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## Genetic Structure and Effective Animal Population Size in the Conditions of Anthropogenic Changed Landscape.

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### ABSTRACT

On the basis of DNA-markers (*RAPD*, *ISSR*) it has been estimated the state of gene pools and determined the effective size of population of six species of animals inhabited in the conditions of anthropogenic changed landscape of the south of Central Upland (Russian). The following animal species have been involved into study: *Bradybaena fruticum*, *Chondrula tridens*, *Cepaea vindobonensis*, *Helicopsis striata* (Mollusca), *Eurygaster integriceps* (Insecta) and *Apodemus flavicollis* (Mammalia). The results have shown high genetic variability and large values of effective population size of common species in comparison with very protected species. It has been advanced the theory according to which genetic processes in the populations of endangered species are the best indicators of seral processes to be expected using, for these purposes, eurybiotic common species, that actively adapt to anthropic effect.

**Key words:** animal population, genetic structure, effective size, anthropic –changed landscape.

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### INTRODUCTION

The explored area, situated in the south of Central Upland (the Belgorod Region, Russia) refers to old-developed territories with high degree of anthropogenic load. The most acute problem is here maintenance of ecological balance, and also preservation of biological and landscape diversity.

Very important problem being solved in the course of monitoring measures, carried out in the aspect of nature conservation, is the assessment of effective population size of the species of interest, that is, the minimal size necessary for species survival.

**The aim of the research** is to estimate the state of gene pools and to determine the effective size of common population and much protected species of animals inhabited in the south of Central Upland, on the basis of genetic structure data.

### METHODS

In the present work 6 population species, belonging to three different classes: Mollusca - *Bradybaena fruticum* (23 populations), *Chondrula tridens* (21 populations), *Cepaea vindobonensis* (11 populations), *Helicopsis striata* (19 populations); Insecta - *Eurygaster integriceps* (5 populations); Mammalia - *Apodemus flavicollis* (11 populations) have been studied. It is no accident that these species have been selected. Some of them are background species that are used as indicators of anthropogenic influence (*Br. fruticum*, *Ch. tridens*, *A. flavicollis*) [1-5], one species is vermin of crops (*E. integriceps*), two more species refer to very protected animals that have been recorded into the regional Red Book (*H. striata*, *C. vindobonensis*) [6, 7].

The analysis of genetic structure of populations was conducted using polymerase chain reaction (PCR) - methods RAPD (Random amplified polymorphic DNA) and ISSR (Inter simple sequence repeats). The following primers OPF 8, OPC 8, OPA 1, OPA 10, It 1, SAS 1, SAS 3, UBC 811, UBC 827 were used for the analysis. Amplification was carried out in thermocyclers MJ Mini and My Cycler (Bio-Rad, the USA). The products of PCR were divided by means of 2% agarous gel electrophoresis. The received data were processed using the programs GenAlEx, POPGENE 32.

### THE MAIN BODY

**Table 1: Average indicators of genetic diversity and population differentiation of the studied species (M±m)**

Species	N	P%	Ae	He	Ish	Nei model		AMOVA (P=0,01)			R
						Gst	Nm	Vap/Vwp (%)	Φst	Nm	
<i>Br. fruticum</i>	903	73.8 ±2.2	1.34 ±0.05	0.206 ±0.028	0.318 ±0.039	0.254	1.47	30/70	0.298	0.708	0.014 ±0.063
<i>Ch. tridens</i>	1006	84.5 ±3.3	1.42 ±0.05	0.250 ±0.025	0.381 ±0.034	0.177	2.33	19/81	0.185	0.954	-0.057 ±0.069
<i>H. striata</i>	694	69.8 ±5.3	1.34 ±0.06	0.207 ±0.030	0.317 ±0.042	0.358	0.895	60/40	0.404	0.360	0.571 ±0.051
<i>C. vindobonensis</i>	488	62.1 ±5.0	1.20 ±0.04	0.177 ±0.020	0.284 ±0.028	0.226	1.712	24/76	0.261	0.716	-0.085 ±0.135
<i>A. flavicollis</i>	145	85.2 ±5.8	1.46 ±0.06	0.280 ±0.027	0.425 ±0.037	0.296	1.191	17/83	0.165	1.265	-0.163 ±0.144
<i>E. integriceps</i>	121	88.75±5 .4	1.33 ±0.29	0.218 ±0.038	0.351 ±0.207	0.119	3.70	13/87	0.131	3.001	0.184 ±0.322

Note: P % – percent of polymorphous locus, Ae – effective number of alleles, Ish - Shannon index, He – expected heterozygosity, R – coefficient of correlation between logarithms of geographical distances (Dg) and indices of logarithms of pair assessment gene flow (Nm) between populations

At the first stage the level of genetic diversity of the studied populations and degree of their subdivision are assessed (table 1). According to the obtained data the most degree of variability is possessed

by the common species *A. flavicollis*. The least indicators of heterozygosity are marked in very protected species *H. striata* and *C. vindobonensis*.

