====== **PLANT GENETICS** ======

Genetic Variation in Six Species of the Genus *Oxytropis* DC. (Fabaceae) from Kamchatka Peninsula

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Abstract—Using the isozyme analysis, genetic variations in six species of the genus *Oxytropis* DC. (Fabaceae) from Kamchatka was assessed. It was demonstrated that diploid species from the section *Arctobia* were characterized by a low level of variations typical of endemic plant species. At the same time, polyploid species from the *Orobia* section demonstrated very high values of the heterozygosity parameters (H_o varied from 0.200 to 0.274). It has been suggested that the level of polymorphism of the oxytropes from Kamchatka was shaped as a result of the interaction of a number of factors, among which the most important are the ecological confinedness of the species, the specific features of the reproductive system, and gene drift. In the species of *Orobia* section, it is also the presence of the polyploid genome.

INTRODUCTION

Kamchatka Peninsula is a unique region of the northwest Pacific, an area of high volcanic activity. In the volcanic deserts, formed after the eruptions there are the factors, limiting revegetation. These factors include chemical aggressiveness of the outbursts, disturbance of moisture conservancy in the near-surface soil layer, mobility of the loose material particles, and others. Despite this, on juvenile materials of ashy slag deposits, the formation of primary plant succession is initiated [1]. A substantial role in the substrate overgrowing is played by pioneer plants. The biological features of these plants ensure their successful population and survival on materials of volcanic eruptions. Among the pioneer plants of Kamchatka volcanoes, special attention is paid to the representatives of the genus *Oxytropis* DC. [2, 3]. Due to specific features of their root system, oxytropes are active fixers of loose volcanic substrates. Oxytropes settle in the places lacking soil cover and, as a result of their activity, favorable soil conditions for the settlement of other plants are formed [2, 4]. Symbiosis of oxytropes with nodule nitrogen-fixing bacteria also leads to the settlement of other plants. In terms of plant biodiversity conservation in regions with a complex ecological situation [2], analysis and conservation of the gene pools of the species formed under stressful conditions of volcanic ecosystems of Kamchatka is very important.

The flora of Kamchatka Peninsula contains 12 species of the genus *Oxytropis* [5], which belongs to two sections [6, 7]. *Arctobia* section (four species) is a metaarctic amphi-Beringian section with mostly diploid species, which represents the ancient cryophile (high-mountain) evolutionary lineage of the genus; the small chromosome number is indirect evidence of the antiquity of the section [6]. The *Orobia* section (eight species) is characterized by polyploidy and hybridogenesis (reticulate evolution). It is suggested that the increased variety of the chromosome number is associated with later evolution due to climatic changes and orogeny in the boreal zone. Moreover, high chromosome ploidy in many species of the section indirectly points to its secondary origin [6].

Kamchatka representatives of the genus *Oxytropis* are the herbaceous perennials, arctalpine and boreal-montane species confined to rocky habitats, dry gravelly mountain tundra, gravelly scree, dry shingles, ash and slag fields, coastal sands (Table 1). All of these species require light, and are adapted to the high-mountain conditions and to growing on loose substrates. They also can populate lava flows, as well as ash and slag fields [5–7]. At the same time, these species are characterized by low competitive ability and, with overgrowing of volcanic substrates, they are gradually replaced by other species [2, 4]. Oxytropes are obligate entomophilous outcrossing plants not capable of apogamic seed reproduction. Most of the species disperse their seeds like ballista plants; quite often, the fruits fall off in whole with seeds, and are dispersed by wind and water [6].

The objective of the present study was to examine the genetic diversity of six species of the genus *Oxytropis* from Kamchatka (*Arctobia* section: subsection Kamtschaticae Jurtz., *O. kamtschatica* Hult.; subsection Revolutae Jurtz., *O. exserta* Jurtz., *O. revoluta* Ledeb.; *Orobia* section, *O. erecta* Kom., *O. evenorum*

