Acta Botanica Hungarica 60(3–4), pp. 263–278, 2018
DOI: 10.1556/034.2018.1

GENETIC DIVERSITY AND POPULATION GENETIC STRUCTURE OF THE ENDANGERED KAZAKH ENDEMIC *OXYTROPIS ALMAATENSIS* (FABACEAE)

Sh. Almerekova[1,2], Zs. Lisztes-Szabó[3], N. Mukhitdinov[1], M. Kurmanbayeva[1]
K. Abidkulova[1] and G. Sramkó[4,5]*

[1]*Department of Biodiversity and Bioresources, Al-Farabi Kazakh National University Almaty*
Al-Farabi, 71, Kazakhstan; E-mails: shyryn_89@mail.ru, Meruyert.Kurmanbayeva@kaznu.kz
[2]*Institute of Plant Biology and Biotechnology, Almaty, Timiryazev street 45, Kazakhstan*
[3]*Isotope Climatology and Environmental Research Centre, Institute for Nuclear Research,*
Hungarian Academy of Sciences, H-4001 Debrecen, Pf. 51, Hungary
E-mail: lisztes-szabo.zsuzsanna@atomki.mta.hu
[4]*MTA-DE 'Lendület' Evolutionary Phylogenomics Research Group*
*H-4032 Debrecen, Egyetem tér 1, Hungary; *E-mail: sramko.gabor@science.unideb.hu*
[5]*Department of Botany, Faculty of Science and Technology, University of Debrecen*
H-4032 Debrecen, Egyetem tér 1, Hungary

(Received 14 December, 2017; Accepted 19 January, 2018)

The central Asian narrow endemic species *Oxytropis almaatensis* is a highly endangered plant with a very restricted distribution in the Tian Shan Mountains. In this study, we present the basic conservation genetic characteristics of this species based on a DNA fingerprinting approach in order to provide yardsticks for official conservation agencies to develop an informed conservation strategy. The three currently known populations with two allopatric subpopulations at each site were sampled in the Trans-Ili Alatau Mountains (S Kazakhstan) and subject to AFLP analysis using four primer combinations. This was supplemented by flow cytometry of plants with remarkably different body sizes to check for possible ploidy differences. The presence or absence of AFLP bands was used in downstream analyses utilising various population genetic approaches. Genetic diversity of *O. almaatensis* was found to be on the upper end of the spectrum typical for other outcrossing species of similar life-history characteristics. Most of the genetic variation was attributable to within (sub) population variance, and we also found a remarkable gene flow between the populations. However, the geographically closer populations were found to be more close to each other genetically, and population differentiation showed the same pattern with a significant isolation by distance. Similar patterns were not found for subpopulations of the geographically more close populations, and the subpopulations living along the same river valley were found to be genetically more cohesive. Flow cytometry did not reveal any difference in DNA content between the small and large forms of the species. All these results suggest the presence of two separate populations at the three localities of this species. Conservation efforts should focus on these two populations, and, given the relatively high genetic diversity within each population, both *ex situ* and *in situ* conservation measures can be effectively carried out based on the currently known populations of this narrow endemic species.

Key words: AFLP, endemic species, genetic diversity, population structure

INTRODUCTION

Central Asian mountain ranges associated with the Qinghai–Tibet Plateau (QTP), including the Pamirs and the Tian Shan Mountains (Favre *et al.* 2015), played a major role in the formation and evolution of the xeric Eurasian temperate flora (Manafzadeh *et al.* 2017). Although most research focus on the southern QTP (Qiu *et al.* 2011, Wen *et al.* 2014), the Tian Shan Mountains also emerge as a biodiversity hotspot on a global level (Kier *et al.* 2005), and could also significantly contribute to the formation of our modern flora (Favre *et al.* 2015). Indeed, this mountain range might have played an important role in the evolution of drought-tolerant taxa (Shahi Shavvon *et al.* 2017), such as *Oxytropis* DC. (Candolle 1802).

This genus with *ca* 450 species worldwide has the largest number of species in central Asia (Malyshev 2008), where 154 species are restricted to the region thus representing exceptionally high levels of endemicity (44%) (Grubov 2003). Such an endemic species in the genus is *Oxytropis almaatensis* Bajt. (Baitenov 1961), which has a very limited distribution in the Trans-Ili Alatau range (Fig. 1) of the Tian Shan Mts (Zakirova *et al.* 2014); currently, it is only reported from the Great Almaty Gorge, Small Almaty Gorge (Abidkulova *et al.* 2016) and had been known from the Syugaty and Toraigyr mountains (Baitenov 1961). Given this very restricted distribution and the threat

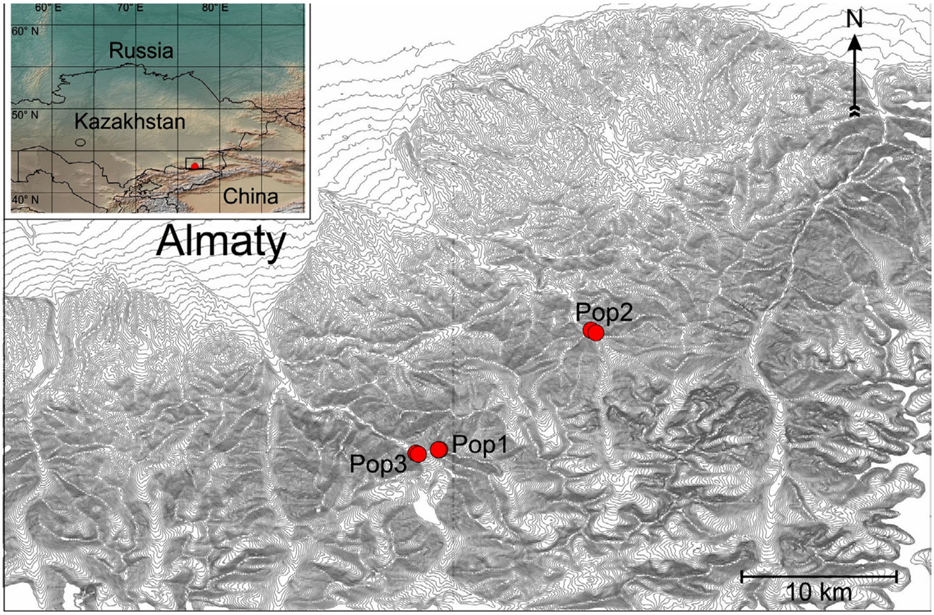

Fig. 1. Location of the currently known and studied populations of *Oxytropis almaatensis* in the Tian Shan Mts in S Kazakhstan as displayed on an elevation map with contour lines. The insert shows the location of the sampled population in a larger geographic context