The analysis of molecular dispersion (AMOVA) [8] has shown that the greatest disconnection between populations is inherent in the very protected species *H. striata*, where the index of differentiation is  $\Phi_{st}=0,404$ , and the level of gene flow  $Nm=0,360$  of the individuals for a generation. At that, the ratio of interpopulation dispersion ( $V_{ap}$ ) and intrapopulation dispersion ( $V_{wp}$ ) has accounted for 60/40%, that considerably differs from the indices of other species.

The analogous assessment has been obtained on the basis of the model suggested by M. Nei [9], according to which the greatest disunity of the studied species is possessed by *H. striata* ( $G_{st}= 0,358$ ,  $Nm=0,895$ ).

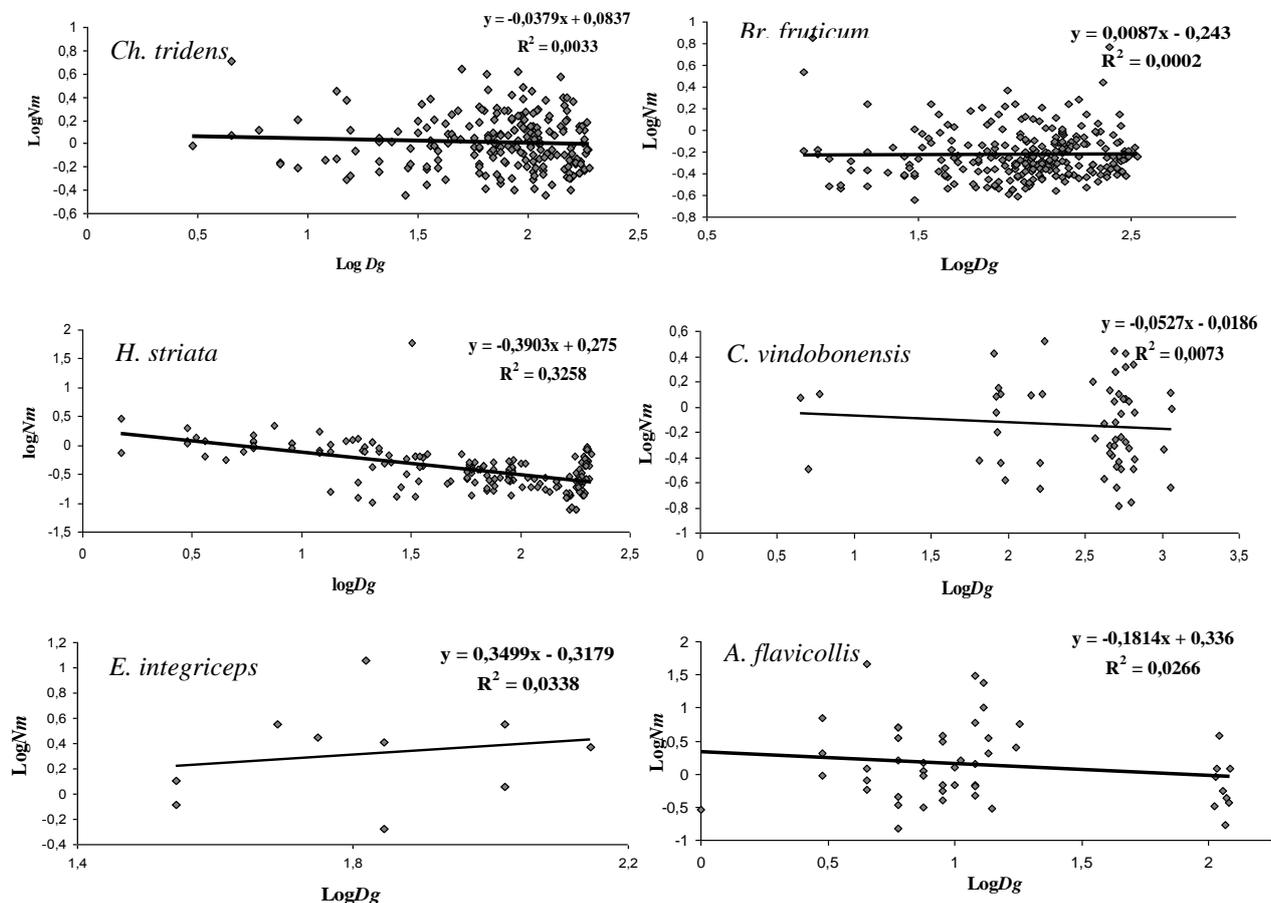
And in both cases the least indicators of subdivision of populations are typical for vermin of crops *E. integriceps*.

