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PLANT GENETICS

Divergence of *Oxytropis* Species from the Section *Verticillares* (Fabaceae) of Steppe Flora of Baikal Siberia Based on Analysis of Chloroplast DNA

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Abstract—The analysis of nucleotide sequence polymorphism of chloroplast DNA *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers in the *Oxytropis lanata*, *O. myriophylla*, *O. oxyphylla*, *O. selengensis*, and *O. stukovii* (section *Verticillares*) of the steppe flora of Baikal Siberia demonstrated that the populations are characterized by a high haplotype (0.634–1.000) and relatively low (0.0003–0.0045) nucleotide diversity. Statistically significant values of genetic distances between the populations of the same species change from 0.19382 to 0.43449. Marker nucleotide substitutions and indels were detected in *O. lanata*, *O. gracillima*, *O. interposita*, *O. pumila*, and *O. mongolica*, indicating a significant divergence of their chloroplast genomes. A weak differentiation ($\Phi_{ST} = 0.137$, $P < 0.0001$) between *O. oxyphylla, O. selengensis, O. stukovii* and *O. bargusinensis* and also low values of genetic distances (0.08939–0.32339) (corresponding to interpopulation distances), absence of differentiating molecular markers, formation of a single haplogroup in the median network, and presence of common haplotypes indicate that these species are a genetically homogeneous group probably formed as a result of relatively recent and rapid divergence from the common ancestor, as well as a high degree of interspecies hybridization.

Keywords: Oxytropis, Fabaceae, genetic diversity, species divergence, haplotypes, chloroplast DNA, Baikal Siberia

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INTRODUCTION

The steppe flora occupies a special position in the vegetation of Baikal Siberia [1–4]. The steppes make up only 7–9% of the region area [4], and the steppe complex is the most floristically rich and includes 666 species and subspecies of vascular plants, while the mountain and forest complexes include 550 and 638, respectively [3]. The steppe regions are mainly found in the forest-steppe zone and become zonal only in the south of Baikal Siberia [1]. The steppe ecosystems are the center of the newest endemism and relict of Inner Asia [4, 5]. The largest number of taxa as a part of the steppe flora of Baikal Siberia was registered for the genus *Oxytropis* DC. (30 species and one subspecies); out of them, nine species (*O. bargusinensis* Peschk., *O. lanata* (Pall.) DC., *O. lasiopoda* Bunge, *O. myriophylla* (Pall.) DC., *O. oxyphylla* (Pall.) DC., *O. prostrata* (Pall.) DC., *O. selengensis* Bunge, *O. stukovii* Palib., *O. turczaninovii* Jurtzev) belong to the section *Verticillares* DC. (=*Baicalia* Bunge) of the subgenus *Oxytropis* of the genus *Oxytropis* DC. [3]. The species of the section *Verticillares* (*O. oxyphylla* is a typical species) are characterized by a verticillate location of leaflets and represent a rather sharply isolated group formed on the basis of a promising macromutant according to Polozhii [6]. It is believed that the section *Verticillares* is derivative from other *Oxytropis* species [7, 8]; in particular, it represents one of the branches in the section *Orobia* Bunge evolution [7]. Mountain-steppe species of the section, occurring mostly in Transbaikalia, are characterized by a number of traits (for example, unilocular pods and significant ecological amplitude) indicating a lack of high specialization [9]. On the basis of this, the author suggested that the steppes of Transbaikalia and adjacent regions of Northwest Mongolia are the center of origin of the species of the section *Verticillares*, separation of which occurred in the Early Pleistocene in more southern regions, and favorable conditions in subsequent cryoxerotic stages contributed to further development of species of this section and their movement to the north.

Some species of the section *Verticillares* are close to the members of the section *Orobia*, less often to species of the sections *Xerobia* Bunge and *Polyadena* Bunge. So, according to G.A. Peshkova [10], *O. bargusinensis* originated from mixing between *O. turczaninovii* (*Verticillares*) and *O. sylvatica* (Pall.) DC. (*Orobia*); *O. tompudae* M. Pop. (*Verticillares*) is also close to the latter species, while *O. oxyphylla* hybridizes with *O. leptophylla* (Pall.) DC. (*Xerobia*) and with *O. ircutensis* M. Pop. (=*O. baicalia* (Pall.) Pers.) section *Orobia*. V.N. Siplivinskii [11] believed that *O. interposita* Sipl. is intermediate between the *Verticillares* and *Polyadena*. L.I. Malyshev [12] noted that the crossing of species related to the same section is observed more often, as a result of which races (subspecies), varieties, and ecological forms could arise. According to the authors studying the flora of Siberia [10, 13, 14], within the section *Verticillares*, *O. oxyphylla* easily hybridizes with *O. lanata*; *O. prostrata* is probably a hybrid between them that lost pubescence on the pods; *O. stukovii* is an intermediate species between *O. prostrata* and *O. mongolica* Kom.; *O. selengensis* can be a form of *O. oxyphylla* with small flowers.