posed by grazing in their habitats (Zakirova *et al.* 2014), it is included in the Red Book of Kazakhstan, and on the list of rare and endangered species (act nr. 1034 31/10/2006; http://ru.government.kz/docs/p061034~2.htm). This high conservational importance has already prompted studies that explored the demographic structure of *O. almaatensis* populations (Abidkulova *et al*. 2016) and the anatomical features of this rare species (Almerekova *et al.* 2016). In their 2015–2016 field survey of demographic structure of six subpopulations in different habitats ('coenopopulations'), Abidkulova *et al*. (2016) have found a worryingly low number of young plants, and only two subpopulations displayed a bimodal spectrum of age groups with both young vegetative and adult, generative plants. However, the population genetic structure and potential genetic link between the populations have not been studied yet.

Conservation genetics can provide us with the necessary information on the population genetic background of *Oxytropis almaatensis* to maximise the retention of genetic diversity and minimise the risk of inbreeding, one of the main goals of this research area (Frankham *et al.* 2004), thus enabling us to make informed decisions on practical conservation of this endangered species (Avise 2008). Molecular genetic methods, which provide enough resolution at the population level, can be used effectively to study the population genetic background of plant populations (Nybom 2004). An appropriate such technique is Amplified Fragment Length Polymorphism (AFLP) analysis, one of the most widely used DNA fingerprinting techniques in plants that does not require *a priori* sequence information (Bensch and Åkesson 2005, Mueller and Wolfenbarger 1999, Weising *et al.* 2005). This approach has already been successfully utilised to solve conservation genetic questions at the population level (e.g. da Silva *et al.* 2016, Gaudeul *et al.* 2000, Prohens *et al.* 2007, Szczepaniak and Cieślak 2006) even within the genus *Oxytropis* (Schönswetter *et al.* 2004).

In the current study, we employ the AFLP technique to (i) explore the genetic diversity of *Oxytropis almaatensis* and (ii) to seek genetic variation between the populations in a spatial context at the sites currently known in the Trans-Ili Alatau range of the Tian Shan Mts. We were also interested to see (iii) if there is any sign of barrier to gene-flow between the subpopulations of this rare species at each location. In addition, we also check DNA content of individuals with contrasting organ sizes to (iv) trace for a sign of polyploidisation within the species.

MATERIAL AND METHODS

The study species

Oxytropis almaatensis is a predominantly outcrossing perennial species (Zakirova *et al.* 2014) with a relatively long lifespan (Abidkulova *et al.* 2016), and a habitat preference for rocky outcrops at montane elevations around

2,500 m a.s.l. (Zakirova *et al.* 2014). Although extensive studies aimed at the reconstruction of phylogenetic relationships of the genus (Archambault and Stromvik 2012, Artyukova and Kozyrenko 2012, Kholina *et al.* 2016, Shahi Shavvon *et al.* 2017), it is not surprising that this species was not included in any of them. As we can deduct from its overall morphology, *O. almaatensis* is apparently similar to *O. glabra* (Lam.) DC., a highly polymorphic species with a large distribution in the mountains associated with the QTP but with a distinct ecological preference for wet habitats (Grubov 2003). According to our recent results (Almerekova *et al.* 2017) this morphological similarity does not reflect evolutionary relatedness. Another peculiar characteristics of the species is the presence of individuals in the populations with organs twice as large as the others. This can be an indication of (auto)polyploidy (Lavania 2013), and there are reported cases in *Oxytropis* with different ploidy levels (Artyukova and Kozyrenko 2012, Artyukova *et al.* 2011, Kholina *et al.* 2009, 2013).

Plant materials and DNA extraction

Three populations of *Oxytropis almaatensis* were sampled south to Almaty (SE Kazakhstan) up in the Trans-Ili Alatau range of the Tian Shan Mountains in 2016 (Table 1). Because all of these populations occupy areas of slightly contrasting habitat characteristics (rocky outcrop on cliffs with an open vegetation *vs.* sedimental parts below the cliffs with a more closed vegetation), a subsampling was designed to represent each habitat type (aka 'coenopopulation') in the analyses. The three populations were represented in this study by 13, 16, 13 individuals, respectively, with the subpopulations evenly divided within the populations (Table 1). All samples (2–4 cm^2 of the leaf) were put into air-tight sachets filled with silica gel at the sampling site. Total genomic DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987) as detailed in Sramkó *et al.* (2014). Quality and concentration of the extracted DNA was estimated using a NanoDrop ND-1000 instrument (Thermo Scientific, USA) and stored at 4 °C until use.

