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The Genetic Structure of Populations of the Specially Protected *Androsace kozo-poljanskii* Ovsz. Species under the Conditions of the Southern Central Russian Upland Based on DNA Markers

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Abstract—Based on DNA markers (Inter simple sequence repeats (ISSR)) the state of the gene pools of 10 populations (438 individuals) of specially protected relict plant species *Androsace kozo-poljanskii* Ovsz. seu *Androsace villosa* subsp. *koso-poljanskii* Fed. in the Southern Central Russian Upland is studied. The data demonstrate a low level of genetic heterogeneity of the populations ($I_{sh} = 0.217 \pm 0.011$; $He = 0.131 \pm 0.007$), as well as a low degree of the genetic fragmentation ($\Phi_{st} = 0.136$, $G_{st} = 0.091$), despite the strong geographical isolation. An analysis of multilocus genotypes (by Chao1-bc and the 1st order jackknife methods) revealed the group with potentially high and low numbers of genetic combinations. There is a low correlation between the logarithms of the gene flow level and the geographic distances between populations ($r = -0.276 \pm 0.141$), indicating the impairment of the isolation model by distance and strengthening of the role of stabilizing selection. We propose that the species studied in the past settled mainly along the river valleys. The effective population size (*Ne*), calculated based on the subdivision index and regression equations, is in the range of 4.9–20.4 individuals.

Keywords: herbaceous plant, relict species, population gene pool, anthropogenically changed landscape

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INTRODUCTION

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

The goal of this study is to analyze the state of the gene pools of the population of the especially protected herbaceous plant of the Kozo-Polansky rock jasmine (*Androsace kozo-poljanskii* Ovcz.[1]), which inhabits the southern part of Central Russian Upland.

Kozo-Polansky rock jasmine is an endemic species in the Central Russian Upland. This species belongs to the so-called mountain-alpine flora, formed on the territory of the Central Russian Upland during the glacial epoch. This species grows on the tops and slopes of Cretaceous hills, usually on open or semigrassy areas, preferring an open grassy canopy. In the territory of the study area, the species is represented by local populations, clearly separated from each other by distinct territories. *A. kozo-poljanskii* is listed in the Red Books of Russia and the Belgorod Region (the limiting factors include intensive grazing and development of chalk quarries). It occurs in the Voronezh, Kursk, and Belgorod oblasts in the basin of the right hand tributaries of the Middle Don, in the upper reaches of the Oskol, and the Seversky Donets (Vinogradov, Golitsin, 1954; Golitsyn, 1956).

MATERIALS AND METHODS

The leaves of the plants collected from ten points of the Belgorod oblast (Fig. 1, Table 1) were used for the study. The plants were collected in May 2013. All the collected samples were stored in the cryobank of the laboratory of population genetics and genotoxicology of Belgorod National Research University. In total 438 *A. kozo-poljanskii* plants from ten populations were examined by the DNA loci (see Fig. 1, Table 1).[2]

Variability analysis was carried out using a polymerase chain reaction by the *Inter simple sequence*

[1] In some sources this species is considered to be a subspecies of *Androsace villosa* subsp. *koso-poljanskii* Fed.

[2] Due to the fact that *A. kozo-poljanskii* in the places of growth is represented by separated groups of vegetative origin, each group was regarded as a single entity.