Table 1. Species of examined *Oxytropis* genus

Species, 2n	Distribution	Ecotopes	Sampling localities
O. exserta, 2n = 16	Anad.-Penzh., Kol., Okhot., Kamch., Nor. Kur.; **endemic**	On shingles, gravelly slopes and rocky outcrops	Southeastern Kamchatka, Mutnovsky volcano, outskirts of geothermal station under construction, along rocky slopes in sub-Alpine belt (**EXS**)
O. kamtschatica, 2n = 16	Chuk., (s.), Anad.-Penzh., Kor., Kamch.; **endemic, rare species**	In mountain tundra, nival patch meadows, old lava flows and slag fields of volcanic origin, on gravels	1. Southeastern Kamchatka, southern slope of Avacha volcano, Lava Pad', on volcanic sand and slag in sub-Alpine belt (**KAS**). 2. Klyuchevskoy volcano, the region of Podkova bald peak, slag field on a flat plot of eastern slope (**KKS**)
O. revoluta, 2n = 16	FER: Kor., Kamch., Nor. Kur.; Nor. Am. (Aleut Islands)	In high-mountain belt, in fell tundra and dwarf-shrub tundra, nival patch meadows, old lava flows and slag fields	1. Tolbachinsky volcano, region of Vysokaya Mountain, flat plots on slag field (**RTS**). 2. Klyuchevskoy volcano, the region of Podkova bald peak, tephra regions between blocks of lava (**RKS**). 3. Mutnovsky volcano, outskirts of geothermal station, in mountain tundra (**RVM**)
O. evenorum, 2n = 4x = 32	Anad.-Penzh., Kol., Okhot., Kamch., Upper-Zey., Amg., Ussur.; **endemic**	On rocky debris slopes, gravels, river terraces, in shrub tundra	Central Kamchatka, 11 km to the south of the settlement of Esso (Bystrinsky region), southern slopes of Gargachan pass, the belt of white birch forests, dry hummocky meadow, sometimes turning into shrub tundra
O. erecta, 2n = 6x = 48	Kamch.; **endemic**	On rocky slopes, lava flows, slag fields, marine terraces	Southeastern Kamchatka, outskirts of Petropavlovsk-Kamchatsky, right bank of Khalaktyrka River near the mouth, coastal conifer shrub
O. ochotensis, 2n = 8x = 64	Chuk., (s.), Anad.-Penzh., Kor., Kamch.; **endemic**	On gravelly slopes and tops of mountains in forb-shrub tundra	Klyuchevskoy volcano, the region of Podkova bald peak, fresh areas of tephra under the rocks and overhanging blocks of ancient lava, 1080 m above the sea level

The species names and ecological confinedness are given according to [7], chromosome numbers [15−17].

Jurtz. et Khokhr., *O. ochotensis* Bunge) based on the isozyme analysis. *O. exserta* and *O. kamtschatica* are endemic to the territory, which encompasses the northern part of the coast of the Sea of Okhotsk and the adjacent regions, including Koryakia, Kamchatka, and the Northern Kuril Islands [7]. *O. revoluta* is distributed over the same territories (the habitats of *O. kamtschatica* and *O. revoluta* characterize Okhotia as a special area [8]), but its range includes the coastal area of Alaska and Aleut Islands [6, 7]. *O. erecta* is endemic to Kamchatka Peninsula [7, 9]; *O. evenorum* is endemic to the region of Kolyma−northern coast of the Sea of Okhotsk [6, 7]; *O. ochotensis* is considered to be endemic to the Northeast of Russia [10] (Table 1). In our earlier studies, the data on the genetic diversity of the two species of the genus *Oxytropis*, *O. retusa* Matsum. and *O. chankaensis* Jurtz. were obtained [11−13], along with preliminary data on the level of allozyme polymorphism in a number of oxytrope species from Siberia and the Russian Far East [14]. The species examined were characterized by a wide range of allozyme variation, from very low (*O. retusa*, $P = 14.0\%$, $H_e = 0.069$ [11]) to very high (*O. chankaensis*, $P = 42.9\%$; $H_e = 0.301$ [12]). The data of the present study indicate a medium level of genetic diversity in the species of the *Arctobia* section, and to increased heterozygosity characteristic of the polyploid species of the *Orobia* section.

