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REGULAR PAPER

Genetic structure and conservation status of *Astragalus subrecognitus* (Fabaceae): a very rare and narrow endemic species

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Background and aims – Narrow endemics, characterized by small distribution areas and small effective population sizes are prone to extinction due to low genetic variation caused by genetic bottlenecks and drift together with the danger of habitat loss. *Astragalus subrecognitus* is an endemic species that only occurs in northwestern Iran in mountain steppe at higher elevations above the Qezel Ozan River in the province Zanjan.
Methods – In this study, we investigated the genetic structure of the populations of *A. subrecognitus* using inter simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP) as molecular markers.
Key results – We detected two genetic groups, which do however not concur with the geographic origins east and west of the Qezel Ozan River. Extant gene flow between these populations is rather unlikely given their distance of c. 70 km and the lack of suitable habitats for this species in lower areas of the valley in between.
Conclusions – From this we conclude that we see an older pattern of geographic isolation that was followed by an exchange of genotypes during a time when migration through the valley was possible. Still, admixture between both genetic clusters was found only in one individual, indicating that broadening of allelic diversity by hybridization between individuals belonging to different genetic clusters might be a slow process. Due to the small size of the population, sparse inhabited area, and also very slow or even lack of regeneration in some places we consider *A. subrecognitus* according to IUCN Red List criteria as 'endangered' species.

Keywords – *Astragalus subrecognitus*; conservation; endemic species; genetic variability; ISSR; SRAP.

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INTRODUCTION

Astragalus L. (Fabaceae) is with 2500–3000 species and more than 250 sections the largest genus of flowering plants in the World (Lock & Simpson 1991; Podlech & Zarre 2013). The species mostly occur in semiarid and arid mountainous areas of South America and the Northern Hemisphere. *Astragalus* is particularly diverse in southwestern Asia where about 1000–1500 species occur (Maassoumi 1998). Section *Hymenostegis* Bunge, one of the spiny sections within *Astragalus*, has its centre of distribution in Iran, particularly in the northwestern parts of the country (Bagheri et al. 2014). Local endemism plays an important role in the biology of this section (Zarre & Podlech 1996; Bagheri et al. 2017) and nearly all of its members have very narrow distribution areas. Narrow endemism combined with small effective population sizes might result in depletion of genetic diversity in such species, restricting their ability to cope with changing environmental conditions.

One of the endemic sect. *Hymenostegis* species is *Astragalus subrecognitus* Bagheri, Maassoumi & F.Ghahrem., a small perennial shrub (Bagheri et al. 2011). *Astragalus subrecognitus* grows very slowly and is a weak competitor, although by forming cushions it thrives well in wind-blown areas, thus avoiding competition with many other plants. The species seems not adapted to specific soil types but is overall rare in more rocky areas. It is entomophilous and seems to be pollinated mostly by bees. The inflorescence is usually elongated reaching above the cushion surface. When seeds are mature, the pods easily separate from the inflorescences. Given these structures and also based on our field observations, it seems that seed dispersal of this species is mainly by wind. *Astragalus subrecognitus* propagates most probably only through seeds, as we did not find signs of vegetative reproduction in this species. It occurs with few individuals in a restricted part of the Iranian Zanjan province. Its habitats are mountain steppes at elevations of 2100–2800 m in the vicinity of the Qezel Ozan River. As the river and bottom of the valley is 900–1600 m below the *A. subrecognitus* populations, and the valley floor is densely populated and used for agriculture, it effectively subdivides the species' distribution area in a western and eastern population.

Studies of genetic diversity within *Astragalus* species are still rare. For example, Anand et al. (2010) found two distinct genotype groups in Indian *A. rhizanthus* Royle ex Benth. using a set of anonymous molecular markers. Morris et al. (2016) developed and characterized microsatellites in the endangered *A. bibullatus* Barneby & Bridges, while inter-simple sequence repeats (ISSR) was used to analyse the narrow endemics *A. oniciformis* Barneby (Alexander et al. 2004) and *A. crassicarpus* Nutt. (Rogenski et al. 2009). Due to the few results regarding genetic diversity in endemic *Astragalus* species it is currently not possible to generalize results and to draw conclusions for conservation measurements. This makes additional studies of genetic diversity within *Astragalus* species necessary particularly for the endemic species.

