Relationship between genetic and phenotypic variations in natural populations of perennial and biennial sagebrush

Oyundelger Khurelpurev[1], Lisa Grossmann[2], Veit Herklotz[3], Dörte Harpke[4], Batlai Oyuntsetseg[5], Karsten Wesche [3], and Christine Ritz[3]

[1]Senckenberg Museum für Naturkunde Görlitz
[2]Ministry for Rural DEvelopment Environment and Agriculture Brandenburg
[3]Senckenberg Museum of Natural History Görlitz
[4]Leibniz-Institut fur Pflanzengenetik und Kulturpflanzenforschung Gatersleben
[5]National University of Mongolia

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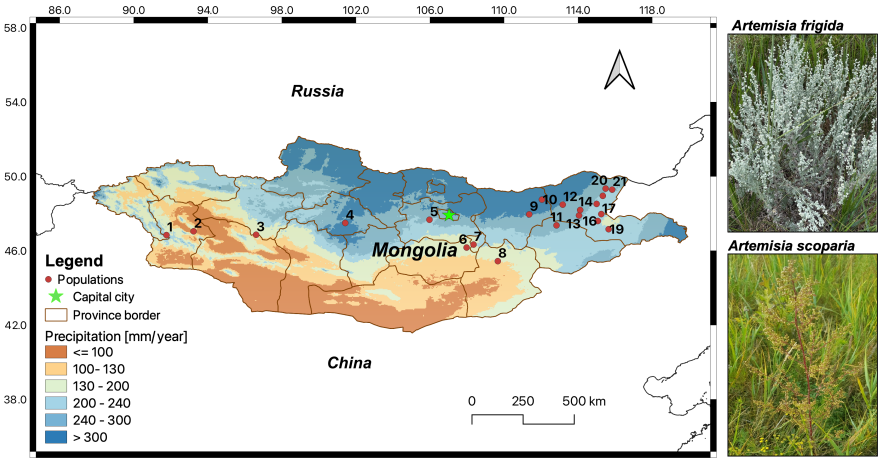
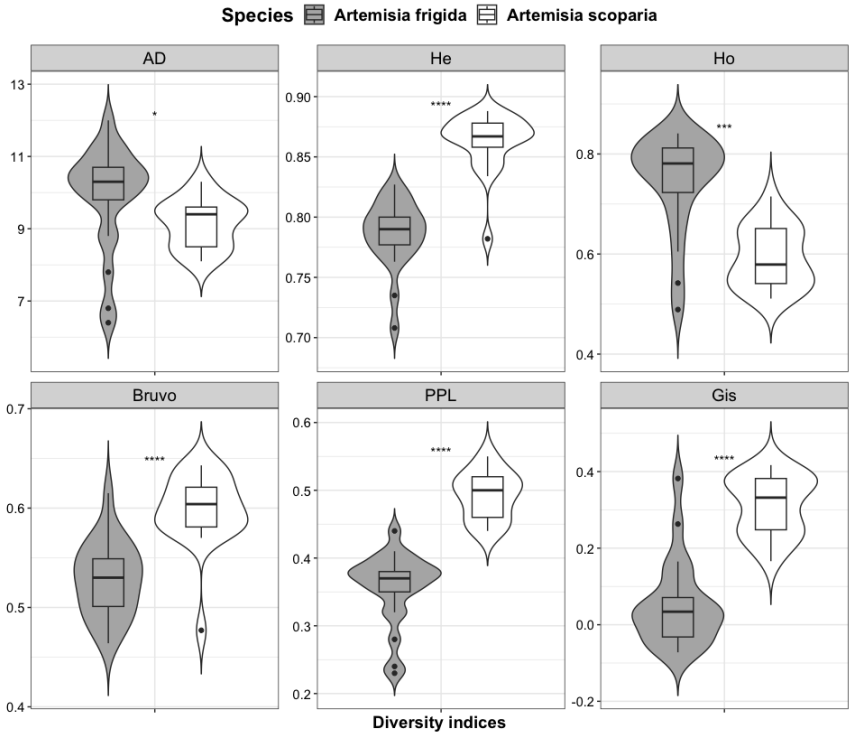
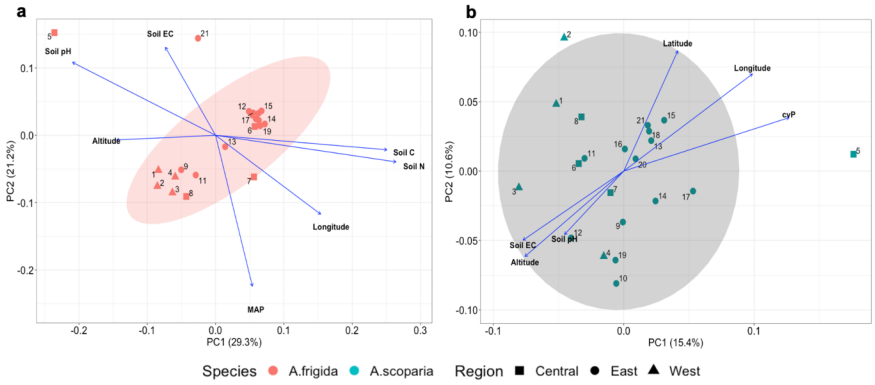
Abstract

Plant responses to environmental heterogeneity depend on life-history traits, which could relate to phenotypical and genetic characteristics. To elucidate this relationship, we examined the variation in population genetics and functional traits of short- and a long-lived Artemisia species that are co-occurring in the steppes of Mongolia. Mongolian steppes represent stressful, waterlimited habitats demanding phenotypic modifications in the short term and/or genetic adaptation in the long term. However, detailed knowledge is missing about both plant phenotypic and genetic differentiation and their inter-relationships in temperate grasslands. Here, we investigated 21 populations of the widely distributed subshrub A. frigida and the herbaceous biennial A. scoparia. Genetic variation was assessed with newly developed Simple Sequence Repeats (SSRs) markers. Functional trait data was collected from each individual, and data on environmental variables was collected for each population. We detected significantly higher genetic diversity in the biennial species (H E =0.86) compared to the perennial (H E =0.79). For both species, the largest share of genetic variation was partitioned within populations (96%). Population genetic structure in the biennial A. scoparia was weak, while the perennial A. frigida showed some spatial genetic structure, which was impacted by geographical factors, soil nutrients, and precipitation. Morphology-related functional traits (i.e., plant height) were predominantly associated with environmental variables rather than with genetic variation, while physiology-related traits (i.e., specific leaf area) were partly genetically determined.