MATERIALS AND METHODS

The isozymes were examined using 3−4-week-old seedlings of the species examined. The seeds were collected at natural habitats on Kamchatka Peninsula (Table 1). The chromosome numbers were determined by N.S. Probatova using the samples from the populations studied [15, 16], except for *O. evenorum* (sample from Yakutia) [17]. The enzymes were extracted immediately before electrophoresis by means of the homogenization of the leaf tissues (about 100 mg) in liquid nitrogen with an addition of 200 μL of extracting 0.1 M phosphate buffer (pH 7.4) containing 10 mM ascorbic acid; 1 mM EDTA; 1% PVP-40; 1% Triton X-100; and 1% β-mercaptoethanol. Electrophoresis was performed in horizontal 13% starch gel with an addition of 10% sucrose. The three buffer systems used included Tris−citrate (pH 6.2), Tris−citrate (pH 7.8), and Tris−EDTA−borate (pH 8.6). Histochemical staining of the enzyme activity zones was performed using standard techniques [18]. A comparative analysis of the species from section *Arcobia* was carried out using 13 enzymes, presumably encoded by

20 loci, among which eight monomorphic (*Adh*, *Gdh-1*, *Gpi-1*, *Mdh-2*, *Me*, *Pgm-2*, *Pgm-3*, *Skdh-2*) and 12 polymorphic genes were identified (Table 2). The loci were enumerated in descending order of electrophoretic mobility of the enzymatic zones controlled. Alleles were designated in accordance with the electrophoretic mobility relative to the most abundant variant, the mobility of which was taken as 1.00. Due to the absence of some loci in different species, these loci were not included in the comparative analysis. However, the polymorphism indices in each species were determined taking into account all loci identified in this species (in different species the loci *Aat*, *Ald*, *Gdh-2*, α-*Gpd*, *Mdh-4* were active). The polymorphism indices (P_{95}), mean number of alleles per locus (*A*), and mean observed (H_o) and expected (H_e) heterozygosity were calculated according to the standard methods [18, 19]. Since the mobilities of some different alleles coincided (for instance, that for the slow allele of the *Mdh-1* locus and for the fast allele of the *Mdh-2* locus, and others) for two highly polyploid species, hexaploid *O. erecta* and octoploid *O. ochotensis*, the exact identification of heterozygous genotypes with consideration of the gene dose was complicated. For instance, unambiguous interpretation of variability at the *Mdh-2*, *Fe-3*, and other loci, and respectively, the determination of allele frequencies and H_e, was impossible in all samples. Because of this, for these species, only H_o was calculated. The statistical treatment of the data was performed as described earlier [12]. Genetic distances between the species of *Arctobia* section (D_N) were calculated according to Nei [20]. Clusters were constructed by the unweighted pair group method with the arithmetic mean (UPGMA); the data were treated using the DISPAN software program [21].

RESULTS AND DISCUSSION

Among the enzymatic systems examined, in six species of *Oxytropis* no differences were observed in electrophoretic profiles of the *Adh*, *Gdh-1*, *Gpi-1*, *Me*, *Pgm-2* and *Skdh* monomorphic loci. Loci *Mdh-2* and *Pqm-3*, which are monomorphic for the species of the *Arctobia* section (Table 2), appeared to be polymorphic for the species of the *Orobia* section. Furthermore, the *Mdh-2* locus with two alleles was highly polymorphic for all species of the section (Fig. 1). The *Pgm-3* locus was monomorphic in *O. ochotensis*, while in *O. erecta* and *O. evenorum*, the low frequency of the slow allele of this locus was observed. The *Mdh-1* locus was polymorphic for the species of the *Arctobia* section, while in the species of another section, it was invariant. The *Mdh-4* monomorphic locus was only identified in the species of the *Orobia* section (Fig. 1). Previously, this locus was detected in *O. chankaensis*, the tetraploid species of Baicalia section [12]. Taken together, an analysis of 13 enzymatic systems identified 40 allelic variants at 20 structural loci (Table 2). In

