Karyology of the Species Oxytropis lessingiana Knjasev (Fabaceae) Endemic to the South Urals

N A Kalashnik, A A Muldashev, O A Elizaryeva and N V Maslova
Ufa Institute of Biology, Ufa Federal Research Centre, Russian Academy of Sciences, prospect Oktyabrya, 69, Ufa, 450054, Russia

E-mail: kalash.ufa@mail.ru, cyto.ufa@mail.ru

Abstract. Studies have been performed on the karyotype Oxytropis lessingiana Knjasev endemic to the South Urals. It is recognized that the number of chromosomes in the somatic tissue of the species under investigation is 2n=48 and these metacentric and submetacentric chromosomes are of a small size (1.30±0.02 to 2.59±0.05 μm). The variability of morphometric chromosome parameters is characterized by very low, low and average values of the coefficient of variation. The highest variability is observed in the absolute chromosome length and the lowest one in the relative chromosome length. The differences found between the karyotype O. lessingiana and those of previously studied closely related species may attest to a particular (probably species-level) status of the research object.

1. Introduction
The issues on species formation and systematics of endemic species of the genus Oxytropis DC of the series Approximatae Knjasev growing on the eastern slope of the South Urals are of great interest [1]. Three species (O. approximata Less., O. gmelinii Fisch. ex Boriss., O. sibujensis Knjasev) vicarizing relative to each other are spread from the Ilmen Ridge on the north (Chelyabinsk District) to the southern extremity of the Irendyk Ridge on the south (Republic of Bashkortostan) in the aforesaid consecutive order. The fourth species of the series (O. hippolyti Boriss.) occurs outside the Urals, mainly within the Bugulma-Belebey Upland. These species have a very close relationship to each other and are often linked by transitional populations according to one or another characteristic feature. The differences among the species are primarily dealt with the pubescence pattern and the shape of fruits. The co-growth of these species is not recorded in nature; however, between the species of O. approximata and O. gmelinii on the north of the Uchaly hills there is a zone with their transitional forms described by Knyzev [2] as a nothospecies (O. × lessingiana Knjasev) resulting from introgressive hybridization. Later on, nevertheless, considering its vast natural habitat comparable to that of O. gmelinii as well as the distribution features of these races, Knazyev [1] spoke about the probability of the antithetic hypothesis, according to which O. lessingiana should be treated as a stabilized species and the ancestor of daughter derivatives – O. gmelinii and O. approximata.

Currently, there is no single understanding of the boundary between these species. The same populations are taken by different authors for O. gmelinii or O. lessingiana [1]. In addition, the problem becomes even more difficult due to the fact that some populations have individuals tending to belong to one or another presumable parental species. The determination of the origin of these races presents not only theoretical, but also practical interest in regard to the forthcoming reissue of the Red Data Book of
the Republic of Bashkortostan in the near future. The existing version of the Red Data Book [3] treats all the above-listed Oxytropis species as rare and endemic species, with the exception of O. lessingiana considered to be a nothospecies at that time.

The determination of the O. lessingiana status necessitates its further integrated study, including the use of various techniques, one of which is the karyological analysis. It should be noted that previously karyological studies have already been conducted within the Urals on some closely related Oxytropis species (O. approximata, O. gmelinii, O. hippolyti) with the number of chromosomes determined [4] and the morphology described [5, 6]. No karyological studies were conducted concerning O. lessingiana. In connection with the above-mentioned problems we suppose it to be interesting to perform a study precisely on this problem.

Thus, our research is focused on determining the number and morphology of chromosomes in O. lessingiana with a case study of the population located on the spurs of the Kamshal Ridge of the Uchaly hills (Republic of Bashkortostan, Uchaly District, at a distance of 2-3 km north-northeastward from the village of Kaypkulovo). The population is located in the upper part of the hill on the south-western slope of the ridge, its growing conditions representing a stony steppe.

2. Materials and methods
The meristematic tissue from the tips of seedling roots was used as a material for karyological analysis. The coloring of the material was made in a 1% aqueous solution of aceto-hematoxylin [7]. Squeezed micropreparations were examined using a microscope BIMAM R-13 equipped with a x40 objective, a x7 eyepiece and x2.5 caps. In total we explored more than 100 cells at the metaphase stage, and chromosome average statistical parameters were assessed through analyzing 12 metaphase plates.