To analyse the genetic variability of *A. subrecognitus* we are employing ISSR and sequence-related amplified polymorphism (SRAP) markers. ISSRs are microsatellite-based markers that employ the ubiquity of microsatellite repeats in the eukaryotic genomes to PCR-amplify stretches of DNA in-between microsatellite loci. This marker class has been widely used in population studies because they are variable, reliable and fast compared to other molecular markers (Wolfe & Liston 1998; Harris 1999; Esselman et al. 1999; Camacho & Liston 2001; McGlaughlin et al. 2002; Smith & Bateman

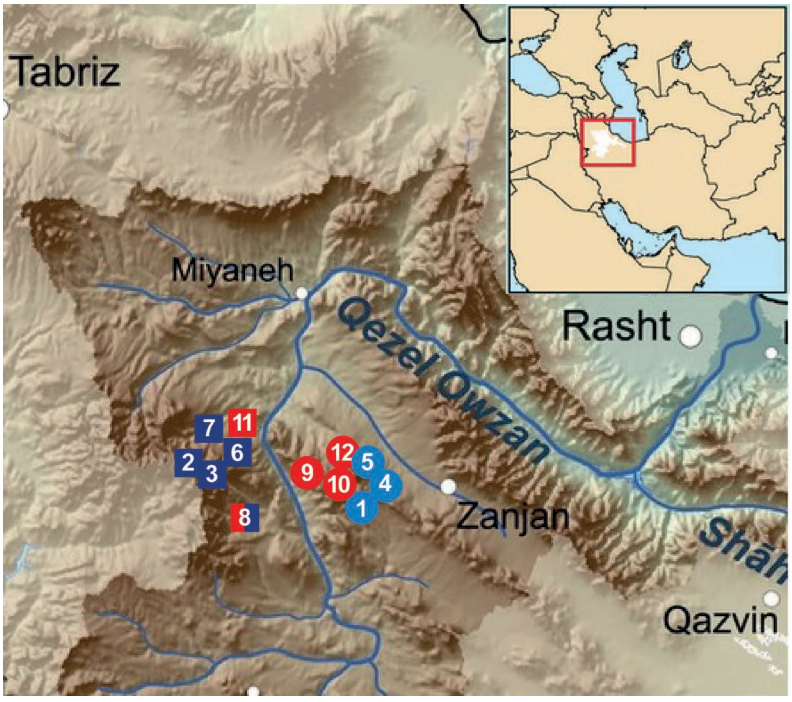

Figure 1 – Geographic positions of the 12 collection sites. Dots and squares represent individuals from the eastern and western sides of the Qezel Ozan River, respectively. Colours depict affiliation to genetic groups according to figure 2.

Table 1 – **Data of examined individuals of *Astragalus subrecognitus* in this study.**

Ind. No.	Herb. No.	Collector	Locality	Individuals observed in each sample location	W/E of river	Altitude (m)	Geographical coordinates
1	100334b	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, summit of Mount Damerlu, South slope	2	W	2750	36°40′25.6″N 47°58′04.0″E
2	8960	Bagheri	Zanjan: 14 km from Mahneshan to Pari, Holotype	1	E	2110	36°43′56.83″N 47°33′13.07″E
3	98278	Bagheri	Zanjan: Mahneshan to Pari	3	E	2200	36°44′1.63″N 47°33′12.55″E
4	100076	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, 6 km toward summit of Mount Damerlu	1	W	2530	36°39′09.9″N 48°00′00.3″E
5	100386a	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, summit of Mount Damerlu	2	W	2730	36°40′39.8″N 47°58′48.6″E
6	98279	Bagheri	Zanjan: Mahneshan to Pari	1	E	2200	36°44′7.72″N 47°33′2.38″E
7	6811	Bagheri	Zanjan: Mahneshan to Pari	1	E	2200	36°43′57.91″N 47°32′59.69″E
8	98281	Bagheri	Zanjan: Dandi to Aqkand	1	E	2300	36°41′9.06″N 47°35′25.68″E
9	100113	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, summit of Mount Damerlu	2	W	2830	36°40′57.9″N 47°58′06.2″E
10	100334a	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, summit of Mount Damerlu, South slope	1	W	2740	36°40′25.6″N 47°58′04.0″E
11	6808	Bagheri	Zanjan: Mahneshan to Pari	3	E	2300	36°44′16.16″N 47°33′4.48″E
12	100119	Mahmoodi	Zanjan: 45 km on the road from Zanjan to Dandi, Morassa village, summit of Mount Damerlu	1	W	2810	36°41′24.1″N 47°57′35.8″E