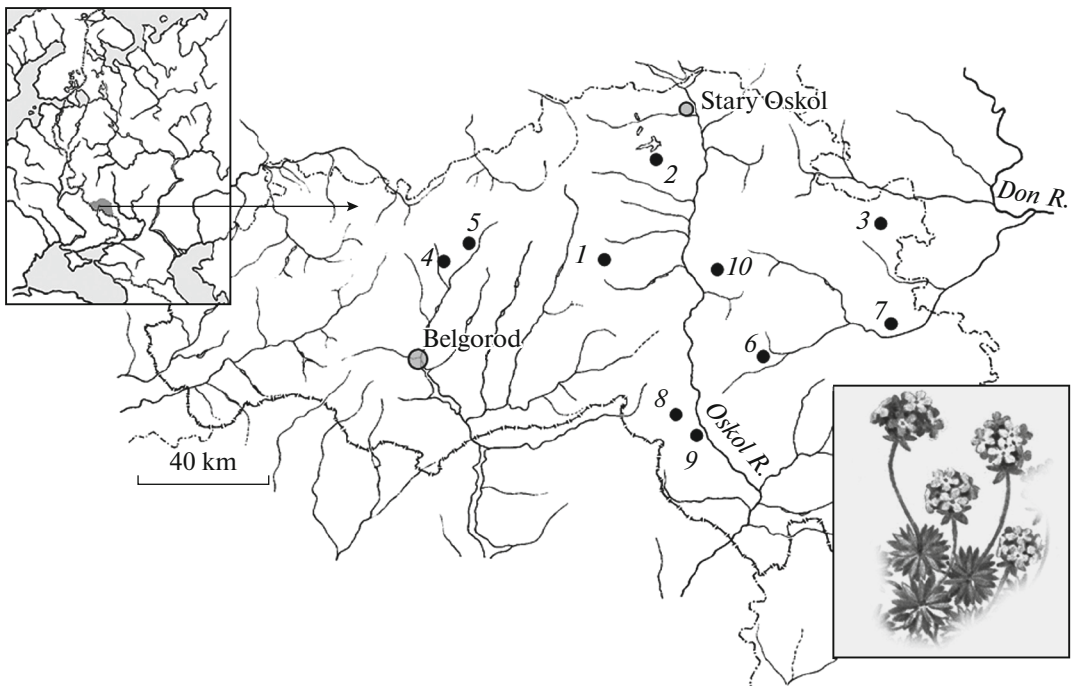

Fig. 1. Points of collection of *Androsace kozo-poljanskii* in study area

repeats (*ISSR*) method (Zietkiewicz et al., 1994). Two primers were used for the analysis: *IT1* (5'-$(CA)_8$GT-3') and *UBC820* (5'-$(GA)_8$C-3'). Amplification was performed using MJ Mini and MyCycler thermal cyclers (Bio-Rad, United States). The reaction was performed in 25 μL, containing 20 ng of genomic DNA, a PCR buffer (67 mM Tris-HC1 (pH 8.8), 16 mM $(NH_4)_2SO_4$, 5 mM β-mercaptoethanol, 7 mM EDTA,

Table 1. Description of collection points

Point	Description of the point	Coordinates
Khmelevoe	Korochanskii district, vicinity of Khmelevoe village	50°53′160″ N 37°27′246″ E
Yamskaya Step’	Protection zone of Yamskaya Steppe protected area	51°10′260″ N 37°37′510″ E
Svistovka	Krasnenskii district, vicinity of Svistovka village	50°58′691″ N 38°45′766″ E
Ozerovo	Yakovlevskii district, vicinity of Ozerovo village	50°51′048″ N 36°39′136″ E
Gnezdilovka	Prokhorovskii district, vicinity of Gnezdilovka village	50°52′679″ N 36°57′051″ E
Novokhutornoe	Krasnogvardeiskii district, vicinity of Novokhutornoe village	50°33′890″ N 37°11′261″ E
Belaya Gora	Alekseevka city, Belaya Gora tract	50°38′506″ N 38°39′447″ E
Pogromets	Volokonovskii district, vicinity of Pogromets village	50°21′052″ N 37°49′355″ E
Konoplyanovka	Valuiskii district, vicinity of Konoplyanovka village	50°19′497″ N 37°52′184″ E
Balka Khanova	Novooskol’skii district, Balka Khanova	50°45′466″ N 38°02′423″ E

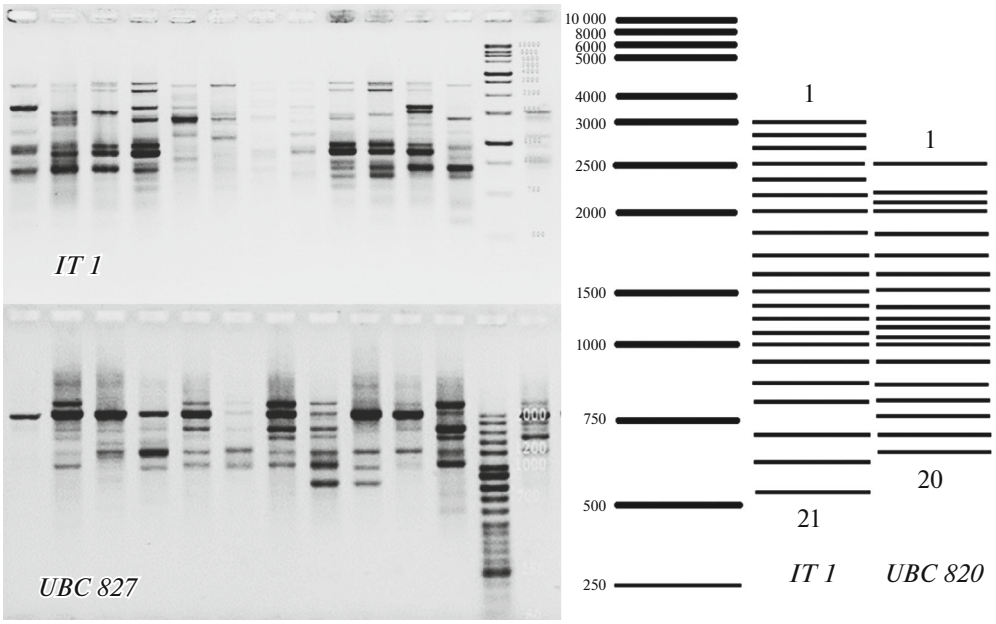

Fig. 2. DNA-patterns on left, decoding of DNA-patterns of *Androsace kozo-poljanskii* on right (only first and last loci are indicated by numbers).