Relationship between genetic and phenotypic variations in natural populations of perennial and biennial sagebrush

Khurelpurev Oyundelger[1*], Lisa Großmann[2], Veit Herklotz[1], Dörte Harpke[3], Oyuntsetseg Batlai[4], Karsten Wesche[1,5,6] and Christiane M. Ritz[1,5,6]

[1] Department of Botany, Senckenberg Museum of Natural History Görlitz, Görlitz, Germany
[2] Ministry of Agriculture, Environment and Climate Protection of the State of Brandenburg, Potsdam, Germany
[3] Leibniz institute of Plant Genetics and Crop Plant Research, Seeland, Germany
[4] Department of Biology, School of Arts and Sciences, National University of Mongolia, Ulaanbaatar, Mongolia
[5] Chair of Biodiversity of Higher Plants, International Institute Zittau, Technical University Dresden, Zittau, Germany
[6] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Corresponding author*: oyundelger.kh@gmail.com

Abstract

Plant responses to environmental heterogeneity depend on life-history traits, which could relate to phenotypical and genetic characteristics. To elucidate this relationship, we examined the variation in population genetics and functional traits of short- and long-lived *Artemisia* species that are co-occurring in the steppes of Mongolia. Mongolian steppes represent stressful, water-limited habitats demanding phenotypic modifications in the short term and/or genetic adaptation in the long term. However, detailed knowledge is missing about both plant phenotypic and genetic differentiation and their inter-relationships in temperate grasslands. Here, we investigated 21 populations of the widely distributed subshrub *A. frigida* and the herbaceous biennial *A. scoparia*. Genetic variation was assessed with newly developed Simple Sequence Repeats (SSRs) markers. Functional trait data was collected from each individual, and data on environmental variables was collected for each population. We detected significantly higher genetic diversity in the biennial species (H_E=0.86) compared to the perennial (H_E=0.79). For both species, the largest share of genetic variation was partitioned within populations (96%). Population genetic structure in the biennial *A. scoparia* was weak, while the perennial *A. frigida* showed some spatial genetic structure, which was impacted by geographical factors, soil nutrients, and precipitation. Morphology-related functional traits (i.e., plant height) were predominantly associated with environmental variables rather than with genetic variation, while physiology-related traits (i.e., specific leaf area) were partly genetically determined.

Keywords: growth-form, *Artemisia*, inter-relationship among variations of genetic, functional traits and environment

1. Introduction

It is widely acknowledged that a species' genetic diversity and its variation are associated with life-history traits, such as life form, breeding system, seed dispersal mechanism, and geographical range (Hamrick & Godt 1996; Nybom & Bartish 2000; Reisch & Bernhardt-Römermann 2014). Species with outcrossing and mixed-mating systems tend to have higher levels of genetic variation than selfing species (Nybom 2004). Short-lived, non-woody, self-compatible, and early-successional species, i.e., annuals/biennials, are characterized by higher genetic variation between populations, but lower genetic variation within populations. In contrast, long-lived, woody, outcrossing, and late-successional species, i.e., many perennials, have higher genetic variation within populations (Reisch & Bernhardt-Römermann 2014). However, comparative studies such as that of Heeleman *et al.* (2015) found lower within-population variation in perennial *Eriocephalus africanus* L. than in the annual species *Hemimeris racemosa* (Houtt.) Merrill. A comparison of perennial and annual wild species of the genus *Oryza* L. discovered that perennial species had higher population level genetic diversity but less genetic variation among populations than annuals (Zhou *et al.* 2008).

Even within a species, plant phenotypic variation is often high. Functional trait plasticity related to morphology (e.g., plant height), (eco)physiology (e.g., specific leaf area), and life history (e.g., flowering time and seed traits) was found to be under genetic control in some model plants (Locascio *et al.* 2009; Hughes *et al.* 2019). Several studies detected correlations between phenotypic traits (morphological and functional trait variation) and genetic variation (Waitt & Levin 1998; Karbstein *et al.* 2019; Csilléry *et al.* 2020). In particular, Waitt & Levin (1998) presented a meta-study demonstrating a positive correlation between the genetic and phenotypic character traits of 27 species. However, trait variation does not necessarily coincide with genetic variation, especially if the trait is completely plastic (Chevin & Hoffmann 2017). Plasticity, i.e., phenotypic modification, allows for long term adaptation to the local environment and/or short term (reversible) responses. However, how genetic diversity and intraspecific functional traits interact at the population level, particularly in natural environments, remains poorly understood.

Artemisia L. (sagebrush) is a large and diverse genus that comprises over 500 taxa of annuals/biennials, perennial herbs, and shrubs or subshrubs distributed across temperate regions of the northern hemisphere (Riggins & Seigler 2012). Many species are clearly wind-pollinated; however, some indication of insect pollination was observed (colorful capitula and sticky pollen; Vallès & McArthur 2001). *Artemisia* spp. inhabits arid, semi-arid and mesic environments spanning deserts to tundras, and their range of phenotypic diversity is broad (morphological, (eco)physiological, and reproductive traits), as is their range of ploidy levels ($2n$=16 or 18 up to $2n$=144; Sanz et al. 2008). Although the genus offers ample opportunities for comparison, studies on genetic diversity and life history traits are hardly available. Al-Ajmi *et al.* (2021) compared seven species of *Artemisia* and found a positive interspecific correlation between similarities in genetic variation among species. However, we do not know of any study that addressed intraspecific variation in traits and genetic structures.

