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ON THE ISSUE OF THE GENETIC STRUCTURE OF SPECIES FOUND IN THE RELIC COMMUNITIES OF CENTRAL RUSSIAN UPLAND SOUTHERN TERRITORIES

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Abstract

This paper performs a comparative analysis of population genetic structure concerning specially protected species of terrestrial mollusk Helicopsis striata Müller and the herbaceous plant Androsace kozo-poljanskii Ovsz. included in the relic communities of Central Russian Upland southern territories. A low level of allelic diversity and the small values of effective number are revealed, which confirm their vulnerable status. It was found that all studied species use different strategies for survival in fragmented landscape conditions.

Key words: Terrestrial mollusk, Herbal plant, Relict species, Genetic structure.

Introduction

The assessment of vulnerable species populations includes a set of studies concerning various aspects of their biology. One important aspect of this approach is the study of population gene pools of these species, allowing predict the future course of genetic fluctuations and to assess the chances of survival of these species in the biosphere with a varying degree of probability.

The purpose to this work is the comparative analysis of population conditions concerning two specially protected species - herbaceous plant Androsace kozo-poljanskii Ovsz.1, the terrestrial mollusk Helicopsis striata Müller living in Central Russian Upland southern territories.

These species make the part of the mountain-alpine communities, developed on the territory of the Central Russian Upland during the glacial epoch. On the territory of study region both species are represented by local populations, clearly separated from each other by considerable extened territories [1, 2].

Methods

The studies were conducted in 10 populations of A. kozo-poljanskii and 9 populations of H. striata. The analysis of population genetic structure was performed using polymerase chain reaction (PCR) - RAPD methods (Random amplified polymorphic DNA) and ISSR (Inter simple sequence repeats). The following primers were used for analysis: OPA 1, It 1, UBC 811, UBC 820. Amplification was performed in thermocyclers MJ Mini and MyCycler (Bio-Rad, USA) were used for analysis. PCR products were separated using electrophoresis in 2% agarose gel. The processing of the data was performed using GenAlEx and POPGENE 32 program.

Main part

The averaged values of genetic variation indicators in both types of populations are presented in Table 1. According to obtained information, the studied populations have a lower level of genetic diversity in comparison with other species in the study area [3-7]. This may be explained by a strong isolation, the limited dissemination opportunities, as well as by the habitation in specific habitats of chalky denudations. Besides, A. kozo-poljanskii is capable of vegetative propagation, which also negatively affects the level of its population polymorphism. H. striata really surpassed A. kozo-poljanskii by the level of expected heterozygosity (He), and an effective number of alleles (Ae). An opposite result is obtained by the percentage of polymorphic loci and an average number of alleles per locus. Shannon index showed no significant differences between two species..

Table 1: Average values of genetic variation in *H. striata* and *A. kozo-poljanskii* populations.

Species	N	N_L	%P	A	A_e	I_{sh}	H_e
H. striata	333	34	50.65 ±6.23	1.134 ±0.053	1.253 ±0.020	0.230 ±0.016	0.153 ±0.011
A. kozo-poljanskii	438	41	67.07 ±2.31	1.356 ±0.046	1.187 ±0.012	0.217 ±0.011	0.131 ±0.007

Note: N – the number of analyzed individuals, N_L – the number of analyzed loci, P – the percentage of polymorphic loci, A – an average number of alleles per locus, Ae – an effective number of alleles, I_{sh} - Shannon index, He – expected heterozygosity.

Then we calculated the level of population differentiation for both species by molecular dispersion analysis (AMOVA) [8] (Table. 2, 3). The obtained data showed significantly higher dissociation of snail populations H. striata (Fst = 0.576) compared with the populations of A. kozo-poljanskii (Fst = 0.136). This is also evidenced by the ratio of intra and inter-population variability (Fig. 1). H. striata has 58% of interpopulation differences, and A. kozo-poljanskii has 14% of corresponding differences.

Table 2: The result of molecular dispersion analysis (AMOVA) according to DNA loci in A. kozo-poljanskii populations.