O. erecta, a total of 33 allelic variants were identified; in *O. exserta* and *O. kamtschatica*, by 31 loci in each; in *O. revoluta*, *O. evenorum*, and *O. ochotensis*, by 28 loci in each. Qualitative allele composition at homologous loci of the species examined was almost identical. The rare alleles identified in the species of the *Arctobia* section included *Idh-2*$^{1.20}$ in all species with a frequency of 0.023−0.057; *Idh-2*$^{0.80}$ in *O. exserta* (0.014), and *6-Pgd-2*$^{1.20}$ in populations of *O. revoluta* (Table 2) with a weight-average frequency of 0.082. A total of four species-specific alleles were identified, including *6-Pgd-2*$^{1.20}$ and *Aco*$^{0.85}$ (*O. revoluta*), *Mdh-1*$^{1.15}$ (*O. kamtschatica*), and *Mdh-1*$^{0.85}$ (*O. exserta*). Alleles identified in a single species of the *Arctobia* section (*Aco*$^{1.15}$, *Gpi-2*$^{0.92}$, and *Idh-2*$^{0.80}$, in *O. exserta*; *Fe-2*$^{1.05}$ and *Fe-2*$^{0.90}$, in *O. revoluta*; *6-Pgd-2*$^{0.75}$, in *O. kamtschatica* (Table 2)) were also detected in the *Orobia* section (Fig. 1). At the same time, allele *Fe-3*$^{0.80}$, identified in *O. exserta* and *O. kamtschatica*, was found only in *Arctobia* section. The differences between the populations of *O. kamtschatica* deserve special attention. In the population from Avacha volcano, at two loci, *Gpi-2* and *Lap*, the change in the allele was observed. In addition, only in this population of rare allelic variants of the *Fe3*, *Idh-2*, and *Mdh-1* loci were detected. At the same time, in the population from the Klyuchevskoy volcano, rare allelic variants were found at the *Mdh-3* and *6-Pgd-2* loci (Table 2). A comparative analysis of allelic diversity showed that the species differed in allele frequencies, and no fixation of alternative alleles was observed. However, despite the considerable similarity of the genetic structures of the populations examined, which was determined by a great number of common alleles, many of which are characterized by high frequencies in all populations, almost every population of the species examined was to a certain degree original in the allele number, composition, and frequency.

In the species examined, the values of the genetic polymorphism indices were greatly variable (Table 3). Specifically, 20−47% of the species genes were in polymorphic state (P_{95}); the number of alleles per locus (*A*) for different species constituted 1.40−1.61; observed heterozygosity (H_o) varied from 0.096 to 0.274. The similarity of the observed and expected heterozygosity values suggested that the populations of the species examined were in the state close to the equilibrium. The exception was *O. evenorum*, where the heterozygote deficiency was observed.

The species of *Arctobia* section were characterized by the medium level of genetic variation. The polymorphism index values observed in *O. exserta* (P = 42.9%, H_e = 0.156) were close to the values described for endemic outcrossing plants (P = 54.4%; H_e = 0.142) and herbaceous legumes (P = 53.0%; H_e = 0.160) [22]. At the same time, the values of these indices observed in *O. kamtschatica* (at the species level, P = 35.0%; H_e = 0.108) were lower. Minimum values of the variation parameters were found in the popula-

Table 2. Allele frequencies in populations of three *Oxytropis* species from *Arctobia* section

| Locus	Allele	Population					
		EXS	KAS	KKS	RTS	RKS	RVM
Aco	1.15	0.206	—	—	—	—	—
	1.00	0.794	1.000	1.000	1.000	1.000	0.667
	0.85	—	—	—	—	—	0.333
Acp-2	1.00	0.421	0.500	0.627	1.000	1.000	1.000
	0.30	0.578	0.500	0.373	—	—	—
Adh	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Fe-2	1.05	—	—	—	0.467	0.333	0.447
	1.00	1.000	1.000	1.000	0.533	0.500	0.474
	0.90	—	—	—	—	0.167	0.079
Fe-3	1.20	—	0.083	—	—	—	—
	1.00	0.937	0.750	1.000	1.000	1.000	1.000
	0.80	0.063	0.167	—	—	—	—
Gdh-1	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-1	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-2	1.00	0.328	0.222	0.968	1.000	1.000	1.000
	0.92	0.281	—	—	—	—	—
	0.84	0.391	0.778	0.032	—	—	—
Idh-2	1.20	0.057	0.053	—	0.023	—	—
	1.00	0.929	0.947	1.000	0.977	1.000	1.000
	0.80	0.014	—	—	—	—	—
Lap	1.05	—	0.100	0.080	0.155	0.250	0.100
	1.00	1.000	0.400	0.770	0.845	0.750	0.780
	0.95	—	0.500	0.150	—	—	0.120
Mdh-1	1.15	—	0.105	—	—	—	—
	1.00	0.214	0.895	1.000	1.000	1.000	1.000
	0.85	0.786	—	—	—	—	—
Mdh-2	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	1.40	0.529	—	0.033	—	—	—
	1.00	0.471	1.000	0.967	1.000	1.000	1.000
Me	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	1.05	0.343	0.088	0.355	0.171	0.067	0.240
	1.00	0.657	0.912	0.645	0.829	0.933	0.760
Pgm-2	1.00	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-3	1.00	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgd-1	1.00	0.850	1.000	1.000	1.000	1.000	1.000
	0.90	0.150	—	—	—	—	—
6-Pgd-2	1.20	—	—	—	0.107	0.053	0.020
	1.00	1.000	1.000	0.871	0.893	0.947	0.980
	0.75	—	—	0.129	—	—	—
Skdh-2	1.00	1.000	1.000	1.000	1.000	1.000	1.000