3 mM $MgCl_2$, 0.25 mM dNTP, 0.5 μM primer, and 1 unit Taq DNA polymerase (inhibited for a hot start). The reaction was performed under the following conditions: hot start—2 min/94°C, 40 cycles (denaturation—30 s/94°C, primer annealing—30/55°C, synthesis—2 min/72°C), additional synthesis—10 min/72°C, and cooling—up to 4°C

PCR products were separated by electrophoresis in a 2% agarose gel using a TAE-Buffer (cooled to +4°C), 10 V/cm for 45 min. The blocks were stained with ethidium bromide.

Binary matrices were composed according to the patterns of the amplified fragments obtained during electrophoresis, where the presence of the band was designated as 1 (allele *p*) and its absence was designated as 0 (allele *q*).

In this species, we detected 41 loci: 21 species were detected using the *IT1* primer and 20 species were detected using the *UBC 820* primer. The obtained DNA patterns and their decoding are shown in Fig. 2.

The data were processed using the GenAlEx (Peakall, Smouse, 2001), POPGENE 32 (Yeh et al., 2000), and MEGA5 programs (Tamura et al., 2011).

RESULTS AND DISCUSSION

At the first stage, the Ewens-Watterson neutrality test (Ewens, 1972; Watterson, 1978; Manly, 1985) of the used loci showed that for most alleles (an average of 91%) there were no statistically significant differences between the observed homozygosity according to Hardy–Weinberg and the homozygosity expected in a neutral process (Table 2).

Estimates of the expected heterozygosity of different loci and the level of subdivision of the population, obtained according to M. Nei's model (Nei, 1975) (Table 3), can also give some idea of the degree of selective processes in the groups studied using the DNA markers used.

It is known that the average G_{st} values correspond to the level of genetic differentiation in a selectively neutral process. In this case, loci with higher G_{st} values are most probably subjected to the effect of disruptive

Table 2. Results of Ewens-Watterson test. Numbers of loci for which observed homozygosity differs from expected homozygosity ($p < 0.05$) are shown. Data were calculated based on 1000 simulations of main sample

Population	Loci		% of neutral loci
	It1	*UBC 820*	
1	3	13	95.1
2	14	15, 20	92.7
3	4, 8	8, 10, 14	87.8
4	no	14, 15	95.1
5	20, 21	1, 7	90.2
6	14, 19, 21	11, 13, 14, 15, 18	80.5
7	6, 14	no	95.1
8	20	11, 15, 17	90.2
9	5	8, 16, 17	90.2
10	16	9	95.1
Average value			91.2 ± 1.5

Table 3. Indices of genetic differentiation of studied *Androsace kozo-poljanskii* for DNA loci (according to Nei, 1975)

Primer	Locus	*Ht*	*Hs*	*Gst*	*Nm*
It1	1	0.221	0.138	0.376	0.831
	2	0.212	0.181	0.146	2.919
	3	0.002	0.002	0.010	49.165
	4	0.039	0.038	0.025	19.252
	5	0.125	0.120	0.037	13.076
	6	0.189	0.172	0.092	4.958
	7	0.248	0.215	0.133	3.269
	8	0.201	0.197	0.019	26.150
	9	0.238	0.225	0.054	8.836
	10	0.262	0.254	0.030	16.292
	11	0.249	0.237	0.046	10.294
	12	0.248	0.233	0.063	7.455
	13	0.227	0.210	0.075	6.172
	14	0.066	0.064	0.021	23.815
	15	0.255	0.245	0.042	11.533
	16	0.321	0.304	0.053	8.989
	17	0.158	0.151	0.043	11.278
	18	0.190	0.179	0.057	8.342
	19	0.086	0.083	0.044	10.919
	20	0.088	0.077	0.129	3.368
	21	0.165	0.149	0.098	4.608
UBC 820	1	0.035	0.030	0.140	3.065
	2	0.297	0.271	0.089	5.105
	3	0.025	0.024	0.056	8.376
	4	0.192	0.157	0.182	2.255
	5	0.020	0.020	0.027	18.271
	6	0.403	0.385	0.046	10.446
	7	0.101	0.080	0.207	1.916
	8	0.084	0.079	0.061	7.711
	9	0.050	0.048	0.047	10.251
	10	0.059	0.056	0.046	10.464
	11	0.213	0.154	0.276	1.314
	12	0.340	0.315	0.074	6.232
	13	0.068	0.066	0.041	11.672
	14	0.021	0.020	0.026	18.528
	15	0.049	0.048	0.030	16.115
	16	0.011	0.011	0.033	14.544
	17	0.051	0.045	0.112	3.949
	18	0.002	0.002	0.010	49.165
	19	0.018	0.017	0.037	13.118
	20	0.025	0.024	0.043	11.033
Average		0.143 ± 0.012	0.130 ± 0.010	0.091	5.022

Gst is proportion of interpopulation genetic diversity in general diversity, *Ht* is expected ratio of heterozygous genotypes in whole population, *Hs* is average value of intrapopulation diversity for all subpopulations, *Nm* is average gene flow per generation.

