Contrasting effects of local environment and grazing pressure on the genetic diversity and structure of *Artemisia frigida*

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Abstract

Drylands count among the most globally extensive biomes, and while many desert and dry rangeland ecosystems are under threat, genetic structures of dryland species are still rarely studied. *Artemisia frigida* is one of the most widely distributed plant species in the temperate rangelands of Eurasia and North America, and it also dominates in many habitats of Mongolia due to its tolerance to low temperatures, drought and disturbance. Local environmental conditions and grazing pressure can influence species performance and affect spatial patterns of genetic diversity in contrasting ways, and our study set out to evaluate such effects on the genetic diversity and structure of *A. frigida*. We first developed new species-specific Simple Sequence Repeats (SSRs) markers using whole genome sequencing. We then analysed 11 populations of *A. frigida* that had been sampled along a large climatic gradient in Mongolia, which were sub-structured according to three levels of grazing intensity. Estimates of genetic diversity at the population level were high ($H_O = 0.56$, $H_E = 0.73$) and tended to increase with higher precipitation and soil nutrient availability. Grazing had no effect on genetic diversity, however, a high number of grazing-specific indicator alleles was found at grazed sites. Genetic differentiation among populations was extremely low (global $G_{ST} = 0.034$). Analysis of Molecular Variance revealed 5% variance between populations along the climatic gradient, with 3% of the variance being partitioned among different grazing intensity levels. We found no relationship between geographic and genetic distances, and thus no isolation by distance in this widely distributed species. The relatively low genetic structuring suggests that considerable gene flow exists among *A. frigida* populations across the rangelands of Mongolia, in spite of the pervasive grazing in the region.

Keywords *Artemisia frigida* · Microsatellites · Population genetics · Mongolian rangeland

Introduction

The study of genetic changes in natural populations in response to environmental conditions, land use and their respective interactions has become a major research topic (Grant and Grant 2002; Sork 2018). Populations' and species' responses to local selection pressures and associated long-term survival is strongly influenced by their prevailing level of genetic diversity and its partitioning between and within populations (Li et al. 2018; Pazouki et al. 2016). A population's genetic diversity depends on a variety of factors including the species' geographical distribution, reproductive system and ecological traits (Nybohm 2004). Among more continuously distributed and outcrossing species, strong gene flow promotes genetic diversity and counteracts genetic differentiation among populations. For more isolated populations and/or self-fertilizing species where gene

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flow is more restricted, local genetic diversity can recede due to inbreeding and genetic drift as well as increased differentiation among populations (Kimura 2020). Moreover, the extent of gene flow between populations might not only depend on geographic distance but also on isolation patterns defined by environmental conditions (Sexton et al. 2014). Where gene flow occurs between populations from different environments, adaptation to local environmental conditions can be stalled; in contrast, where gene flow is prevalent across more similar environments, it may facilitate local adaptation by increasing population sizes and introducing new beneficial alleles (Sexton et al. 2014). Hence, climatic conditions can have a strong influence on natural selection among populations. In addition to such interactions with the local environment, human land use practices such as livestock grazing can have direct and compounding effects on a population's genetic structure as well as its genetic diversity. For some species, land use can have positive effects on genetic diversity: either directly as a result of herbivore damage accelerating mutation rates (Marcotrigiano 2000); indirectly following diaspore transport across long distances (epizoochory) (Bläß et al. 2010); or by providing safe sites for germination due to grazing-induced gaps in vegetation (Frank 2005). On the other hand, adverse effects of grazing on genetic diversity can occur as a result of decreasing effective population sizes or restrictions in gene flow following animal consumption of whole plants or damage to flowers or fruits (Suoto and Tadey 2019), or by the spread of selectively favoured genotypes adapted to grazing. Thus, understanding a population's genetic variability and sub-population structure with respect to both abiotic constraints and human land use can be critical to the successful conservation and management of its genetic resources. Drylands are particularly relevant in this respect, because they cover 30–40% of the global terrestrial surface, and they are subject to harsh climatic conditions and often intensively grazed. However, there is a definitive lack of studies on population genetics among dryland species (Greenville et al. 2017).

Central Asia hosts one of the world's most extensive dryland regions. Climate, soil conditions and grazing are among the key variables controlling plant species occurrence and abundance in this region (von Wehrden and Wesche 2007; von Wehrden et al. 2009). Central Asia includes Mongolia, northern and western China and the Tibetan Plateau, which together comprise a total of ca. 5 million km² of steppe habitat (Wesche et al. 2016), representing more than half of the entire Palearctic steppe biome. Grasslands cover ~80% of Mongolia and have been subjected to a number of studies assessing the impact of its extreme continental climate (Fernández-Giménez and Allen-Diaz 1999; Shinoda et al. 2010; Sternberg 2008), its > 4000 year old history of nomadic pastoralism (Herrero-Jáuregui and Oesterheld 2018; Hoshino et al. 2009), and the interacting effects of

climate and grazing on vegetation (Ahlborn et al. 2020; Bat-Oyun et al. 2016; Hoshino et al. 2009; Khishigbayar et al. 2015; von Wehrden et al. 2012). However, only a few studies have investigated grazing effects on population genetics of Central Asian rangeland species, and they yielded contrasting results: Wang et al. (2004) reported that for Inner Mongolia, intensive grazing led to declining genetic diversity in populations of *Artemisia frigida* Willd. Due to plant loss and consequent reduced sexual reproduction, gene flow and size of gene pool. Likewise, Shan et al. (2006) reported that increased grazing pressure resulted in stronger divergence among populations and decreased genetic diversity in *Stipa grandis* P.A. Smirn as a result of the smaller effective population sizes. In contrast, for *Stipa glareosa* P.A. Smirn., overall genetic diversity was lower in less-grazed populations (Oyundelger et al. 2020), and moderate grazing even promoted genetic diversity in *Stipa krylovii* Roshev. and *S. grandis* (Peng et al. 2015) as a result of higher availability of free soil patches for seed germination.

Such contrasting results underline the possible interaction of different effects, particularly combinations of climatic and land use factors influencing the genetic constitution of rangeland species (Wang et al. 2006; Wu et al. 2010; Zhao et al. 2006). Some studies have indeed assessed the effect of environmental factors on population genetics. For instance, Yang et al. (2013) found that for *Caragana* Fabr. in Central Asia, variation in precipitation had stronger negative effects on genetic connectivity than isolation by distance. Likewise, Zhang et al. (2018) and Jiang et al. (2019) demonstrated that total annual and winter precipitation levels were crucial in shaping the population structure of *Dactylis glomerata* L. and *Ammoiptanthus mongolicus* (Komarov) Cheng respectively, since low precipitation restricts plant reproductive success.