Population codes are given in Table 1.
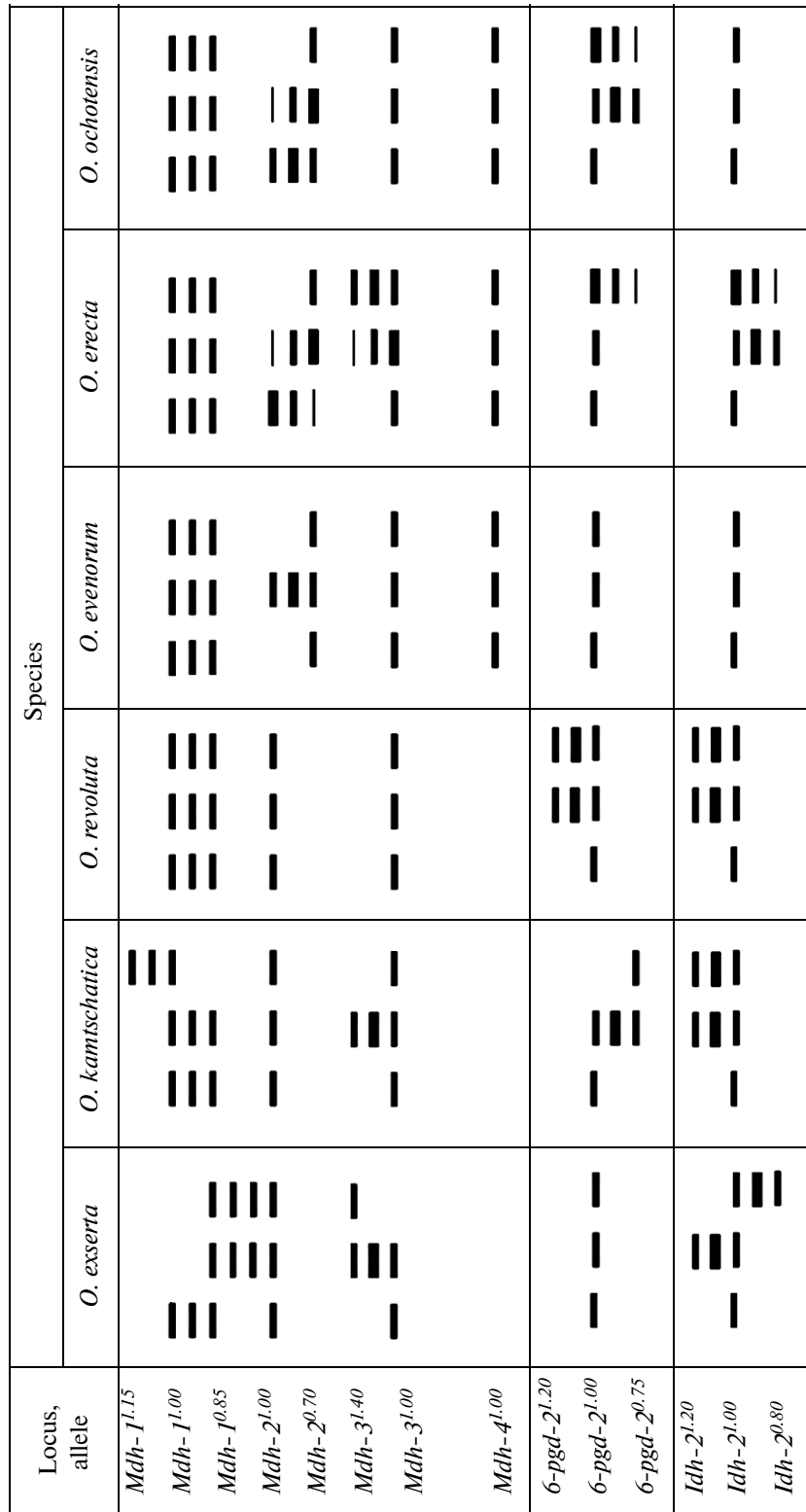
Fig. 1. Schematic electrophoregrams of three enzymatic systems in species of genus *Oxytropis* (alleles of *Mdh–1* locus have three–banded phenotype with bands of identical stain–ing intensity; in the other loci, three–banded phenotype corresponds to heterozygotes).