Table 4. Indices of genetic heterogeneity of *Androsace kozo-poljanskii* populations according to ISSR markers

Point no.	*N*	Frequency of alleles		*P* (%)	*A*	*Ae*	I_{sh}	*He*
		p	*q*					
1	45	0.138	0.862	58.54	1.195 ± 0.153	1.241 ± 0.048	0.240 ± 0.040	0.154 ± 0.028
2	45	0.128	0.872	68.29	1.390 ± 0.143	1.236 ± 0.047	0.247 ± 0.037	0.154 ± 0.026
3	33	0.110	0.890	65.85	1.341 ± 0.147	1.199 ± 0.038	0.227 ± 0.035	0.139 ± 0.023
4	45	0.104	0.896	65.85	1.317 ± 0.150	1.212 ± 0.042	0.232 ± 0.036	0.143 ± 0.025
5	45	0.094	0.906	73.17	1.463 ± 0.140	1.188 ± 0.033	0.230 ± 0.032	0.137 ± 0.021
6	45	0.072	0.928	63.41	1.268 ± 0.152	1.139 ± 0.031	0.170 ± 0.032	0.101 ± 0.021
7	45	0.086	0.914	78.05	1.560 ±0.131	1.174 ± 0.033	0.219 ± 0.030	0.127 ± 0.020
8	45	0.087	0.913	60.98	1.244 ± 0.151	1.142 ± 0.029	0.183 ± 0.030	0.106 ± 0.019
9	45	0.099	0.901	78.05	1.586 ± 0.126	1.168 ± 0.025	0.227 ± 0.028	0.131 ± 0.018
10	45	0.102	0.898	58.54	1.195 ± 0.153	1.173 ± 0.038	0.199 ± 0.034	0.121 ± 0.023
Average value		0.092	0.898	67.07 ± 2.31	1.356 ± 0.046	1.187 ± 0.012	0.217 ± 0.011	0.131 ± 0.007

N is number of analyzed individuals, *P* is percentage of polymorphic loci, *A* is average number of alleles per locus, *Ae* is effective number of alleles, I_{sh} is Shannon index, *He* is expected heterozygosity.

Table 5. Result of analysis of molecular variance (AMOVA) for DNA loci in *Androsace kozo-poljanskii* populations

Source of variability	*df*	*SS*	*MS*	*V*	%	Φ_{st}	*p*	*Nm*
Between populations	9	277.877	30.875	0.616	14	0.136	0.001	1.588
Inside populations	428	1677.354	3.919	3.919	86			
Total	437	1955.231	34.794	4.535	100			

selection, and loci with a low subdivision index are subjected to the influence of stabilizing selection (Dynamics of population gene pools ..., 2004). According to the received data, the highest differentiation between populations was recorded for the loci *It1-1* and *UBC820-7*, *-11*. In this case, the *It1-16* and *UBC820-6*, *-12* loci were the most variable.

The values of the genetic heterogeneity indices are presented in Table 4. The data demonstrate that according to all the indices of genetic diversity, the populations were not significantly different from each other (at $p = 0.05$). Thus, we observe a certain similarity between the geographically isolated groups of rock jasmine.

It can be assumed that in the recent past, the population areas of this plant in the study area were much larger and an intensive exchange of genetic information between groups took place, leading to the formation of a subdivided panmictic population in the study area, which corresponded more to the island model. Then, as a result of climate change and the influence of humans, there was a fragmentation of the species population into isolated groups, up to the complete cessation of the genetic exchange between them. However, the rate of decrease in the heterozygosity level caused by the drifting of genes due to inbreeding could be the same in the studied populations after their isolation. The differences were probably leveled by the large number of individuals in the studied groups (all studied populations were represented by extensive thickets, hundreds of meters long, including thousands of groups).

The genetic proximity of the studied *A. kozo-poljanskii* populations was also demonstrated by the analysis of molecular dispersion (AMOVA, Excoffier et al., 1992) (Table 5). Only 14% of the variability was due to interpopulation differences, the subdivision index $\Phi_{st} = 0.136$, and the gene flow level between populations $Nm = 1.588$ individuals per generation. An even weaker genetic differentiation of the studied rock jasmine groups was demonstrated by the analysis based on Nei's model (Nei, 1975) (see Table 3), according to which the subdivision index (G_{st}) was 0.091, and $Nm = 5.022$ individuals per generation.

It should be noted that according to the "shifting balance theory of evolution" (Wright, 1970), a gene flow of 1–2 individuals per generation is required in order to maintain panmixia in the metapopulation. In our case, in spite of the high *Nm* values, it is hardly reasonable to discuss an intensive gene exchange between isolated rock jasmine groups (the interpopulation geographic distances here vary from 4 to 140 km). Such a common similarity of the genetic structure can probably be explained either by similar vectors of natural selection, occurring in the populations linked by a

Table 6. Pairwise estimates of genetic differentiation (Φ_{st}) between studied *Androsace kozo-poljanskii* populations according to ISSR-loci

Point	1	2	3	4	5	6	7	8	9	10
1		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2	0.185		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3	0.243	0.163		0.001	0.001	0.001	0.001	0.001	0.001	0.001
4	0.204	0.101	0.160		0.001	0.001	0.001	0.001	0.001	0.001
5	0.127	0.116	0.112	0.078		0.001	0.001	0.001	0.001	0.001
6	0.229	0.157	0.101	0.165	0.091		0.001	0.001	0.001	0.001
7	0.264	0.181	0.083	0.167	0.121	0.064		0.001	0.001	0.001
8	0.221	0.153	0.066	0.139	0.092	0.070	0.075		0.001	0.001
9	0.196	0.100	0.045	0.094	0.063	0.044	0.067	0.043		0.001
10	0.219	0.099	0.203	0.113	0.091	0.166	0.177	0.182	0.113	

Under diagonal, Φ_{st} estimates are shown, and level of their significance is shown above diagonal.

single landscape structure or by their relatively recent dissociation.