Karyological analysis was performed according to Turkov et al. [8], morphometric chromosome types were determined according to the classification put forward by Grif and Agapova [9]. Calculations of major statistical characteristics were carried out according to standard methods [10]. The degree of variation in the traits under investigation was determined using the Mamaev’s scale of the degrees of variability [11].

3. Results and discussion
Based on our research we have found out that O. lessingiana in the population under investigation contains the number of chromosomes 2n=48 in the somatic tissue of the majority of the studied cells; however, there are cells with fewer chromosomes (2n=36–44). Microphotographs of metaphase chromosomes are given in figure 1. The results of statistical processing of morphometric chromosome parameters (absolute length, relative length and centromeric index) are given in table 1. The average total length of a diploid set is 90.07± 2.26 µm, and the coefficient of variation of this indicator is 12.95%.

It has been determined that in conformity with the classification we used [9], 22 pairs of chromosomes are metacentric (Ф>40%) and two pairs of chromosomes are determined as submetacentric (30<Ф<40%). It should also be noted that three more pairs of chromosomes are close to the submetacentric type, since the centromeric index only slightly exceeds 40%. Variations of morphometric chromosome parameters are characterized by very low (CV<7%), low (CV=8–12%) and average (CV=13–20%) values of the coefficient of variation. The highest variability is observed in the absolute chromosome length and the lowest one in the relative chromosome length (table 1).

Average statistical parameters make it possible to construct the karyogram of the population under investigation shown in figure 2.

Our results show that judging by its chromosome set O. lessingiana has both similarity and difference from previously studied closely related species [5, 6]. The similarity is primarily observed in the number of chromosomes which is the same among all the species (2n=48).
Differences are observed in the chromosome length varying in the range of 1.86±0.05 to 2.89±0.06 µm in O. approximata; 1.84±0.03 to 2.84±0.06 µm in O. gmelini; 1.77±0.04 to 2.82±0.05 µm and 1.30±0.02 to 2.59±0.05 µm in O. hippolyti. That is to say, in O. lessingiana the chromosomes of smaller size than in its close relatives. The average total length of the diploid chromosome set in O. lessingiana
is also of a small value than in the previously studied species. It should be noted that the karyotype of O. lessingiana is more differentiated by the morphological chromosome types, since it includes chromosomes of both metacentric and submetacentric types.

![Figure 2](image_url)

**Figure 2.** The karyogram of Oxytropis lessingiana. The rating scale equals 1 µm.

Many researchers think that variations in the karyotypes represent the initial stage of intraspecific divergence and speciation. The detailed study on these variations plays an important role in the understanding of evolutionary paths and mechanisms in different systematic groups [12]. The Oxytropis species were studied by many authors. A lot of research works are devoted to determining the number of chromosomes. So, when studying representatives of the genus Oxytropis growing in the South-East Altay, Plennik points out to the great diversity in the number of chromosomes within the sections Janthina, Orobia, Polyadena, Xerobia, Leucotriche and Baicalia [13]. In the sections that include ecologically similar species, the numbers of chromosomes are identical (diploid or polyploid), whereas in the sections with the most highly expressed species-specific adaptation to different ecological niches (for instance, under the most extreme environments of mountain depressions, alpine-steppe and alpine-tundra belts) include the species with a higher and more diversified level of ploidity. This researcher also notes that the overwhelming majority of endemic species of this genus are polyploids. This opinion is confirmed by other authors based on their case studies of various taxa [14-17]. Many authors also think that polyploid races and species are phylogenetically younger than diploids [18, 19].

In view of the foregoing, we believe that the differences between the O. lessingiana karyotype and those of previously studied closely related species may attest to a particular (probably, special-level) status of the research object. The features revealed in the O. lessingiana karyotype (high level of ploidity, small chromosomes, differentiation by morphological chromosome types) attests to the fact that O. lessingiana is a younger species compared to O. gmelinii and O. approximata. Accordingly, this confirms the first Knyazev’s hypothesis about more recent origin of O. lessingiana as a result of introgressive hybridization [2].

4. Conclusions

The karyological studies showed that the number of chromosomes in the somatic tissue of O. lessingiana is 2n=48 and these metacentric and submetacentric chromosomes are of a small size (1.30±0.02 to 2.59±0.05 µm).