Another counteracting factor for plant reproductive success in Central Asian drylands is soil nutrient availability (Baranova 2018; Ronnenberg and Wesche 2011). Only a few studies have demonstrated that soil nutrient availability affects genetic variation in plant populations: Stevens et al. (2007) found that high nutrient-soil conditions increased growth (i.e., diameter, height and total biomass) in *Populus tremuloides* Michx., which led to increased genotypic variability in growth. Elsewhere, Reisch et al. (2020) revealed a clear impact of soil nutrient conditions on clonal diversity and genetic variation in alpine populations of *Carex nigra* L., which increased with phosphorous concentration but decreased with potassium concentration.

Our study region of Mongolia is ideal for testing the contrasting effects of environmental conditions and grazing pressure. Mongolia experienced a strong revival of pastoralism at the beginning of the 1990s, when the privatization and growth of livestock rearing prompted discussions on potential degradation of communal rangelands. Since the nineties,

the number of livestock has doubled to ca. 71 million (NSO 2020), which has had tremendous effects on both vegetation and soil (Jamsranjav et al. 2018). Besides grazing effects, climatic conditions such as annual precipitation, its variability and the occurrence of droughts have strongly influenced vegetation distribution and land degradation (Ahlborn et al. 2020; Dai 2011; Fatichi and Ivanov 2014). Intensive grazing in mesic steppes can lead to invasions by more drought-adapted species (xerophytisation) (Zemmrach et al. 2010). In temperate grasslands, intensive grazing has been shown to promote more drought-adapted *Artemisia* species such as *A. frigida* (Bai and Romo 1996; Jinhua et al. 2005), however, it is not clear whether certain ecotypes of this species were more successful than others. *Artemisia frigida* Willd. is a perennial, outbreeding and wind-pollinated species that is one of the most widely distributed rangeland plants (McArthur and Jeffrey 2004). While the species tolerates a large range of climatic conditions including cold and drought, seed production and germination of the species are largely dependent on climate, since in years of sufficient precipitation, plant growth and seed production can be promoted (Bai et al. 1995; Ronnenberg et al. 2007). As such, this drought-adapted species was chosen for the present study as it is well suited for studying the effects of environmental and grazing pressures on its population genetics, such that the results may inform rangeland management and conservation practice in Mongolia.

Recent developments in molecular science approaches have vastly enhanced the understanding of genetic variation and adaptation in many important, yet non-model, species. Non-coding DNA regions (introns) are highly variable and consequently widely used in population genetic and phylogeographic studies (Pleines et al. 2009). Microsatellites (i.e. SSRs) are commonly used as powerful markers in population genetics based on their high polymorphism and co-dominant inheritance (Kalia et al. 2011; Vieira et al. 2016). The SSRs still remain an important tool in the genomic era due to its cost-efficiency and the possibility to manually screen loci, which reduces error as the analysis relies on a relatively small number of loci compared to the high-throughput involved in sequencing fingerprints in e.g. RADseq (Hodel

et al. 2016). As a neutral marker, SSR cannot trace positive or negative responses of genotypes to selection; however, it can provide some insight into the overall distribution of certain genotypes, which can be characterized by favourable suits of traits under unknown (multigenetic) control.

Our first aim was therefore to develop species-specific SSR markers for *A. frigida* using whole genome sequencing, and then to validate them. In order to investigate genetic variation from larger to smaller scales (i.e. between and within populations to between sites) along a local grazing gradient, we conducted SSR analysis on eleven populations, which represented the first genetic study of *A. frigida* in Mongolia. We hypothesized the following: (i) Genetic diversity in populations of this drought and disturbance adapted species is high, but, because of wind-facilitated genetic exchange in the vast steppe plains, we expect moderate genetic structuring in space; (ii) Genetic differentiation is higher in those populations where grazing pressure is more pronounced than in non-grazed (least-grazed) populations, as a result of adapted genotypes and/or accelerated genetic exchange; and (iii) Genetic differentiation among populations increases with increasing distance in our study area due to the large geographical distance and strong environmental gradient from north to south.

Materials and methods

Study species: *Artemisia frigida* Willd. (Asteraceae)

Prairie sagewort is an aromatic perennial sub-shrub that is 20 to 60 cm in height (McArthur and Jeffrey 2004) and occurs primarily on fixed and semi-fixed silty-sandy rangelands. The entire plant is covered in dense silvery pubescence (Fig. 1a), and the lower stems are often strongly branched and woody. The species most likely evolved during the Pleistocene-Holocene (Yurtsev 1987), and it currently has an Amphi-Beringian distribution range, growing mainly in the steppe and prairie rangelands of Alaska, western Canada, the United States, Siberia, Kazakhstan, Mongolia and northern China (Harvey 1981). Prairie sagewort is a prolific seed

Fig. 1 **a** *Artemisia frigida* in its typical habitat in Mongolia, and **b** its distribution in Central Asia (samples were only collected from Mongolia; map drafted by Jan Treiber)

producer, with each 2.5 cm length of inflorescence containing approximately 1000 seeds (Harvey 1981). In addition to sexual reproduction, it also has a clonal growth strategy (Jinhua et al. 2005). The species was initially considered to be diploid ($2n=2x=18$; Garcia et al. 2004), however, Wan et al. (2011) identified a tetraploid cytotype ($2n=4x=36$) from an Inner Mongolian population, and Pellicer et al. (2010) confirmed the existence of tetraploid cytotypes in a Russian population. *Artemisia frigida* is one of the first perennials to become established on disturbed sites, and it tends to increase in abundance with overgrazing (Coupland 1950; Sarvis 1941) since it can tolerate harsh climatic conditions as well as mechanical disturbance. In Mongolia, it occurs throughout the country (Fig. 1b), and it serves as important forage for livestock (Jigjidsuren et al. 2015; Li et al. 2012).