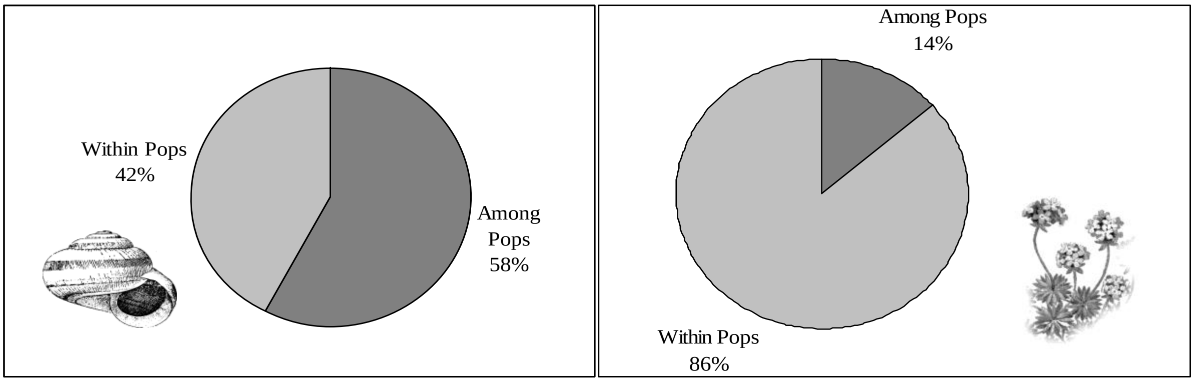
Variability source	*df*	*SS*	*MS*	*V*	Φ_{st}	*P*	*Nm*
Between populations	9	277.877	30.875	0.616	0.136	0.001	1.588
In populations	428	1677.354	3.919	3.919			
Total	437	1955.231	34.794	4.535			

The level of gene flow between A. kozo-poljanskii groups proved to be more than one and reached 1,588 of a unit per generation.

It is worth noting that according to "the theory of evolution with the shifting equilibrium" [9], in order to maintain panmixia in metapopulation the following gene flow is required: 1-2 units per generation.

Table 3: The result of molecular dispersion analysis (AMOVA) according to DNA loci in H. striata populations.

Variability source	*df*	*SS*	*MS*	*V*	Φ_{st}	*P*	*Nm*
Between populations	8	888.112	111.014	2.985	0.576	0.001	0.184
In populations	324	711.560	2.196	2.196			
Total	332	1599.673	113.210	5.181			



H. striata *A. kozo-poljanskii*

Fig. 1. The ratio of intra- and inter-population variability in *H. striata* and *A. kozo-poljanskii* populations.

A similar result was obtained based on Nei model [10] (Table. 4), where intrapopulation diversity level was really higher among H. striata. Also this species has a high proportion of interpopulation gene diversity (Gst = 0.476).

Thus, the terrestrial mollusk populations of H. striata have higher intra- and inter-population genetic diversity than the herbaceous plant A. kozo-poljanskii.

First of all, this fact can be explained by the presence of snails with only sexual reproduction among populations, while the plant demonstrates the alternation of sexual and asexual (vegetative) reproduction. Secondly, a weak genetic differentiation of plant populations is the result either of their recent separation, or an active transition of seeds and pollen by wind.

Snails, being slowly moving animals, despite the auxiliary migration mechanisms (for example, using birds), however, have less capacity for genetic information exchange between the strongly isolated populations, the geographical distances between which may vary from 4 to 140 km.

Table 4: Genetic variability and *H. striata* and *A. kozo-poljanskii* population differentiation indicators (according to Nei [8]).