The variability of morphometric chromosome parameters is characterized by very low, low and average values of the coefficient of variation. The highest level of variations is observed in the absolute chromosome length, the lowest one in the relative chromosome length.

The differences revealed between the O. lessingiana karyotype and those of previously studied closely related species may attest to a particular (probably, special-level) status of the research object.
Acknowledgments
The research was performed under Government Contract No. 075-00326-19-00 of the Ministry of Education and Science of the Russian Federation according to theme No. AAAA-A18-118022190060-6 using the equipment base of the Centre for Collective Use "Agidel" (Ufa Federal Research Centre, Russian Academy of Sciences.).

References
[1] Knazyev M S 2015 Fabaceae Lindl. of the Urals: Species formation, geographical distribution, ecohistorical formations: DrSc Thesis in Biology (Ekaterinburg) p 40
[2] Knazyev M S 2001 Notes on systematics and chorology of the species of the genus Oxytropis (Fabaceae) in the Urals. 3. Types of affinity in Oxytropis campestris Botanical Journal 86 (1) 79–87
[3] Red Data Book of the Republic of Bashkortostan. V. 1. Plants and fungi 2011 B.M. Mirkin (ed.). 2nd edition amended and revised. (Ufa: MediaPrint) p 384
[4] Filippov E G, Kulikov P V, Knazyev M S 1998 Numbers of chromosomes in the species of the genus Oxytropis (Fabaceae) in the Urals Botanical Journal 83 (6) 138–9
[5] Arslanova L R, Kalashnik N A 2009 Karyology of the South Ural species of the genus Oxytropis DC. Bulletin of the Orenburg University 6 43–5
[6] Arslanova L R 2015 Morphological and karyological variability of rare South Ural species of the genus Oxytropis DC. PhD Thesis in Biology (Ufa) p 18
[7] Pausheva Z P 1980 Workshop on plant cytology (Moscow: Kolos) p 304
[8] Turkov V D, Guzhov Yu L, Shelepina G A, Kishmariya Ya Sh, Kometiani D G 1988 Plant chromosome research on the issues of selection, cell engineering and genetic monitoring. (Moscow: People’s Friendship University of Russia) p 64
[9] Grif V G, Agapova N D 1986 On the methods of describing plant karyotypes Botanical Journal 71(4) 550–3
[10] Zaytsev G N 1973 Methodology of biometric calculations. Mathematical statistics in experimental biology (Moscow: Nauka) p 256
[11] Mamaev S A 1973 Types of intraspecific variability of woody plants (a case study of the family Pinaceae in the Urals) (Moscow: Nauka) p 284
[12] Dmitrieva S A, Parfenov V I 1991 Karyology of the flora as a basis for cytological monitoring: A case study of the Berezina Biosphere Reserve (Minsk: Nauka i tekhnika) p 231
[13] Plennik R Ya 1976 Morphological evolution of Fabacea in the South-East Altay (Novosibirsk: Nauka) p 215
[14] Malakhova L A 1971 Numbers of chromosomes and karyotypes of some high mountain plants of the West Sayans and South-East Altay: PhD Thesis in Biology. 03.00.05 (Novosibirsk) p 24
[15] Kartashova N N, Malakhova L A, Plennik R Ya 1968 On the introduction of diploid and polyploid Astragalus and Oxytropis species in the South-East Altay Abstracts for presentations at the Conference on Studying and Developing Plant Resources of the USSR Novosibirsk pp 230–1
[16] Kartashova N N, Malakhova L A 1970 Karyological characteristics of some Fabacea species in the South-East Altay Issues on the integrated research of geographical region and methods for studying local lore in school: Proceedings of Science & Research Conference Novokuznetsk pp 22–6
[17] Kartashova N N 1973 Karyological characteristics of some representatives of the family Asteraceae Dumort. in the West Sayans Bulletin of the Tomsk Department of the Russian Botanical Society 6 172–81
[18] Sokolovskaya A P, Strelkova O S 1960 Geographical distribution of polyploid plant species in the Eurasian Arctic Botanical Journal 45 (3) 369–81
[19] Tolmachev A I 1964 Progression phenomena and conservatism in the evolution of the Arctic flora Bulletin of the Leningrad University 3 21–33