2002). SRAP, in contrast, aims for the amplification of coding regions of DNA with primers targeting ubiquitous motifs of open reading frames (Li & Quiros 2001). These markers have proven to be robust and highly variable (Robarts & Wolfe 2014). While ISSR loci are distributed throughout the entire genome, SRAP amplifies fewer and functional regions. Thus, ISSR markers might be more informative than SRAP in closely related taxa. We here use these dominant markers to infer the amount and distribution of genetic structure within *A. subrecognitus*, i.e. we want to evaluate if the river valley functions as a barrier to gene flow between the subpopulations resulting in even smaller effective population size for the species.

MATERIAL AND METHODS

Taxon sampling

We regularly monitored the occurrence of *A. subrecognitus* individuals during the years 2008–2017. Few young individuals were found within the population, which indicates on going natural regeneration, however the number of seedlings is low compared to mature individuals and seedlings are not present at all stands. The area where the species thrives is its type locality and some neighbouring regions from both sides of the Qezel Ozan River in Zanjan province, Iran (fig. 1). Leaf material was collected from a single individual at each of the 12 known stands of the species. Individual numbers at these stands range from one to three specimens but mostly only one individual is present. Herbarium vouchers were taken by removing a flowering twig without destroying the plants. They were deposited in the herbarium TARI. Sample information is given in table 1.

DNA extraction and amplification

Total genomic DNA of individuals of *A. subrecognitus* was extracted from leaf tissue using the method of Gawel & Jarret (1991). For performing ISSR analysis, 40 ISSR primers (Blair et al. 1999) were tested, from which eight (supplementary file 1) amplified reliably detectable and polymorphic DNA fragments from genomic DNA of *A. subrecognitus* accessions. The PCR amplification for ISSR was performed in 15 µL volume with 250 nM of primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1 U Taq polymerase (Ampliqon), and 50–100 ng of genomic DNA. The PCR was carried out at 95°C for 4 min for initial denaturation, 40 cycles of 1 min denaturation at the primer-specific annealing temperature (supplementary file 1), ending with an extension step of 2 min at 72°C, followed by a final extension step of 10 min at 72°C. PCR products were screened on 2% agarose gels stained with ethidium bromide under UV light.

For SRAP we used nine primer combinations (supplementary file 2) designed by Li & Quiros (2001). The PCRs

for SRAP were performed in 25 µL reaction volumes containing Taq 2× Master Mix Red (Ampliqon), 0.1 µM of each forward and reverse primer (supplementary file 2), 50 ng DNA template and 20 µL nuclease-free water. The PCR program conducted with the following cycle profile: 5 min of initial denaturation at 94°C followed by 5 cycles of 1 min denaturing at 94°C, 1 min annealing at 35°C and 1 min of extension at 72°C. After these, 35 cycles of 1 min denaturing at 94°C and 1 min annealing at 50°C ending with an extension step of 5 min at 72°C, followed by a final extension step of 10 min at 72°C. PCR products were screened as before.

Data analysis

For both marker classes the presence (1) or absence (0) of DNA fragments amplified in PCR was scored and both datasets were combined in a single binary data matrix. Genetic similarities were calculated based on Nei and Li distances (Nei & Li 1979) in PAUP* version 4.0a163 (Swofford 2002). The Neighbor-Joining (NJ) algorithm was used in PAUP for clustering and to calculate a phenogram that shows relationships between the studied individuals. Branch support was evaluated by 1000 bootstrap re-samples using identical settings as before. The correlation between genetic distances and geographic distances (r) was measured using the Mantel test (Mantel 1967) implemented in GenAlEx version 6.5 (Peakall & Smouse 2006). Principal coordinates analysis (PCoA) was performed using GenAlEx. To assess genetic variability detected in both datasets, basic parameters including Nei's gene diversity index (h), Shannon index (I) and percentage of polymorphic loci (PPL) were calculated from the data using Popgene version 1.32 (Yeh et al. 2000).