Artemisia frigida Willd. and *A. scoparia* Waldst. & Kit. are both outbreeding and wind pollinated species (Vallès et al. 2011) with a range of phenotypic variations. In this study, we aimed to test the effects of environment on genetic variation and genetic structure of the short-lived biennial *A. scoparia* and the long-lived sub-shrub *A. frigida*, which are co-occurring in the steppes of Mongolia. The flora of Mongolia lists 103 native *Artemisia* species (Baasanmunkh *et al.* 2022), among which species growing in dry steppes and forest steppes are the most numerous. Mongolia has one of the world´s largest steppes, covering 1.2 million km^2 and being home to thousands of steppe species (Munkhzul *et al.* 2021; Baasanmunkh *et al.* 2022). The continuous plain steppe of Mongolia allows for sufficient genetic exchanges between plant populations, as shown by former studies on the perennial grass *Stipa glareosa* P.A.Smirn. (Oyundelger *et al.* 2020) and on *Artemisia frigida* (Oyundelger *et al.* 2021, 2023). In these studies, we detected moderate genetic structuring, which was mostly attributed to the differences in climate and edaphic conditions of the local populations rather than the geographical distance. However, the present study covers an even larger area of Mongolia, ranging from the western Altai Mountains to the eastern Mongolian Steppes. Specifically, we aimed to answer the following questions: i) How do genetic diversity and population structure differ between the two *Artemisia* species? ii). Do environmental factors relate to the genetic variation of the species across the Mongolian steppe? iii). Are functional traits related to genetic diversity and/or abiotic habitat heterogeneity?

2. Material and methods

2.1. Study species: *Artemisia frigda* and *A. scoparia*

Perennial prairie-sage (*A. frigida*) has the largest natural range within its genus, being distributed across the North American prairie and the Eurasian steppe, whereas *A. scoparia* is a biennial species widely distributed from Central Europe to East Asia. Species' ranges overlap in Inner Asia and specifically in Mongolia, where they are common steppe plants (Hilbig 1995). They share the same breeding system (outbreeding) and dispersal mechanism (wind), yet differ in their life form (biennial herb vs. perennial subshrub). The perennial *A. frigida* grows primarily in mountains, hillsides, and ruderal sites in steppes (Tkach *et al.* 2008). It bears a dense silvery pubescence and has woody ascending stems that are usually strongly branched (Fig. 1). The biennial *A. scoparia* is found in riverbanks, as well as in ruderal sites in steppes and semi-deserts. Its stems are initially pubescent, becoming glabrous and strongly branched in the middle and upper parts (Fig. 1). *Artemisia frigida* and *A. scoparia* are pioneer plants at sites disturbed by grazing and also occur in the early recovery stages of abandoned land that underwent severe soil erosion (Jiao *et al.* 2013; Wang *et al.* 2022). Both species have high seed yields and small seeds (*A. frigida*: 0.106 g and *A. scoparia*: 0.047 g) that are easily propagated by wind and are then buried into soils (Yi *et al.* 2019).

Artemisia scoparia belongs to the subgenus *Dracunculus* Besser representing the most basal lineage of *Artemisia* (clade divergence in 17.6 ± 2.1 Mya), while *A. frigida* is part of the subgenus *Absinthium* DC. (clade node 6.8 ± 0.8 Mya; Sanz *et al.* 2011; Hussain *et al.* 2019).

Artemisia frigida comprises diploids (2n = 2*x* = 16) as well as tetraploids (2n = 4*x* = 36; Pellicer *et al.* 2010; Korobkov *et al.* 2014). In *A. scoparia*, mostly diploid cytotypes were observed (2n = 2x = 16 or 18; Pellicer *et al.* 2010); yet 2n = 4x = 32 or 36 have also been reported from Slovenia, Siberia, and recently from the Western Himalayas (Kawatani 1964; Amel'chenko 1979; Gupta *et al.* 2014).

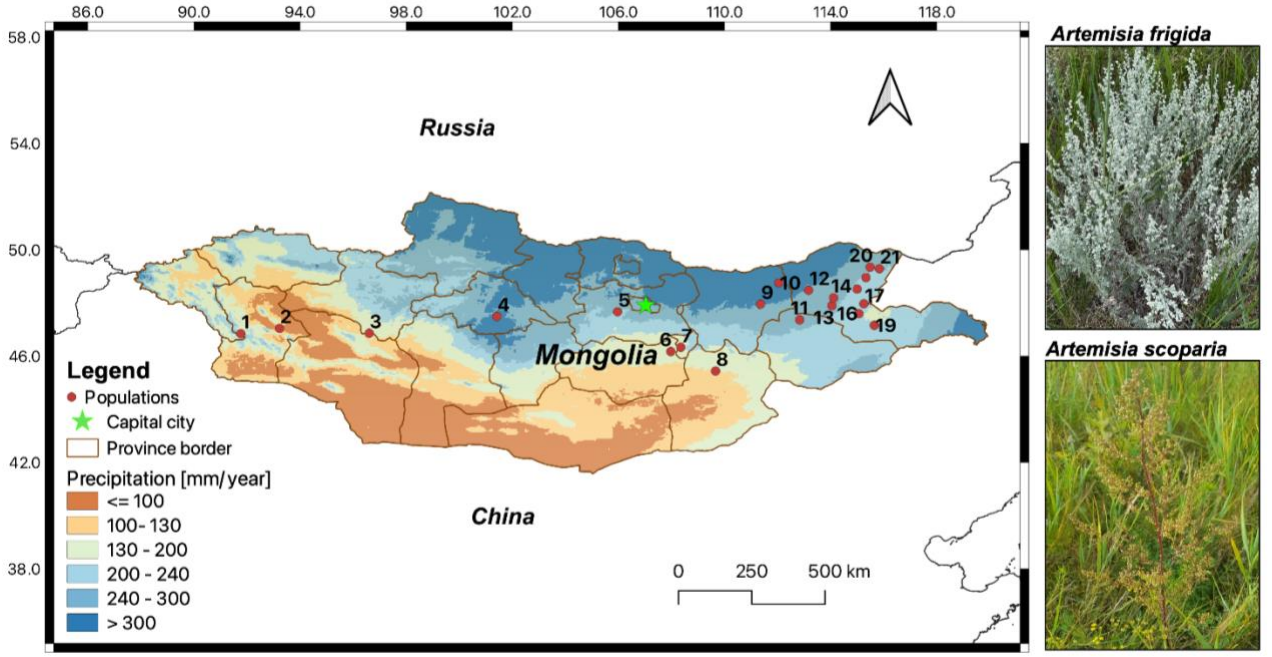


Figure 1. Study area with locations of 21 populations sampled for *A. frigida* and *A. scoparia* across Mongolia. Precipitation data were derived from Fick & Hijmans (2017).