Study design

Our study was conducted in central and southern Mongolia between June and September 2018, covering eleven populations along a 600 km north to south gradient with 100–300 mm S/N differences in mean annual precipitation (Table 1; Fig. 2a). Population no.2 (hereafter Pop2) was sampled from Hustai National Park (NP), which lies slightly west of the gradient line and was chosen to avoid the capital city Ulaanbaatar (Fig. 2a). Nested within this gradient, sub-populations were sampled along local transects radiating away from herders' camps or wells, which represented different levels of grazing intensity (150 m: heavily-grazed; 750 m: moderately-grazed; 1500 m: least-grazed) (Fig. 2b). Distances among sub-populations differing in grazing intensity were based on previous successful applications in the region (Earl 2012; Oyundelger et al. 2020; Sasaki et al. 2008) and elsewhere (Besnier and Glover 2013; Peper et al. 2011). However, grazing gradients reflect also small-scale spatial gradients, and therefore could not be directly distinguished from small-scale effects caused by isolation by distance. Pop2 was different because it was the only population where a fenced-off plot was available. The 10 m × 10 m large-mammal enclosure was built in 2003 and meets the criteria of a permanent sampling site for monitoring phenology (Tserendulam et al. 2018). In Hustai NP, the main grazers were Przewalski horse, marmot and red deer, while all other populations were grazed by goat, sheep, cattle or horse.

Sample collection and analyses

Field collection

Fresh leaf material was collected from a total of 361 individuals and stored in silica gel. For each population, representative herbarium specimens were deposited at Herbarium Senckenbergianum Görlitz. For the sub-populations, 12

Table 1 Characteristics of the study sites (localities, population code, coordinates, altitude, steppe habitat type, mean annual precipitation (MAP), coefficient of variation of annual precipitation (cvP), sample size (N) and genetic diversity indices estimated for each population (AD—allele diversity, EfN—number of effective allele, PPL (%)—Percentage of Polymorphic Loci, H_O —Observed Heterozygosity, H_E —Expected Heterozygosity, G_{IS} —Inbreeding Coefficient and Bruvo index among samples within a given population)

Locality, province	Pop code	E	N	Altitude (m.a.s.l)	Habitat	MAT (°C)	MAP (mm)	cvP	N	AD	EfN	PPL (%)	H_O	H_E	G_{IS}	Bruvo index
Guntin Davaa, Tuv	Pop1	106.74402	48.14746	1291	MoS	-0.29	304.0	1.30	36	14	6	47.0	0.43	0.74	0.43	0.63
Hustai National Park, Tuv	Pop2	105.93008	47.69145	1325	MoS	0.14	204.5	1.41	34	12	5	42.2	0.53	0.75	0.29	0.58
Khushigtin Khundii, Tuv	Pop3	106.83702	47.59631	1447	DryS	-0.23	175.4	1.44	36	16	7	53.0	0.50	0.74	0.33	0.56
Guntin Davaa, Tuv	Pop4	106.63280	47.33764	1650	MoS	-1.28	145.8	1.53	36	15	5	47.6	0.49	0.73	0.33	0.62
Guntin Davaa, Tuv	Pop5	106.55361	47.06427	1439	DryS	0.35	177.5	1.53	36	16	6	52.4	0.60	0.73	0.17	0.61
Bayantsagaan, Tuv	Pop6	106.63921	46.79678	1392	DryS	1.00	138.6	1.58	15	11	6	37.7	0.61	0.73	0.17	0.56
Hayaa, Dundgovi	Pop7	106.64373	46.56192	1345	DeS	1.45	124.8	1.65	24	14	6	47.3	0.59	0.74	0.20	0.54
Deren, Dundgovi	Pop8	106.54400	46.36338	1389	DeS	1.50	131.3	1.54	36	16	6	52.1	0.61	0.70	0.12	0.53
Saintsagaan, Dundgovi	Pop9	106.29334	45.91903	1468	DeS	1.74	111.3	1.73	36	15	6	50.6	0.62	0.71	0.13	0.52
Luus, Dundgovi	Pop10	105.85641	45.62553	1425	DeS	2.30	119.7	1.53	36	16	7	52.7	0.61	0.76	0.20	0.58
Dund Saikhan Nuruu, Umnugovi	Pop11	103.59987	43.74417	2171	DryS	0.58	188.0	1.32	36	13	5	41.6	0.56	0.67	0.16	0.51
Mean										14	6	47.7	0.56	0.73	0.23	0.57

Steppe habitat types: MoS - mountain steppe, DryS - dry steppe and DeS - desert steppe

Fig. 2 **a** Map of 11 study sites (corresponding to Table 1) showing mean annual precipitation totals; contour lines indicate elevation. Rainfall data were derived from CHELSA and elevation data from TanDEM-X—Digital Elevation Model (German Aerospace Center (DLR) 2018); map drafted by Jörg Lorenz. **b** Experimental design

individuals of *A. frigida* were sampled within a 10 m × 10 m plot, from which plant community composition and total vegetation cover of vascular plants were also recorded to characterize the community properties. Plots were then classified by steppe type according to a standard classification for Mongolian steppe vegetation (Tuvshintogtokh 2014). In addition, a sample of topsoil (0 ± 5 cm depth) with fine plant roots and some humic layer was taken at each plot to examine nutrient availability.

Soil preparation and analyses

Soil samples were initially dried in the laboratory for 18 h at 75 °C before being sieved (2 mm coarse screen) and analysed further. Soil pH and electrical conductivity (EC; as a proxy for salinity) were measured in the laboratory with a pH/EC meter (water: soil volume = 5:1). Plant available P, Mg, Ca, K, Al were extracted using the Olsen P method (22 °C, 200 rpm for 30 min; Sims 2000) followed by ICP determination. Nutrient contents in these extractions were measured by spectrometry (ICP-OES, Institute of Soil Science, Hannover University). By drying samples at 105 °C for 24 h, the rest water was determined. The carbonate content was first assessed using a simple test with 10% HCl, and samples showing a reaction were further analysed using a calcimeter following Scheibler's method (ON L 1084-99, 1999). Total N and C concentrations were

to study the relative effect of precipitation and grazing in Mongolian steppes. Eleven study sites were visited along a north to south gradient, and at each site sub-populations were sampled along local transect representing different grazing intensities (A–C, initial draft C. Römermann)

analysed using a CN analyser (Vario Pyro Cube; Elementar, Langensfeld, DE). The remaining water was used to calibrate the nutrient contents per gram of soil and the carbonate content to correct C/N measurements.

Climate data extraction

Twenty years (1994–2013) of meteorological data including mean annual temperature (MAT), mean annual precipitation (MAP), mean spring temperature (SpringT; March–May), mean summer temperature (SummerT; June–August) and mean summer precipitation (SummerP) were retrieved for each locality from the high resolution CHELSA_V1 dataset (Karger et al. 2017). The coefficient of variation of annual precipitation (cvP) was estimated based on the retrieved MAP data, and it was also used as predictor, since cvP is a critical driver of rangeland dynamics (von Wehrden et al. 2012).