The degree of genetic proximity between the studied *A. kozo-poljanskii* populations was also demonstrated by the pairwise estimates of the genetic differentiation F_{st} (Table 6). According to the obtained data, the group from the vicinity of Khmelevoe village was the most original and different from other populations (point *1*).

A similar result was demonstrated by cluster analysis based on genetic distances (Nei, 1978) by the unweighted pair group method (UPGMA) (Fig. 3), as well as the Debets polygons plotted for frequencies of the *q*-allele (Fig. 4). In this case, a pattern is revealed. The highest genetic similarity was observed between groups located in the same river valleys or tributaries. For example, the Pogromets (no. *8*) and Konoplyanovka (no. *9*) points are located in the Oskol River valley, Ozerovo (no. *4*) and Gnezdilovka (no. *5*) are located in the Seversky Donets River valley, and Belaya Gora (no. *7*) and Novokhutornoe (no. *6*) are located in the Tikhaya Sosna River valley. In this case, a fairly isolated group from the vicinity of Svistovka village (no. *3*) was in the Oskol cluster. The groups inhabiting the basin of Oskol River, the populations of the Yamskaya Steppe (no. *2*) and Balka Khanova (no. *10*) were also genetically close (although remote from other groups (nos. *8* and *9*) in this basin), and the population of Khmelevoe (no. *1*) based on the allele frequencies was at a distance from all the studied groups.

Nevertheless, despite the location described above, the geographic proximity of the populations was not the decisive factor determining the genetic proximity. The relatively high genetic distance was observed both between geographically closed and remote groups. This conclusion was supported by the linear regression plot (Fig. 5), which showed a weak correlation ($r = -0.276$) between the logarithms of the geographical distances *G* between populations and the logarithms of the pairwise gene flow rates *Nm* between them, calculated based on the pairwise differentiation indices F_{st}. This demonstrates the impairment of the isolation model by the distance in the population structure of *A. kozo-poljanskii* and the strengthening of the role of the stabilizing selection in these populations.

In this respect, the following should be noted. The revealed genetic proximity between the populations belonging to the same river basins may indicate that in the past the expansion of rock jasmine probably took place along the river valleys, resulting in the formation of panmictic groups confined to these elements of landscape[3]. After further fragmentation of the population areas, the remaining groups retained a similar allele set. As a result, the populations located nearby but in different river valleys were more genetically distinct than the more geographically remote populations located in the same valley. In addition, it cannot be ruled out that the transfer of pollen and seeds along the river valleys is currently taking place, causing a limited gene flow between the rock jasmine populations that were integrated a long time ago and are now divided. However, the latter conclusion requires a certain amount of caution and experimental confirmation of the possibility of transferring the seeds and pollen of this plant such long distances.

Due to the fact that the samples of a limited abundance containing only a small part of the population allele fund was used for the analysis of the viability of the *A. kozo-poljanskii* populations, we performed a multilocus analysis of the variation. For this analysis, multilocus combinations for each of the 438 individuals were calculated. Then, in each group, we estimated the total number of multilocus genotypes (N_{MLG}) and the number of unique multilocus genotypes (N_{MLG-1}), i.e., the combinations detected in a single sample. In the future, based on the distribution of the frequencies of multilocus genotypes, the potential genetic diversity expected for the increase in the sample size up to infinity (N_{max}) was calculated for each population. The

[3] The transfer of seeds and pollen through mountainous watersheds was probably less intensive.