DNA measurement using flow cytometry (FCM)

The plant material for FCM analysis was collected in 2016 from the second population; at each site, two plants, one with relatively small organs ('small') and one with relatively large organs ('large') were brought to the lab alive to Debrecen (Hungary). DNA ploidy level was estimated from fresh leaf tissues using a Becton Dickinson FACScan flow cytometer (with Ar laser lamp: 488 nm). Leaf tissues of the analysed samples and the internal standards *Capsicum annuum* L. 2C = 6.32 pg (Moscone *et al.* 2003) and *Bellis perennis* L. 2C = 2.30 pg (Olszewska and Osiecka 1983) were chopped using a razor blade in a plastic

Table 1
Sampled populations of *Oxytropis almaatensis* plants and basic statistics of Nei's genetic diversity (expressed as expected heterozygosity) values assessed from 403 AFLP loci using Lynch and Milligan method as implemented in AFLP-SURV

Population	Geocoordinates [°]		Size	Nr of polymorphic loci (>5%)	Nei's gene diversity (H_e)	SE (H_e)
	N	E				
Pop1.1	43.08083	76.99314	6	256	0.2545	0.00941
Pop1.2	43.08076	76.99242	7	259	0.24032	0.00931
Pop2.1	43.14124	77.06976	8	283	0.24399	0.00905
Pop2.2	43.13993	77.07226	8	265	0.237	0.00929
Pop3.1	43.07906	76.98105	7	277	0.24957	0.00893
Pop3.2	43.07846	76.9823	6	258	0.2526	0.00939
Total			42		0.2463	0.00286

Petri dish containing 1 ml of ice-cold Galbraight's buffer (Doležel *et al.* 2007). The suspension was filtered through 40 µm nylon mesh to remove tissue debris and incubated for at least 10 min on ice. Isolated nuclei in filtered suspension were stained with propidium-iodide in 1 ml of buffer. The relative fluorescence intensity was recorded for 10,000 particles ten times for every species. Sample/standard ratios were calculated from the means of fluorescence values visualised using the FCS Express 4 software (De Novo Software). Fluorescence intensity values of *Oxytropis almaatensis* samples were compared with each other and the amount of nuclear DNA of the unknown sample was calculated with mean values as follows: sample 2C DNA value (pg) = sample 2C mean peak position / standard 2C mean peak position * standard 2C DNA value (pg).

AFLP fingerprinting

The AFLP procedure was performed as described in detail in Mosolygó-L. *et al.* (2016) using separated restriction and ligation. In short, 250 ng of total genomic DNA – as quantified by Qubit v.3.0 fluorimeter (Thermo Scientific) – was digested with EcoRI and TruI restriction enzymes, then EcoRI and TruI adaptors were ligated onto the fragments. The bands were selected from the ligate in two rounds of PCR amplification: first, a pre-selective polymerase chain reaction (PCR) was performed, which was followed by a second, selective amplification. In this latter step, we used fluorescently-labelled *Eco*RI selective primers, and normal *Tru*I selective primer. In an initial screening of selective primers using 13 primer combinations four primer combinations gave apparently variable, sharp bands: 1.) 6-FAM-*Eco*RI-AAG+*Tru*I-CAC; 2.) 6-FAM-*Eco*RI-AAG+*Tru*I-CTA; 3.) VIC-*Eco*RI-ACA+*Tru*I-CAC; 4.) PET-*Eco*RI-AGC+*Tru*I-CTA, which were chosen for the final analyses.

The final PCR products were diluted and separated on a 310 Genetic Analyzer (Applied Biosystems) together with a GeneScan-500 LIZ size standard (Applied Biosystems). AFLP raw fragment data were collected and sized using GeneScan v.3.7 software (Applied Biosystems). Only well-scorable and unambiguous fragments were scored above the fluorescence value of 250 between the length of 50–500 bp using the peak-calling algorithm of GeneScan. Twelve samples from a total of 42 were analysed twice to test for reproducibility of the AFLP analysis. The sized fragments were finally converted into a binary matrix of presence (1) and absence (0).

Statistical analysis

The AFLP dataset was first imported into AFLP-SURV v.1.0 (Vekemans 2002), which was used to carry out basic genetic diversity analyses at the subpopulation level using the approach of Lynch and Milligan (1994), which uses Nei's gene diversity (i.e. expressed as average expected heterozygosity) as a measure of genetic diversity. Then, pairwise population matrices were calculated for Nei's genetic distance (Nei's D) and standard genetic differentiation (Wright's F_{ST}) assuming each subpopulation to be a separate entity. The latter measure was also calculated globally (i.e. to test genetic differentiation among the populations) using 9999 permutations to test the significance of the result. The genetic distance matrix was subject to cluster analysis using chord distances, whereas the genetic differentiation matrix was subject to Principal Coordinate Analysis (PCoA) using Chord distance measure, both analyses as implemented in PAST v.2.17c (Hammer *et al.* 2001). The N_m index of the gene flow (based on G_{ST}) was calculated using PopGene 3.2 (Yeh *et al.* 1999). Finally, the genetic matrix was subject to Analysis of Molecular Variance (AMOVA) as implemented in GenAlEx v.6.5 (Peakall and Smouse 2012), and the relationship between geographic and genetic distances to test for correlation between these two distances via a Mantel test also as implemented in GenAlEx.

RESULTS

AFLP fingerprinting and population structure of O. almaatensis

The AFLP profiles of the 42 individuals generated 403 AFLP loci with four primer combinations of which an average of 266 were polymorphic (66%). The overall gene diversity, expressed as expected heterozygosity calculated using the approach of Lynch and Milligan (1994), was found to be 0.2463±0.00286 (H_e±S.E.) (Table 1). The value of overall genetic differentiation among the (sub)populations showed the existence of a significant genetic structure (F_{ST} = 0.123; $p \leq 0.001$; i.e. the actual populations are more genetically differentiated than random assemblages of the individuals). When considering Nei's genetic distances between the subpopulations (Fig. 2), subpopulations of the geographically most distant (Fig. 1) Pop2 are separate from the rest of the studied populations with high statistical certainty. This was also shown by the significant relationship between geographic and genetic distances as shown by the Mantel test (R = 0.661, p = 0.021), whereas this has changed to non-significant when we confined the analysis only to subpopulations of Pop1 and Pop3 (R = 0.722, p = 0.269). The genetic relationship between the subpopulations of Pop1 and Pop3 remained statistically unresolved, but their close genetic proximity is evident (Fig. 2). The populations are grouped similarly in the genetic space based on pairwise genetic differentiation (Fig. 3); here, the subpopulations of the same population show proximity to each other, and Pop2 shows remarkable differentiation from the rest. Within the group of Pop1 and Pop3 subpopu-