Molecular markers of nuclear and chloroplast genomes are widely used to study genetic variability, population structure, species identification, phylogenetic relationships, and divergence of the *Oxytropis* species and other members of the Fabaceae family [15–26]. Thus, based on the analysis of chloroplast DNA (cpDNA) *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers, we studied the genetic diversity and population divergence of the endemic species *O. chankaensis* Jurtz. [15], *O. glandulosa* Turcz. [20], and *O. bargusinensis*, *O. interposita*, and *O. triphylla* (Pall.) Pers. [21]. Previously, no similar studies were conducted for *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. selengensis*, *O. stukovii*, *O. pumila* Fisch. ex DC., and *O. gracillima* Bunge from section *Verticillares*.

The aim of the present work was to study the genetic diversity of natural populations and the degree of divergence of the chloroplast genome in the *Oxytropis* species from the section *Verticillares* of the steppe flora of Baikal Siberia according to polymorphism of *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA.

MATERIALS AND METHODS

One hundred thirty-five plants from natural populations of *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. selengensis*, *O. stukovii*, *O. pumila*, and *O. gracillima* section *Verticillares* of the subgenus *Oxytropis* of the steppe flora of Baikal Siberia, Mongolia, and Altai were material for the study (Table 1). To estimate the divergence of species of this section, we also included in phylogenetic analyses previously obtained nucleotide sequences of *O. mongolica* [19], *O. bargusinensis*, *O. interposita*, and *O. tompudae*, as well as *O. glabra* (Lam.) DC. [21] section *Mesogaea* Bunge of the subgenus *Phacoxytropis* Bunge as an outgroup (Table 1). The names of species, sections and subgenera are accepted according to L.I. Malyshev [27].

The methods of DNA isolation, amplification, and direct sequencing of intergenic spacers *psbA–trnH, trnL–trnF*, and *trnS–trnG* are given in our previous works [19, 21, 28]. Nucleotide sequences of forward and reverse chains were determined on an ABI 3130 genetic analyzer (Applied Biosystems, United States) and assembled using the Staden Package software package [29]. For each sample, sequences of three regions were aligned manually using the SeaView v. 4 program [30]. Haplotype (h) and nucleotide (π) diversity, pairwise genetic distances (F_{ST}), level of differentiation and distribution of genetic variability within and between the populations (Φ_{ST} fixation index, analysis of the molecular variation, AMOVA), and population stability tests (Tajima's D and Fu's Fs) were calculated by means of the Arlequin v. 3.5 program [31]. The analysis of the distribution of pairwise nucleotide differences (mismatch distribution) were conducted in the DnaSP v. 5.0 program [32]. Genealogical relationships of haplotypes were analyzed by a median-joining (MJ) method in the NetWork v. 5.0 program [33], encoding each deletion or insertion (regardless of their size) as a single mutation event.

The work was carried out using equipment of the Joint-Use Center "Biotechnology and Genetic Engineering" at the Federal Scientific Center of the East Asia Terrestrial Biodiversity (Far Eastern Branch, Russian Academy of Sciences).

RESULTS

Sequence polymorphism analysis of intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of cpDNA demonstrated that the length of each of the regions in the studied samples is different owing to the presence of insertions/deletions (indels) and mono- and dinucleotide repeats. The length of combined sequences of three regions in 135 representatives of the *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. selengensis*, *O. stukovii*, *O. pumila*, and *O. gracillima* was 2475 sites after the alignment; out of them, 2349 were monomorphic and 22 variable. Ten nucleotide substitutions were parsimony informative. The analysis of the obtained matrix detected 63 haplotypes (V1–V63); out of them, three haplotypes were common: V27 in the *O. oxyphylla*, *O. selengensis*, and *O. stukovii*; V31 in the *O. oxyphylla* and *O. stukovii*; V38 in the *O. oxyphylla* and *O. selengensis* (Table 1).

