Hematopoiesis of Anurans: Specific Features of Species Adaptogenesis in Recent Ecosystems

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Abstract—Studies on the hematological parameters of amphibians along the gradient of anthropogenic transformation of the environment have revealed significant changes in the blood of animals exposed to the influences of urbanization and pollution. Qualitative differences in the adaptive strategies (as applied to hematopoiesis) of three species of the genus *Rana* are considered. It is shown how differences in population polymorphism of closely related species can affect these species upon anthropogenic transformation of the environment.

Adaptoogenesis, one of the most important properties of animate matter, proceeds in diverse ways not only among different taxonomic groups, but also within related species and even populations. Therefore, a comparative analysis of hematological characteristics in three species of the genus *Rana* (*R. temporaria*, *R. arvalis*, and *R. ridibunda*) provides a means of revealing the species specificity of adaptation pathways at the level of one of these organism’s systems.

It is known that such a dynamically responsive tissue as blood reflects any functional changes occurring in a living organism. This is clearly manifested in amphibians, the first terrestrial vertebrates, whose dependence on environmental conditions is well known.

Studies of hematological indices of amphibians are relatively few, and most of them are either experimental laboratory investigations or else deal with a certain specific problem (Michurina et al., 1999). In addition, it is typically only adult animals that are used in such studies (Tarasenko and Tarasenko, 1988; Isaeva and Vyazov, 1997), with but a few rare exceptions (Syuzyumova et al., 1984; Syuzyumova and Grebennikova, 1987). The number of studies on the hematology of amphibians inhabiting anthropogenically transformed territories is relatively small (Chernysheva and Starostin, 1997; Isaev and Vyazov, 1997; Afonin, 1999; Toktamyssova, 2000), and virtually none of them deals with changes in hematological parameters under the effects of urbanization. This paper presents results obtained at the first stage of studies on certain features of hematopoiesis in anurans living in an anthropogenically transformed urban environment.

A large amount of information has been accumulated to date, but its analysis and interpretation within the framework of conventional zoological approaches does not allow researchers to progress any further than merely proposing elegant theories. To find out what factors are responsible for the observed authentic phenomena, more precise methods must be used.

MATERIALS AND METHODS

Data on the hematological indices of juvenile *Rana ridibunda*, *R. arvalis*, and *R. temporaria* frogs from populations variously affected by urbanization and pollution were collected in the city of Yekaterinburg from June to the end of September of 2000, 2001, and 2002. Postmetamorphic juveniles in the first two weeks of their life on land were collected in the immediate vicinity of water bodies where their larval development took place. Depending on the degree of anthropogenic impact, four zones were distinguished within the territory of a big industrial city, with places inhabited by amphibians being confined to a certain zone (Vershinin, 1980a). This zoning was based on the degree of urban development, taking into account the density of buildings, number of stories, and other relevant parameters, including the degree of pollution resulting from household and industrial waste. The zonal position of a habitat was determined mainly from the degree of general anthropogenic transformation of the corresponding area, rather than from its topographic location.

Zone I: city area (mainly in the central part) with multistory buildings, massive asphalt pavement, and water bodies with a high degree of industrial pollution; small streams are channeled through pipes. Zone II: regions of multistory buildings and areas of land development, wastelands, and bare soils; small water bodies are heavily polluted. Zone III: regions with low buildings (mainly, private houses with orchards and gardens), wastelands, and parks; the biotopes of this zone often adjoin park forests. Zone IV: the park-forest belt of the city. The habitats of this zone suffer mainly from recreational load.
A plot located 23 km away from Yekaterinburg was chosen as a control. The results of hydrochemical analysis of spawning reservoirs, which was performed in the course of this work, confirmed that the above zonal classification is acceptable in studies of this kind.

Juvenile *R. arvalis* frogs from the population inhabiting the southwestern shore of Lake Berdanish (the Southern Ural Reserve, near the Experimental Research Station of the Mayak Production Association, Chelyabinsk oblast) were kindly provided by M.V. Chibiryak. This lake is in the area of the Eastern Ural Radioactive Trace (EURT), where the initial density of radioactive contamination (by $^{90}$Sr) was 500–1000 Ci/km$^2$ and the current density is 320–350 Ci/km$^2$.

