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Anomalies of the smooth newt *Lissotriton vulgaris* (Linnaeus, 1758) in European and the East Uralian parts of its distribution area

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The article presents data derived from 38 year-long observations of morphological abnormalities in populations of smooth newts living in the Eastern (Asian) slope of the Southern and Middle Urals. The data were compared with literature data on variants and frequencies of morphological anomalies of *Lissotriton vulgaris* in European populations. The spectrum of anomalies in the European part differs from that in the East Uralian part of the distribution area by 9 unique features while also 9 features were shared.

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INTRODUCTION

It is well known that the phenotypic realization of genotypes differ among habitats due to different environmental conditions (Gilbert 2001). This has been documented for the development of several amphibian species in the wild (e.g., *Hyla chrysoscelis* Cope, 1880 and *Scaphiopus* Hoolbrook, 1836). Moreover, understanding these genetic environmental interactions may be critical in the scientific evaluation of agricultural and industrial policies (Gilbert 2001).

Because the capsule of amphibian eggs provides only limited protection and because of the exposure to external environmental factors throughout development, amphibian eggs and larvae are prone to develop morphological anomalies that easily can be observed and often persist within amphibian populations. Presence of morphologically deviating variants is a normal manner of morphogenesis for any biological species (Kovalenko 2003).

Various reasons for the development of anomalies exist, e.g. mutations, parasites, inbreeding depression, developmental stress, abnormal regeneration (Dubois 1979; Borkin & Pikulik 1986; Hebard & Brunson 1963; Talvi 1994, Henle *et al.* 2017*a*). Adverse environmental condition can lead to an increase of the frequency of morphological anomalies (Cooke 1981; Henle *et al.* 2017*b*). Pollution may cause thyroid dysfunction, which in turn may lead to anomalies, arising as a result of developmental deviations and atypical regeneration, it may lead to retardation of morphogenetic processes through proliferation suppression (Syuzyumova 1985) and it also can influence the intensity of metabolic processes (Tokar' *et al.* 1991). One of the remarkable features of Urodela (including the smooth newt) is the regeneration ability that animals keep during their entire life as well as the accumulation of abnormalities in populations. That's why the subject of our investigation is the spectra of morphological abnormalities in populations of smooth newts from European and Asian parts of its distribution area and their comparative analysis.

STUDIED SPECIES

The smooth newt, *Lissotriton vulgaris* (Linnaeus, 1758), is a common and widespread urodele species of the family *SALAMANDRIDAE* Goldfuss, 1820. Its distribution area reaches from Britain in the West to Eastern Siberia in the East and from southern Norway in the North to the Northern Balkans in the South. In Europe, the smooth newt is very common (Kuzmin 1999) (with the exceptions of southern France, Spain, Portugal, northern Scandinavia and Russian and Ukrainian steppes). In contrast, in Asia (Ural - Siberian part of the area) *L. vulgaris* is restricted in its distribution (Skorinov *et al*. 2008).

MATERIAL AND METHODS

We analyzed externally visible anomalies of *L. vulgaris* detected in the European part of the species' distribution area based on literature analyses (see results section for references) and long-term data for populations from the South (Chelyabinsk region) and Middle Ural (Yekaterinburg region) collected by us from urban and natural territories between 1977 and 2015. Yekaterinburg is situated in the Asian part of Russia near the border between Europe and Asia. In total, 29 adults were collected from the Chelyabinsk and 958 and 587 juvenile specimens from Yekaterinburg region, respectively. For variants of anomalies we followed the terminology of Henle *et al.* (2017*c*). The degree of overlap of the spectra of anomalies between juveniles and adults in the Asian part of the distribution area was calculated with the modified Morisita Index (Hurlbert 1978):

$$C_N = \frac{2\,(\sum n_{ix}\, n_{iy})}{\sum(n^2_{ix} + n^2_{iy})},$$

n_{ix} is the share of anomaly i in sample X;
and n_{iy} is the share of anomaly i in sample Y.

RESULTS AND DISCUSSION

In populations of *L. vulgaris* inhabiting the eastern slopes of the South and Middle Urals we found eight variants of anomalies in juvenile and 12 variants in adult newts during 38 years of research (Table 1).

The total frequency of anomalies in juveniles is 11.58 % against 21.69 % in adults, with the difference being significant ($\chi^2 = 25.08$; $\alpha = 0.001$; d.f. = 1).

The most frequent variant of anomalies both in adults and juveniles was ectrodactyly. In adults, the second most frequent one was polydactyly followed by neoplasm and then ectromely. In juveniles the second most common type of anomaly was ectrodactyly, then polydactyly, ectromely and syndactyly with tail defects.

The anomaly related to the retardation of parts of the hyobranchial system - we called it ceratobranchiale (Table 1) - is a form of limited (residual) neoteny or paedogenesis (Roček 1996; Djorovic & Kalezic 2000). An elongated distal part of the first ceratobranchiale is a unique type of anomalies that was found for the first time in 2015 by D.L. Berzin (fig. 1a). The cause of this phenomenon is unknown but probably derived from local environmental conditions. Variants of "ecological morphology" as a result of incomplete metamorphosis was also described in *Notophthalmus viridescens* (Reilly 1987); it was attributed to the elongation of the juvenile period in permanent ponds and to a gradually finishing metamorphosis.