All populations were characterized by relatively low nucleotide and high haplotype diversity, except the SEL population of *O. selengensis* (Table 2). According to the AMOVA results, more than 75% of all genetic variability is due to the intrapopulation component in *O. lanata* ($\Phi_{ST} = 0.245$, $P < 0.0001$) and *O. oxyphylla* ($\Phi_{ST} = 0.229$, $P < 0.003$). The extremely low and sta-

Table1. List of *Oxytropis* species, population codes, location information, haplotypes, and GenBank accession numbers for nucleotide sequences of cpDNA intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG*

Species	Population code	Sample origin (number of samples)	Haplotype	GenBank accession numbers		
				psbA–trnH	*trnL–trnF*	*trnS–trnG*
O. lanata (Pall.) DC.	LAN1	Republic of Buryatia, neighborhood of Unegetei village (20)	V1–V18	**LT994841–LT994858**	**LT994895–LT994912**	**LT994949–LT994966**
	LAN2	Republic of Buryatia, Barguzinskii district, neighborhood of Urzhil village (15)				
	LAN3	Mongolia, Central aimag, neighborhood of Argalant sum (5)				
O. myriophylla (Pall.) DC.	MYR1	Transbaikal krai, Mogoituiskii district, neighborhood of Tsugol village (5)	V19–V26	**LT994859–LT994866**	**LT994913–LT994920**	**LT996002–LT996009**
	MYR2	Republic of Buryatia, Khorinskii district, neighborhood of Udinsk village (21)				
	MYR3	Republic of Buryatia, Eravninskii district, neighborhood of Komsomol'skoe village (8)				
O. oxyphylla (Pall.) DC.	OXY1	Transbaikal krai, Aginskii district, environs of Lake Nozhii (5)	V27–V51	**LT994867–LT994891**	**LT994921–LT994945**	**LT996010–LT996034**
	OXY2	Republic of Buryatia, Selenginskii district, neighborhood of Novoselenginsk village (16)				
	OXY3	Republic of Buryatia, Eravninskii district, neighborhood of Komsomol'skoe village (8)				
	OXY4	Mongolia, Bulgan aimag, neighborhood of Rashaant sum (4)				
O. selengensis Bunge	SEL	Republic of Buryatia, Zaigraevskii district, neighborhood of Unegetei village (18)	V27, V38, V52–V56	**MH174937, LT996052–LT996057**	**LT996045–LT996051**	**LT996038–LT996044**
O. stukovii Palib.	STUK	Transbaikal krai, Aginskii district, environs of Lake Nozhii (4)	V27, V31, V57	**LT994892–LT994894**	**LT994946–LT994948**	**LT996035–LT996037**
O. gracillima Bunge	GRAC	Mongolia, Central aimag, neighborhood of Argalant sum (5)	V58–V62	**MH174938, LT996058–LT996061**	**LT996062–LT996066**	**LT996067–LT996071**
O. pumila Fisch. ex DC.	PUM	Altai Republic, Kosh-Agachskii district, Chuiskaya steppe (1)	V63	**MH174939**	**LT996251**	**LT996252**
O. mongolica Kom.	MONG	Tyva Republic, Tandinskii district, western shore of Lake Khadan (1)	H63*	LN898501	LN898613	LN898625
O. bargusinensis Peschk.	BARG	Republic of Buryatia, Kurumkanskii district, valley of Alla River (15) and Barguzinskii district, neighborhood of Urzhil village (15)	H11–H22**	LT856473–LT856478; LT856483–LT856488	LT856506–LT856511; LT856516–LT856521	LT856539–LT856544; LT856549–LT856554
O. interposita Sipl.	INTER	Republic of Buryatia, Kurumkanskii district, valley of Alla River (14)	H23–H27**	LT856489–LT856493	LT856522–LT856526	LT856555–LT856559
O. tompudae M. Pop.	TOMP	Republic of Buryatia, Kurumkanskii district, neighborhood of Maisk village (8)	T1–T6**	LT856566–LT856571	LT856579–LT856584	LT856592–LT856597
O. glabra (Lam.) DC.	GLAB	Republic of Buryatia, Ivolginskii district, neighborhood of Orongoi village (1)	G1**	LT856572	LT856585	LT856598

Accession numbers of sequences obtained in this study are highlighted in bold.
* Haplotype from [19]. ** Haplotype from [21].

Table 2. Genetic diversity and population stability in *Oxytropis* species according to cpDNA data