2.2. Study design and sampling

Sampling was carried out along a broad-scale longitudinal precipitation gradient from western to eastern Mongolia during the summers of 2018 and 2019 (Fig. 1). Fresh leaf materials were collected from 21 populations where both species co-occurred. For each population, representative herbarium specimens were deposited at Herbarium Senckenbergianum Görlitz (GLM). As a result, we sampled thirteen eastern (E) populations and four western (W) and four central (C) populations across various steppe vegetation types (Table 1).

At each site, 15 individuals per species were sampled within a 10 m × 10 m plot. Within these plots, plant community composition and total cover (%) of vascular plants were recorded, and a sample of top soil (1 – ±5 cm depth) with fine plant roots and the humic layer was collected. Soil samples were separated from litter, debris, and after shifting through a 2 mm sieve the following measurements were conducted in the laboratory: pH value, electrical conductivity (EC, as a proxy for salinity), plant available P, N%, organic C%, and C/N ratio. All results refer to oven-dried soil (75 °C, 18 h). Moreover, plots were classified into different steppe types according to "The steppe vegetation of Mongolia" (Tuvshintogtokh 2014) based on our sampling location, which was also validated by our field-based plant community composition data.

Three functional traits were measured in the same individuals sampled for molecular data. In the field, 'height of inflorescence (HI)' (if plants were flowering), 'height of vegetative part (HV)', and leaf area for the trait 'specific leaf area (SLA)' were measured. The HI was determined as the height from ground level to the tip of the highest inflorescence, and the HV as height of one randomly selected vegetative branch per plant. In *A. frigida*, vegetative and generative shoots differ in length. Thus, both heights were chosen as traits. *Artemisia scoparia* does not develop sterile shoots, and thus only the HI was applicable. For SLA, two fresh leaves were taken from each individual (30 leaves per site) and scanned using a Conrad P-573 handheld document scanner. Scanned pictures were later analyzed with ImageJ (Abràmoff *et al.* 2004) to determine the leaf area. Leaves were then air-dried for more than a month, and biomass weight was measured with a Mettler Toledo XP6 balance in the laboratory. The SLA was then calculated by dividing leaf area by dry mass (Perez-Harguindeguy *et al.* 2013). Population level trait data and their correlation matrices, indicating their independence are provided in Suppl. Table 1).

Meteorological data of 20 years (mean annual temperature (MAT), mean annual precipitation (MAP), and mean spring temperature (March-May), mean summer temperature, and mean summer precipitation (June-August) between 1994 – 2013) were retrieved for each locality from the high-resolution CHELSA_V1 dataset, which has the advantage to capture interannual precipitation variation (Karger et al 2017). The coefficient of interannual variation of annual precipitation (cvP) was estimated based on the retrieved MAP data and was also used as a predictor since cvP is a critical driver of rangeland dynamics (von Wehrden *et al.* 2012).

2.3. Molecular analyses and microsatellite marker development

Two randomly selected individuals of each species from two distinct populations were used to develop new SSR markers by applying whole genome sequencing (WGS). A previous study by Oyundelger *et al*. (2021), gives detailed steps for DNA extraction, library preparation, quality control and bioinformatics in SSR development. Raw sequencing data were submitted to the NCBI Sequence Read Archive (SRA) and made publicly accessible under BioProject: PRJNA680535.

A total of 20 and 21 SSR markers were then tested for optimization in *A. frigida* and *A. scoparia*, respectively, using randomly selected samples from more than ten populations containing 8–16 samples. Furthermore, cross-checking of markers for both species was performed, and ten SSR markers published for *A. frigida* in the master thesis of Wang (2011) were tested with our samples in parallel. Based on reproducibility and polymorphism, 11 markers were chosen for each species. Detailed information on SSR markers of *A. frigida* can be found from Oyundelger *et al.* (2021). Information about species-specific SSR markers for *A. scoparia* developed for this study are presented in Table 2. Amplifications of a total of 22 SSR markers were performed in a volume of 12.5µl, and customized PCR reaction mixtures and cycling programs were used (see PCR details from Suppl. Table 2). Individuals of all 21 populations from both species exhibited a maximum of four alleles per locus, indicating prevailing tetraploidy (see Suppl. Table 3 for ploidy information).

Table 1. Characteristics of the study sites: (population code, localities, main climatic variables, steppe vegetation type and region).