Table 3. Main genetic polymorphism parameters for six species of *Oxytropis* genus

Species, population	N_i	N_l	$P_{95}, \%$	A	A_p	H_o	H_e
Section Arctobia							
O. exserta	35	21	42.9	1.57	2.22	0.142	0.156
O. kamtschatica	81	20	35.0	1.55	2.22	0.098	0.108
Avacha volcano	19	20	35.0	1.45	2.29	0.122	0.114
Klyuchevskoy volcano	62	20	20.0	1.35	2.17	0.087	0.083
Population average		20	27.5	1.40	2.23	0.105	0.099
O. revoluta	133	20	25.0	1.40	2.33	0.096	0.089
Tolbachinsky volcano	88	20	20.0	1.25	2.00	0.069	0.067
Klyuchevskoy volcano	20	20	20.0	1.25	2.25	0.074	0.064
Mutnovsky volcano	25	20	20.0	1.35	2.40	0.104	0.089
Population average		20	20.0	1.28	2.22	0.082	0.073
Section Orobia							
O. evenorum	43	20	40.0	1.40	2.00	0.200	0.280
O. erecta	151	22	47.8	1.61	2.08	0.274	—
O. ochotensis	77	18	38.9	1.44	2.29	0.204	—

In the lane with the species names, the polymorphism parameters of the species level are demonstrated; N_i, N_l, the number of accessions and loci examined; $P95$, $\%$, polymorphism level with consideration of the $P95\%$ criterion; A, the number of alleles per locus; A_p, the number of alleles per polymorphic locus; H_o, observed heterozygosity; H_e, expected heterozygosity.

tions of *O. revoluta*. These values were almost two times lower than in endemic *O. exserta*. It seems likely that the low level of polymorphism observed in the species of this section was formed due to the interaction of a number of factors. The ability of the Kamchatka oxytrope to only exist under certain climatic and edaphic conditions leads to the fact that populations of these species are isolated, located far away from each other, and are often fragmented. The displacement of oxytrope by more competitive species due to volcano overgrowth [2, 4] causes fluctuations in the population number, accompanied by the loss of part of the gene pool. It seems likely that, for an oxytrope with low-level polymorphism, peculiarities of their breeding system (sexual type of reproduction and outcrossing) represent the most important source for the maintenance and renewal of the variability reserve. However, these peculiarities cannot explain the differences between the species (minimum polymorphism level in more abundant species compared to endemic species). In this case, forms of plant life may be important. *O. exserta* and *O. kamtschatica* are loose-tussock forms. In cenoses, individual plants of these species are located some distance away from one another. At the same time, due to intensive shoot branching, low clumps of *O. revoluta* form large mats. This considerably increases the probability of inbreeding, which leads to the loss of allelic diversity. The events of species evolutionary history can also influence the level of polymorphism [23]. It has been suggested that that the species of the Revolutae subsection

originated from the initial type of the entire section, subsection Kamtschaticae (the plants with the shape of *O. kamtschatica* and partly of *O. exserta*); the separation of *O. revoluta* from the ancestral form *O. exserta* occurred during the formation of Southern Koryak–Kamchatka (Late Cenozoic) mountain area, hich had experienced intensive glaciation in Pleistocene. It seems likely that in this case the species conservation was possible along the eastern (Oceanside) margin of foot glaciers [6]. It can be suggested that the low polymorphism level observed could be the consequence of the gene drift (founder effect or bottleneck). The low polymorphism level described for the species of *Arctobia* section is is determined by their rather narrow ecological confinedness, the weak competitive ability of pioneer plants, population fragmentation, and the influence of gene drift.