Another type unique for the Asian part of the distribution area is hernia of lungs and ovaries in adult animals that were distinctly visible externally (fig. 1b, 1c respectively). Neoplasm, such as melanoma (fig. 1 d), was also found only in Asia: in urban populations of *L. vulgaris* in Yekaterinburg.

Table 1. Variants and frequencies of abnormalities of *Lissotriton vulgaris* in the Urals.

Variants of Anomalies	Juveniles *N* total = 587		Adults *N* total = 982	
	n anomalies	%	*n* anomalies	%
Anophthalmy	0	0	1	0.1
Ceratobranchiale	0	0	4	0.41
Edema	1	0.17	0	0
Pigment abnormalities (partial)	1	0.17	7	0.71
Hernia	1	0.17	4	0.41
Neoplasm	0	0	14	1.43
Brachymely	0	0	1	0.1
Ectromely	3	0.51	12	1.22
Taumely	0	0	2	0.2
Polydactyly	4	0.68	53	5.32
Syndactyly	2	0.34	6	0.6
Ectrodactyly	54	9.2	104	10.6
Tail defects	2	0.34	4	0.41

The spectrum of morphological anomalies increased from juvenile to adult smooth newts, with anophthalmia, neoplasm, brachymely, and taumely being present only in adults. The skeletal ones of these anomalies may accumulate in populations due to abnormal regeneration (Henle *et al*. 2017*c*). The overlap of the spectrum of anomalies of juvenile and adult newts by the Morisita Index is C = 0.883.

For the European part of the smooth newt's distribution 18 different types of anomalies were reported (Table 2). The most frequent variant of anomaly in adults was the same as in the Urals – ectrodactyly: from 1.8 % to 5.7 % (Litvinchuk 2014; Smirnov 2014). The second most frequent one was phalangeal bone thickness – 2.7 % (Litvinchuk 2014) followed by polydactyly: from 0.9 % (Litvinchuk 2014) to 1.5 % (Smirnov 2014) and tail bifurcation – 1 % (Smirnov 2014). The total frequency of anomalies in adults was 9 % (n = 111) and 5.5 % (n = 170) in adult newts from the Leningrad region (Elizavetino) and from Western Ukraine, respectively.

The comparison of the spectra of anomalies found in European and Asian populations showed that nine variants were shared between the two regions, nine were observed only in Europe and four only in the Eastern Ural (Table 2).