Pop code	Locality and province	Longitude	Latitude	Altitude [m]	MAT [°C]	MAP [mm]	Summer temp. [°C]	Summer prec. [mm]	cvP [%]	Steppe type	Region
1	Munkhkhairkhan, Khovd	91.765	46.841	1781	-6.1	147	8.9	87	33	MoS	W
2	Center of Khovd, Khovd	93.228	47.044	1355	2.6	115	19.5	77	42	DrS	W
3	Taishir Soum, Gobi-Altai	96.605	46.860	2009	-1.6	172	14.7	105	29	DrS	W
4	Khotont Soum, Arkhangai	101.421	47.492	1608	-1.3	300	14.3	198	24	MoS	W
5	Hustai National Park, Tuv	105.968	47.666	1264	0.9	167	18.3	118	24	MoS	C
6	Tsagaandelger, Dundgovi	107.975	46.170	1280	2.2	117	19.9	82	42	DrS	C
7	Choir, Dundgovi	108.350	46.331	1270	1.9	135	19.7	92	40	DrS	C
8	Altanshiree, Dundgovi	109.660	45.438	1007	3.8	126	21.9	84	31	DeS	C
9	Batnorov, Khentii	111.357	47.955	1078	0.5	286	18.8	194	25	DrS	E
10	Norovlin, Dornod	112.044	48.751	1020	0.6	277	18.6	192	29	DrS	E
11	Hulunbuir, Khentii	112.831	47.364	1008	0.4	231	18.6	164	37	DrS	E
12	Tsagaan-Ovoo, Dornod	113.167	48.480	1009	1.4	240	19.6	166	34	DrS	E
13	Bulgan, Dornod	114.046	47.896	961	1.5	238	19.9	163	46	DrS	E
14	Bayantumen, Dornod	114.109	48.190	991	1.4	210	19.7	142	44	DrS	E
15	Choibalsan, Dornod	114.997	48.519	847	1.5	209	20.3	141	49	DrS	E
16	Matad, Dornod	115.062	47.598	761	2.1	184	20.5	130	52	DrS	E
17	Matad, Dornod	115.250	47.971	1075	1.4	198	19.9	139	53	DrS	E
18	Choibalsan, Dornod	115.331	48.953	909	1.0	231	19.9	153	46	DrS	E
19	Shar-Khudag, Dornod	115.646	47.157	1011	1.5	199	19.7	144	52	DrS	E
20	64n toochig, Dornod	115.485	49.344	650	1.3	232	20.4	154	45	DrS	E
21	Otor pasture, Dornod	115.837	49.288	821	1.1	239	20.3	159	44	DrS	E

MAT – mean annual temperature, MAP – mean annual precipitation, Summer temp. – summer mean annual temperature, Summer prec. – summer mean annual precipitation, cvP – coefficient of variation of interannual precipitation, MoS – mountain steppe, DrS – dry steppe, DeS – desert steppe, W – western, C – central, E – eastern region of Mongolia, coordinates are in WGS84

Table 2. Characterization of eleven polymorphic microsatellite markers used in this study for *Artemisia scoparia.* Details on SSR markers for *A. frigida* can be found in Oyundelger *et al.* (2021).

No.	Locus	Repeat motif	Primer sequences (5´-3´)	Ta (°C)	Allele size range (bp)	Fluorescent dye	PCR type
1	*Arcs2*	(GT)9	F: TGTAAAACGACGGCCAGTTCTC CTTTCTGATTCATTGG	55	585-620	6 FAM	Multiplex
			R: CGAGATGAATTTGCGTCAT				
2	*Arsc12*	(TGT)9	F: TGTAAAACGACGGCCAGTGGAC ATTTGAATGATGTTCG	55	200-265	6 FAM	
			R: AAGTCTTCCGCCAGCTATA				
3	*Arsc7*	(TG)11	F: TGTAAAACGACGGCCAGTTGT CCATCAAGATACCTATGC	55	520-560	VIC	Multiplex
			GGTTATCGCCTCTCATTTG				
4	*Arsc11*	(ACA)8	F: TGTAAAACGACGGCCAGTGAAC GGGAAGATTACAAGC	55	130-180	VIC	
			R: CACCAATATTACCTGGTGTG				
5	*Arsc18*	(ATG)8	F: TGTAAAACGACGGCCAGTACAC TGGAAAGCTATGTGC	55	610-660	PET	Multiplex
			R: CGAGTCACAGTCATGGTC				
6	*Arsc19*	(TGA)8	F: TGTAAAACGACGGCCAGTCCT CAAACCTTGAAAGATAGC	55	350-400	PET	
			R: CCGTATGAGTTAAGCAATCAG				
7	*Arsc17*	(TGA)8	F: TGTAAAACGACGGCCAGTAATG GATTATGTTGATAGCCA	55	135-160	6 FAM	Singleplex
			R: CAAGTTCCGTTGACTCG				
8	*Arsc14*	(ATA)8	F: TGTAAAACGACGGCCAGTATG CACATAATATCCGAGC	55	270-325	VIC	Singleplex
			R: GTGCTGAGACCGAATGC				
9	*Arsc20*	(ACA)14	F: TGTAAAACGACGGCCAGTGAC ACCCATAGACAGGAGC	55	~500	NED	Singleplex
			R: GTCAGCTCGAAGCTTTCC				
10	*Arsc21*	(TGT)8	F: TGTAAAACGACGGCCAGTTGC CTTTGCAACAATTAAC	55	110-128	NED	Singleplex
			R: GCTGCAAACATTACGTAAGC				
11	*Ch468*	NA	F: TGTAAAACGACGGCCAGTTAG GGTTGCAGAAGATAAAC	55	160-236	PET	Singleplex
			R: GCTTCTTCACTTCCTACTAAAG				

2.4. Statical analyses

Analysis of genetic diversity and population structure

To compare the genetic diversity within each species, we employed two programs, which allowed handling of microsatellite data for polyploids and species with mixed ploidy: GenoDive v.3.04 (Meirmans 2020) and the R-package *Polysat* v. 1.7 (Clark & Jasieniuk 2011) in R v.4.0.3 (R Core Team 2020). Estimators of genetic diversity comprised allelic diversity (AD), percentage of polymorphic loci (PPL), observed heterozygosity (H_O), expected heterozygosity (H_E) and inbreeding coefficient (G_{IS}), all of which were calculated using GenoDive. Bruvo distances were computed with the R-package *Polysat* v.1.7 (Bruvo et al., 2004). Using the R-package *vegan* (Oksanen et al., 2007), we calculated the mean Bruvo distance among individuals for any given

population (hereafter 'Bruvo index'; see detail in Oyundelger *et al.* (2021)), which was then used as a surrogate for genetic diversity (See Suppl. Table 4 for the genetic diversity indices). A paired T-test was used to determine the significance of the difference in genetic diversity indices between two species.

