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Role of Heterochronies in the Formation of the Morphological Features of Amphibians in Urban Areas

V. L. Vershinin[a, b, *] and S. D. Vershinina[a, **]

[a] Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, 620144 Russia

[b] Ural Federal University, Yekaterinburg, 620002 Russia

*e-mail: vol_de_mar@list.ru

**e-mail: s_verchok@list.ru

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Abstract—Morphogenesis evolves through evolutionary modifications in ontogeny under environmental factors. The same genotype can serve as a basis for different phenotypes, depending on environmental conditions. Changes in the rate and direction of cell proliferation can lead to phenotype modifications following Thompson's transformation theory. When acceleration and hypermorphosis are superimposed, the resulting vector can lead to recapitulations or the formation of some variants that can seriously differ from the phenotypic norm. Gene expression and other forms of morphogenetic signals are influenced by environmental factors, which have a multidirectional effect on the rate and vectorization of morphogenetic processes. Amphibian ontogenesis depends on the temperature regime, geochemical background, diet, and density of larval populations, which influence the rate and duration of separate developmental stages. The high heterogeneity of the urban environment and the presence of gradients of a number of conditions lead to heterochronic changes in the resulting vectors of amphibian morphogenesis under urban conditions towards the dominance of hypomorphoses. On the whole, the proportion of variants of morphological deviations based on heterochronies is similar (22.2–35.3%) for different amphibians, while their frequencies are more taxon-specific. The variants of deviations of ontogenesis under urbanization conditions can be considered as a model of the main directions of its destabilization during evolutionary transformations.

Keywords: heterochrony, urbanization, amphibians, morphological anomalies, morphogenesis

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INTRODUCTION

The problems of morphogenetic evolution have been a topical issue for researchers since the time of Aristotle, who believed that form, motion, and matter did not exist independently of each other (see Coonen, 1962). The term *heterochrony*, introduced by E. Haeckel, meant the acceleration or deceleration of certain stages of ontogeny with respect to the time of their occurrence according to phylogeny (see Russel, 1916). Changes in the rate and direction of cell proliferation can lead to phenotype modifications (Meyen, 1977) according to the "growth rules", or Thompson's transformations (Thompson, 2000).

Heterochrony is a relatively safe way of evolutionary transformation of ontogenesis, since it is related only to the time of the occurrence and duration of main events, rather than to changes in their sequence. These ontogenetic deviations have a lower effect on the survival rate of individuals and do not cause dramatic disturbances in the canalization of separate processes; however, they can ultimately lead to significant changes in the morphology (Alberch, 1981). The amplification of initially small changes at early stages of ontogenesis can further significantly influence the defining features during development.

The resulting vector ("overlap" = interference) of acceleration and hypermorphosis can lead to recapitulations in morphological features (Alberch et al, 1979) or to the formation of variants that can strongly differ from the phenotypic norm ("wild-type"). Thus, evolutionary changes in the duration of the *HoxD 12–13* expression lead to polyphalangy. *HoxD* genes are responsible for the number of fingers, membrane formation, and high variability of limb regions (Cohn and Bright, 1999). Changes in the timing of gene expression can induce evolutionary innovations in the morphological features (Bush and Zachgo, 2007). At the same time, similar effects may also be determined by the epigenetic regulation of gene function, i.e., by the expression of a normal gene in an unusual place or at an unusual time (Battulin, 2014). Thus, analysis of rapid evolutionary changes in the foot shape in Italian cave salamander populations from an artificial cave did not reveal significant genetic differences between these populations and the nearby forest population (Salvidio et al., 2015).

Morphological patterns are formed during the complex interactions of gene expression products and other morphogenetic signals (according to the reaction-diffusion Turing model), which, like a mixing console, make it possible to control the creation of a definitive morphological structure (Kondo and Miura, 2010; Sheth et al., 2012). Environmental factors serve as mediators of morphogenetic processes and have a multidirectional effect on their vectorization and rate (Vershinin, 2018).

The synergism of morphogenetic factors (the pattern of gene expression, epigenetic marking, and external inductors) determines its diversification under real environmental conditions (Gilbert, 2000; Stocum, 2000; Carlin et al., 2013). This shows that the ecology of morphogenesis is an integral part of the knowledge about its evolutionary transformations. The transformation of the patterns of amphibian morphogenesis in the gradient of an urban environment is an important aspect, that makes it possible to obtain new data on the ecological mechanisms of ontogenetic evolution.