Detection of SSRs, primer design and marker validation

Library preparation and quality control

Two individuals of *A. frigida* selected randomly from two distinct populations (collection ID: P8_15 and P9_17;

BioSample accession numbers: SAMN16882070 and SAMN16882071) were used to develop new SSR markers by applying whole genome sequencing (WGS). High molecular weight DNA was extracted using the Mag-Bind® Plant DNA DS kit (Omega Bio-tek, Norcross, USA). Genomic DNA was quantified using a Qubit 4 Fluorometer (Life Technologies), and library preparation and sequencing were conducted in the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). As an input, 1.0 µg DNA (~20 ng/µl) per sample was used for covarizing (Covaris S220, Duty Factor: 8%, Peak Incident Power: 160, Cycles Per Burst: 200, Time: 60 s), and BluePippin (Sage Science) was used to produce fragments with an average size distribution of 450 base pair (bp) lengths. Library preparation was carried out as described by Meyer and Kircher (2010), and fragment size distribution was evaluated on an Agilent BioAnalyzer High Sensitivity DNA Kit (Agilent Technologies, Inc). DNA concentration was measured using the Qubit DNA Assay Kit in a Qubit 2.0 Fluorometer (Life Technologies). Finally, the DNA concentration of the library was checked by a quantitative polymerase chain reaction (qPCR) run, allowing for a targeted quantification of fragments with adaptor sequences on both ends before cluster generation, which was run on Illumina cBot following Illumina's recommendation. Paired-end sequencing (2×250 bp) of the clusters was performed on the Illumina HiSeq 2500 platform, with a 1% Illumina PhiX library used as an internal control. The resulting 50 million raw reads were checked with FASTQC for quality control (Andrews 2010) and trimmed with Trimmomatic v0.39 (Bolger et al. 2014). WGS raw sequencing data were submitted to the NCBI Sequence Read Archive (SRA) and made publicly accessible under BioProject (accession number PRJNA680535; available at: <https://www.ncbi.nlm.nih.gov/sra/PRJNA680535>).

Bioinformatics and SSR development

In order to identify any low coverage contigs, the velvet v1.2.10 (Zerbino 2010) de novo genome assembler was utilized (kmer size: 45; maximum coverage parameter: 500; minimum contig length: 300 bp). Putative SSR markers were detected in the contigs of the assembled nuclear genome using the MISA tool with default parameters (Thiel et al. 2003). The contigs possessing SSR markers were compared between both *A. frigida* assemblies using blast + v2.7.1 (Camacho et al. 2009), and only contigs with differences in their repeat length were selected for further analysis. Back mapping of the raw reads against contigs containing di- and tri-nucleotide SSR motives were conducted on Bowtie2 v2.2.4 (Langmead et al. 2009), further examined by converting the mapping results and extracting the mapped contigs with SAM tools v1.2 (Li et al. 2009) and visualized in Geneious® v. 10.2.6 (<https://www.geneious.com>). Editing

was not necessary since the reads were correctly mapped to the contigs. Primers were designed using the ThermoFisher tool using default parameters 18–22 nucleotides long, a GC content of ca. 40–50% (Tm between 58–60 °C) and, where possible, G/C-rich at the 3'-end. Heterodimers of the primers were checked and 40 primers that met the requirements were preferentially selected to produce a PCR product in the range of 100–600 bp, allowing for multiplex PCR. Primers were then synthesized by Metabion International AG, with the forward primer of each set being tailed with a M13 sequence (TGT AAA ACG ACG GCC AGT; according to Schuelke 2000) to facilitate dye-labelling. The nucleotide sequences of the primer pairs used for each microsatellite are shown in Table 2. The loci named 'Arfi' are the markers developed in this study, and the loci names starting with 'Ch' are from the master thesis of Wang (2011).

Application of SSR markers

Genomic DNA was extracted according to the ATMAB protocol (Doyle and Doyle 1987) with modifications by Ziegenhagen (1990). Developed SSR markers were tested with randomly chosen samples from the 11 populations. A total of 40 primer pairs were run as an initial screening for 8–16 samples. After PCR optimization, seven of these loci proved to be reproducible and were reused with the same set of samples to receive positive controls (for details about primers see Table 2). In addition, ten SSR markers published in the master thesis of Wang (2011) were tested with our samples in parallel. Potential duplications between both markers' sources were checked beforehand. Based on reproducibility and polymorphism, four loci published by Wang (2011) were chosen in addition to our seven newly developed markers. Thus, a total of 11 SSR amplifications were performed in a total volume of 12.5 µl, and different PCR reaction mixtures and cycling programs were used (Suppl. 1 Table 1). Fragment sizes were determined by the central laboratory of the Senckenberg Biodiversity and Climate Research Center (SBiK-F, Germany) using an ABI3730 sequencer with LIZ600 size standard (Life Technology). The program Genographer version 2.1.4 (Benham 2001) was used for the initial screening of the microsatellite data, and Geneious® 10.2.6 (<https://www.geneious.com>) was used to score allele sizes. To estimate genotyping error, DNA amplification and fragment scoring were replicated for the randomly selected (8–16) samples (Hoffman and Amos 2005). In addition, each plate included two to three positive controls, which ensured that all the replications provided identical results. The number of microsatellite alleles per locus was compatible with the proposed tetraploidy, since individuals displayed a maximum of four alleles per locus. Due to a lack of information on allele dosage in the polyploids, only the allelic phenotypes (and no genotypes) could be assessed. Thus, deviations

Table 2 Characterization of eleven polymorphic microsatellite markers used in the population genetic study of *Artemisia frigida*