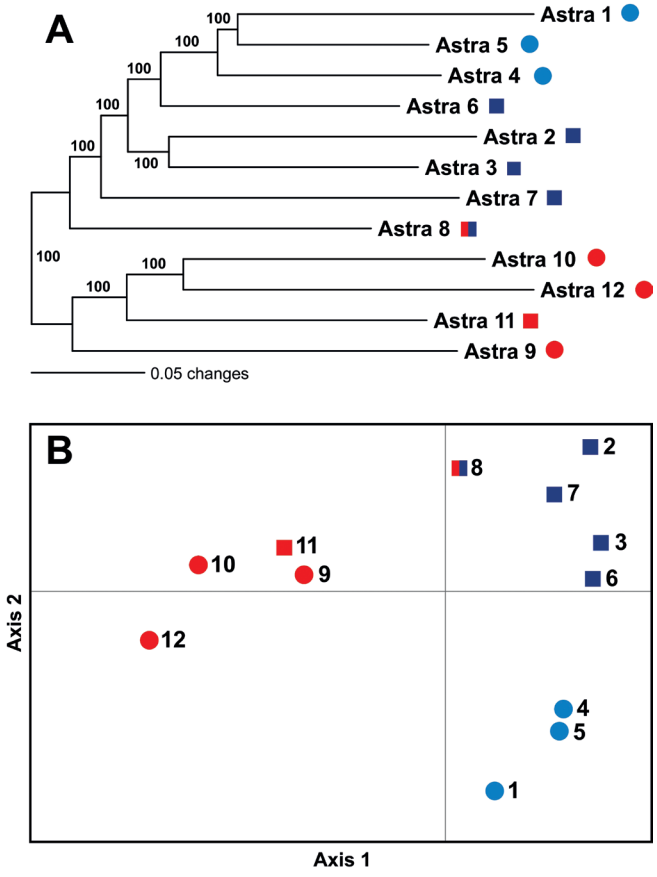

Figure 2 – **A**. Unrooted Neighbor-Joining tree of combined ISSR and SRAP markers of the individuals of *Astragalus subrecognitus*. Numbers along branches provide bootstrap support values. **B**. Principal coordinates analysis (PCoA) 2D plot based on the same dataset. Percentage of variation explained in PCoA is 21.79% and 20.71% for first and second axis, respectively. Dots and squares indicate the geographic origin of individuals from the eastern and western sides of the Qezel Ozan River (fig. 1), while colours depict group affiliation in the NJ and PCoA analysis.

RESULTS

A total of 260 bands for the combined ISSR and SRAP dataset were obtained (125 bands for ISSR, 135 bands from SRAP). The number of bands per ISSR marker ranged from 11 to 25 with an average of 18 bands per primer. The highest and the lowest numbers of bands for the ISSR markers were produced from the primer ISSR 6 (25 bands) and ISSR 880 (11 bands), respectively (supplementary file 1). The number of bands per primer combination for SRAP ranged from 6 to 26 with an average of 16 bands per primer combination. The highest and the lowest numbers of bands were detected by the primer combinations Me2+Em3 (26 bands) and Me3+Em2 (6 bands), respectively (supplementary file 2). A comparison of genetic information detected by ISSR and SRAP is provided in supplementary file 3.

The NJ analysis of genetic distances resulted in two clades separating individuals 1–8 (clade 1) from 9–12 (clade 2; fig. 2A). These genetic groups do however not concur with the geographic origins of the individuals east and west of the Qezel Ozan River, as individual 11 of clade 2 was collected in the east while 9, 10 and 12 originated from the west of the valley. This clustering is also reflected in the PCoA plot (fig. 2) where the two main groups were separated along axis 1. PCoA axis 2 separates in addition individuals from the westernmost stands west of the river (1, 4, 5) from the remaining individuals of clade 1 (2, 3, 6, 7). In the NJ tree these westerly individuals form a monophyletic group within clade 1 and are deeply embedded within the group of individuals derived from the slopes east of the river. Individual 8 is at the basal position within clade 1 and possesses some bands otherwise occurring in the individuals of clade 2. This intermediate position is also visible in the PCoA plot and is interpreted by us as the result of hybridization between members of both main clades.