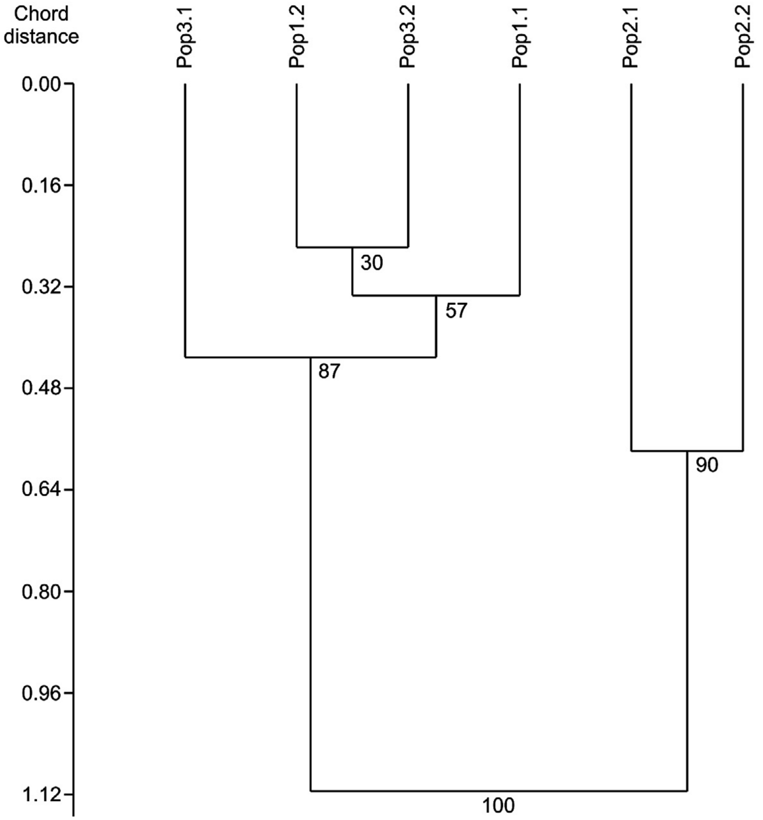

Fig. 2. Genetic relationship between the sampled (sub)populations displayed as a dendrogram based on pairwise values of Nei's genetic distance calculated by the approach of Lynch and Milligan (1994). Figures next to the branches are statistical support values resulting from 999 bootstraps

lation Pop3.1 is somehow distanced from the rest of this group. Estimation of gene flow (N_m) from G_{ST} was calculated for all 42 individual plants of the three populations. Gene flow among the three populations was 5.437 individuals per generation. The partitioning of genetic variability in an AMOVA (Table 2) showed the overwhelming majority of the variability to be associated with intra-population variability (94%), with a significant but relatively low level of genetic differentiation both among six subpopulations ($\Phi_{PT} = 0.058$, $p \geq 0.01$) and three populations ($\Phi_{PT} = 0.049$, $p \geq 0.001$).

DNA content of different morphotypes

The FCM analysis of different morphotypes (i.e. plants with comparatively large *vs.* small organs) showed no difference in their DNA content (Fig. 4). Thus, FCM analysis did not reveal any intraspecific variation or difference in level of ploidy. The CV of the samples and the standards (*Bellis perennis* L. and *Capsicum annuum* L.) were below 3.00 in every case. The amount of nuclear DNA was 3.58 pg compared with internal standard of *Capsicum annuum*,

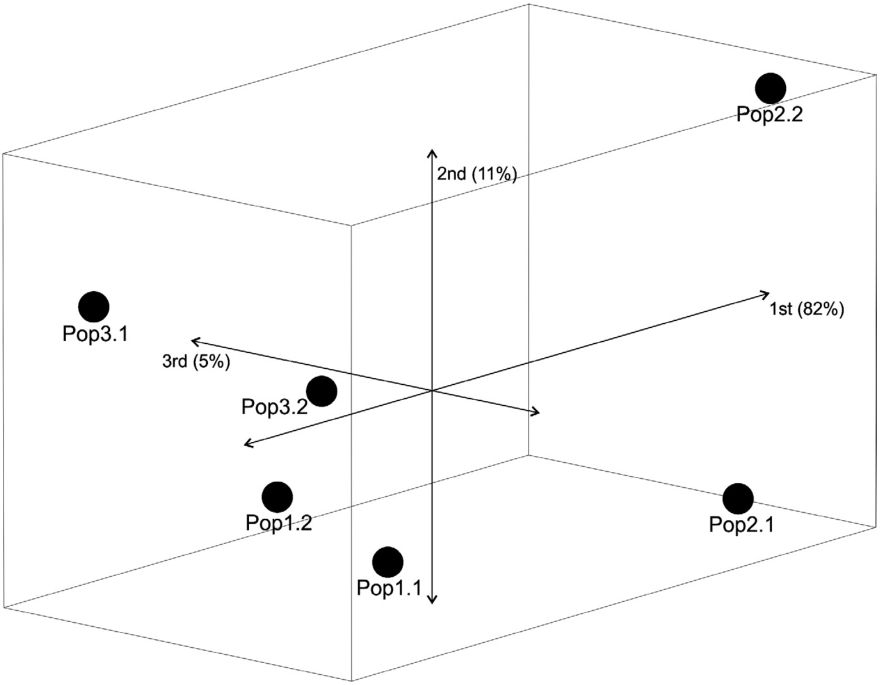

Fig. 3. Genetic differentiation between the studied (sub)populations displayed as a principal coordinate analysis (PCoA) plot drawn from pairwise F_{ST} values transformed using chord distance. The variation depicted by the first three axes are shown next to the corresponding axis

Table 2
Partitions of genetic variability in the studied subpopulations and populations of *Oxytropis almaatensis* as shown by an AMOVA

Source of variation	df	SS	MS	Est. var.	%	Φ_{PT}
Among subpops	5	315.295	63.059	2.730	6	0.058 ($p > 0.01$)
Within subpops	36	1586.014	44.056	44.056	94	
Total	41	1901.310		46.786	100	
Among pops	2	153.449	76.724	2.291	5	0.049 ($p > 0.001$)
Within pops	39	1747.861	44.817	44.817	95	
Total	41	1901.310		47.108	100	

and it was 3.34 pg calculated based on *Bellis perennis* as an internal standard. To summarise, nuclear DNA content of *Oxytropis almaatensis* was evaluated between 3.34 and 3.58 pg.