No.	Locus	Repeat motif	Primer sequences (5′–3′)	Ta (°C)	Allele size range (bp)	Florescent dye	PCR type	References
1	<i>Arfi2</i>	(CA) ₁₉	F: TGT AAA ACG ACG GCC AGT TAG ACAATAAGCACACAGAACC R: GAGAGCTAGAGGGATCATTACG	55	467–552	VIC	Multiplex	This paper
2	<i>Arfi15</i>	(AAG) ₁₄	F: TGT AAA ACG ACG GCC AGT GTACCGGTGGATACCTTACG R: CCTAATCCAACACACAAGAA	55	207–300	VIC		This paper
3	<i>Arfi5</i>	(AC) ₁₁	F: TGT AAA ACG ACG GCC AGT TCCACATGCATGACACTC R: CAGTTGGAAGTATGGGTTTG	55	230–310	6 FAM	Multiplex	This paper
4	<i>Arfi13</i>	(GAT) ₁₂	F: TGT AAA ACG ACG GCC AGT ACACGCAGAGATGATGAGT R: CACAATACCAGCCTTTGTC	55	406–520	6 FAM		This paper
5	<i>Arfi16</i>	(TCA) ₁₀	F: TGT AAA ACG ACG GCC AGT GGTTCTTTCATCAAGTCCA R: ATTGCCAATGAACATCCAG	55	200–275	PET	Singleplex	This paper
6	<i>Arfi14</i>	(ACA) ₈	F: TGT AAA ACG ACG GCC AGT TGTTATGTTGCCCTGTG R: GCATGTTAGACCCCTTGA	55	222–335	NED	Singleplex	This paper
7	<i>Arfi19</i>	(CAA) ₈	F: TGT AAA ACG ACG GCC AGT GTCTAAATCTGAAGGCGG R: CAACTCATCCCATGGTCAC	55	140–318	VIC	Singleplex	This paper
8	<i>Ch459</i>	NA	F: TGT AAA ACG ACG GCC AGT TTACCAAATGCTCCATTGAC R: TTGGAAGCACCATGTGAC	42	103–200	6 FAM	Singleplex	Wang (2011)
9	<i>Ch468</i>	NA	F: TGT AAA ACG ACG GCC AGT TAGGGTTGCAGAAGATAAAC R: CTTTAGTAGGAAGTGAAGAAGC	58	160–226	NED	Singleplex	Wang (2011)
10	<i>Ch477</i>	NA	F: TGT AAA ACG ACG GCC AGT GGGACCCAATTTACCGAAAG R: CAAAGAATGGGTAAAGCTCAGTG	58	103–140	VIC	Singleplex	Wang (2011)
11	<i>Ch484</i>	NA	F: TGT AAA ACG ACG GCC AGT TCGTTCAAACGCGTACACAC R: AGCATGCATGAAGAAACAAGC	58	146–240	PET	Singleplex	Wang (2011)

from the Hardy–Weinberg equilibrium could not be calculated, nor could the frequency of null alleles be estimated (Dufresne et al., 2014).

Analysis of genetic variation and population structure

Population genetic structure

Population structure was estimated by Bayesian clustering with STRUCTURE v. 2.3.4 conducted in R-package *ParallelStructure* (Besnier and Glover 2013). To choose the model with the best fitting number of clusters (K), Evanno's method was employed with Structure Harvester (Earl 2012; Evanno et al. 2005). Results of Bayesian clustering were illustrated using Distruct v. 1.1 (Rosenberg 2004). To examine the

distribution of genetic variation between populations and sub-populations, Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) was performed in R-package poppr (Kamvar et al. 2014) based on the individual level Bruvo distance matrix estimated in R-package Polysat v. 1.7 (Clark and Jasieniuk 2011; Suppl. 2 Table 1). The Bruvo metric was developed specifically to assess microsatellite data in polyploids and it considers allelic dosage in ambiguous polyploid genotypes using a step-wise mutation model (Bruvo et al. 2004; Dufresne et al. 2014). As such, we used the Bruvo distance wherever possible and estimated mean Bruvo distances between population and sub-population levels using the 'meandist' function in R-package vegan (Oksanen et al. 2007; Suppl. 2 Table 2 & 3). To facilitate comparisons with other studies, we also estimated pairwise G_{ST} (Nei and Chesser 1983) for (sub-) population levels in Polysat (Supl.

2 Tables 4 and 5). Principal Coordinate Analysis (PCoA) was conducted to assign individuals to populations based on (i) sub-population level mean Bruvo, and (ii) individual level Bruvo genetic distances using the R-package ape (Paradis et al. 2019). On the sub-population level ordination, environmental variables were fitted *post hoc* using vegan (Oksanen et al. 2007) and ggplot2 (Wickham 2011) to reveal environmental variables and grazing intensities that could be significantly associated with the population's genetic structure. Environmental variables covered geography (latitude, longitude, and altitude), climate (MAT, SpringT, SummerT, MAP, SummerP and cvP), soil nutrient content (P, Mg, Ca, K, Al, C, and N) and vegetation type (mountain steppes, dry steppes, and desert steppes). Data on geographic and climatic variables are given in Table 1, and vegetation cover, plant species richness and analysed soil nutrient values are provided in Suppl. 1 Table 2.

In order to evaluate the number of alleles specific to certain grazing levels, data were exported from Polysat as a dominant marker matrix (presence/ absence of alleles; Suppl. 2 Table 6) and used for Indicator Species Analysis (ISA) implemented in R-package indicpecies (De Caceres and Jansen 2014). The analysis was based on: (i) the frequency of a given allele within a given groups (grazing level), and (ii) the frequency distribution across groups (represented by the share of overall occurrence found for a given group). The resultant frequencies for (i) and (ii) were multiplied and the result tested for significance using a permutation test, which examined whether alleles were significantly associated with a certain group. By taking the entire dataset into account, the ISA ensured that alleles potentially associated with grazing did not simply reflect small-scale isolation by distance effects.

Genetic diversity

Genetic diversity among and within populations was assessed using GenoDive v. 3.04 (Meirmans 2020) and the R-package Polysat v. 1.7 (Clark and Jasieniuk 2011; R Development Core Team 2018), both of which offer tools for handling microsatellite data at any ploidy level with allele dosage correction. First of all, diversity and polymorphism of the employed markers were analysed using G-statistics in GenoDive. Next, based on the individual level Bruvo distance, we developed a surrogate for gene diversity by calculating mean Bruvo distances among individuals for any given population using the 'mrpp' function in R-package vegan (Oksanen et al. 2007). As a result, we obtained a single value for each population (hereafter 'Bruvo index'), which we used as a proxy for genetic diversity. In addition, allele diversity (AD, mean number of alleles per locus), effective number of alleles (EfN, number of alleles in a population weighted for their frequencies), percentage of polymorphic loci (PPL, %), observed heterozygosity

(H_O), expected heterozygosity (H_E) and inbreeding coefficient (G_{IS} ; analogous to F_{IS}) were estimated in GenoDive for all (sub-) populations such that comparisons with other studies could be made (Table 1). To overcome the problem of higher diversity in polyploids compared to diploids, we estimated the expected heterozygosity (H_E , gene diversity) according to Nei (Meirmans 2020), representing a generic method that is independent of ploidy level. Observed heterozygosity (H_O) was based on allelic phenotypes (e.g. AB), with both full and partial heterozygosity being taken into account (possibilities of genotypes AAAB, AABBB or ABBBB) to allow for direct comparisons between H_O and H_E (for further information see Meirmans 2020). Correlations among different genetic diversity indices are given in Suppl. 1 Table 3.