MATERIAL AND METHODS

The purpose of this research was to carry out a comparative analysis of the spectra of morphological anomalies in young-of-the-year individuals of four anuran species of the family Ranidae and two salamander species of the order Urodela from the same region, and assess the proportions of deviant forms based on heterochronies, as well as their contribution to the specific ontogenetic features and definitive morphological features of these species in the urbanization gradient and possible causes of the resulting differences.

The material was collected from 1977 to 2018 from amphibian populations living in the gradient of the urban environment of Yekaterinburg and the adjacent forest areas on the eastern slope of the Middle Urals. The variants of morphological anomalies and the frequency of their occurrence were analyzed in young-of-the-year Siberian salamander—*Salamandrella keyserlingii* (646 ind.), common newt—*Lissotriton vulgaris* (742 ind.), grass frog *Rana temporaria* (4892 ind.), moor frog *R. arvalis* (17 613 ind.), and marsh frog *Pelophylax ridibundus* (2122 ind.). We used modern terminology (Henle et al., 2017) and the author's methodology (Vershinin, 2015) in the analysis.

The typification of urban areas (Vershinin, 1980) was based on their degree of development by humans and their degree of pollution:

(I) The central part of the city with multistory buildings, massive asphalt coverings, water bodies with severe industrial pollution, and small rivers and streams drained by pipes. There are no amphibians in this zone.

(II) Areas with multistory buildings, including territories being developed, waste ground, sites with open soil, and small ponds with high levels of pollution.

(III) Low-rise building areas generally occupied by private sector houses with gardens and kitchen gardens, as well as by waste grounds and parks. The biotopes of this zone often adjoin forest parks.

(IV) A forest park belt of the city. The habitats of this zone are generally under the influence of recreational pressures. The control is a forest area at a distance of 23 km from Yekaterinburg, where there is no urbanization effect.

The presence of the urbanization gradient corresponding to our typification is annually confirmed by hydrochemical analyses of spawning water bodies that are carried out at the Laboratory of Physicochemical Analyses, Ural State Mining University.

RESULTS AND DISCUSSION

Analysis of the spectra of morphological anomalies in young-of-the-year individuals of the studied amphibian species revealed 26 variants: nine of them in Siberian salamander, eight of them in common newt, 23 of them in moor frog, 17 of them in grass frog, and 12 of them in marsh frog.

In turn, the number of deviation variants based on heterochronies was three for Siberian salamander (brachymelia, ectromelia, and ectrodactyly), two for common newt (ectromelia and ectrodactyly), 6 for moor frog (brachycephaly, the absence of eyelids, opercular chamber defects, brachymelia, ectromelia, and ectrodactyly), six for grass frog (brachycephaly, the absence of eyelids, opercular chamber defects, brachymelia, ectromelia, and ectrodactyly), and 3 for marsh frog (opercular chamber defects, ectromelia, and ectrodactyly).

In most of the studied species, the proportion of heterochronies is one-fourth to one-third of the total number of deviation variants (Table 1).

On the whole, the occurrence of heterochronies in the total number of animals is low among anurans of the family Ranidae (0.67–1.97%), being significantly lower than that among the Urodela species (Table 2).

The occurrence of heterochronies increased in the urbanization gradient for the three most common species in the urban area (common newt and moor and marsh frogs). Significant differences were recorded between the young-of-the-year individuals of common newt and frog from each separate urban zone and from the forest suburban area (Table 2).

Water bodies in urban areas accumulate a large amount of mineral and organic substances at high concentrations (Vershinin, 2011; Vershinin et al., 2015), which significantly distinguishes these habi-

Table 1. Occurrence of heterochronies in young-of-the-year individuals of the studied species

Species	Proportion of heterochronies in the total number of variants (in %)	% of the total number of animals
S. keyserlingii	33	5.1
L. vulgaris	22. 2	8.76
R. arvalis	26.09	0.99
R. temporaria	35.3	0.67
P. ridibundus	31.1	1.97

tats from forest ones. According to the accepted classification (Molchanova et al., 2009), they are brackish with respect to the total mineralization level (over 500 mg/dm^3). Along with the direct effect of high mineralization on the quality of larval morphogenesis (von Dassow and Dawidson, 2011), the pollution effect is largely similar to the effects of increased density (Voigt, 1991) and is expressed in growth retardation (Grefner and Slepyan, 1989).