In the Mantel tests for correlation of genetic with geographic distances, the ISSR data revealed low correlation ($r = 0.274$, $p = 0.030$), while SRAP data found no correlation between genetic distance and geographic distance ($r = 0.111$, $p = 0.180$). For the combined dataset the corresponding values were $r = 0.274$ and $p = 0.010$ (supplementary file 4).

DISCUSSION

Based on our data, ISSR resulted in slightly more polymorphisms (97.60%) than SRAP (93.33%; supplementary file 3). Anand et al. (2010) also indicated high polymorphism (93.07%) of ISSR bands across the different genotypes of

A. rhizhanthus. Although in our case the overall number of fragments is higher in the SRAP compared to the ISSR dataset. With the combined dataset it was possible to discern all individuals (fig. 2A). Although our samples covered all sites of the species' occurrence (fig. 1), at each location just one individual was collected as individual numbers are low (1–3) per site. Moreover, in a broad sense, samples from each side of the river could be considered as separate populations. The low number of studied individuals could cause a problem regarding detection of population structure but here evaluation of genetic diversity is in conflict with conservation of the species, which made us sample with lowest impact on

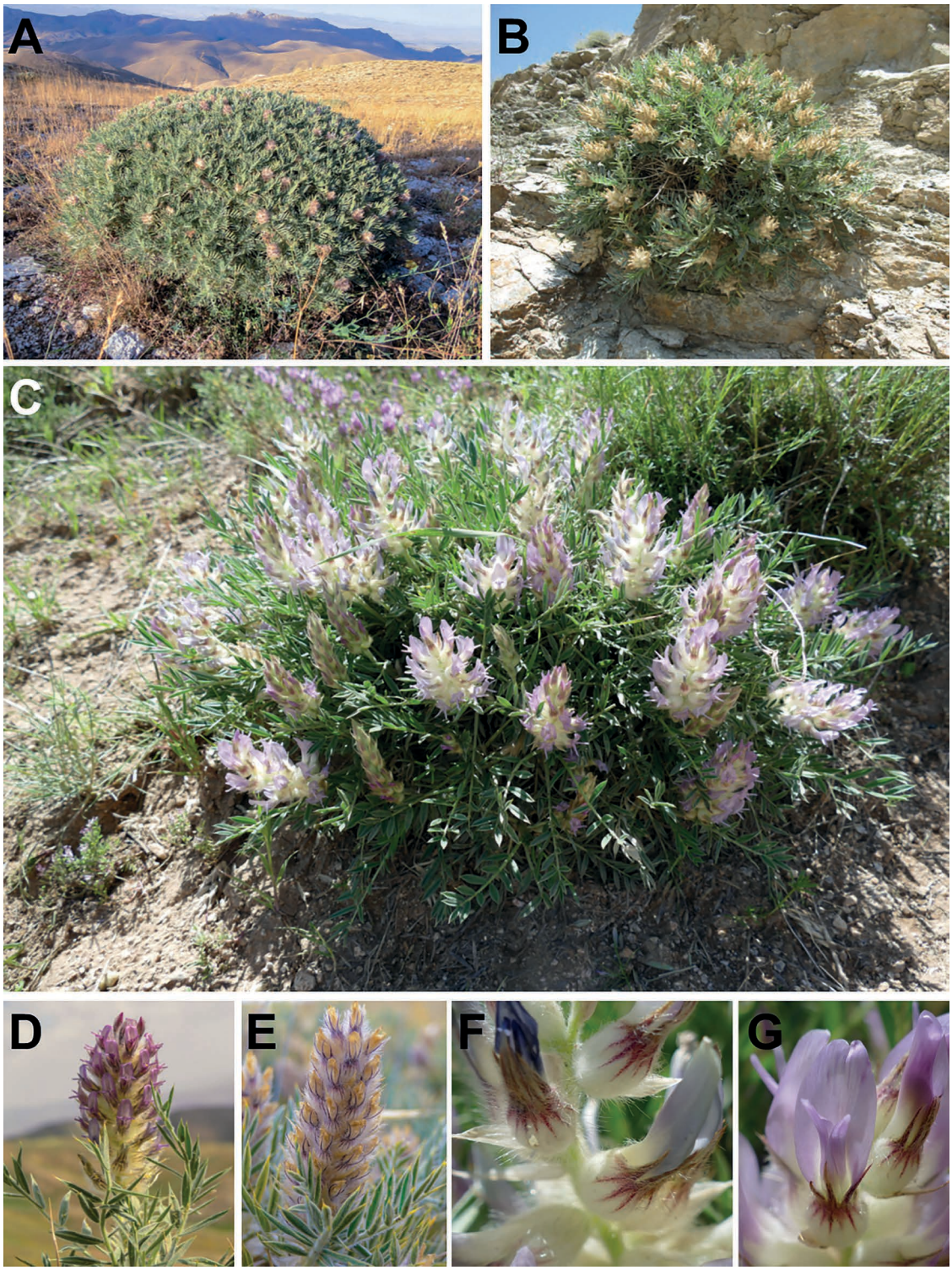

Figure 3 – *Astragalus subrecognitus*. **A**. Habitat in open steppe vegetation. **B**. Individual on rocky outcrop. **C**. Habit in natural setting. **D**–**G**. Inflorescences and flowers of accessions in different stages. Photographs by M. Mahmoodi (A, D, E) and A. Bagheri (B, C, F, G).

the study sites. This is not an unusual issue among rare and narrow endemic taxa. For example, Gaafar et al. (2014) used 5 populations to represent *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood, Ma et al. (2015) used 16 samples to study the genetic diversity of *Cotoneaster schantungensis* G.Klotz, Turchetto et al. (2016) used 13 populations for the rare species *Petunia secreta* Stehmann & Semir, and Cánovas et al. (2015) used seven populations of the endemic species *Viola cazorlensis* Gand. In our case, where we included 12 specimens of *A. subrecognitus*, we sampled all occurrence sites and about 50% of the known individuals of the species, which makes our results probably still representative.

We assume that current gene flow between the westerly and easterly populations is rather unlikely given the horizontal distance of c. 70 km in between and lack of populations low in the valley. However, during colder climate cycles in the past as, e.g., 20 000 years ago, the species' distribution might have been at lower elevations so that the river valley could be bridged by *A. subrecognitus*. Afterwards, with climate warming the population climbed the slopes on both