Blood samples from each animal were collected to prepare blood smears (stained by Romanovsky–Giemsa) and take differential blood counts in a standard hemocytometer chamber following the standard procedure. In addition, standard morphological and morphophysiological indices were recorded (Shvarts et al., 1968). A total of 584 animals were studied. The results were processed statistically by multivariate ANOVA and regression analysis using the Statgraph program package.

### RESULTS AND DISCUSSION

Analyzing the results, significant differences in blood counts were revealed at the interspecific, species, interzonal, and population levels. Interspecific differences (Table 1) concerned the numbers of neutrophils ($F = 3.9, p = 0.02$), small lymphocytes ($F = 12.6, p < 0.0001$), large lymphocytes ($F = 19.3, p < 0.0001$), eosinophils ($F = 5.65, p = 0.0038$), basophils ($F = 6.55, p = 0.0016$), erythrocyte precursors ($F = 7.07, p = 0.0009$), and erythrocytes ($F = 12.84, p < 0.0001$).

In *R. arvalis*, significant differences at the interzonal level (Table 2) were revealed in the proportions of leukocytes ($F = 3.515, p = 0.0155$), lymphocytes ($F = 5.197, p = 0.016$), and eosinophils ($F = 4.918, p = 0.0025$).

In *R. ridibunda*, individuals of different forms proved to differ in the number of monocytes ($F = 10.73, p = 0.0002$). In *R. temporaria* ($n = 127$), interzonal differences were revealed in the proportions of leukocytes ($F = 11.5, p < 0.0001$), segmented neutrophils ($F = 3.5, p = 0.018$), large lymphocytes ($F = 3.45, p = 0.018$), total lymphocytes ($F = 3.02, p = 0.03$), and erythrocyte precursors ($F = 2.64, p = 0.05$).

An increase in the number of eosinophils in both the population from zone IV and the control population (Table 2) may be explained by parasite invasions, which are often more widespread in “natural” than in urban populations of amphibians (Dubinina, 1950; Lebedinskii and Ryzhkova, 1994); it is known that an increase in the number of eosinophils in the peripheral blood is a component of the immune response to invasion (Chernysheva and Starostin, 1997).

Differences in hematological parameters conditioned by the intrapopulation genetic polymorphism that was manifested in the presence of the dorsomedial stripe (the striata morph), or by its absence, were revealed in *R. arvalis* and *R. ridibunda*.

In juvenile *R. arvalis* of the striata morph (below, referred to as striated) from urban populations (zones II, III, and IV; data of 2000), the blood was light pink (Vershinin and Starovoienko, 2001) and contained a significantly greater proportion of erythrocyte precursors than the blood of juveniles from the control population: $70.8 \pm 5.7$ (here and below, $X \pm S_X$) vs. $51.2 \pm 3.7\%$ ($F = 8.3, p = 0.007; n = 34$). A similar situation was observed in 2002 (Fig. 2), but no such differences were revealed in 2001.

As follows from Table 3, significant differences in a series of indices between striated and nonstriated animals persisted for two years.

The proportion of neutrophils was greater in striated than in nonstriated juveniles: in *R. arvalis*, $10.1 \pm 0.79$ vs. $7.37 \pm 0.56\%$ ($F = 7.995, p = 0.005$); in *R. ridibunda*, $18.5 \pm 2.4$ vs. $12.2 \pm 2.4\%$ ($F = 5.1, p = 0.03; n = 76$), respectively. This is evidence that the capacity of the hematopoietic system for producing phagocytes is higher in the striated animals of both species. In a technogenically polluted environment, when the neutrophils in the blood of striated frogs increased, the number of eosinophils also increased (Table 3).
The trophil lineage of hematopoiesis is suppressed (Chernysheva and Starostin, 1997), this factor is very important. In *R. ridibunda*, the blood of striped individuals contains greater proportions of basophils ($F = 4.8, p = 0.003$) and large lymphocytes ($F = 8.5, p = 0.005$), and has a higher total lymphocyte content ($F = 1.1, p = 0.01$).

It is known that sharp changes in the environment entail a drastic increase in the content of erythrocyte precursors in the blood of juvenile frogs (Syuzyumova and Grebennikova, 1978; Grebennikova, 1979). The initially higher content of immature erythrocytes in striped juvenile *R. arvalis* (in 2000 and 2002) may provide for the enhancement of erythropoiesis and, therefore, higher survival rates at the beginning of land life under conditions of urbanization and pollution. It should also be noted that the amplitude of changes in blood indices depending on the degree of urbanization is significantly greater in striped individuals (Fig. 1).

