

SHORT
COMMUNICATIONS

Genetic Differentiation of *Rhododendron aureum* Georgi at Nuclear Microsatellite Loci

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Abstract—SSR analysis of *Rhododendron aureum* Georgi in eight populations (63 samples) from Siberia (West and East Sayans) and Kamchatka was performed. Polymorphism of five SSR loci showed a high level of genetic diversity in all the populations (mean values $H_o = 0.667$, $H_e = 0.638$). The analysis of molecular variation (AMOVA) revealed that about 16% of the genetic diversity of *R. aureum* occurs in interpopulation differences ($F_{st} = 0.161$, $p = 0.001$). The Bayesian approach in the STRUCTURE program revealed that the species has a heterogeneous genetic structure—populations from Kamchatka form a separate cluster and show high homogeneity, and populations from Western and Eastern Siberia are of mixed origin. The obtained results indicate the prospects of using this type of markers for population-genetic studies of *R. aureum*.

Keywords: SSR markers, population structure, genetic variability, microsatellites

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Rhododendron aureum Georgi (Ericaceae) is an evergreen bushy plant commonly found in alpine and subalpine highlands up to 2700 m above sea level in *Pinus pumila* undergrowth. It may also often be found in the upper parts of the forest zone, marshes, and low bush tundra. It is represented by different ecobiomorphological forms that vary from low bush of about 30 cm to bushy plants that may reach 2 m in height, depending on the habitat, owing to its flexible life form [1].

It has a wide geographic range that encompasses Western Siberia (Altai), Eastern Siberia (Sayan, Baikal and Transbaikalian regions, and Eastern Yakutia), and the Far East, including Chukotka, Kamchatka, Sakhalin, and Kuril and Commander Islands. An isolated location in Putoran Mountains is also known. Outside of Russia, it is also found in Mongolia, Korea, China, and Japan.

Rhododendron aureum is the main component of highland ecosystems. It possesses the landscape forming function under severe climatic conditions, supports ecological balance, forms a specific soil microbiome, and prevents soil erosion [2]. Together with a variety of alpine species, *R. aureum* is considered as a model indicator of climatic fluctuations that took place in the past and are taking place at the present time [3, 4]. Moreover, the plant raw material of this species contains many pharmacologically valuable biochemical properties [5]. Taken together with its high decorative properties, this circumstance causes

anthropogenic pressure on some of its populations. The species is included in the Red Books of several subjects of Russia, including Kemerovo oblast, Chita oblast, Zabaykalsky krai, and Agin-Buryat okrug.

Both population and genetic studies of *R. aureum* are necessary in order to assess the adaptive potential of this species under changing environmental conditions, as well as to estimate the status of populations in order to develop measures to protect their resources. These studies are also fundamentally important as they are aimed at the supplementation to the list of wide-area model plants, the genetic structure of which reflects historical changes in climate, making them suitable for the reconstruction of plant dynamics in Northern Eurasia in the Pleistocene–Holocene [6, 7].

There are numerous studies of genetic and population diversity of different species of the *Rhododendron* genus [8–12, etc.]. However, little is known about *R. aureum* [13–17]. In the present study, we used the multilocus analysis of nuclear DNA microsatellites. The genetic diversity of *R. aureum* was previously studied by this type of markers only in single populations of Japan, China, and Sakhalin [15–17]. Studying the structure of variability on a larger amount of samples will identify the genetic diversity of *R. aureum* and clarify the formation of its range from a biogeographical point of view.

The goal of this work was to assess the parameters of genetic variability and population differentiation

Table 1. The parameters of genetic diversity of microsatellite loci in the populations of *Rhododendron aureum*

No.	Population	Coordinates, north latitude/east longitude	N	N_a	N_e	H_o	H_e	$F \pm SE$
1	Krasnoyarsk krai, Western Sayan, Ergaki, Tarmazakovsky bridge	52°85′/92°41′	8	4.20	2.68	0.650	0.616	-0.059 ± 0.086
2	Krasnoyarsk krai, Western Sayan, Stone Town (Kamenny Gorodok)	52°53′/92°54′	10	3.40	2.35	0.540	0.565	0.030 ± 0.144
3	Western Sayan I	52°81′/93°12′	10	4.00	2.70	0.620	0.607	-0.020 ± 0.067
4	Western Sayan II	51°59′/93°07′	4	3.60	2.89	0.750	0.644	-0.178 ± 0.064
5	Buryatia, Eastern Sayan, border with Tuva Republic	52°30′/98°78′	4	3.60	2.81	0.750	0.613	-0.115 ± 0.282
6	Tuva Republic, Eastern Sayan, Obruchev Range	52°03′/94°07′	11	4.40	2.71	0.655	0.618	-0.077 ± 0.133
7	Kamchatka krai, Avachinsky volcano	53°21′/158°75′	10	5.20	3.79	0.780	0.710	-0.116 ± 0.118
8	Kamchatka krai, Vokchazhets Mountain	53°07′/157°93′	6	4.60	3.91	0.667	0.733	0.093 ± 0.088
	Mean			4.13 (0.180)	2.98 (0.142)	0.667 (0.031)	0.638 (0.015)	-0.055 ± 0.047

N —population size, N_a —mean number of alleles per locus, N_e —effective number of alleles per locus, H_o —observed heterozygosity, H_e —expected heterozygosity, F —Wright's fixation index, SE—standard error.

level of *R. aureum* in Siberia and Kamchatka using nuclear microsatellite loci.