It was revealed (Vershinin, 2011) that water bodies in the multi-story building zone differ in the composition of microscopic algae; this contributes to the retardation of growth rates (Kupferberg et al, 1994) and affects the morphogenesis of developing larvae, which is reflected in the composition (biochemistry) of their nutrition (Kanazawa, 1981; Naess et al., 1995). Since the ontogeny of amphibians depends on the temperature regime (Hertwig, 1898; Crozier, 1926), the increase in the temperature of spawning water bodies in the zone of multistory buildings by an average of 3°C (Vershinin, 2015) promotes the acceleration of their embryonic development (Vershinin, 1997).

High mineralization of the water bodies of the residential part of the city accelerates the ossification of the cranial sutures of young-of-the-year individuals of common newt (Vershinin et al., 2019). On the other hand, the presence of pollutants in water bodies affects the morphogenesis of common newt: they increase the number of elements in the modular structures of its skeleton (Vershinin et al., 2016), thereby contributing to partial neoteny (Vershinin and Berzin, 2018). It is well known that anthropogenic changes in environmental chemistry affect the hormonal system (Kashiwagi et al., 2009); in turn, the interference of thyroid hormones and endocrine disruptors disturbs the normal course of ontogenetic events, which is reflected in the emergence of morphological abnormalities (McDaniel et al., 2008; Murphy et al., 2006; Reeder et al., 1998; Skelly et al., 2010).

Therefore, the existing factors in the urban area have a multidirectional effect on the rate of growth and development. Most often, they result in hypomorphosis and paedomorphosis; i.e., retardation prevails over acceleration in this case. The addition of the multidirectional vectors that accelerate embryogenesis and decelerate the larval development (and vice versa) lead to the formation of a new definitive morphological structure.

Similarly to evolutionary changes in the level of adaptations (Shvarts, 1980), adaptive transformations of ontogenesis tend to minimize energy expenditures. The energy assimilation efficiency in this process shows similar values for each level of consumer organization according to the Ricklefs' rule (Riklefs, 1979; Shvarts, 1976). It is known that heterochronies are one of the most energy-saving and structurally efficient ways of ontogenesis transformation, since evolution itself is an energy-saving process and functions by "reworking the old" (Jacob, 1977).

Therefore, it is indicative that the proportion of heterochronies in the total spectrum of deviation variants in the studied amphibian species is 22.2–35.3%. Their frequencies in the total number of the studied animals are more taxon-specific (Tables 1 and 2) and differ from those in the anurans of the family Ranidae and salamanders; within the order Urodela, the family Salamandridae differs from the family Hynobiidae (the basic taxon in the system of the first terrestrial tetrapods, where even the morphogenesis of the finger arc is more archaic, whereas its pattern in Salamandridae is similar to the developmental transformations of anurans, which, in turn, are similar to amniotes with respect to this feature (Vorobyeva and Hinchliffe, 1996; Hinchliffe, 2002).

Table 2. Occurrence of heterochronies in the urbanization gradient (% of the total number of animals)

Species	Multistory buildings	Low-rise buildings	Forest-park zone	Suburban area
S. keyserlingii	–	7.34	4.41	7.89
L. vulgaris	7.77 ($\chi^2 = 5.31$; $p < 0.02$)	8.43 ($\chi^2 = 5.9$; $p < 0.015$)	14.10 ($\chi^2 = 13.43$; $p < 0.0002$)	1.47
R. arvalis	1.17 ($\chi^2 = 360.24$; $p < 0.0001$)	1.1 ($\chi^2 = 219.38$; $p < 0.0001$)	1.15 ($\chi^2 = 434.76$; $p < 0.0001$)	0.51
R. temporaria	1.40	0.64	0	1.30
P. ridibundus	1.85	3.49	2.18	0

Urbanization conditions involve changes in ontogenesis, which are determined by the interference of the multidirectional vectors of morphogenesis, as well as by the disturbance of the initial correlations and coordinations. These irregular deviant phenomena within highly heterogeneous urban areas simulate situations of large-scale evolutionary events or global biocenotic crises.

CONCLUSIONS

Urbanization conditions involve changes to the resulting vectors of morphogenesis due to an increase in the proportion of heterochronies dominated by hypomorphoses.

The proportion of heterochronies as one of the most energy-saving and structurally efficient ways of ontogenesis transformation is probably related to the efficiency of using assimilated energy at a certain trophic level.

Therefore, the number of variants of morphological deviations based on heterochronies is generally similar between different amphibians, while their frequencies are more taxon-specific.

The deviations of ontogenesis under urbanization conditions can be considered as a model of the main vectors of its destabilization during evolutionary transformations.

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