Individuals of the striata morph, which feature a light dorsomedial stripe, are always present in *R. arvalis* populations. Experiments with crossing (Shchupak, 1977) showed that this character is controlled by one autosomal gene *striata* with two alleles and that the allele determining the presence of the stripe is fully dominant. Similar data were obtained in hybridization experiments with Japanese frogs *R. limnocharis* (Moriwaki, 1952) and North American brown frogs *R. sylvatica* (Browder et al., 1966). Thus, striata is an effective marker for allowing specialists to study phenotypic manifestations of changes in the genetic structure of populations.

Color polymorphism has been reported for many amphibian species and is of a genetic nature, which determines the high probability of physiological differences between individuals of different morphs. Neutral polymorphism in amphibian populations, as well as in populations of other taxonomic groups, is relatively rare (Gray, 1984). As a rule, certain variants gain selective advantage under specific conditions.

It has repeatedly been noted that the frequency of striped individuals in *R. arvalis* populations in Yekaterinburg increases along with the strength of anthropogenic impact (Vershinin, 1980b, 1982a, 1983).

The presence of nonstriped animals in these populations is probably explained by a high viability of heterozygotes phenotypically manifested as the striata

| Table 2. Interzonal differences in hematological indices ($X \pm S_X$) of frogs |
|-----------------|-----------------|-----------------|-----------------|---------------|
| Index           | Zone II         | Zone III        | Zone IV         | Control       |
| R. arvalis      |                 |                 |                 |               |
| Lymphocytes, %  | ($n = 87$)      | ($n = 58$)      | ($n = 95$)      | ($n = 96$)    |
|                 | $80.5 \pm 1.6$  | $72.7 \pm 2.1$  | $73.2 \pm 1.6$  | $72.8 \pm 1.6$|
| Monocytes, %    | $1.9 \pm 0.2$   | $1.5 \pm 0.3$   | $1.5 \pm 0.2$   | $2.6 \pm 0.22$|
| Eosinophils, %  | $4.2 \pm 1.1$   | $6.5 \pm 1.2$   | $9.8 \pm 1.0$   | $8.0 \pm 1.0$  |
| Leukocytes (total) | $91989.7 \pm 7001.6$ | $76333.8 \pm 8575.2$ | $110901.5 \pm 6700.3$ | $96656.9 \pm 6665.3$ |
| R. ridibunda     |                 |                 |                 |               |
| Monocytes, %    | $1.85 \pm 0.3$  | $5.2 \pm 0.7$   | $1.7 \pm 0.4$   | No data       |
| Immature erythrocytes, % | ($n = 29$) | ($n = 46$) | ($n = 32$) | ($n = 21$) |
| Lymphocytes, %  | $52.9 \pm 4.2$  | $47.8 \pm 3.6$  | $62.3 \pm 4.4$  | $46.8 \pm 5.0$|
| large           | $50.5 \pm 2.8$  | $45.5 \pm 2.3$  | $52.8 \pm 3.0$  | $39.9 \pm 3.3$|
| small           | $79.9 \pm 2.6$  | $75.0 \pm 2.2$  | $81.2 \pm 2.8$  | $70.1 \pm 3.1$|
| Segmented neutrophils, % | $7.9 \pm 1.2$ | $8.9 \pm 0.96$ | $6.3 \pm 1.3$ | $11.9 \pm 1.3$ |
| Leukocytes (total) | $105003.5 \pm 10149.9$ | $75738.4 \pm 8148.1$ | $131374.9 \pm 9662.5$ | $151104.9 \pm 11927.7$ |

Fig. 1. Proportions of total neutrophils in different morphs of *R. arvalis* frogs depending on the degree of urbanization (2000–2002).
morph. In populations of natural ecosystems, under lower anthropogenic pressure, the proportion of striated individuals is usually lower. The animals of this morph apparently gain selective advantage in *R. arvalis* and *R. ridibunda* populations inhabiting anthropogenically transformed, polluted territories. Changes in phenotype frequencies under the impact of urbanization, including increase in the proportion of striated individuals (Zhukova et al., 1986; Gogoleva, 1989; Kolyakin, 1993), is characteristic of frogs of the genus *Rana* and representatives of other anuran families (Peskova, 1995).