DISCUSSION

The level of AFLP polymorphism and genetic variation of O. almaatensis

The narrow endemic Kazakh species *Oxytropis almaatensis* is currently only known from three populations in the Trans-Ili Alatau range of the Tian Shan Mts, where our study was carried out. The application of the AFLP for studying the genetic diversity in *O. almaatensis* was rather successful as 266 polymorphic loci (66%) were generated using four primer combinations. This result is comparable with previously published reports of other, herbaceous species (Gaudeul *et al.* 2000, Prohens *et al.* 2007, Schönswetter *et al.* 2004) and confirms robustness of the AFLP approach in the assessment of intraspecies genetic variation. The AFLP-based genetic diversity of this species (Table 1) seems to be satisfyingly high ($H_e = 0.2463$; i.e. there is a chance of *ca* 25% to randomly choose a heterozygote from the populations). This value is more-or-less directly comparable (Nybom

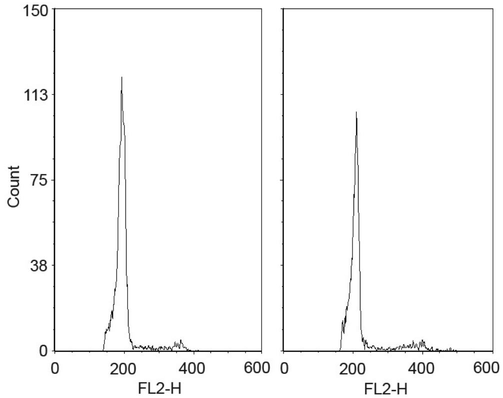

Fig. 4. Histograms of flow cytometric analysis of nuclei from different morphotypes ('small' left, 'large' right) of *Oxytropis almaatensis* leaves. The x axis shows relative fluorescence, whereas the y axis stands for number of events detected

2004) to similar measures in Far Eastern *Oxytropis* species, *O. chankaensis* using allozymes (Kholina *et al.* 2009) and RAPDs (Artyukova *et al.* 2011), who reported similar values in that similarly narrow endemic *Oxytropis* species, which were interpreted as high. More direct comparison can be made with the data of Chung *et al.* (2004), who used similar fingerprinting approach as we did for the study of the supposed endemic variety *O. campestris* var. *chartacea* (Fasset) Barneby in North America. They found H_e values ranging from 0.145 to 0.189, which were interpreted as high given the uppermost reported limit of $H_e = 0.23$ found in *Eryngium alpinum* (Gaudeul *et al.* 2000). Thus, *O. almaatensis* the Kazakh endemic species displays a high level of genetic diversity – a surprising finding given the narrow endemicity of this species that deserves further study.

The genetic relationship between the populations (Fig. 2) mirrored their overall spatial relationship (see Fig. 1); the most remote population (Pop2) was the farthest from the rest, and within the remaining samples there was no clear pattern. This suggests a simple isolation-by-distance (IBD) relationship between the populations as demonstrated by the Mantel test. Between the subpopulations of the two, geographically close populations (Pop1 and Pop3) the genetic relationship is not clear, and we could not find a significant IBD either. This suggests that these populations are connected to each other, and they are not isolated. Therefore, the three currently known populations of *Oxytropis almaatensis* effectively behave as two populations; conservation efforts thus should focus on these two entities: the populations at Great Almaty Gorge and the population at Shymbulak.

Population structure analysis

According to our AMOVA results (Table 2), most of the variation was explained by the within population component, thus indicating a weak isolation between the (sub)populations. Population differentiation of six subpopulations based on AMOVA results (Table 2) revealed that over 94% of the total genetic variation is partitioned within the subpopulations. Similar results were obtained in the assessment of three populations as only 5% contributed to interpopulational genetic diversity. This is further corroborated by the low Φ_{PT} values, which is typical for outbreeding perennials (Nybom 2004). The level of genetic variation within each population was equally high in different spatial parts of the populations. Therefore, subpopulation genetic structure can be ruled out as a causative effect of high intraspecies diversity. The survey of relevant literature is suggesting that F_{ST} is ranging from 0.2 in outbreeding to 0.5 in inbreeding species (Bussell 1999, Gaudeul *et al.* 2000, Loveless and Hamrick 1984). Based on this assumption *O. almaatensis* ($F_{ST} = 0.123$) fits well to outbreeding species ranges, and the N_m index of gene flow with 5.4 individuals per generation firmly supports this conclusion. Thus, it is possible that high intraspecific diversity is a consequence of spatial gene dispersal ensured by seed or pollen dispersal. The high population genetic diversity might be a consequence of the long lifespan of the species (Nybom 2004), but can also be influenced by the habitat heterogeneity (i.e. the presence of 'coenopopulations'), which is to be studied further in the future.

Population 2 is growing in a different gorge apart from Pop1 and Pop3 in a *ca* 9 km distance. Therefore, putative explanations for the low interpopulation diversity index can be explained by: i) the existence of intermediate populations growing between the two gorges, and this can be supported by a positive relationship between genetic divergence and geographic distance determined by Mantel test; and/or ii) high outbreeding rates within a population of this perennial species. Although the above explanations are not mutually exclusive, all previous collecting efforts failed to find intermediate populations between Pop1&3 and Pop2. Thus, it is possible that even isolated populations of *O. almaatensis* may maintain a relatively high level of genetic variation due to a random mating system and high outbreeding rates.