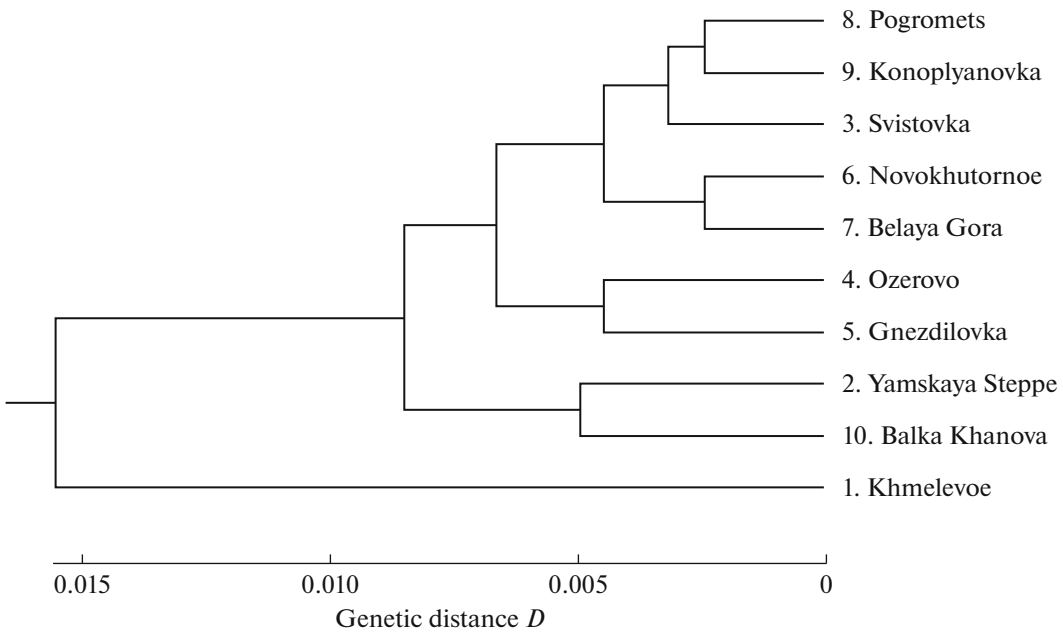

Fig. 3. Dendrograms of genetic distances according to Nei (Nei, 1972) (UPGMA) between *Androsace kozo-poljanskii* populations.

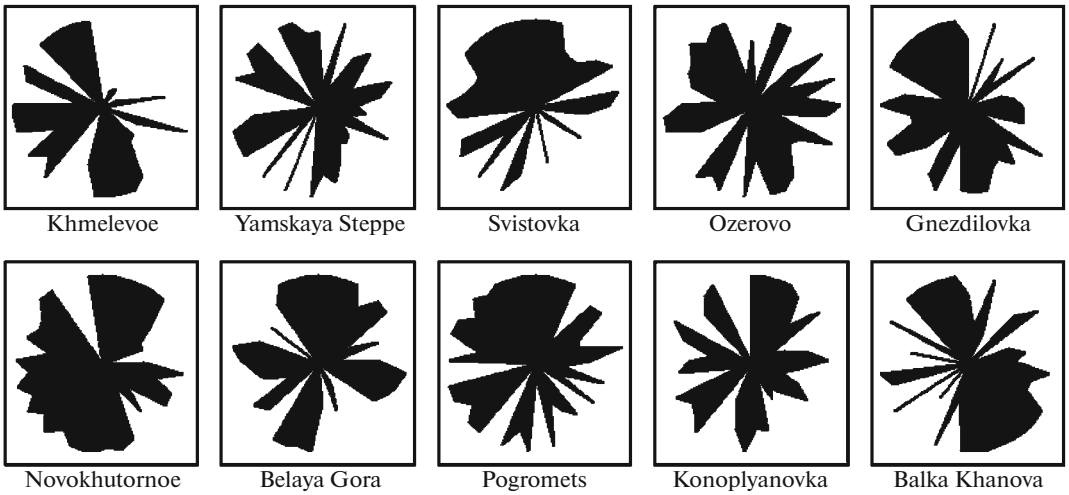

Fig. 4. Debets's polygons, constructed based on aggregated frequencies of *q*-allele of 41st locus of DNA in *Androsace kozo-poljanskii* populations.

analysis was carried out using two nonparametric methods: the Chao1-bc method (bias-corrected form for the Chao1) (Chao, 2005) and the *Ist order jackknife* method (Burnham and Overton, 1978). All the calculations were carried out using the SPADE program (Chao and Shen, 2009). The results of the analysis of the multilocus genotypes are presented in Table 7.

The analysis demonstrated that the total number of multilocus genotypes, the number of unique combinations, and accordingly, the maximal possible number of combinations was significantly higher for the *It1* primer. It is worth noting that I.I. Schmalhausen (1968) called allelic diversity the "mobilization reserve" of the population, providing it with a more stable existence over time. According to our data, among the studied populations, the potentially richest gene pool was characteristic for groups from the vicinity of Khmelevoe village (no. *1*) and from the protected area of the Yamskaya Steppe (no. 2). The most monomorphic group was from Balka Khanova (no. *10*).

The multilocus genotypes obtained using *UBC 820* was uniform. Among the populations studied for this primer, the Belaya Gora (no. *7*) population was the most polymorphic, and the Gnezdilovka population (no. *5*) was the most uniform.

It should be noted that significant differences between the populations were not obtained as a result of unifying combinations with the use of both primers.