Species and population code	Diversity (SD)		Population stability test	
	haplotype	nucleotide	Tajima's *D*	Fu's *F*s
O. lanata				
LAN1	0.895 (0.051)	0.0010 (0.0006)	−0.84303 ns	−4.90331*
LAN2	0.895 (0.052)	0.0010 (0.0006)	−1.00161 ns	−3.44483*
LAN3	0.900 (0.161)	0.0017 (0.0012)	−0.81650 ns	−1.55426 ns
In total	0.923 (0.023)	0.0012 (0.0007)	−1.16189 ns	−12.82981*
O. myriophylla				
MYR1	0.700 (0.218)	0.0003 (0.0003)	0.00000	−0.82920 ns
MYR2	0.767 (0.057)	0.0007 (0.0005)	0.00000	−0.52438 ns
MYR3	0.821 (0.101)	0.0008 (0.0005)	0.00000	−0.03355 ns
In total	0.786 (0.042)	0.0007 (0.0005)	0.00000	−1.6968 ns
O. oxyphylla				
OXY1	0.900 (0.161)	0.0007 (0.0005)	−0.97256 ns	−1.40478 ns
OXY2	0.958 (0.036)	0.0023 (0.0013)	−0.46739 ns	−3.75465*
OXY3	0.964 (0.077)	0.0045 (0.0026)	0.50437 ns	0.11267 ns
OXY4	1.000 (0.177)	0.0042 (0.0029)	1.45884 ns	−0.18978 ns
In total	0.981 (0.012)	0.0034 (0.0018)	−1.20403 ns	−11.32100**
O. selengensis, SEL	0.634 (0.127)	0.0009 (0.0006)	−1.51077*	−1.09311 ns
O. stukovii, STUK	0.833 (0.222)	0.0005 (0.0005)	0.00000	−0.65789 ns
O. gracillima, GRAC	1.000 (0.126)	0.0042 (0.0027)	−0.81650 ns	−0.31199 ns

SD, standard deviation; * $P < 0.05$; ** $P < 0.001$; ns, not significant. See population code in Table 1.

tistically insignificant fixation index value (Φ_{ST} = 0.008, $P > 0.37$) in *O. myriophylla* indicates the absence of differentiation between the populations. The analysis of genetic distances (F_{ST}) between the populations of the same species demonstrated (Table 3) that F_{ST} values between all *O. myriophylla* populations and between the pairs of *O. oxyphylla* OXY1–OXY2 and OXY3–OXY4 populations are very small and statistically insignificant, while the lowest (0.19382) and the largest (0.43449) statistically significant ($P < 0.0001$) F_{ST} values were determined between the *O. lanata* populations.

To reconstruct demographic histories of the populations, the analysis of the frequency distribution of pairwise nucleotide differences between the haplotypes was carried out for all populations, except for the *O. myriophylla* and *O. stukovii* populations (since there are no nucleotide substitutions in their sequences). The histograms demonstrate (Fig. 1) that significant differences between the expected and observed distribution of pairwise nucleotide differences and bimodal character of the observed distribution were detected only in the OXY3 and OXY4 populations. In all other populations, as well as in the combined samples of each of *O. lanata* and *O. oxyphylla*, two curves coincide or demonstrate relatively good coincidence and have a unimodal distribution pattern (Fig. 1). Tajima's *D* and Fu's *F*s values (Table 2) in most populations (except for OXY3) are negative, but statistically significant Tajima's *D* values were registered only for the SEL population of *O. selengensis*, while Fu's *F*s, for the LAN1 and LAN2 populations of *O. lanata* and OXY2 of *O. oxyphylla* and for combined samples of each of the species.

To estimate the divergence of the *Oxytropis* species section *Verticillares*, previously obtained sequences of the samples of *O. mongolica* [19], *O. bargusinensis*, *O. tompudae*, and *O. interposita* [21] were included in the analysis (Table 1). The length of combined sequences of 188 samples of 11 species was 2484 sites; out of them, 2336 were monomorphic and 33 variable. Fifteen nucleotide substitutions were parsimony informative. Interspecific genetic distances (F_{ST}) are given in Table 4. High and statistically significant F_{ST} values were determined between the pairs of the *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. gracillima*, *O. interposita* with *O. tompudae*; high and insignificant F_{ST} values were determined between all pairs generated by each of the *O. pumila* and *O. mongolica* with all others (Table 4). Genetic distances between the pairs *O. oxyphylla–O. stukovii*, *O. selengensis–O. stukovii*, and *O. oxyphylla–O. bargusinensis* are very small and

Table 3. Genetic distances (F_{ST}) between populations of *Oxytropis lanata*, *O. myriophylla*, and *O. oxyphylla* according to cpDNA data

Population code	LAN1	LAN2	LAN3	MYR1	MYR2	MYR3	OXY1	OXY2	OXY3
LAN2	0.19382*	–							
LAN3	0.19868**	0.43449*	–						
MYR1	0.72032*	0.70231*	0.73799*	–					
MYR2	0.72416*	0.69208*	0.76576*	–0.01255 ns	–				
MYR3	0.70647*	0.67393*	0.72246*	0.04762 ns	0.01166 ns	–			
OXY1	0.91232*	0.91079*	0.89928*	0.94643*	0.92185*	0.92278*	–		
OXY2	0.85033*	0.83463*	0.81535*	0.81025*	0.84937*	0.81492*	0.07287 ns	–	
OXY3	0.79317*	0.76259*	0.69164*	0.67702*	0.78910*	0.70269*	0.30425**	0.28229*	–
OXY4	0.84281*	0.82252*	0.74437*	0.73904*	0.82958*	0.75674*	0.32269**	0.28509**	–0.01926 ns

* $P < 0.0001$; ** $0.009 < P < 0.05$; ns, not significant. Significance level determined based on 1023 permutations. See population code in Table 1.