The analysis included data obtained from 63 plant samples, which included four populations (32 plants) from Western Sayan, two populations (15 plants) from Eastern Sayan, and two populations (16 plants) from Kamchatka. The locations and geographical coordinates are shown in Table 1. Leaves dried in silica gel were used for DNA analysis. DNA was isolated by the CTAB method using the standard type protocol for plant tissues [18]. Loci developed for *R. aureum* (*RD4*, *RD8*, *RDE12*) [16] and *R. brachycarpum* (*RA20*, *RA31*) have been analyzed. The PCR conditions are described in [16, 17]. The amplification fragment length was assessed by a Genetic Analyzer 3130 (Applied Biosystems, United States) in the presence of the S-450 molecular weight standard (Gordiz, Russia). The chromatograms were analyzed with the GeneMapper v. 4.0 program.

The parameters of genetic diversity, Wright's F-statistics, population differentiation, analysis of the hierarchical structure variability (AMOVA), PCA analysis of the genetic distance template, and comparative analysis of the genetic and geographical distances with the Mantel test were carried out with the GenAIEx 6.5 program [19]. The analysis of the genetic structure of the all samples studied without taking into account their population belonging was performed in the STRUCTURE 2.2 program using the Bayesian clusterization approach with Markov chains [20]. This

method divides all samples into a certain number of clusters (K) on the basis of data on genotypes, estimating the probability of a sample belonging to a certain cluster. The algorithm was repeated five times for K values varying from 2 to 5, using the "admixture" model, which takes into account the apparently mixed origin of the samples under the condition of independent frequencies of alleles between the clusters, with the number of iterations being 100 000. The first 10 000 iterations were omitted. The optimal group number was chosen with the Structure Harvester program [21], which utilized the method proposed in [22].

All microsatellite loci selected demonstrated variability in the populations studied. The number of alleles revealed in the loci in all populations varied from 6 to 13. The *RD4* locus was represented by 6 alleles; *RD8*, 13 alleles; *RDE12*, 9 alleles; *RA20*, 10 alleles; and *RA31*, 11 alleles. In total, the analysis of five microsatellite loci allowed us to identify 49 allelic variations, nine of which were represented in all populations. The mean number of alleles per locus in populations varied from 3.40 to 5.20; the effective number of alleles varied from 2.35 to 3.91. The maximal values of these parameters were found in the populations from Kamchatka. The values of the observed and expected heterozygosity were high and varied from 0.540 to 0.780 and 0.565 to 0.733, respectively. The mean values of these parameters were $N_a = 4.13$, $N_e = 2.98$, $H_o = 0.667$, and $H_e = 0.638$.