sites of the valley and became isolated (again). This hypothesis of secondary contact of formerly subdivided and already differentiated populations of *A. subrecognitus* can explain the occurrence of members of the major genetic clusters within the extant eastern and western subpopulations. Although the recognition of admixture can be partly masked by the dominant nature of the markers we used, gene flow between the genetic clusters we found seems to be a slow process, as only one individual showed clear indications of hybridization (8) despite the presence of plants from the genetic groups on both sites of the river. Other taxa in Fabaceae also showed little gene flow between populations. Cires et al. (2013) analysed genetic diversity of *Genista legionensis* (Pau) M.Lainz and found low rates of gene flow among populations (Nm = 0.3874). Similarly, Cires et al. (2018) indicated low rates of gene flow among populations and low differentiation between extant populations of *Genista sanabrensis* Berm., Castrov. & Casaseca. Yang et al. (2017), in a study of population differentiation of *Lotus sessilifolius* L. using microsatellite markers, revealed low gene flow levels between adjacent populations. Thus, the values detected in *A. subrecognitus* seem to be no exception within the family. Our results mean on the one hand that the populations of *A. subrecognitus* might be able to retain their genetic diversity for the time being and that genetic drift might play currently no major role in this species, despite the few existing individuals of the species. On the other side, the small effective population size is further enhanced by the subdivision of the population by the river valley and the absence of extant gene flow between subpopulations. From this, it would be interesting to find out how old the individuals can become and what the actual reproduction rate within these populations is. Such demographic data would be of high additional importance to finally infer the possible future fate of species with very small population sizes.

Preliminary conservation status

Astragalus subrecognitus (fig. 3) only exists at two localities with c. 70 km maximum distance in northwestern Iran. The known area of occupancy (AOO) is only 28 km^2, and the extent of occurrence (EOO) is 108 km^2. There are no protection strategies for the region of the species in effect. Furthermore, there is pressure on habitats in this region caused by overgrazing that leads to high risk of habitat loss and fragmentation for *A. subrecognitus*. Regarding the low number of individuals of *A. subrecognitus*, we infer that conservation measurements for this endangered and narrow endemic species might be crucial. Hence, following the IUCN Red List criteria (IUCN 2012) *A. subrecognitus* is categorized as 'Endangered' (EN: B1ab(iii)+2ab(iii)).