Table 4. Genetic distances (F_{ST}) between *Oxytropis lanata* (1), *O. myriophylla* (2), *O. oxyphylla* (3), *O. selengensis* (4), *O. stukovii* (5), *O. gracillima* (6), *O. bargusinensis* (7), *O. interposita* (8), *O. tompudae* (9), *O. pumila* (10), and *O. mongolica* (11) according to cpDNA data

	1	2	3	4	5	6	7	8	9	10
2	0.67563*	–								
3	0.78156*	0.77768*	–							
4	0.88894*	0.91770*	0.10515*	–						
5	0.88734*	0.92734*	0.00520 ns	–0.01037 ns	–					
6	0.81469*	0.84348*	0.76361*	0.89158*	0.82679*	–				
7	0.86259*	0.88186*	0.08939**	0.32339*	0.31773**	0.87579*	–			
8	0.74145*	0.72343*	0.74864*	0.89490*	0.88309*	0.71406*	0.86487*	–		
9	0.70156*	0.72838*	0.70864*	0.87429*	0.83348*	0.67759*	0.84445*	0.67524*	–	
10	0.91186 ns	0.94797 ns	0.59389 ns	0.88512 ns	0.93778 ns	0.78205 ns	0.79854 ns	0.90017 ns	0.83033 ns	–
11	0.87122 ns	0.90659 ns	0.78899 ns	0.94483 ns	0.97028 ns	0.68323 ns	0.91268 ns	0.85661 ns	0.76984 ns	1.00000 ns

* $P < 0.0001$; ** $0.009 < P < 0.02$; ns, not significant. Significance level determined based on 1023 permutations.

statistically insignificant (except for the latter), while F_{ST} values between the *O. oxyphylla*–*O. selengensis*, *O. selengensis*–*O. bargusinensis*, and *O. stukovii*–*O. bargusinensis* are at the level of interpopulation values (Tables 3 and 4). The analysis of molecular dispersion detected a weak differentiation between the species *O. oxyphylla*, *O. selengensis*, *O. stukovii*, and *O. bargusinensis* ($\Phi_{ST} = 0.137$, $P < 0.0001$); the main portion of all genetic variability (more than 86%) is due to intraspecific variability and only about 13% is due to interspecific.

To determine genealogical relationships between the haplotypes, haplotypes of *O. mongolica* (H63), *O. bargusinensis* (H11–H22), *O. interposita* (H23–H27), *O. tompudae* (T1–T6), and *O. glabra* (G1) as an outgroup were added to the matrix of identified haplotypes of seven studied *Oxytropis* species (Table 1). The total length of aligned sequences of 92 haplotypes of three cpDNA regions was 2580 sites; out of them, 2156 were monomorphic and 44 variable. Ten nucleotide substitutions were parsimony informative. Four common haplotypes were detected: V27 haplotype, which was present in *O. oxyphylla*, *O. selengensis*, and *O. stukovii*, was identical to the H16 haplotype of *O. bargusinensis*; V31 haplotype identified in *O. oxyphylla* and *O. stukovii* was identical to H22 haplotype of *O. bargusinensis*; V38 haplotype common in *O. oxyphylla* and *O. selengensis* was identical to H14 haplotype of *O. bargusinensis*; V42 haplotype of *O. oxyphylla* was identical to H11 haplotype of *O. bargusinensis* (Table 1). The median network in which all haplotypes of *O. lanata*, *O. myriophylla*, *O. gracillima*, and