*Rana temporaria*, a sympatric species closely related to *R. arvalis*, has no striated morph, which may be one of the factors accounting for the low tolerance of this species to anthropogenic transformation and pollution. Quantitative dominance of *R. arvalis* over *R. temporaria* has repeatedly been noted in anthropogenic landscapes (Garanin, 1964; Astradamov, 1973; Toporkova, 1973).

Electrophoretic and karyological data clearly show that the group of brown frogs is of East Asian origin (Nishioka et al., 1992) and that *R. arvalis* separated from the common phyletic stem earlier than *R. temporaria*. In the latter species, the striata morph was apparently lost in the course of phylogeny due to its stronger connection with water. Under conditions of hypoxia or anoxia, *R. temporaria* frogs can significantly reduce their metabolic rate, but wintering individuals usually withstand hypoxia due to skin respiration (Boutilier et al., 1997). These frogs winter at the bottom of a water body, and it is known that the striata morph is most vulnerable to oxygen deficiency (Shwarts and Ishchenko, 1968).

Differences in adaptation to the abiotic environment between *R. arvalis* and *R. temporaria* are insignificant and are probably explained by their evolution via different pathways (Severtsov et al., 1998). Adaptation to respiration under conditions of insufficient oxygen supply in amphibians is achieved by means of increase in the amount of blood and hemoglobin content (Chugunova, 1974).

At the population level, adaptive responses of the hematopoietic system to factors of the urbanized environment are qualitatively different in *R. arvalis* and *R. temporaria*. As noted above, the number of immature erythrocytes in the blood of juvenile frogs from urban populations is markedly increased. The frequency of the striata morph in urban *R. arvalis* and *R. ridibunda* populations is relatively high (Tables 4, 5). This morph has a number of genetically determined physiological features (Vershinin, 2002), including a high initial level of erythrocyte precursors in the blood. This feature, combined with their high responsiveness, protects striated frogs from hematopoietic depression (cf. Figs. 2 and 3). Such a mode is more energy-efficient, because this is an adaptation of a higher level. This hypothesis is confirmed by the weak multiplicative correlation ($R = 0.35$, $F = 13.5$, $p < 0.0001$) between the proportion of erythrocytes and the liver index in juvenile

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**Table 3. Differences in blood indices (%) of the striata morph in *Rana arvalis* and *R. ridibunda* frogs (2000–2002)**

<table>
<thead>
<tr>
<th>Indices</th>
<th>striata</th>
<th>Nonstriated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. arvalis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n = 125$</td>
<td>$n = 246$</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>young</td>
<td>3.65 ± 0.35</td>
<td>4.6 ± 0.26</td>
</tr>
<tr>
<td>total</td>
<td>10.1 ± 0.79</td>
<td>7.37 ± 0.56</td>
</tr>
<tr>
<td><em>R. ridibunda</em></td>
<td>$n = 21$</td>
<td>$n = 55$</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>young</td>
<td>16.6 ± 2.0</td>
<td>11.3 ± 1.2</td>
</tr>
<tr>
<td>total</td>
<td>18.5 ± 2.4</td>
<td>12.2 ± 1.4</td>
</tr>
<tr>
<td>Basophils</td>
<td>10.5 ± 1.1</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>large</td>
<td>18.8 ± 4.6</td>
<td>34.4 ± 2.7</td>
</tr>
<tr>
<td>total</td>
<td>66.1 ± 3.2</td>
<td>75.6 ± 1.8</td>
</tr>
</tbody>
</table>

Fig. 2. Proportion of immature erythrocytes in juvenile *R. arvalis* frogs (2000).

Fig. 3. Proportion of Immature erythrocytes in juvenile *R. temporaria* frogs (2001).
Table 4. Frequency (%) of the striata morph in the urban territory

<table>
<thead>
<tr>
<th>Age group</th>
<th>Zone II</th>
<th>Zone III</th>
<th>Zone IV</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>42.05 (n = 88)</td>
<td>42.86 (n = 42)</td>
<td>34.75 (n = 127)</td>
<td>22.02 (n = 109)</td>
</tr>
<tr>
<td>Juveniles</td>
<td>44.73 (n = 2611)</td>
<td>37.55 (n = 514)</td>
<td>29.26 (n = 3835)</td>
<td>19.51 (n = 2466)</td>
</tr>
</tbody>
</table>

R. arvalis

R. temporaria (over the whole study period). It is interesting that a similar model of correlation between the proportion of immature erythrocytes and the liver index ($R = -0.62, F = 8.17, p = 0.01$) is only observed in juvenile R. ridibunda from the park-forest zone, where the striata morph is absent.