All representatives of the examined *Orobia* section were polyploids, which are generally characterized by increased polymorphism indices [12, 24, 25]. Maximum values of variation parameters observed in hexaploid *O. erecta* endemic Kamchatka species ($P = 47.8\%$; $H_o = 0.274$), were close to those reported for tetraploid *O. chankaensis* ($P = 42.9\%$; $H_e = 0.301$) [12]. The plants from the species of *Orobia* section tested belonged to one life form (were dense tussocks) and were similar in breeding type. It seems likely that the differences in the levels of polymorphism between the species of this section can be associated with the location of the sampling sites. It is assumed that central populations are characterized by a greater reserve

of genetic diversity compared to marginal populations [26]. The sampling sites of the representative of arctic flora *O. ochotensis* (Klyuchevskoy volcano) and the Kolyma—northern coast of the Sea of Okhotsk species *O. evenorum* (outskirts of the settlement of Esso) are located at the southeastern margins of their ranges. The depletion of marginal populations, along with the intensification of the selection processes in the suboptimal conditions at the range margins [23] can have a certain influence on shaping the level of polymorphism in the populations of the species examined. The Kamchatka endemic species *O. erecta* is compactly located in central and southeastern parts of the peninsula. The examined population of this species (outskirts of Petropavlovsk-Kamchatsky) is located rather close to the center of the species distribution range and, as a result, is probably characterized by a higher level of variation. The characteristic feature of many polyploid species is increased heterozygosity [12, 24, 25]. This is also typical of species of the *Orobia* section, the level of heterozygosity of which is two times higher than in species of the *Arctobia* section (Table 3). A combination of two or more variants of the same enzyme can supplement the heterozygous plants with higher flexibility in unfavorable conditions. For instance, there are data on the better adaptation of heterozygotes to the conditions of ecological stress [23, 24]. It can be suggested that, for the species examined living under severe climate conditions, in places with a destroyed soil cover, under the conditions of sharp temperature fluctuations and the drying influence of strong winds, the high level of heterozygosity has an adaptive value.

Based on the allele-frequency data, genetic distances were determined for species of the *Arctobia* section (Table 4). The D_N values ranged from 0.0003 between the populations of *O. revoluta* to 0.117 between the population of *O. revoluta* from Mutnovsky volcano and *O. exserta*. The mean value for the sam-

Table 4. Genetic distances (D_N) between the populations of three *Oxytropis* species from section *Arctobia*

Populations	EXS	KAS	KKS	RTS	RKS
KAS	0.082				
KKS	0.077	0.046			
RTS	0.111	0.076	0.022		
RKS	0.114	0.071	0.023	0.0003	
RVM	0.117	0.080	0.027	0.006	0.007

ples examined constituted $D_N = 0.057 \pm 0.041$. The differentiation analysis of species from this examined section revealed no distinct subdivision into clusters, which corresponds to traditional subsections. The dendrogram shows that populations of *O. revoluta* and *O. kamtschatica* group in one cluster, while the population of *O. exserta* occupied a separate position (Fig. 2). The similarity between two populations of *O. revoluta* (RKS and RTS) can be explained by the fact that they are located close to each other and probably had or still have a common gene pool. The possibility of past gene exchange between sympatric populations of *O. revoluta* and *O. kamtschatica* (RKS and KKS) can be also hypothesized.