We paid special attention to subpopulations ('coenopopulations') of this species at the three sampling sites by treating each subpopulation occupying different habitats (i.e. Pop1.1, Pop2.1, Pop3.1 on rocky outcrops of cliffs; Pop1.2, Pop2.2, Pop3.2 on scree-slope and sediments below the cliffs) as separate entities during the genetic analyses. If we examine our results in all three studied populations it becomes evident that the most important isolating factor between the subpopulations is distance. Pop2, which is *ca* 10 km away from Pop1 and Pop3 (Fig. 1), is the most separate one (see Figs 2 and 3). Within the geographically more proximate Pop1 and Pop3 (distance between them is *ca* 1 km and they lay in the same river valley at different elevations), subpopulations Pop1.2 and Pop3.2 are somewhat closer to each other (Figs 2 and 3). This suggests a stronger connection between the river valley subpopulations, which might be explained by gene-flow via seed dispersal by the Big Almaty River, the main watercourse of the valley.

Implication for conservation

Oxytropis almaatensis is a local endemic and rare plant included in the red book of Kazakhstan (Zakirova *et al.* 2014). Therefore, the maintenance of genetic diversity is a very important aspect and can be related to the adoption of a conservation strategy for this endangered species. This strategy recently gained growing importance as severe weather phenomena (e.g. hurricane-force winds) has become increasingly typical in the Tian Shan Mts (Kelgenbaev *et al.* 2016) and together with constant anthropogenic pressure (Kokoreva *et al.* 2013) may severely threaten the biodiversity of the Tian Shan Mts. Mountain ecosystems maintaining high levels of plant diversity and endemism are especially vulnerable to climate change (Munson and Sher 2015, Thuiller *et al.* 2005). Also, small sizes of *Oxytropis almaatensis* populations may reflect an ability to grow in spatially restricted environments, which can easily lead to a rapid decline of these isolated populations. On the other hand, such restricted and small populations may be subject to either *in situ* or *ex situ* preservation as a conservation strategy. Our genetic and cytometric results indicated that most of the genetic diversity could be preserved *ex situ* by conservation of sampled seeds even from a single population. Since we most likely deal with plants at the same ploidy level, there is no need to handle large individuals as separate entities. Therefore, in case of the risk of extinction, the species could be re-established with individuals of population conserved in seed banks (Nuez *et al.* 2004, Prohens *et al.* 2007). Taking into account high gene flow even between distant populations and low population structure, the maintenance of the genetic variation within *O. almaatensis* can be successfully achieved through *in situ* conservation if human pressure in the habitats where populations are growing decrease. Thus, measures for conservation of *O. almaatensis* using both *in situ* and *ex situ* strategies can be implemented.

*

Acknowledgements – We express our thanks to Levente Laczkó for his kind assistance in the lab, and to Tamás Malkócs for his comments on the manuscript. We are grateful to our reviewers for their constructive comments. Second author was supported by the European Union and the State of Hungary, co-financed by the European Regional Development Fund in the project of GINOP-2.3.2.-15-2016-00009 'ICER'. The work of GS was supported by the "János Bolyai Scholarship" (BO/00001/15) of the Hungarian Academy of Sciences. Instrumental and infrastructural support was received from the Hungarian Scientific Research Fund (OTKA PD109686) to GS.

REFERENCES

Abidkulova, K. T., Mukhitdinov, N. M., Ametov, A. A., Ivashchenko, A. A., Almerekova, S. and Ydyrys, A. (2016): Особенности структуры ценопопуляций редкого, эндемичного растения Заилийского Алатау Oxytropis almaatensis Bajt. [The features of cenopopulations structures of rare, endemic plant species Oxytropis almaatensis Bajt. from Trans-Ili Alatau mountains]. – *Exp. Biol.* **68**: 24–33. [in Russian]

Almerekova, Sh., Mukhitdinov, N. and Abugalieva, S. (2017): Phylogenetic study of the endemic species Oxytropis almaatensis (Fabaceae) based on nuclear ribosomal DNA ITS sequences. – *BMC Plant Biol.* **17**: 173. https://doi.org/10.1186/s12870-017-1128-x

Almerekova, Sh. S., Mukhitdinov, N. M. and Kurmanbayeva, M. S. (2016): Biometric data of anatomical structure of vegetative organs of rare, narrowly endemic species Oxytropis almaatensis Bajt. in Trans-Ili Alatau mountains (Kazakhstan). – *Exp. Biol.* **68**: 4–13.

Archambault, A. and Stromvik, M. V. (2012): Evolutionary relationships in Oxytropis species, as estimated from the nuclear ribosomal internal transcribed spacer (ITS) sequences point to multiple expansions into the Arctic. – *Botany-Botanique* **90**: 770–779. https://doi.org/10.1139/b2012-023

Artyukova, E. V. and Kozyrenko, M. M. (2012): Phylogenetic relationships of Oxytropis chankaensis Jurtz. and Oxytropis oxyphylla (Pall.) DC. (Fabaceae) inferred from the data of sequencing of the ITS region of the nuclear ribosomal DNA operon and intergenic spacers of the chloroplast genome. – *Russian J. Genetics* **48**: 163–169. https://doi.org/10.1134/s1022795411110032

Artyukova, E. V., Kozyrenko, M. M., Kholina, A. B. and Zhuravlev, Y. N. (2011): High chloroplast haplotype diversity in the endemic legume Oxytropis chankaensis may result from independent polyploidization events. – *Genetica* **139**: 221–232. https://doi.org/10.1007/s10709-010-9539-8

Avise, J. C. (2008): *The history, purview, and future of conservation genetics*. – In: Carroll, S. P. and Fox, C. W. (eds): Conservation Biology. Evolution in action. Oxford University Press, Oxford, pp. 5–15.