Furthermore, we performed a Linear Mixed Model Analysis implemented in R-package lme4 (Bates et al. 2007) to contrast the effects of local environment and grazing pressure on genetic diversity. Based on the data distribution and variables, the Bruvo index was used as a response variable. Significant environmental variables revealed by PCoA (MAT, MAP, cvP, latitude soil P and Ca) were used as continuous predictors, while 'grazing' was included as a factor. The response variable was log-transformed, and all predictors were scaled to zero mean-unit-variance prior to modelling to make effect sizes comparable. Starting with the most comprehensive model, we conducted model simplification by dropping the least relevant models, and all complex models that did not result in significantly better results were rejected. We plotted residuals of the final parsimonious model to check for possible deviations from normality and reasonable distribution of variances.

Relationship between genetic and spatial distances

The relationships between genetic and geographical distances (Euclidean distances) were analysed for 11 populations of *A. frigida* using the Mantel test (10,000 randomizations) in the R-package vegan (Oksanen et al. 2007). An additional Mantel test was conducted between genetic distance and differences in plant community composition (Bray–Curtis distance of log-transformed vegetation cover). Correlation with geographical distance was based on population level mean Bruvo distance, while sub-population level mean Bruvo distance was used for correlation with plant community composition based on the available data for each grazing level within populations. All analyses were conducted in R 3.5.1 (R Development Core Team 2018).

Results

Microsatellite marker profile

The 11 SSR markers proved to be highly polymorphic, with an overall of 76% polymorphic information across all primers (Table 3). The locus *Arfi16* was less polymorphic than others, however, it did not have a negative impact on analyses, according to pre-analyses excluding the locus (data not shown). Therefore, further analyses were based on all available 11 loci. The most polymorphic loci were *Arfi2* and *Arfi15*, as shown by the number of effective alleles (EfN) and the expected heterozygosity (H_E ; Table 3). The 11 loci amplified in *A. frigida* yielded a total of 317 alleles across all samples. The total number of alleles (Num) was high in all the loci, albeit the effective number of alleles differed substantially.

Population genetic structure

The coefficient of genetic differentiation across all populations was low (Global $G_{ST}=0.034$, $p<0.001$), with genetic variation being partitioned to 5% among populations and 3% between sub-populations (among different grazing intensity levels), and the largest share resided between individuals (92%, $p<0.001$, AMOVA; Table 4). The STRU CTURE analysis suggested the 11 populations form two genetic clusters (best fitting $k=2$) that are not completely segregated, although Pop2 and Pop10 tended to show some genetic admixture (Suppl. 1 Fig. 1). The PCoA also revealed a similar pattern as the STRU CTURE analysis, with the first two axes explaining only about 14% of the genetic variation (Fig. 3). Furthermore, PCoA ordination with *post hoc* fitted predictor variables revealed significant correlations between

Table 4 AMOVA for 11 *A. frigida* populations, and different grazing intensity levels within populations

Source of variance	Df	Sum Sq	Variance component	% Total	Φ_{ST}
Between populations	10	8.93	0.02	4.8***	0.08
Between sub-populations	20	8.10	0.01	3.3***	0.03
Within sub-populations	330	94.73	0.29	92***	0.05
Total	360	111.76	0.31	100	

Df - degrees of freedom, Sum Sq - sum of square, Φ_{ST} - Phi statistics, p-values based on 999 permutations

genetic structure and environmental variables (MAT, MAP, cvP and latitude) and thus major habitats, but no main effect of grazing on genetic similarity was found (Fig. 3 & Suppl. 1 Fig. 2).

According to the ISA, some indicator alleles were associated with a certain grazing level across widely spaced populations, i.e. some alleles were prone to occur at only one grazing level, and/ or were absent at other levels, irrespective of their location along the main climatic gradient (Suppl. 1 Table 4). A total of 63 alleles were significantly indicative, which corresponds to 20% of all alleles. The heavily-grazed sites had 31 indicator alleles, which was more than three times that found in the least-grazed sites. In addition, 23 alleles were indicative of moderately grazed sites, indicating that certain alleles are associated with higher grazing intensity.

Genetic diversity

Populations along the main climatic gradient differed with respect to their genetic diversity (Table 1). The observed heterozygosity (H_O) among populations ranged from 0.43

Table 3 Characteristics of the 11 employed markers using G-statistics based on Nei from GenoDive (PIC—Polymorphic Information Content, Num—number of alleles, EfN—number of effective alleles, H_O —Observed Heterozygosity, H_E —Expected Heterozygosity, G_{ST} —Fixation Index, G_{IS} —Inbreeding Coefficient and P-values were calculated using G_{ST} (Nei))

Locus	PIC	Num	EfN	H_O	H_E	G_{ST}	G_{IS}	P-value
<i>Arfi2</i>	0.95	29	13	0.72	0.94	0.01	0.37	0.01
<i>Arfi5</i>	0.65	32	2	0.36	0.56	0.04	0.29	0.01
<i>Arfi13</i>	0.78	29	3	0.50	0.71	0.03	0.16	0.01
<i>Arfi14</i>	0.84	28	4	0.33	0.80	0.05	0.21	0.01
<i>Arfi15</i>	0.94	28	12	0.73	0.93	0.01	0.24	0.01
<i>Arfi16</i>	0.25	18	1	0.16	0.17	0.01	0.59	0.01
<i>Arfi19</i>	0.79	42	3	0.65	0.72	0.02	0.12	0.01
<i>Ch459</i>	0.86	35	7	0.78	0.86	0.02	0.09	0.01
<i>Ch468</i>	0.90	34	8	0.73	0.88	0.02	0.10	0.01
<i>Ch477</i>	0.66	13	3	0.55	0.68	0.02	0.16	0.02
<i>Ch484</i>	0.80	29	4	0.62	0.74	0.05	0.16	0.01
Overall	0.76	29	5	0.56	0.75	0.03	0.23	0.01

The loci named ‘*Arfi*’ were developed in this study, and the loci names starting with ‘*Ch*’ were taken from the master thesis of Wang (2011)