Coefficients of genetic differentiation (F_{ST} and G_{ST}) were estimated using *Polysat* (Suppl. Table 5). Population genetic structure was further analyzed with Principal Coordinate Analysis (PCoA) using population-wise F_{ST} distance using the R-package *ape* (Paradis & Schliep 2019). In order to reveal environmental variables that were significantly associated with population genetic structure of the species, environmental and vegetation variables were fitted *post hoc* on the ordination using *vegan*, and plots were visualized with *ggplot2* (Wickham 2011).

To examine the partitioning of genetic variation between and within 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 with *Polysat.*

Relationship between genetic and spatial distances

To assess the overall relationship between genetic and spatial distances, Mantel tests between genetic distance (linearized population level pairwise F_{ST} (Fst/(1-Fst))) and geographic distances (Euclidean distances) were computed through 10,000 randomizations using the R-package *vegan* (Oksanen et al., 2007). Further Mantel tests were then conducted between genetic distances and a) climatic differences (Euclidean distance of centred and standardized climatic variables); b) distance of soil indicator variables (Euclidean distance of centred and standardized variables), and c) differences in plant community composition (Bray-Curtis's distance based on log-transformed species' cover).

Relationships of functional trait variation with genetic and environmental patterns

We estimated population-level means and coefficients of variation (CV) for trait variables, the latter as the ratio of standard deviation to mean. We checked collinearity among traits (mean and CV) with Pearson´s coefficient (Suppl. Table 1) using the R-package *corrplot* (Wei *et al.* 2021). As correlation coefficient values (r) of the mean and CVs were below ~ $\left|.7\right|$, we did not exclude particular functional traits.

To assess whether functional traits are related to environmental heterogeneity and genetic diversity, we fitted linear models (Dobson & Barnett 2018) with mean and CV of traits as the dependent variables. We again used *corrplot* for an exploratory analysis of associations among measures of genetic diversity. As a result, H_E was chosen as main response variable, as it had the highest correlation and depends less on population history (e.g., bottlenecks) compared to the other indices (Rosenberg 2004; Szczecińska *et al.* 2016). For the predictors, we first checked correlations among environmental variables to select representative variables based on their importance and independencies ($r<\left|.7\right|$; See Suppl. Table 6 for the data and their correlations). As a result: MAP, MAT and cvP for climate; altitude for topography, and soil C/N ratio for soil nutrient contents were initially used as predictors for the models.

All predictors were first scaled to zero mean – unit variance (z-scores) to make effect sizes comparable. The response variable: cvIH of *A. frigida* was log-transformed due to its non-normal distribution; other response variables (cv and means) were in normal distribution, and thus no transformation was done. We then conducted model simplification by dropping the least relevant variables from linear models until a null model with intercept only. Models were compared using ANOVA, the summary was used to estimate significances and to choose the most parsimonious models. Lastly, plotting was used to check residuals of the models for possible deviations from normality and reasonable distribution of variances.

3. Results

3.1. Comparison of genetic diversity between the perennial and biennial *Artemisia*

The overall polymorphic information content (PIC) of newly developed species-specific SSR markers was high (PIC=0.77 and 0.84) for both *A. frigida* and *A. scoparia*. Paired T-test revealed that proxies of genetic diversity differed between two the *Artemisia* species (Fig. 2). Specifically, H_E, Bruvo, PPL and G_{IS} of the biennial *A. scoparia* was significantly higher than in the perennial *A. frigida*. In contrast, AD and H_O were larger in the perennial than the annual species, yet with lower significance. Details for estimators of genetic diversity are presented in Suppl. Table 4.

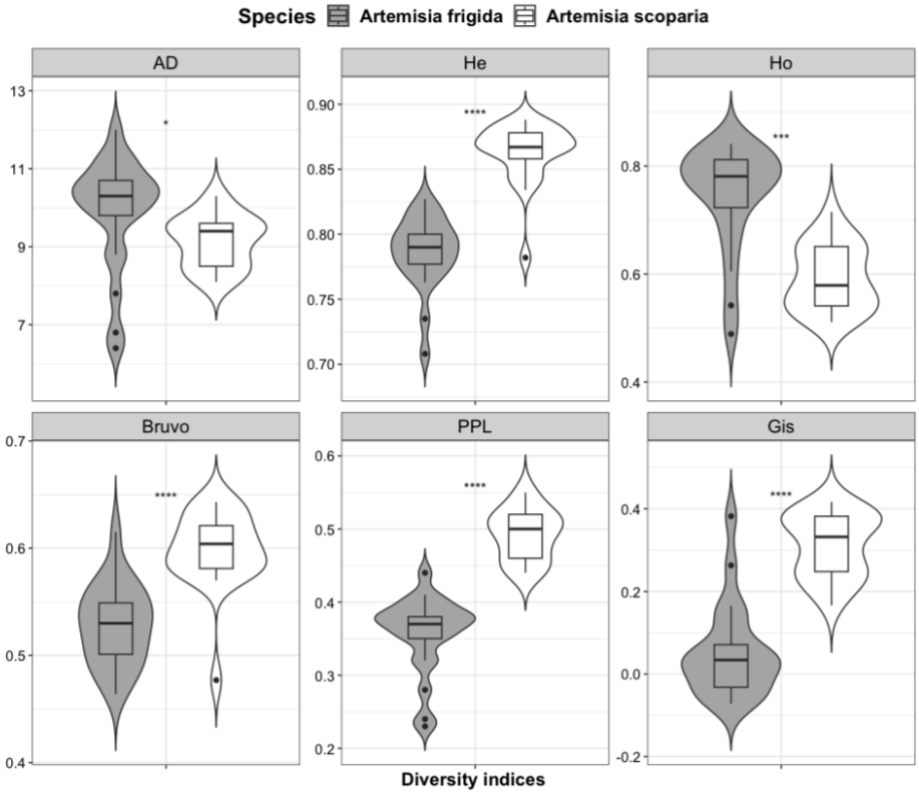


Figure 2. Violin boxplots of genetic diversity indices of the perennial *A. frigida* (N= 304) and the biennial *A. scoparia* (N=303) (AD – Allelic diversity, H_E–expected heterozygosity, H_O – observed heterozygosity, Bruvo – Bruvo index, PPL – percentage of polymorphic loci and G_{IS} – inbreeding coefficient). Significance codes: $p \leq 0.0001$ '***'; $p < 0.001$'***'; $p < 0.05$'*'.