In R. arvalis from zone II, the multiplicative correlation between the proportion of erythrocyte precursors and the liver index had the following parameters: in 2000, $R = 0.52 (F = 4.4, p = 0.05)$; in 2002, $R = 0.85 (F = 12.8, p = 0.01)$; and over the whole study period, $R = 0.35 (F = 11.3, p = 0.0017)$. In 2002, its coefficient in the zone of multistory buildings was $R = 0.91 (F = 28.9, p = 0.0017)$, while coefficients in the other zones were significantly lower: even in the control, $R = 0.72 (F = 18.1, p = 0.0006)$.

Thus, differences in polymorphism between closely related species may lead to significant qualitative differences in the mechanisms of adaptation to new environmental factors. This, in turn, can have a strong effect on the direction of microevolutionary transformations in the recent biosphere.

Specificity of adaptive processes is most strongly expressed under an extreme influence of new environmental factors. Analysis of results obtained in the EURT area revealed a significant increase in the proportion of monocytes: $9.3 \pm 0.7\% (n = 7)$ vs. $2.5 \pm 0.2\% (n = 100)$ in the control population ($F = 24.1, p < 0.0001$). At the same time, the examined animals displayed no signs of leukosis, as proportions of the majority of other white blood cells were within the statistical norm. The higher total leukocyte content, $167.330.3 \pm 26.195.9 (n = 7)$ vs. $99.422.9 \pm 6930.8 (n = 100)$ ($F = 3.39, p = 0.005$), is apparently explained by an increase in the number of monocytes, which were necessary for enhancing phagocytosis of the products of tissue destruction caused by radiation. A similar reaction (monocytosis) was reported for R. ridibunda exposed to chemical pollution (Isaev and Vyazov, 1997).

Analysis of intrapopulation polymorphism has revealed a number of features that provide evidence for a directed alteration of genetic structure (an increase in the proportion of striated frogs to 63.3\%, compared to 18.2\%) under the effect of new environmental factors. This alteration is preadaptive (Vershinin, 1997) and occurs in R. arvalis populations inhabiting polluted territories (Vershinin, 1987). High embryonic mortality, with only $11.82 \pm 2.36\%$ of embryos surviving to developmental stage 30 (Pyastolova et al., 1996), and selective elimination of juveniles with a low level of metabolic processes (Pyastolova and Vershinin, 1999) indicate that high levels of radionuclide pollution remain a serious factor affecting the population and leading to directed alteration of its genetic structure. That is, we deal with an “elementary evolutionary phenomenon” (Timofeeff-Ressovsky et al., 1973). Adaptation of the population to environmental conditions existing in the EURT develops via a selective mortality of physiologically different phenotypes (these differences have a preadaptive character); and adaptatiogenesis is far from reaching its final stage.

Thus, the observed species specificity of hematological indices is largely related to differences in polymorphism between the representatives of the genus Rana studied in this work.

Differences at the interzonal level are a result of adaptive transformations at different levels that have occurred in amphibian populations under the effects of urbanization and pollution.

Rana arvalis and R. ridibunda frogs of the striata morph have similar genetically determined physiological features. Hence, striated and nonstriated individuals of both species differ in their responses to the maximal level of transformation and pollution.

Studies on the dynamics of hematopoiesis in juvenile frogs of three species are interesting not only from a pragmatic standpoint, i.e., for the assessment of environmental quality. In our opinion, it is much more important to study the diversity of population responses in different species inhabiting extremely heterogeneous biotopes of urbanized territories. The theoretical significance of the data obtained consists in the elucidation of the mechanisms responsible for adaptive and preadaptative transformations of the population on the basis of genetic heterogeneity of individuals.
Thus, intraspecific polymorphism largely accounts for the successful existence and reproduction of populations in diverse environments and may influence their further evolution.

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SPELL: 1. adaptatiogenesis