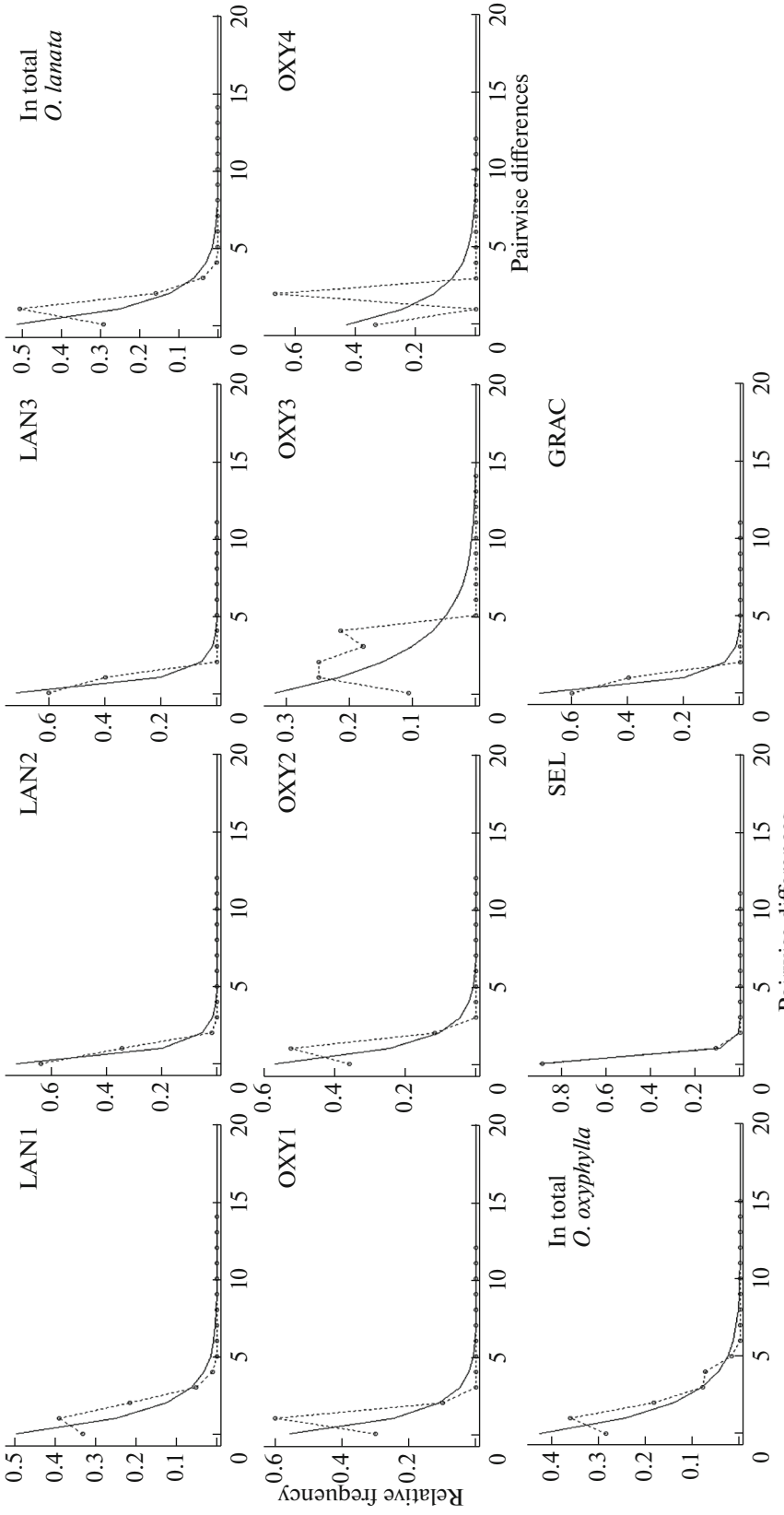

Fig. 1. Distribution of frequencies of pairwise differences between nucleotide sequences of cpDNA intergenic spacers in populations of *Oxytropis* species. Dashed line demonstrates the observed distribution; solid line, the expected distribution with a model of constant population size. See population code in Table 1.

O. interposita and five out of six haplotypes of *O. tompudae* form haplogroups according to the species affiliation is presented in Fig. 2. All *O. lanata* haplotypes have two marker nucleotide substitutions (A in position 1196 and C in position 2291); the *O. interposita* haplotypes have three (T in positions 1287 and 1723, G in position 1513); the *O. gracillima* haplotypes have one (T in position 403) and insertion of five nucleotides (AGTAA in position 1759) absent in all others. V63 haplotype of *O. pumila* is associated with H21 haplotype of *O. bargusinensis* through three mutational steps, two of which (insertion of seven nucleotides (GAATTAT, positions 1435–1441) and deletion of five nucleotides (ACCTT, positions 1796–1800)) distinguish *O. pumila* from all others. H63 haplotype of *O. mongolica* is associated with V26 haplotype of *O. myriophylla* through ten mutational steps; out of them, six nucleotide substitutions (T in positions 158 and 1382, A in positions 1457 and 1765, G in position 1704, and C in position 1715), insertion of six nucleotides (TATATC, positions 1383–1388), and deletion of two nucleotides (AT, positions 1146–1147) distinguish *O. mongolica* from all others. One large haplogroup is formed by all haplotypes of *O. oxyphylla*, *O. selengensis*, *O. stukovii*, and *O. bargusinensis* and by T6 haplotype of *O. tompudae*. It contains well-defined "star" structures with one of the common haplotypes V27, V31, V38, and V42 in the center, which are connected to each other through a single mutational step (Fig. 2), indicating a recent origin of the populations on the studied part of the area.