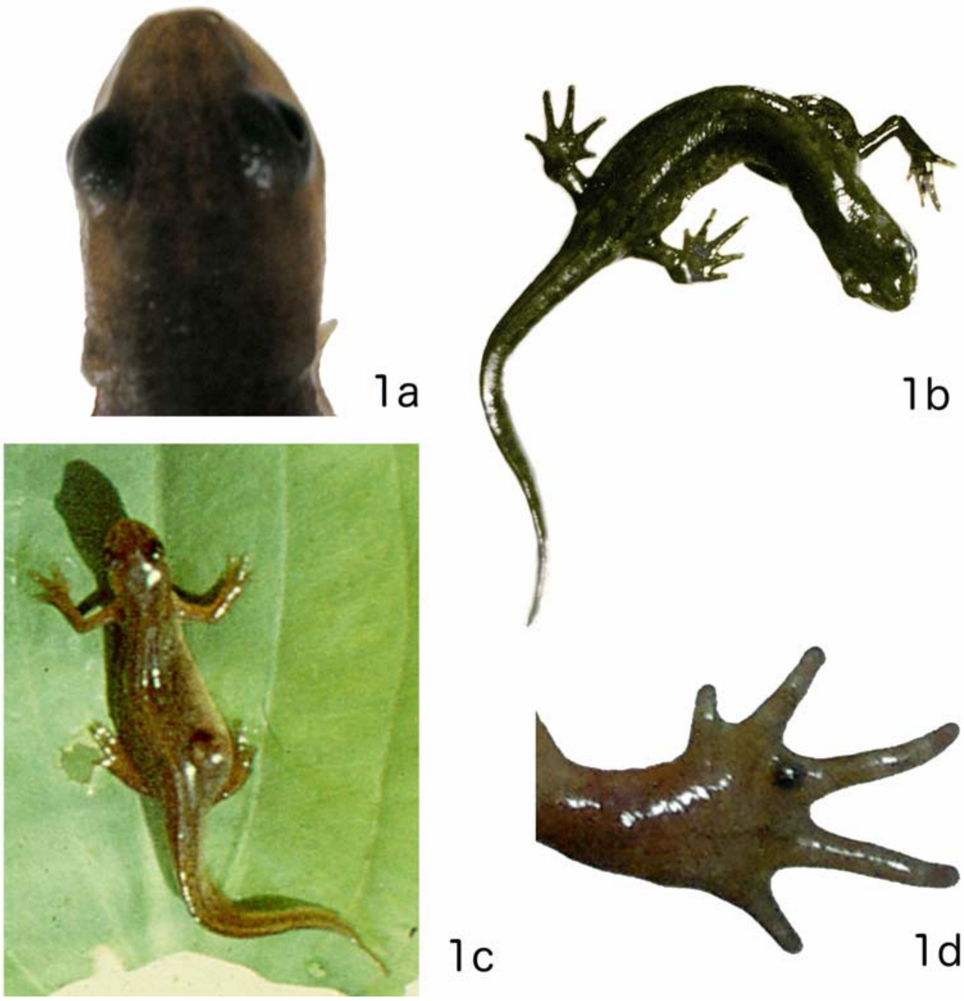

Figure 1. Some of unique abnormalities of *L. vulgaris* from Asian part of the area. (a) ceratobranchiale; (b) hernia of lung; (c) hernia of ovarium; (d) neoplasm (melanoma).

Table 2. Types of anomalies reported for *Lissotriton vulgaris* of the European and Asian parts of the distribution area.

Variants of Anomalies	Europe	Asia
Anophthalmy (Spemann 1904)	+	+
Black eye (iris depigmentation) (Benl 1965)	+	-
Scull deformities (Wolterstorff 1925)	+	-
Ceratobranchiale	-	+
Edema (Beebee & Griffiths 2000; Wolterstorff 1925)	+	+
Pigment abnormalities (partial) (Rimpp 2007)	+	+
Albinism (Stöck 1998; Modesti *et al*. 2011; Smirnov 2014)	+	-
Melanism (Suffert 1949)	+	-
Hernia (Vershinin 2002)	-	+
Neoplasm (Vershinin 2002)	-	+
Brachymely (D'Amen *et al*. 2006; Smirnov 2014)	+	+
Ectromely (Vershinin 2002)	+	+
Taumely (Vershinin 2002)	-	+
Polymely (Litvinchuk 2014; Smirnov 2014)	+	-
Syndactyly (Litvinchuk 2014)	+	+
Webbing between digits (Roberts & Verrell 1984)	+	-
Polydactyly (Litvinchuk 2014; Smirnov 2014)	+	+
Ectrodactyly (Litvinchuk 2014; Smirnov 2014)	+	+
Abnormal thickness of phalangeal bones (Litvinchuk 2014)	+	-
Tail defects (Griffiths 1981)	+	+
Tail bifurcation (Smirnov 2014)	+	-
Neoteny (Priemel 1917; Schreitmüller 1923; Schreitmüller *et al*. 1923; Procter, 1941; Benl, 1965; Litvinchuk *et al.* 1996; Ostrovskikh 2003; Pisanenetz 2007*a*, *b*; Covaciu-Marcov & Cicort-Lucaciu 2007; Gvoždík *et al*. 2013)	+	-

We suppose that the differences can be related to the complicated phylogenetic relations of smooth newts in Central Europe (Kalezic 1984; Babik *et al*. 2005). *L. vulgaris* is a polytypic species that consists of several subspecies (Skorinov & Litvinchuk 2013). Skull morphology shows that the smooth newt is characterized by a strongly deme-structured population structure (Ivanovich *et al*. 2013). *L. vulgaris* subspecies diversification is primarily based on morphological characters (Raxworthy 1988, 1990; Schmidtler & Franzen 2004; Ivanović *et al*. 2011; Skoriniv & Litvinchuk 2012). Smooth newts still retain the ability for gene exchange and show a complex intraspecific genetic structure with intraspecific polymorphism (Zieliński 2015).

The main part of the smooth newt's distribution is within the continental European part of Eurasia. The Asian part (nominative subspecies - *L. vulgaris vulgaris*) of its distribution is formed by a narrow belt diminishing eastwards (Kuzmin 1999). Therefore, this part of its distribution area differs from the European one by less numerous populations and lower genetic polymorphism and variability. Significant morphological differences among populations are also related to climatic and ecological differences of habitats. As was shown for *L. vulgaris* from different habitats of the forest park zone of Yekaterinburg by Ishchenko (1966), the particular habitat conditions but not isolation were playing the major role in population morphological divergence. Within the city area, morphology, such as enlargement of female snout-vent length and various proportions, were significantly determined by changes in environmental conditions along the urban gradient (Vershinin 2002, 2014; Vershinin *et al.* 2015). The acceleration of the intergrowth of interparietal sutures of juvenile newts from residential parts of the city was correlated with high mineralization and occurrence of calciphilic organisms in their stomachs (Perekhrest *et al.* 2018). Morphological features of *L. vulgaris* reflect the specificity of local developmental conditions (sizes of a pond, exposition, temperature, newt density, quantity and quality of food resources) (Vershinin 2002; Berzin & Vershinin 2016). We assume that the ecological conditions determined by climate and other environmental factors determine the range of Asian phenotypes, including the morphological anomalies of populations inhabiting the region.

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