The effective size have been assessed by means of two approaches. In the first variant we used the equation of rectilinear regression, based on coefficient of linear function between pair assessment of gene flow ( $Nm$ ) and geographical distance between populations ( $Dg$ ):

$$\log Nm = a + b \cdot \log Dg$$

M. Slatkin [10] has shown that the effective size of populations (for all studied populations in the whole) can be obtained as  $Ne = 10^a$ , where  $a$  – coefficient, received in the equation. As a result of calculations the following values of  $Ne \pm \Delta$  have been received: *Br. fruticum* –  $0,57 \pm 0,26$ , *Ch. tridens* –  $1,26 \pm 0,60$ ; *C. vindobonensis* –  $0,96 \pm 0,60$ ; *H. striata* –  $1,90 \pm 0,80$ ; *E. integriceps* –  $0,480 \pm 0,479$ ; *A. flavicollis* –  $2,17 \pm 1,28$ .

Figure 1. The linear regression of the logarithm of gene flow  $Nm$  between the pairs of populations of



the studied species on the logarithm of geographical distance between them  $Dg$ . Besides, the presence of isolation effect by the distance in population structure  $H. striata$  can be supported with decrease of the role of stabilizing (balancing) selection in the populations of this species for a number of selectively important loci.

According to the obtained data relatively high average value of effective size has been representative for both common species of animals ( $A. flavicollis$  и  $Ch. tridens$ ), and very protected species ( $H. striata$  и  $C. vindobonensis$ ). Besides, confidence intervals, calculated at 95% level of significance, have not shown significant distinction between them in most cases<sup>1</sup>.

It is also worth noting that it is only  $H. striata$  of all studied species that has been marked to have significant correlation between logarithms of geographical distances  $Dg$ , between the groups and logarithms of pair indices of the level of gene flow  $Nm$ , calculated through pair indices of the differentiation  $\Phi_{st}$  ( $R_{DNA}=0,571\pm0,052, t=10,9, p<0,05$ )<sup>2</sup> (figure 1).

Proceeding from these findings one can argue, that population structure of the endangered species  $H. striata$  in the conditions of the south of the Central Upland has the ordered character and corresponds more to isolation effect by distance [11]. This is likely to be connected with peculiarities of biology of this species, being timed exceptionally to communities of chalky outcrops and having limited possibilities of inhabitation in the conditions of very fragment landscape, that does not allow it to form meta-population structure which is distinctive for the common species as  $Br. fruticum$ ,  $Ch. tridens$  and  $A. flavicollis$ .

By calculating effective size via integral model based on the values of index of population subdivision, somewhat different result has been received [12]:

$$F_{st} = \frac{1-t_k}{1+t_k}, \text{ where}$$

$$t_k = \exp - \left\{ \left( \frac{1}{Ne} \right) [\ln(K-0,5) + 0,5772] + \left( \frac{1}{2 \cdot Ne^2} \right) \left[ 1,6449 - \frac{2}{2 \cdot K - 1} \right] + \left( \frac{1}{3 \cdot Ne^3} \right) \left[ 1,202 - \frac{2}{(2 \cdot K - 1)^2} \right] \right\},$$

where  $K$  – number of used populations

In view of the fact that it has been used two other integral and interchangeable indices  $G_{st}$  and  $\Phi_{st}$ , instead of the index  $F_{st}$  for determining the degree of population subdivision in this work, we have seen it feasible to modify the mentioned formula introducing, in turns, the values of these indices. The results of calculations are represented in table 2.

According to the received data the lowest indices of effective size are possessed by the most endangered species  $H. striata$  and  $C. vindobonensis$  (especially, using the index of  $\Phi_{st}$ ).

**Table 2: The values of effective size of populations of different species, calculated on the basis of subdivision indices ( $G_{st}$  and  $\Phi_{st}$ )**

Index	<i>Br. fruticum</i>	<i>Ch. tridens</i>	<i>H. striata</i>	<i>C. vindobonensis</i>	<i>A. flavicollis</i>	<i>E. integriceps</i>
$Ne (G_{st})$	7.3	10.3	4.9	6.6	6.3	15.6
$Ne (\Phi_{st})$	6.2	9.9	4.4	5.7	11.0	14.3

<sup>1</sup> Considerable confidence intervals of effective size of *E. integriceps* are conditioned by few number of studied populations.

<sup>2</sup> This scheme of finding the effect of isolation by distance according to selectively neutral genes between geographically and historically connected populations has been suggested by M. Slatkin [10].

## CONCLUSION

Thus, the studies to be carried out on the basis of DNA-markers (*RAPD*, *ISSR*) allowed to assess the state of gene pools and to determine the effective size of populations of six species of the animals that inhabit in the conditions of the south of the Central Upland.

## SUMMARY

The results of the studies have shown that the very protected animal species in comparison with common and vermin species possess the lowered degree of genetic variability, the highest index values of subdivision ( $\Phi_{st}$  и  $G_{st}$ ) and low indices of effective size. This fact is supported by protective status of these species.

Besides, as a result of stenoecic character of defenceless species, the genetic processes in their populations are likely to be the best indicators of seral processes than to be expected using eurybiotic common species for this purpose, that actively adapt to anthropic factor.

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