SUPPLEMENTARY FILES

Four supplementary files are associated to this paper:

(1) Information on primers, band sizes and numbers of polymorphisms for the ISSR analysis (pdf)

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(2) Information on primer combinations, band sizes and numbers of polymorphisms for the SRAP analysis (pdf)

https://doi.org/10.5091/plecevo.2020.1648.2045

(3) Comparison between genetic information obtained through ISSR and SRAP markers (pdf)

https://doi.org/10.5091/plecevo.2020.1648.2047

(4) Relationship between genetic distance and geographic distance for the combined dataset (pdf)

https://doi.org/10.5091/plecevo.2020.1648.2049

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REFERENCES

Alexander J.A., Liston A., Popovich S.J. (2004) Genetic diversity of the narrow endemic *Astragalus oniciformis* (Fabaceae). *American Journal of Botany* 91(12): 2004–2012. https://doi.org/10.3732/ajb.91.12.2004

Anand K.K., Sivastava R.K., Chaudhary L.B., Singh A.K. (2010) Delimitation of species of the *Astragalus rhizanthus* complex (Fabaceae) using molecular markers RAPD, ISSR, and DAMD. *Taiwania* 55(3): 197–207. https://doi.org/10.6165/tai.2010.55(3).197

Bagheri A., Maassoumi A.A., Ghahremaninejad F. (2011) Additions to *Astragalus* sect. *Hymenostegis* (Fabaceae) in Iran. *Iranian Journal of Botany* 17(1): 15–19.

Bagheri A., Maassoumi A.A., Rahiminejad M.R., Brassac J., Blattner F.R. (2017) Molecular phylogeny and divergence times of *Astragalus* section *Hymenostegis*: an analysis of a rapidly diversifying species group in Fabaceae. *Scientific Reports* 7(1): 14033. https://doi.org/10.1038/s41598-017-14614-3

Bagheri A., Rahiminejad M.R., Maassoumi A.A. (2014) A new species of the genus *Astragalus* (Leguminosae-Papilionoideae) from Iran. *Phytotaxa* 178(1): 38–42. https://doi.org/10.11646/phytotaxa.178.1.4

Blair M.W., Panaud O., McCouch S.R. (1999) Inter simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 9(5): 780–792. https://doi.org/10.1007/s001220051135

Camacho F.J., Liston A. (2001) Population structure and genetic diversity of *Botrychium pumicola* (Ophioglossaceae) based on ISSR. *American Journal of Botany* 8(6): 1065–1070. https://doi.org/10.2307/2657089

Cánovas J.L., Jiménez J.F., Mota J.F., Sánchez Gómez P. (2015) Genetic diversity of *Viola cazorlensis* Gand., an endemic species of Mediterranean dolomitic habitats: implications for conservation. *Systematics and Biodiversity* 13(6): 571–580. https://doi.org/10.1080/14772000.2015.1079275

Cires E., Pérez R., Bueno A., Fernández Prieto J.A. (2013) Genetic diversity in peripheral and central populations of the Cantabrian endemism *Genista legionensis* (Pau) M. Laínz (Fabaceae). *Anales del Jardín Botánico de Madrid* 70(1): 91–96. https://doi.org/10.3989/ajbm.2313

Cires E., Sanna M., Vázquez V.M., Fernández Prieto J.A. (2018) Genetics and conservation of rare and endemic plants: the case of "*Genista sanabrensis*" (Fabaceae) in the Iberian Peninsula. *Mediterranean Botany* 39(2): 77–87. https://doi.org/10.5209/MBOT.60078

Esselman E.J., Jianqiang L., Crawford D.J., Windus J.L., Wolfe A.D. (1999) Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. *Molecular Ecology* 8(3): 443–451. https://doi.org/10.1046/j.1365-294X.1999.00585.x

Gaafar A.R.Z., Al-Qurainy F., Khan S. (2014) Assessment of genetic diversity in the endangered populations of *Breonadia salicina* (Rubiaceae) growing in the kingdom of Saudi Arabia using inter-simple sequence repeat markers. *BMC Genetics* 15(1): 109. https://doi.org/10.1186/s12863-014-0109-4

Gawel N.J., Jarret R.L. (1991) A modified CTAB extraction procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter* 9(3): 262–266. https://doi.org/10.1007/BF02672076

Harris J. (1999) RAPDs in systematics – a useful methodology? In: Hollingsworth P.M., Bateman R.M., Gornall R.J. (eds) Molecular systematics and plant evolution. Systematics Association: 211–228. London.