Species clustering did not provide unambiguous confirmation of the existing opinion that *O. revoluta* is associated in origin with the initial type of the *Arctobia* section (plants of *O. kamtschatica* type) through *O. exserta* [6]. According to the data of allozyme analysis, populations of the representative of section Kamtschaticae occupy an intermediate position between two species of subsection Revolutae, which suggests the existence of relationships between *O. kamtschatica* and both species. It is noteworthy that, according to the data of the phytogeographic analysis of the *Arctobia* section, realized using GIS technologies [8], the dendrogram of the species distri-
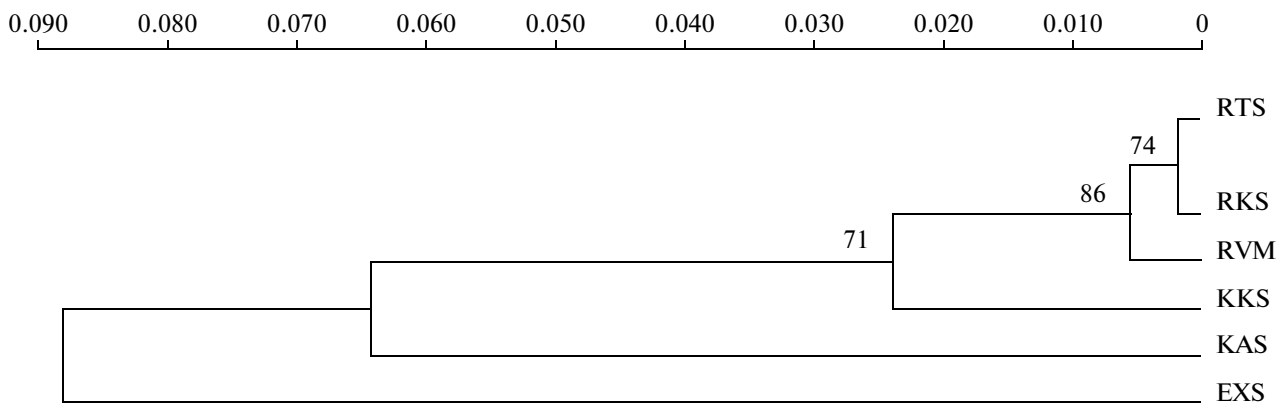

Fig. 2. Dendrogram showing similarity between populations of species of *Oxytropis* genus, *Arctobia* section constructed based on D_N genetic distances (Table 4). The bootstrap support values are demonstrated at corresponding branching nodes.

bution reflects the closeness of *O. revoluta* and *O. kamtschatica* and the isolated position of *O. exserta*. According to the author's opinion, the discrepancy between the taxonomic structure and chorological dendrogram is due to the complex history of the group formation, where geographic distribution is closely associated with ecological confinedness. Taking this into consideration, it cannot be excluded that species clustering based on the isozyme analysis can reflect the convergent similarity of the taxa due to the effect of selection under similar environmental conditions [23]. The level of genetic differences between the species of the *Arctobia* section revealed in the present study was higher than that between the populations or races within the species, and corresponded to the status of closely related species that belong to one subsection in accordance with the scale of genetic distances developed for plants by Shurkhal et al. [27] (between the species from one subsection the D_N values vary from 0.032 to 0.652). The estimate of the divergence time for the *Oxytropis* species examined based on the genetic distance values ($T = 5 \times 10^6 \times D_N$) constitutes about 280 000 years; i.e., it belongs to Middle Pleistocene.

Many attempts were made to resolve the rather complex phylogenetic relationships within the genus *Oxytropis* and for individual species. However, the investigation results often conflicted with traditional taxonomy [28–32]. In the case when these analyses were performed using nuclear ribosomal DNA operon ITS sequences as a marker, it was not always possible to distinguish many closely related species and, in some cases, even species from different sections [13, 31, 32]. The chloroplast genome markers, which have a rather high-resolution capability, were found to be more informative for these purposes [13, 32]. The data on the genetic relationships of the oxytrope of the *Arctobia* section obtained in the present study contribute to the accumulation of information on the phylogeny of the genus *Oxytropis*.

In conclusion, the data on the genetic diversity of six Kamchatka species of the genus *Oxytropis* indicate that diploid species of *Arctobia* section are characterized by the low level of variation, typical of endemic plant species. At the same time, polyploid species of the *Orobia* section demonstrated rather high heterozygosity indices. The level of polymorphism revealed in the populations examined along with their genetic uniqueness and the risk of extinction point to the necessity of preserving the gene pools of all populations of the genus *Oxytropis*, while solving the issues of the plant biodiversity conservation in Kamchatka.

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