Fig. 3 Principle Coordinate Analysis (PCoA) based on population level mean Bruvo distance using 11 SSR markers for the populations of *A. frigida*. Environmental predictors were fitted *post hoc* on the ordination plot (only those that passed $p < 0.05$ according to test with 1000 permutations are shown). MoS - mountain steppe, DryS - dry steppe and DeS - desert steppe

to 0.62 in Pop1 and Pop9 respectively; the expected heterozygosity (H_E) within populations was highest in Pop10 ($H_E = 0.76$). In contrast, allele diversity and number of effective alleles were relatively invariant among all populations. Across all populations, the Average Inbreeding Coefficient was low ($G_{IS} = 0.23$) and Percentage of Polymorphic Loci was 48%, showing a moderate level of polymorphism. The highest Bruvo index was found in Pop1 (Bruvo index = 0.63) and the lowest was in Pop11 (Bruvo index = 0.51). This estimator was used for subsequent statistical tests involving genetic diversity.

Diversity within populations, i.e. among different grazing intensity levels, was moderate (Suppl. 1 Table 5). The highest genetic diversity was found in the heavily-grazed plot of Pop3 (Bruvo index = 0.67), while the lowest was found in the moderately-grazed plot of Pop11 (Bruvo index = 0.46). According to our linear mixed effect model, genetic diversity of *A. frigida* was affected by total summer precipitation and soil phosphorus content (Table 5). Estimates indicate that the Bruvo index increased with higher amounts of summer precipitation and soil phosphorus content. We further checked their interaction effect, but no interaction was found. Moreover, we found no significant grazing effect on genetic diversity or significant differences among different grazing intensity levels in the linear mixed effect model.

Relations between genetic and spatial distances

A Mantel test revealed no correlation of geographical and genetic distances between populations ($r^2 = 0.02$, $p = 0.1$), as such, no indication of isolation by distance across the

Table 5 Summary of Linear Mixed Model assessing the effects of environmental and climatic variables and grazing on Bruvo index as an estimator of genetic diversity of the populations of *A. frigida* in Mongolian drylands

	Estimate	Std. Error	Pr(> t)	
(Intercept)	0.56	0.006	< 0.001	***
Summer precipitation	0.02	0.008	0.04	*
Soil phosphorous	0.02	0.008	0.01	*

The interaction term did not contribute to the model's quality so was skipped. According to model simplification, grazing was not significant

Pr(>|t|) – significance p-value

studied range of 600 km in southern central Mongolia was found. A further test with plant community composition also revealed a negligible correlation ($r^2 = 0.001$, $p = 0.2$). Taken together, there was little evidence to suggest that genetic distances were strongly influenced by simple spatial trends.

Discussion

SSR marker development and application in polyploid *Artemisia*

In population genetics, several molecular markers are proposed and employed. In particular, co-dominant microsatellite markers facilitate the estimation of population genetic parameters in polyploids compared to dominant fingerprint systems (Clark and Jasieniuk 2011). Our newly developed

SSR markers for the widespread polyploid species *A. frigida* has proved reliable and it provides a sound basis for further research, including on other species within the genus, as we could successfully cross-amplify three loci in the rather distantly related annual *A. scoparia* Waldst. et Kit. We also improved cost efficiency, because allele sizes and fluorescent dyes of each marker were adjusted to allow for multiplexing four to seven loci in one well for genotyping.

Polyploidy causes major obstacles for population genetic analysis. It matters whether a taxon is auto- or allopolyploid (Dufresne et al. 2014), however, this is often neither known nor easily detected. Since dosage information is generally missing, complete genotyping is still not feasible with SSR markers, and thus genetic diversity from polyploids may often be overestimated. Few software applications and statistical approaches have been proposed to correct possible bias in polyploids (see Dufresne et al. 2014 for a list). The R-package Polysat (Clark and Jasieniuk 2011) and the software GenoDive (Meirmans 2020) are specifically designed to handle microsatellite data with polysomic inheritance and the need for dosage correction. As such, for the present study, we used them to analyse the at least partly tetraploid samples of *A. frigida*. To ensure consistency, one measure of genetic diversity (Bruvo index) was employed through most of our analyses, with others being provided for cross comparisons with published studies (Table 1). However, it is acknowledged that such comparisons may not always be feasible due to the different marker systems being used. Moreover, in SSR analysis, choosing both diversity metrics and software packages may employ different dosage corrections and mutation models, even for the same index calculation (Dufresne et al. 2014).

Population genetic differentiation

Artemisia frigida is a wide-spread, wind pollinated long-lived perennial that is valued as a highly nutritional rangeland plant in Mongolia (Undarmaa et al. 2015). Our results clearly demonstrated that the large plain steppe of Mongolia supports adequate gene flow between populations. The highest share of molecular variance was found between individuals within sub-populations (92%), while the variance between populations was 5% and among different grazing intensity levels was 3% (Table 4). The overall degree of differentiation across populations was low (Global $G_{ST}=0.034$), which is consistent with another studies on *A. frigida* conducted in Inner Mongolia, China ($D_{ST}=0.05$, Liu et al. 2010, and $F_{ST}=0.01–0.31$, Liu et al. 2012).

In our study, the highest genetical distance was found between Pop2 and others (Hustai NP; $G_{ST}=0.027–0.042$). The only enclosure of the study was located in Pop2 (Pop2C), however, this fenced site was not markedly genetically different from other sites, whereas the other two grazed

sites from Hustai NP differed strongly (Pop2A > Pop2B). Given that the sites in Hustai NP were not different in terms of geography and climate (Table 1), other factors may have influenced the observed genetic structure. Hustai NP lies 100 km from the capital city Ulaanbaatar (UB), and every year, hundreds of herders with their livestock head to the capital city to purchase supplies. As such, due to the proximity of Hustai NP to UB, it is possible that the area experiences relatively higher levels of transiting herds, which may in turn lead to higher diaspore input from more distant sources via epizoochory. The potential for such has been demonstrated for Mongolian drylands, where diaspores can be transported in sheep and goat fur by up to 15 km per day (Bläß et al. 2010). While genetic diversity was lower in grazed sites compared to the enclosure site in Hustai NP (Suppl. 1, Table 5), grazed sites contained a higher number of indicator alleles (see below). It is therefore possible that genotypes introduced by herder movements may only have spread successfully on grazed sites, as the enclosure was devoid of bare soil patches where diaspores might otherwise have germinated (Frank 2005).