3.2. Population genetic variation and relationship with environmental variables

Coefficients of genetic differentiation of the two species across 21 populations were low overall, suggesting that isolation is at most moderate over the distances considered here. However, population differentiation of *A. frigida* was slightly more pronounced (Global F_{ST}= 0.078 and Global G_{ST} = 0.071) than of *A. scoparia* (Global F_{ST}= 0.064 and Global G_{ST} = 0.055). The most genetically distant population was population 5 (Hustai National Park) in both species (dissimilarity data provided in Suppl. Table 5). Analysis of Molecular Variance showed that in both species, highest genetic variation resided between individuals, while genetic variation partitioned among regions was slightly higher in *A. frigida* than *A. scoparia* (0.97% and 0.83%, respectively; Table 3). The ordination plots suggested that there was no pronounced genetic differentiation among steppe types and regions of Mongolia (individual level PCoA in Suppl. Table 7), although *A. frigida* exhibited some population level genetic structure (Fig. 3). In the PCoA ordination of *A. frigida*, the first two axes explained about 50 % of the genetic variation, and some structuring of eastern vs. western populations mixed with central populations was discernable. According to *post hoc* fitting of predictor variables, longitude, altitude, mean annual precipitation (MAP), soil carbon, nitrogen, pH and soil electrical conductivity (EC) showed a significant association with genetic structure (Fig. 3a). In total 26 % of the total genetic variation was explained by the first two axes in the populations of *A. scoparia*, representing more continuous patterns among populations. Main structures along axis 1 and 2 were significantly correlated with altitude, soil pH and EC together with longitude, latitude and coefficient of variation of interannual precipitation (cvP), with western populations being in the upper left (Fig. 3b). The ordinations demonstrated that soil pH and EC, as well as soil C and N, exhibit covariance, as proven by their high correlations (r=0.78 and r=0.99; Suppl. Table 6). Results of *post hoc* fitting predictor variables on the PCoA are provided in the Suppl. Table 8.

Table 3. Summary of Analysis of Molecular Variance (AMOVA) of the perennial *Artemisia frigida* and the biennial *A. scoparia* of 21 populations across Mongolia.

Source of variance	Df	Sum sq	Variance component	% Total		Φ statistic
Artemisia frigida						
Between regions	2	12..56	0.028	0.97	***	0.037
Between populations	18	72.03	0.080	2.69	***	0.027
Within populations	283	806.44	2.850	96.34	***	0.009
Total	303	891.03	2.958			
Artemisia scoparia						
Between regions	2	2.21	0.004	0.83	***	0.035
Between populations	18	11.25	0.013	2.69	***	0.027
Within populations	282	128.68	0.456	96.47	***	0.008
Total	302	142.14	0.473			

Df – degrees of freedom, Sum Sq – Sum of square, % total – percentage of variation

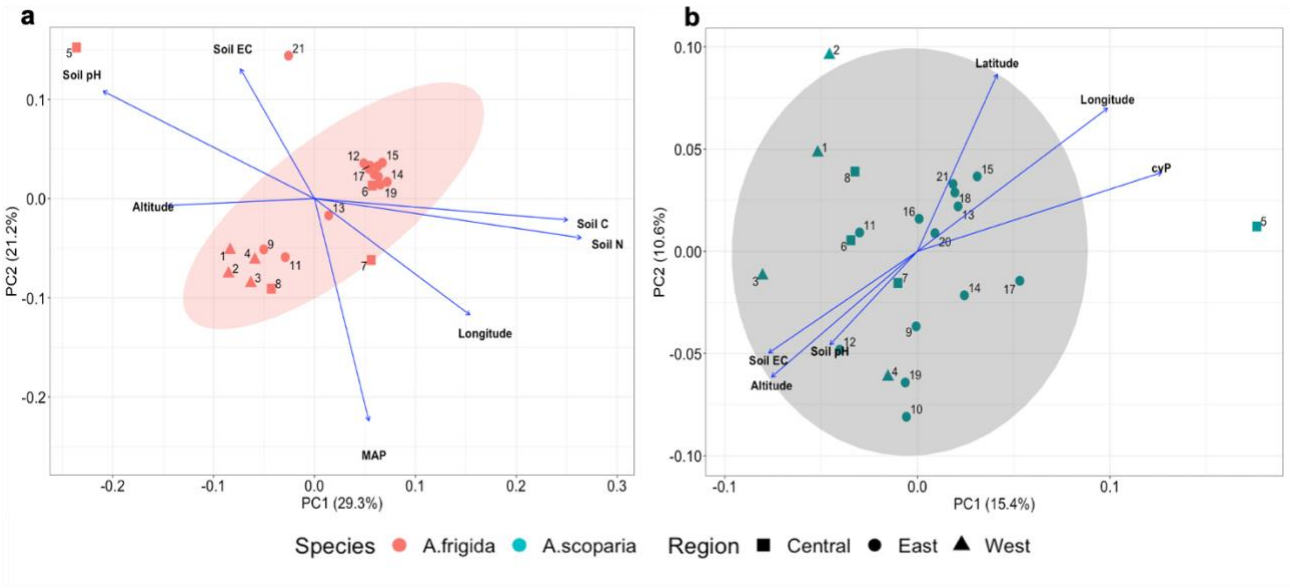


Figure 3. Principal Coordinate Analyses (PCoA) based on F_{ST} distances of the a) perennial *Artemisia frigida* and b) the biennial *A. scoparia* among 21 populations across three regions (east, central and west) of Mongolia. Each symbol represents one population, and 95% confidence intervals are indicated by shaded area. Environmental predictors were fitted *post hoc* on the ordination plot (only those that passed $p < 0.05$ according to a test with 1,000 permutations are shown). Result of *post hoc* analyses indicating the importance of environmental variables are provided in Suppl. Table 8.

