standard morphometric indexes: *Ix*, T./C.in., D.p./C.in.

- Other morphological characteristics: the form of internal metatarsal tubercle, position of the tight articulation (Terentiev, 1950; Berger, 1973).

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