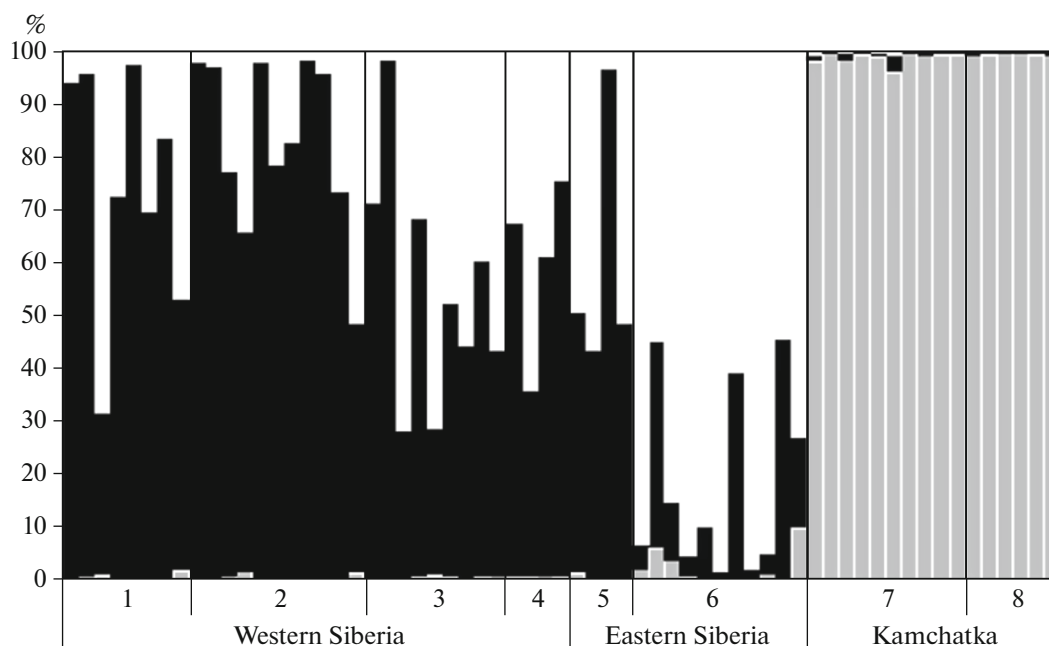


Fig. 1. Probability of the reference of 63 samples of *Rhododendron aureum* to one of the three clusters in accordance with the data of the analysis of five microsatellite loci carried out in the “admixture” assay with the STRUCTURE program. Each vertical column corresponds to one sample. Relative portion of segments of different colors in the column shows the probability of this sample belonging to the corresponding cluster. 1–8—population numbers (see Table 1).

The mean value of the fixation index F was -0.055 , which points to a slight excess of heterozygous genotypes. The mean values of F_{is} and F_{it} , which reflect the inbreeding of a individual with respect to the population and the inbreeding of a individual with respect to the whole species respectively, were -0.059 ($SE = 0.052$) and 0.144 ($SE = 0.053$). The genetic differentiation between all populations analyzed was 16% ($F_{st} = 0.161$, $p = 0.001$) if populations from Kamchatka were taken into account and 7% ($F_{st} = 0.071$, $p = 0.001$) if the latter were excluded. The AMOVA hierarchical analysis showed that the portion of variability between three geographical regions (Western Sayan, Eastern Sayan, and Kamchatka) was 17% of the variability revealed. The remaining variability (83%) was concentrated within the populations. The main contribution to the interpopulational differentiation belongs to the *RDE12* and *RA20* loci (25.0 and 23.6%, respectively).

The Nei's genetic distances (D) [23] between the populations varied from 0.054 to 2.349. According to the Mantel test [24], the genetic distance between the populations shows a statistically significant correlation with geographical distance between them: $r = 0.959$, $p = 0.013$ with populations from Kamchatka and $r = 0.684$, $p = 0.045$ without them. The population from Kamchatka was characterized by the largest distance as compared with the others (the D value varied from 1.246 to 2.349). A low level of difference was found between the populations from Western Sayan (D var-

ied from 0.054 to 0.269). However, it was a little bit higher between two populations from Eastern Sayan ($D = 0.577$). On the whole, the D value between the populations from Western and Eastern Sayan varied from 0.201 to 0.534.

On the basis of genetic distances, the analysis of population structure of the species has been carried out by the method of principal coordinates. On the ordination, the main part of the populations analyzed forms a slightly differentiated group, the populations from Kamchatka are significantly distant. The main part of variability (73.59%) is due to the first axis; the remaining 10.87% belongs to the other axis (figure not shown). Similar results were obtained using the Bayesian algorithm. The highest value of the logarithm of the a posteriori probability was obtained for three groups ($K = 3$). This means that the sample can be divided with maximal probability into three genetic clusters (Fig. 1). All populations from Kamchatka were assigned to the same cluster (Fig. 1). Populations from Western and Eastern Siberia were characterized by mixed genetic features. A tendency to differentiation was observed in the population from Western Sayan (6, Obruchev Range), however, due to the small number of individuals, it cannot be unambiguously interpreted.

Levels of variability and subdivision of the populations of *R. aureum* in Siberia and Kamchatka by the nuclear microsatellite markers are close to the values which were formerly obtained for other species of this

genus. Indeed, the H_e value for the wide-area European species *R. ferrugineum* was 0.29–0.44 in the Pyrenees and 0.37–0.68 in the samples from the Alps and the Apennine Peninsula. The F_{st} value between all samples was 0.095; the variability portion between the regional clusters was 24% [11]. A similar level of heterozygosity was also observed in several other *Rhododendron* species [8–10]. In our study, a high level of genetic diversity was demonstrated even for the small populations (Table 1). Several authors observed a similar situation for endemic and size-restricted populations of this species in China, Japan, and Sakhalin [15–17].

The observed differentiation between the populations from Siberia and the Far East may be explained by the historical division of the common range by large biogeographical barriers, such as the Verkhoyansky and Sikhote-Alinsky mountain ridges. A number of studies of cold-resistant species of the boreal, alpine, and subalpine zones revealed their significant genetic differentiation, associated with the history of the last glaciations [25–28].

The population and genetic study of *R. aureum* from the Siberian and Far Eastern parts of its range showed that microsatellite loci of nuclear DNA are promising markers for wide-scale biogeographical studies of this species.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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