DISCUSSION

According to polymorphism of *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA, our study on the populations of *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. selengensis*, *O. stukovii*, and *O. gracillima* section *Verticillares* revealed a high haplotype (*h* changed from 0.634 to 1.000) and relatively low nucleotide (π from 0.0003 to 0.0045) diversity (Table 2). According to variability of the same regions, the level of genetic diversity in the studied populations is comparable with those in the *O. glandulosa* populations located in Barguzinskii ($h = 0.703$, $\pi = 0.0036$) and Kurumkanskii districts of Buryatia ($h = 0.911$, $\pi = 0.0059$) [20], in endemic *O. bargusinensis* ($h = 0.844$, $\pi = 0.0013$), *O. interposita* ($h = 0.769$, $\pi = 0.0015$), and *O. triphylla* ($h = 0.915$, $\pi = 0.0020$) [21], and also in *O. chankaensis* ($h = 0.718$, $\pi = 0.0005$) according to variability of *psbA–trnH*, *trnL–trnF*, *trnS–trnG*, and *petG–trnP* cpDNA [15]. The combination of high values of haplotype diversity at low values of nucleotide diversity suggests a rapid growth of the population from an ancient population with a low effective population size [34]; at the same time, the time interval should be sufficient to restore haplotype diversity by mutations, but insufficient to accumulate significant differences in the sequences.

The absence of population differentiation in *O. myriophylla* ($\Phi_{ST} = 0.008$, $P > 0.37$), as in *O. bargusinensis* ($\Phi_{ST} = 0.027$, $P > 0.20$) [21], is probably caused by uninterrupted gene exchange through a chain of intermediate local habitats. A unimodal distribution of pairwise nucleotide differences in most of the studied populations of the *O. lanata*, *O. oxyphylla*, *O. selengensis*, and *O. gracillima* (Fig. 1) indicates possible recent demographic expansion [35, 36] or spatial expansion with a high level of gene flow between neighboring populations [37]. Demographic scenarios for combined samples of each of the *O. lanata* and *O. oxyphylla* are also confirmed by high and statistically significant ($P < 0.05$) negative Fu's *F*s values (−12.82981 and −11.32100, respectively). The bimodal character of the observed distribution in the *O. oxyphylla* OXY3 and OXY4 populations indicates that they are likely in demographic equilibrium.

High genetic distances between each of *O. lanata*, *O. myriophylla*, *O. oxyphylla*, *O. gracillima*, *O. interposita* and *O. tompudae* (Table 4), as well as identified marker nucleotide substitutions and indels in *O. lanata*, *O. gracillima*, *O. interposita*, *O. pumila*, and *O. mongolica*, indicate a significant differentiation of their chloroplast genomes. Combining all haplotypes of *O. oxyphylla*, *O. selengensis*, *O. stukovii*, and *O. bargusinensis* and T6 haplotype of *O. tompudae* into a single haplogroup (Fig. 2) is not random. Our previous [21] study on phylogenetic relationships of *O. triphylla*, *O. bargusinensis*, and *O. interposita* with closely related species confirmed the idea (formed on the basis of morphological traits) about the origin of *O. bargusinensis* and *O. tompudae* as a result of intersectional hybridization and demonstrated that the maternal parent for T6 haplotype of *O. tompudae* was close to *O. bargusinensis*, and it could have been *O. oxyphylla* or *O. turczaninovii*. The detected common haplotypes in the *O. oxyphylla*, *O. selengensis*, *O. stukovii*, and *O. bargusinensis* species (Table 1, see Results), the presence of "star" structures in the median network with one of the common haplotypes in center (Fig. 2), weak differentiation between these species ($\Phi_{ST} = 0.137$), which was lower than those between the populations of *O. lanata* ($\Phi_{ST} = 0.245$) and *O. oxyphylla* ($\Phi_{ST} = 0.229$), low values of genetic distances (Tables 3 and 4) (corresponding to interpopulation distances), and the absence of differentiating molecular markers—everything in general indicates a common origin of these four species, their relatively recent divergence, and high degree of interspecific hybridization.