Baitenov, M. S. (1961): *Oxytropis almaatensis Bajt. sp. nova*. – In: Pavlov, N. V. (ed.): Флора Казахстана V. [Flora of Kazakhstan vol. V]. Akademii NAUK Kazakhskoy SSR, Alma-Ata, p. 493. [in Russian]

Bensch, S. and Åkesson, M. (2005): Ten years of AFLP in ecology and evolution: why so few animals? – *Mol. Ecol.* **14**: 2899–2914. https://doi.org/10.1111/j.1365-294x.2005.02655.x

Bussell, J. D. (1999): The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of Isotoma petraea (Lobeliaceae). – *Mol. Ecol.* **8**: 775–789. https://doi.org/10.1046/j.1365-294x.1999.00627.x

Candolle, A. P. de (1802): *Astragalogia, nempe astragali, biserrulae et oxytropidis: nec non phacae, colutae et lessertiae historia iconibus illustrata*. – sumptibus Joann. Bapt. Garnery, Parisiis.

Chung, M., Gelembiuk, G. and Givnish, T. J. (2004): Population genetics and phylogeography of endangered Oxytropis campestris var. chartacea and relatives: arctic-alpine disjuncts in eastern North America. – *Mol. Ecol.* **13**: 3657–3673. https://doi.org/10.1111/j.1365-294x.2004.02360.x

da Silva, L. N., Essi, L., Welker, C. A. D. and de Souza-Chies, T. T. (2016): Assessing the genetic diversity and population structure of the endangered Chascolytrum bulbosum (Poaceae, Poeae) using AFLP markers. – *Biochem. Syst. Ecol.* **68**: 236–242.

Doležel, J., Greilhuber, J. and Suda, J. (2007): Estimation of nuclear DNA content in plants using flow cytometry. – *Nat. Protocols* **2**: 2233–2244. https://doi.org/10.1038/nprot.2007.310

Doyle, J. J. and Doyle, J. L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – *Phytochemical Bulletin* **19**: 11–15.

Favre, A., Päckert, M., Pauls, S. U., Jähnig, S. C., Uhl, D., Michalak, I. and Muellner-Riehl, A. N. (2015): The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. – *Biol. Reviews* **90**: 236–253. https://doi.org/10.1111/brv.12107

Frankham, R., Ballou, J. D. and Briscoe, D. A. (2004): *A primer of conservation genetics*. – Cambridge Univ. Press, Cambridge, 220 pp.

Gaudeul, M., Taberlet, P. and Till-Bottraud, I. (2000): Genetic diversity in an endangered alpine plant, Eryngium alpinum L. (Apiaceae), inferred from amplified fragment length polymorphism markers. – *Mol. Ecol.* **9**: 1625–1637. https://doi.org/10.1046/j.1365-294x.2000.01063.x

Grubov, V. I. (2003): *Plants of Central Asia: plant collections from China and Mongolia. Volume 8b. Legumes. Genus: Oxytropis*. – Science Publishers, Enfield, 110 pp.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2001): PAST: Paleontological statistics software package for education and data analysis. – *Palaeont. Electr.* **4**: 9.

Kelgenbaev, N. S., Besschetnov, V. P. and Mambetov, B. T. (2016): Результаты лесовосстановительных работ в урочище Медеу после стихийного бедствия. [Results of reforestation in Medeo hole after disasters]. – *Vestnik of Kazan State Agrarian University* **39**: 27–29. [in Russian]

Kholina, A. B., Koren', O. G. and Zhuravlev, Y. N. (2009): Genetic structure and differentiation of populations of the tetraploid species Oxytropis chankaensis (Fabaceae). – *Russian J. Genetics* **45**: 70–80. https://doi.org/10.1134/s1022795409010104

Kholina, A. B., Kozyrenko, M. M., Artyukova, E. V., Sandanov, D. V. and Andrianova, E. A. (2016): Phylogenetic relationships of the species of Oxytropis DC. subg. Oxytropis and Phacoxytropis (Fabaceae) from Asian Russia inferred from the nucleotide sequence analysis of the intergenic spacers of the chloroplast genome. – *Russian J. Genetics* **52**: 780–793. https://doi.org/10.1134/s1022795416060065

Kholina, A. B., Nakonechnaya, O. V., Yakubov, V. V. and Koren, O. G. (2013): Genetic variation in six species of the genus Oxytropis DC. (Fabaceae) from Kamchatka Peninsula. – *Russian J. Genetics* **49**: 1021–1029. https://doi.org/10.1134/s1022795413100049

Kier, G., Mutke, J., Dinerstein, E., Ricketts, T. H., Küper, W., Kreft, H. and Barthlott, W. (2005): Global patterns of plant diversity and floristic knowledge. – *J. Biogeogr.* **32**: 1107–1116. https://doi.org/10.1111/j.1365-2699.2005.01272.x

Kokoreva, I. I., Lyssenko, V. V. and Nesterova, S. G. (2013): Влияние рекреационных нагрузок на состояние экосистем Иле-Алатауского национального парка (Северный Тянь-Шань). [Influence recreation loads on ecosystem condition of the Ile-Alatau National Park (the Northern Tian Shan)]. – *KAZNU Bull., Ser. Ecol.* **38**: 191–196. [in Russian]

Lavania, U. C. (2013): Polyploidy, body size, and opportunities for genetic enhancement and fixation of heterozygosity in plants. – *The Nucleus* **56**: 1–6. https://doi.org/10.1007/s13237-013-0075-7

Loveless, M. D. and Hamrick, J. L. (1984): Ecological determinants of genetic structure in plant populations. – *Annu. Rev. Ecol. Syst.* **15**: 65–95. https://doi.org/10.1146/annurev.ecolsys.15.1.65

Lynch, M. and Milligan, B. G. (1994): Analysis of population genetic structure with RAPD markers. – *Mol. Ecol.* **3**: 91–99. https://doi.org/10.1111/j.1365-294x.1994.tb00109.x

Malyshev, L. I. (2008): Phenetics of the subgenera and sections in the genus Oxytropis DC. (Fabaceae) bearing on ecology and phylogeny. – *Contemp. Problems Ecol.* **1**: 440–444. https://doi.org/10.1134/s1995425508040073