Species	*Ht*	*Hs*	*Gst*	*Nm*
H. striata	0.288±0.029	0.151±0.010	0.476	0.550
A. kozo-poljanskii	0.143±0.012	0.130±0,010	0.091	5.022

Gst – the share of interpopulation gene diversity in overall diversity, H_T – an expected share of heterozygous genotypes in all population $\overline{Hs}$ - the average value for all subpopulations of intrapopulation diversity, *Nm*- average gene flow per generation. However, despite a great genetic proximity between the populations of A. kozo-poljanskii, geographical distances were not a decisive factor which determine this similarity. That is, a relatively large genetic distance was observed as between closely located groups, so as between remote ones. This conclusion is supported by the straight regression graph (Fig. 2), which shows a weak correlation (r = 0.276) between the logarithms of geographic distances G, between the populations and logarithms of pairwise gene flow level Nm values between them, calculated by pairwise differentiation indexes Fst. This demonstrates the violation of isolation model by distance in the population structure A. kozo-poljanskii and the strengthening of stabilizing selection role in its populations. A similar regression analysis between LogNm and LogG in the populations of H. striata showed the result close to the average inverse correlation between these parameters (r = 0.411). This fact indicates that the population structure of H. striata in Central Russian Upland southern territories is more orderly and more consistent with the effect of isolation by distance [11].

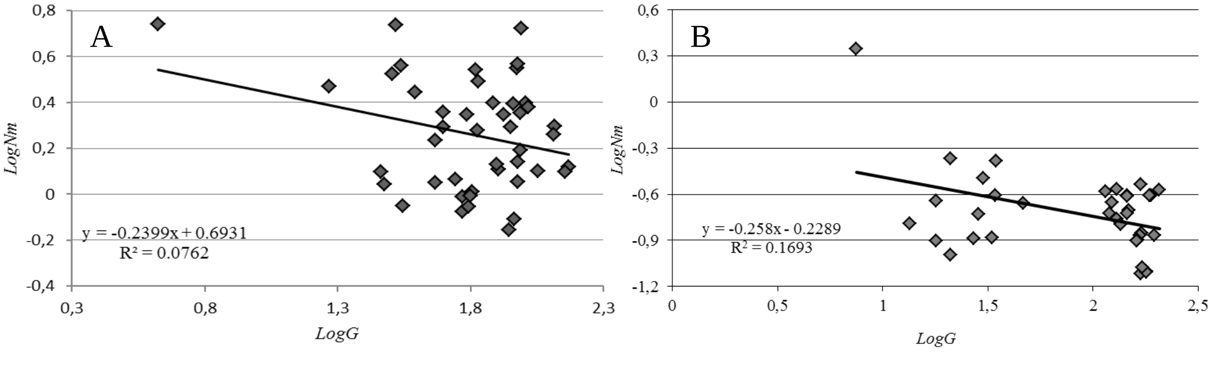


Fig. 2. Linear regression between gene flow logarithm Nm between population pairs and the geographical distance logarithm between them *G*. (A – *A. kozo-poljanskii*, B – *H. striata*).

We also evaluated an effective number of studied species. The calculation is based on linear function ratios between the pairwise estimates of gene flow (Nm) and the geographic distance between populations (G)

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

M. Slatkin [12] showed that an effective population number (for all studied populations in general) can be obtained as $Ne=10^a$, where a is the ratio, obtained in equation. Based on the equations given in Figure 2, an effective number (Ne) of A. kozo-poljanskii was equal to 4.93 units, while H. striata was equal to 1,69 units.

Summary

Thus, the performed studies on the basis of DNA markers (RAPD, ISSR) allowed to assess the state of gene pools and to determine an effective number of two species of populations of specially protected species (shellfish and herbaceous plant) which live in the relic communities of Central Russian Upland southern territories .

Conclusions

The analysis of data concerning the genetic structure of populations among two specially protected species A. kozo-poljanskii and H. striata indicates a low level of allelic diversity in studied groups, which partly confirms their status as endangered species. Low values of an effective number also prove this. However, the specified species belonging to different kingdoms of organisms use different strategies to survival conditions in a fragmented landscape. Snails maintain genetic variability due to sexual process and a balancing selection, and plants rely on an active dissemination, the combination of sexual and asexual reproduction, and the strengthening of stabilizing selection role increase in their populations.

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