On one hand, the results obtained confirm the opinions of the authors (based on morphological traits) that *O. selengensis* can be a form of *O. oxyphylla* with small flowers and prostrate peduncles [13] or an ecological geographic race of *O. oxyphylla* [27] and that some individuals of *O. selengensis* are probably of hybrid origin (since they combine traits of *O. oxyphylla* [14]); on the other hand, they question the assumption

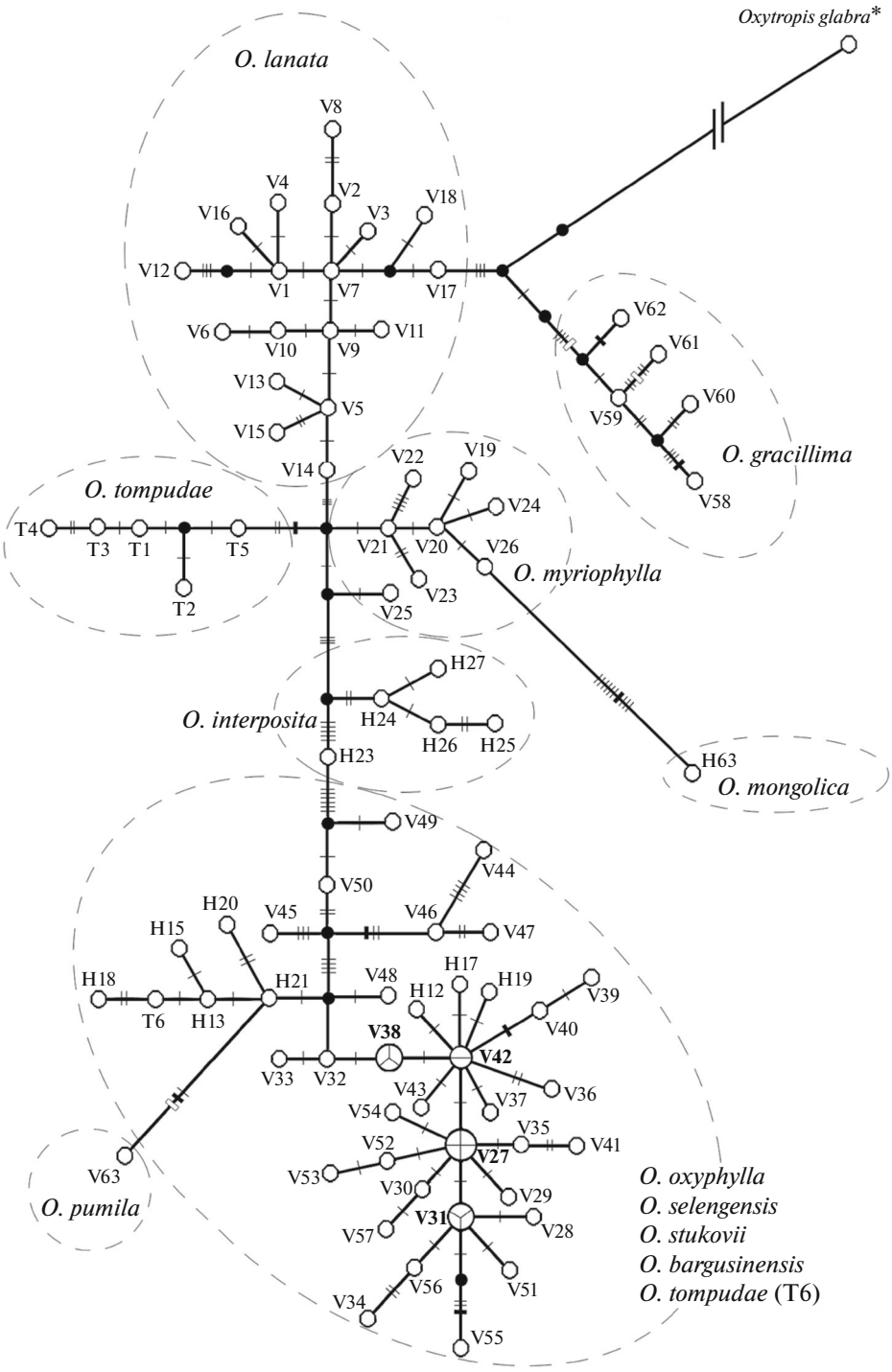

Fig. 2. Genealogical network of haplotypes of *Oxytropis* species constructed using the MJ method. The size of haplotype circles reflects their frequency; small black circles, median vectors; cross thin dashes on branches, number of mutation events; black thick dashes, nucleotide insertion; white thick dashes, nucleotide deletion. The dashed lines combine the haplotypes of one species or group of species. * Mutations for the *O. glabra* used as outgroup are not considered and are not indicated.

that *O. stukovii* is an intermediate race between the *O. prostrata* and *O. mongolica* [13]. To address the issue of the taxonomic status of *O. selengensis* and *O. stukovii*, the study on an extended sample of the plants of the above species is required.

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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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