To reveal the presence of grazing-adapted genotypes, we conducted ISA analysis, which detected some indicator alleles characteristic of certain grazing intensity levels along the large-scale transect (Suppl. 1 Table 5). As a result, almost 50% of significant alleles were indicative of heavily-grazed sites, while 37% was attributed to moderately-grazed sites. Remarkably, most of these significant alleles were found in the Pop2 and Pop6 grazed sites, which further explains the higher genetical differentiation of the grazed sites in the Hustai NP. Given that SSR loci are expected to be evenly scattered throughout the genome and represent neutral non-coding sections of DNA, these data do not provide direct evidence of selection and adaptation. Nevertheless, the high number of indicator alleles found in the heavily- and moderately-grazed sites may point to the evolution of adapted genotypes and thus indicate local adaptive divergence among sub-populations.

Genetic diversity

Levels of genetic diversity of Mongolian populations of *A. frigida* were higher than those found in China based on SSR markers with 0.33–0.58 (H_O) and 0.54–0.68 (H_E ; Liu et al. 2012). In our study, H_O and H_E varied respectively between 0.41–0.68 and 0.64–0.78 at the sub-population level (Suppl. 1, Table 5). A further three studies employed RAPD markers (Random Amplification of Polymorphic DNA) on *A. frigida* in Inner Mongolia, China (Wang et al. 2004; Tao et al. 2008; Liu et al. 2010), each of which detected higher overall shares of polymorphic loci (PPL: 83%, 95% and 79% respectively) across all populations, compared to ours (PPL: 48%) (Table 1). It is however

noted that the data from China originate from profiles of RAPDs as a dominant and completely anonymous marker system. In any case, we revealed relatively higher potential levels of genetic diversity for *A. frigida* than that reported for other similarly widespread and long-lived perennials (e.g. Nybom 2004; Nybom and Bartish 2000).

Our results also reveal that higher amounts of summer precipitation and soil phosphorous promote genetic diversity in *A. frigida* (Table 5), suggesting that environmental conditions have a substantial impact on genetic diversity. In Mongolian rangelands, abiotic constraints exert strong controls on vegetation, where adequate precipitation and soil moisture during the growing season are vital to ensure seed germination of several species, including *A. frigida* (Ronnenberg et al. 2007). It has also been shown that soil carbon and phosphorous have significant positive effects on the establishment of seedlings and plant growth of *Artemisia* species, even under relatively dry conditions (Yang et al. 2015). Successful seed germination promotes reproductive success, resulting in more breeding individuals contributing to the gene pool and a consequent increase in genetic diversity. While previous population genetic studies on *A. frigida* did not specifically investigate the impact of environmental factors on genetic diversity, there are a few studies on other rangeland *Artemisia* shrub and sub-shrub species that indicate that several climatic factors affect genetic diversity. In particular, Huang et al. (2014) found that higher genetic diversity of *A. halodendron* Besser. was associated with a higher range of annual temperatures (intra-annual variation of temperature). Chaney et al. (2017) demonstrated that greater temperature seasonality and higher summer precipitation resulted in a greater probability of population survival of *A. tridentata* Nutt. These results support our findings that higher precipitation promotes genetic diversity in the sageworts.

While we found relatively limited grazing effects on the genetic diversity of *A. frigida*, this was somewhat expected, as the species is adapted to drought and disturbance and/or it may even benefit from mechanical disturbance. *Artemisia frigida* is characterized by a highly variable response to disturbance as it propagates horizontally via stolons (Li et al. 2004), which occupy more space to counteract grazing and the consequent suppression of its apical dominance (Jinhua et al. 2005). Our focus species also produces volatile organic compounds under heavy grazing pressure, which inhibits the growth of grass seedlings and can result in *Artemisia* dominance in heavily grazed areas (Zhao-Jiang et al. 2011). While such results may be interpreted as an adaptation of dryland plants to a long history of grazing, and our findings contrast with those of Wang et al. (2004), who found reduced genetic diversity of *A. frigida* under intensive grazing due to

reductions in plant sexual reproduction by consumption of individuals.

Relations between genetic and spatial distances

With respect to geographical distance (600 km distance), climatic gradient (100–300 mm differences in mean annual precipitation along the S/N gradient) and differences in plant community composition, we had expected to find greater genetic differentiation with larger distance. However, our Mantel tests did not indicate any significant influence of spatial distance or simple spatial processes. Likewise, analysis of three other *Artemisia* species revealed no direct genetic relation with geographic distance (Badr et al. 2012), and similar findings were reported for *Stipa* species in Central Asia (Jing et al. 2013; Zhao et al. 2006).

Conclusions

Rangeland plant genetic resources have been rarely investigated and genetic parameters focusing on conservation are largely absent in land use policies on drylands. While dryland species are crucial for foraging herds and, consequently, the livelihoods and subsistence needs of herder communities, they are often threatened by over-grazing and drought. Our results on *A. frigida* revealed that genetic diversity of this cold-, drought- and disturbance-adapted species in Mongolia was relatively high and genetic differentiation low among eleven populations across a north to south gradient. Furthermore, we observed no severe consequences of grazing on genetic diversity, however, a high number of grazing-specific indicator alleles was found at grazed sites. This indicates that population differentiation may partly be driven by livestock grazing intensity, which may have beneficial effects on the genetic diversity of the species. In addition, sufficient precipitation and soil nutrient availability also promote genetic diversity of *A. frigida* in the water- and nutrient-limited arid lands. We therefore conclude that in spite of the degraded and overgrazed landscapes of Mongolia, sufficient genetic exchange persists among populations, even across larger distances, such that there is no clear indication of fragmentation. With regard to conservation genetics, while *A. frigida* populations in Mongolia are apparently genetically ‘healthy’, the same may not be concluded for the more intensively grazed rangelands of northern China.

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Author contributions All authors contributed to this work, i.e., study conception and design, sample collection and vegetation surveys including species identification were performed by CMR, KW, BO and KO. Library construction and bioinformatics were done by DH and VH. DNA extractions, microsatellite analyses and statistics were done by KO. The first draft of the manuscript was written by KO and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability WGS raw sequencing data is available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA680535, <https://www.ncbi.nlm.nih.gov/sra/PRJNA680535>. Further dataset generated and analyzed during the current study are available from the corresponding authors on reasonable request.

Code availability Codes of R software used for statistical analyses are available upon request from the corresponding author.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Informed consent The authors agree for publication of the paper and all data relevant to this article.

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