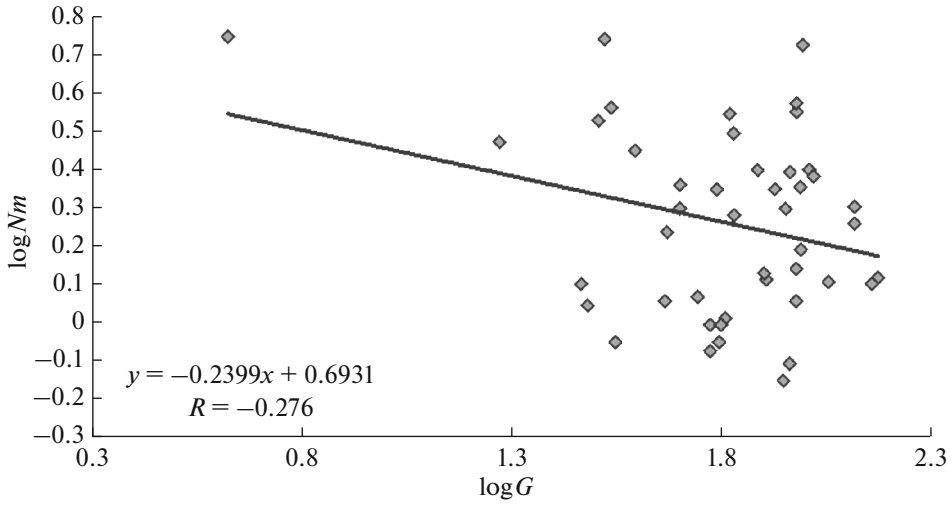

Fig. 5. Linear regression of logarithm of gene flow *Nm* between pairs of *Androsace kozo-poljanskii* populations by logarithm of geographical distance *G* between them.

In fact, in each group, the number of marked combinations (N_{MLG} and N_{MLG-1}) was the same as the sample size, and the maximal possible number of multilocus genotypes tended to infinity.

A very important problem that was solved during the monitoring activities conducted with respect to the environment was the estimation of the effective population size for the studied species, i.e., the minimal abundance required for the survival of a species. From the genetic point of view, the effective population size is the size of the ideal population in which the level of gene drift corresponds to that in the real population (Write, 1931). This understanding of the effective population size allows a more objective approach in develop-

Table 7. Number of detected multilocus genotypes and estimates of potential genetic diversity obtained by different methods for studied *Androsace kozo-poljanskii* populations

Primer	Population	N_{MLG}	N_{MLG-1}	Method			
				Chao1-bc		1st order jackknife	
				$N_{max} \pm SE$	95% CI	$N_{max} \pm SE$	95% CI
It1	1	45	43	1013 ± 291.9	588.0–1770.8	89.0 ± 9.3	74.2–111.4
	2	45	37	1013 ± 291.9	588.0–1770.8	89.0 ± 9.3	74.2–111.4
	3	33	28	545.0 ± 181.0	294.3–1036.3	65.0 ± 7.9	52.8–84.7
	4	44	39	485.5 ± 260.2	194.4–1331.6	86.0 ± 9.1	71.6–108.0
	5	44	42	485.5 ± 260.2	194.4–1331.6	86.0 ± 9.1	71.6–108.0
	6	43	36	310.3 ± 152.6	137.4–800.0	83.1 ± 8.9	69.1–104.6
	7	41	33	171.2 ± 68.8	90.3–385.3	77.2 ± 8.5	64.0–97.9
	8	43	34	310.3 ± 152.6	137.4–800.0	83.1 ± 8.9	69.1–104.6
	9	42	32	843.8 ± 253.6	479.8–1510.5	82.1 ± 8.9	68.1–103.6
	10	36	25	142.3 ± 61.5	73.1–340.9	65.3 ± 7.6	53.8–84.4
UBC 820	1	14	7	21.3 ± 8.0	15.3–55.7	19.9 ± 3.4	16.0–30.9
	2	15	9	20.1 ± 5.3	16.0–42.1	21.8 ± 3.7	17.5–33.4
	3	17	10	27.9 ± 9.3	19.6–63.4	26.7 ± 4.4	21.2–39.5
	4	28	14	65.2 ± 23.4	40.0–143.5	47.6 ± 6.2	38.6–63.9
	5	18	8	29.0 ± 9.4	20.6–64.7	27.8 ± 4.4	22.2–40.7
	6	17	9	34.9 ± 14.8	21.4–90.5	27.8 ± 4.6	21.8–41.1
	7	32	17	125.4 ± 54.4	64.7–302.1	60.4 ± 7.4	49.3–78.9
	8	19	4	44.4 ± 19.6	25.7–116.1	31.7 ± 5.0	25.0–45.8
	9	28	18	69.1 ± 25.5	41.4–153.6	48.5 ± 6.4	39.3–65.2
	10	25	14	55.9 ± 19.6	35.3–122.5	43.6 ± 5.9	35.3–59.4

ing programs for the conservation of species diversity. In addition, this knowledge is very useful for controlling artificial breeding, as well as for studying the evolutionary processes occurring in natural populations.

In our estimate of the effective size, we used two approaches.

The first calculation method is based on the coefficients of the linear function between the pairwise estimates of the gene flow (*Nm*) and the geographical distance between the populations (*G*):

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

M. Slatkin (1993) showed that the effective population size (for all the studied populations as a whole) can be obtained as $Ne = 10^a$, where *a* is the coefficient obtained in the equation. Based on the equation shown in Fig. 5, the effective population size (*Ne*) was 4.9 individuals.