IUCN (2012) IUCN red list categories and criteria. Version 3.1, 2nd Edition. IUCN Species Survival Commission.

Li G., Quiros C.F. (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theoretical and Applied Genetics* 103(2–3): 455–461. https://doi.org/10.1007/s001220100570

Lock J.M., Simpson K. (1991) Legumes of West Asia. Royal Botanic Gardens, Kew.

Ma Y., Qu S., Xu X., Liang T., Zang D. (2015) Genetic diversity analysis of *Cotoneaster schantungensis* Klotz. using SRAP marker. *American Journal of Plant Sciences* 6: 2860–2866. https://doi.org/10.4236/ajps.2015.618283

Maassoumi A.A. (1998) *Astragalus* in the Old World, Check List. Research Institute of Forests and Rangeland, Tehran.

Mantel N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27(2): 209–220.

McGlaughlin M., Karoly K., Kaye T. (2002) Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellate* subsp. *breviflora* (Nyctaginaceae). *Conservation Genetics* 3(4): 411–420. https://doi.org/10.1023/A:1020507416654

Morris A.B., Scalf C., Burleyson A., Johnson L.T., Trostel K. (2016) Development and characterization of microsatellite primers in the federally endangered *Astragalus bibullatus* (Fabaceae). *Applications in Plant Sciences* 4(4): 1500126. https://doi.org/10.3732/apps.1500126

Nei M., Li W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* 76(10): 5269–5273. https://doi.org/10.1073/pnas.76.10.5269

Peakall R., Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources* 6(1): 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Podlech D., Zarre Sh. (2013) A taxonomic revision of the genus *Astragalus* L. (Leguminosae) in the Old World, vols. 1–3. Naturhistorisches Museum, Wien.

Robarts D.W., Wolfe A.D. (2014) Sequence-related amplified polymorphisms (SRAP) markers: a potential resource for studies in plant molecular biology. *Applications in Plant Sciences* 2(7): 1400017. https://doi.org/10.3732/apps.1400017

Rogenski C., Smith M., Esselman E. (2009) ISSR marker diversity of the threatened *Astragalus crassicarpus* var. *trichocalyx* in Illinois populations. *Transactions of the Illinois State Academy of Science* 102(3–4): 149–159.

Smith J.F., Bateman T.A. (2002) Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. *Western North American Naturalist* 62(3): 316–326.

Swofford D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0a150. Sunderland, Sinauer Associates.

Turchetto C., Segatto A.L., Mäder G., Rodrigues D.M., Bonatto S.L., Freitas L.B. (2016) High levels of genetic diversity and population structure in an endemic and rare species: implications for conservation. *AoB Plants* 8: plw002. https://doi.org/10.1093/aobpla/plw002

Wolfe A.D., Liston A. (1998) Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis P.S., Doyle J.J. (eds) Molecular systematics of plants II: 43-86. Springer, Boston.

Yang J.Y., Ojeda D.I., Santos-Guerra, A., Jaén-Molina R., Caujape-Castells J., Cronk Q. (2017) Population differentiation in relation to conservation: nuclear microsatellite variation in the Canary Island endemic *Lotus sessilifolius* (Fabaceae). *Conservation Genetic Resources* 10(2): 219–227. https://doi.org/10.1007/s12686-017-0778-1

Yeh F.C., Yang R., Boyle T.J., Ye Z., Xiyan J.M. (2000) POPGENE 32: Microsoft Windows-based freeware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Edmonton.

Zarre S., Podlech D. (1996) Taxonomic revision of *Astragalus* L. sect. *Hymenostegis* Bunge (Leguminosae). *Sendtnera* 3: 255–312.

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