Manafzadeh, S., Staedler, Y. M. and Conti, E. (2017): Visions of the past and dreams of the future in the Orient: the Irano-Turanian region from classical botany to evolutionary studies. – *Biol. Reviews* **92**: 1365–1388. https://doi.org/10.1111/brv.12287

Moscone, E. A., Baranyi, M., Ebert, I., Greilhuber, J., Ehrendorfer, F. and Hunziker, A. T. (2003): Analysis of nuclear DNA content in Capsicum (Solanaceae) by flow cytometry and Feulgen densitometry. – *Ann. Bot.* **92**: 21–29. https://doi.org/10.1093/aob/mcg105

Mosolygó-L., Á., Sramkó, G., Barabás, S., Czeglédi, L., Jávor, A., Molnár V., A. and Surányi, G. (2016): Molecular genetic evidence for allotetraploid hybrid speciation in the genus Crocus L. (Iridaceae). – *Phytotaxa* **258**: 121–136. https://doi.org/10.11646/phytotaxa.258.2.2

Mueller, U. G. and Wolfenbarger, L. L. (1999): AFLP genotyping and fingerprinting. – *Trends Ecol. Evol.* **14**: 389–394. https://doi.org/10.1016/s0169-5347(99)01659-6

Munson, S. M. and Sher, A. A. (2015): Long-term shifts in the phenology of rare and endemic Rocky Mountain plants. – *Amer. J. Bot.* **102**: 1268–1276. https://doi.org/10.3732/ajb.1500156

Nuez, F., Prohens, J. and Blanca, J. M. (2004): Relationships, origin, and diversity of Galápagos tomatoes: implications for the conservation of natural populations. – *Amer. J. Bot.* **91**: 86–99. https://doi.org/10.3732/ajb.91.1.86

Nybom, H. (2004): Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. – *Mol. Ecol.* **13**: 1143–1155. https://doi.org/10.1111/j.1365-294x.2004.02141.x

Olszewska, M. J. and Osiecka, R. (1983): The relationship between 2 C DNA content, life cycle type, systematic position and the dynamics of DNA endoreplication in parenchyma nuclei during growth and differentiation of roots in some dicotyledonous herbaceous species. – *Biochem. Physiol. Pflanzen* **178**: 581–599. https://doi.org/10.1016/s0015-3796(83)80073-0

Peakall, R. and Smouse, P. E. (2012): GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research, an update. – *Bioinformatics* **28**: 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Prohens, J., Anderson, G. J., Herraiz, F. J., Bernardello, G., Santos-Guerra, A., Crawford, D. and Nuez, F. (2007): Genetic diversity and conservation of two endangered eggplant relatives (Solanum vespertilio Aiton and Solanum lidii Sunding) endemic to the Canary Islands. – *Gen. Res. Crop Evol.* **54**: 451–464. https://doi.org/10.1007/s10722-006-9174-5

Qiu, Y.-X., Fu, C.-X. and Comes, H. P. (2011): Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. – *Mol. Phyl. Evol.* **59**: 225–244. https://doi.org/10.1016/j.ympev.2011.01.012

Schönswetter, P., Tribsch, A. and Niklfeld, H. (2004): Amplified Fragment Length Polymorphism (AFLP) reveals no genetic divergence of the Eastern Alpine endemic Oxytropis campestris subsp. tiroliensis (Fabaceae) from widespread subsp. campestris. – *Plant Syst. Evol.* **244**: 245–255. https://doi.org/10.1007/s00606-003-0096-9

Shahi Shavvon, R., Kazempour Osaloo, S., Maassoumii, A. A., Moharrek, F., Karaman Erkul, S., Lemmon, A. R., Lemmon, E. M., Michalak, I., Muellner-Riehl, A. N. and Favre, A. (2017): Increasing phylogenetic support for explosively radiating taxa: The promise of high-throughput sequencing for Oxytropis (Fabaceae). – *J. Syst. Evol.* **55**: 385–404. https://doi.org/10.1111/jse.12269

Sramkó, G., Molnár V., A., Hawkins, J. A. and Bateman, R. M. (2014): Molecular phylogeny and evolutionary history of the Eurasiatic orchid genus Himantoglossum s. l. (Orchidaceae). – *Ann. Bot.* **114**: 1609–1626. https://doi.org//10.1093/aob/mcu179

Szczepaniak, M. and Cieślak, E. (2006): Genetic variation and structure in natural populations of Melica ciliata and M. transsilvanica (Poaceae) as indicated by AFLP markers. – *Biodiv. Res. Conserv.* **3–4**: 235–239.

Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T. and Prentice, I. C. (2005): Climate change threats to plant diversity in Europe. – *Proceeds Nat. Acad. Sci. USA* **102**: 8245–8250.

Vekemans, X. (2002): *AFLP-SURV version 1.0.* Distributed by the author. – Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Weising, K., Nybom, H., Wolff, K. and Kahl, G. (2005): *DNA fingerprinting in plants. Principles, methods, and applications*. – CRC Press, Boka Raton.

Wen, J., Zhang, J., Nie, Z.-L., Zhong, Y. and Sun, H. (2014): Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. – *Frontiers in Genetics* **5**. https://doi.org/10.3389/fgene.2014.00004

Yeh, F. C., Yang, R., Boyle, T., Ye, Z. and Mao, J. X. (1999): *POPGENE, version 1.32: the user friendly software for population genetic analysis*. – Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, AB, Canada.

Zakirova, P. O., Kazanas, O. D. and Bobrowski, V. P. (2014): *Oxytropis almaatensis Bajt. Красная книга Республики Казахстан. Подлежащие охране сосудистые растения, мхи, печёночники, антоцеротовые и лишайники*. [The red book of the Republic of Kazakhstan. Protected vascular plants, mosses, liverworts, lichens and anthocerotophyta]. ArtPrint XXI, Almaty, pp. 325–326. [in Russian]