A slightly different result was obtained by the calculation of the effective population size using an integrated model based on the population's subdivision index (Wright, 1951):

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

$$\text{where } t_k = \exp-\left\{\left(\frac{1}{Ne}\right)[\ln(K-0.5)+0.5772] + \left(\frac{1}{2Ne^2}\right)\left[1.6449-\frac{K}{2K-1}\right] + \left(\frac{1}{3Ne^3}\right)\left[1.202-\frac{2}{(2K-1)^2}\right],\right.$$

where *K* is the number of used populations.

In order to determine the subdivision index of the populations in this study instead of the F_{st} index, we used two other integral and interchangeable indicators G_{st} and Φ_{st}. We considered that it was possible to modify this formula by introducing the values of these indices by turns. As a result, the effective population size (*Ne*) calculated based on the G_{st} index turned out to be 20.4 individuals, and based on the Φ_{st} index, it was 13.7 individuals, which is much higher than the analogous value calculated from the regression equation (4.9). Nevertheless, the estimates of the effective size are within the range of the effective population size calculated for herbaceous plants (from 3 to 282 individuals) (Silvertown, 1993).

We believe that the obtained results can be useful for monitoring the state of the *A. kozo-poljanskii* populations, as well as for the possible introduction of this species in order to restore it in the elements of the landscape of the southern part of the Central Russian Upland.

REFERENCES

Burnham, K.P. and Overton, W.S., Estimation of the size of a closed population when capture probabilities vary among animals, *Biometrika*, 1978, vol. 65, pp. 625–633.

Chao, A., Species richness estimation, in *Encyclopedia of Statistical Science*, Balakrishnan, N., Read, C.B., and Vidakovic, B., Eds., New York: Wiley, 2005, pp. 7907–7916.

Chao, A. and Shen, T.-J., SPADE, 2009. http://chao.stat.nthu.edu.tw.

Dinamika populyatsionnykh genofondov pri antropogennykh vozdeystviyakh (Dynamics of Population Gene Pools under Anthropogenic Impacts), Altukhov, Yu.P., Ed., Moscow: Nauka, 2004.

Ewens, W., The sampling theory of selectively neutral alleles, *Theor. Pop. Biol.*, 1972, vol. 3, pp. 87–112.

Excoffier, L., Smouse, P.E., and Quattro, J.M., Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data, *Genetics*, 1992, vol. 131, pp. 479–491.

Golitsyn, S.V., To the flora of the eastern wing of the Upper Prioskolie, *Bot. Zh. SSSR*, no. 10, pp. 1428–1438.

Manly, B.F.J., *The Statistics of Natural Selection on Animal Populations*, London: Chapman and Hall, 1985.

Nei, M., Genetic distance between populations, *Am. Nat.*, 1972, vol. 106, no. 949, pp. 283–292.

Nei, M., *Molecular Population Genetics and Evolution*, Amsterdam, 1975.

Peakall, R. and Smouse, P.E., *GenAlEx V5: Genetic Analisis in Excel. Population Genetic Software for Teaching and Research*, Canberra: Australian National University, 2001. http://www.anu.edu.au./BoZo/GenAlEx/.

Shmal'gauzen, I.I., *Faktory evolyutsii. Teoriya stabiliziruyushchego otbora* (Evolution Factors. Theory of Stabilizing Breeding), Moscow: Nauka, 1968

Silvertown, J. and Lovett Doust, J., *Introduction to Plant Population Biology*, Oxford: Blackwell Scientific, 1993.

Slatkin, M., Isolation by distance in equilibrium and non equilibrium populations, *Evolution*, 1993, vol. 47, no. 1, pp. 294–279.

Tamura, K., Peterson, D., Peterson, N., et al., *MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution*, 2011. http://www.kumarlab.net/publications.

Vinogradov, N.P. and Golitsyn, S.V., "Lowered Alps" and thyme fields of the Central Russian Upland, *Bot. Zh.*, 1954, vol. 39, no. 3, pp. 423–430.

Watterson, G., The homozygosity test of neutrality, *Genetics*, 1978, vol. 88, pp. 405–417.

Wright, S., Evolution in Mendelian population, *Genetics*, 1931, Vol. 16, pp. 97–159.

Wright, S., Isolation by distance, *Genetics*, 1943, vol. 28, pp. 114–138.

Wright, S., The genetical structure of populations, *Ann. Eugenics*, 1951, no. 15, pp. 323–354.

Wright, S., *Random drift and shifting balance theory of evolution, Mathematical Topics in Population Genetics*, Berlin: Springer Verlag, 1970, pp. 1–31.

Yeh, F.C., Yang, R., Boyle, T.J., et al., *POPGENE 32, Microsoft Window-based Freeware for Population Genetic Analysis, Version 1.32; Molecular Biology and Biotechnology Centre*, Edmonton: University of Alberta, 2000. http://www.ualberta.ca/~fyeh/popgene_download.html.

Zietkiewicz, E., Rafalski, A., and Labuda, D., Genome fingerprinting by simple sequence repeat (SSR)—anchored polymerase chain reaction amplification, *Genomics*, 1994, vol. 20, no. 2, pp. 176–181.

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