The Mantel tests on association between genetic structures (linearized Fst – Fst/(1-Fst)) with various environmental variable distances revealed an overall negligible relationship with the genetic distances in both species (Suppl. Table 9). Geographic distance and the distance of soil nutrient values in particular showed a significant but weak correlation with the genetic distance of *A. frigida* (r^2 = 0.05*** and r^2 =0.02*). In contrast, *A. scoparia* did not exhibit an isolation by distance effect, while a weak correlation with climatic distance was observed.

3.3. Associations of functional traits with genetic and environmental variations

Results of linear models showed that means as well as coefficients of variation of functional traits in *A. frigida* were associated with climatic and geographic variables, whereas in *A. scoparia* genetic diversity and soil nutrients had a significant relationship with SLA (Table 4). In the perennial *A. frigida*, altitude was positively associated to the physiology related trait (mean SLA), while variations of morphology related traits, cvHI and cvVH were significantly affected by MAT, MAP, and cvP. In the biennial *A. scoparia*, genetic diversity showed an association with mean SLA, and soil nutrient contents with the variation of SLA. With the exception of altitude and cvP, all significant associations were negative (scatter plots with linear regression line of the significant models are provided Suppl. Table 10 and 11).

Table 4. Summary of the retained parsimonious and significant linear models assessing the associations of functional traits of *A. frigida* and *A. scoapria* with the genetic diversity and environmental variables.

	Functional traits	Predictor	Estimate	Std. Error	Pr(>|t|)	
A. frigida	Mean SLA	(Intercept)	0.15	0.006	<0.001	***
		Altitude	0.02	0.006	0.012	*
	CV height of inflorescence	(Intercept)	1.29	0.022	<0.001	***
		MAP	-0.09	0.023	0.002	**
		MAT	-0.08	0.025	0.005	**
		cvP	0.07	0.025	0.01	**
	CV height of vegetative part	(Intercept)	31.78	1.652	<0.001	***
		MAT	-3.86	1.693	0.034	*
A. scoparia	Mean SLA	(Intercept)	0.15	0.007	<0.001	***
		H_E	-0.03	0.007	0.001	***
	CV SLA	(Intercept)	1.61	0.037	<0.001	***
		Soil C/N	-9.06	3.602	0.021	*

Pr(>|t|) – significance p-value. Significance codes: p ≤ 0.001 '***'; p < 0.01'**'; p ≤ 0.05 '*'; p ≤ 0.1 '.'.

4. Discussion

4.1. Population genetic diversity and differentiation of *A. frigida* and *A. scoparia*

Life form and breeding system of plants are known to have a major influence on species' genetic diversity and population genetic structure (see Nybom & Bartish 2000; Reisch & Bernhardt-Römermann 2014; De Kort *et al.* 2021). Our chosen *Artemisia* species both have a wide range of distribution, are wind/water dispersed, outcrossing, and had prevailing tetraploid cytotypes, making a direct comparison of diversity indices possible. Population-level mean values of the genetic diversity in both *Artemisia* species were higher (*A.f*: H_E = 0.79 and *A.s*: H_E = 0.86) than in the review of Nybom (2004) for similar life history traits. The genetic diversity was significantly higher in the biennial *A. scoparia* than in the perennial species, according to four of the six diversity indices (H_E, G_{IS}, Bruvo, and PPL; Fig. 2). This is in line with the study of Balfourier *et al*. (1998), who compared outcrossing annual and perennial ryegrass (*Lolium* L.) species. Probably, the effective population size and recombination rate are higher in the biennial than in the perennial. In short-lived species, recombination rate is higher as a result of their shorter life cycles and smaller genome/ lower DNA content (Brazier & Glémin 2022), which may lead to a higher level of genetic diversity. Indeed, Garcia *et al*. (2004) reported that genome size of *A. scoparia* was the smallest (1C = 1.77 pg) within the studied species, while the genome size of *A. frigida* was 2.63 pg. Furthermore, in *A. frigida*, a smaller number of plants may participate in reproduction, as it is often subject to intensive grazing in natural and permanent pastures, and some individuals may survive vegetatively over several seasons. However, this observation is in contrast to some review studies that compared the genetic diversity of different life forms, utilizing allozyme and RAPD markers (see Hamrick & Godt 1990, 1996; Nybom & Bartish 2000; Nybom 2004) and AFLP markers (Balfourier *et al.* 1998; Reisch & Bernhardt-Römermann 2014). Nonetheless, individual life history traits, as well as genetic markers and diversity indices utilized affect estimates of population genetic diversity, making the direct comparisons among studies somewhat questionable.

Patterns of genetic variation in the two species did not differ much, with spatial differences (among regions) explaining about 1% of the genetic variation, while barely 2-3% variation resided among populations, and the highest variation (more than 95%) was explained by within-population variations (Table 3). Yet, the populations of the perennial *A. frigida* represented some structure illustrated in the PCoA, having fuzzy eastern and western clusters associated with altitude, longitude, amount of precipitation, and soil salinity (Fig. 3a). Patterns in the biennial species were more continuous and impacted by geographical factors, like longitude, latitude, and altitude, as well as the coefficient of interannual precipitation variation (Fig. 3b). Population 5 (Hustai NP) is a geographically central population that, however, represented the greatest genetic distance from others in both species (see PCoA; Fig. 3 and Suppl. Table 5 for differentiation matrices). This pattern has been seen in our former studies (see Oyundelger *et al.* 2021, 2023), and is now supported by the analysis of a second species, indicating this region has a distinct regime of gene flow and/or population connectivity, most likely due to its proximity to the local livestock trade center where animals from all over the country are brought in and may carry seeds.

Only few studies have compared the genetic variation of herbaceous species with different life forms (perennial vs. annual) in the same spatial context (Balfourier *et al.* 1998; Zhou *et al.* 2008; Heelemann *et al.* 2015), but their findings were contradictory: Zhou *et al*. (2008) found the highest molecular variation among populations in the annual (78%) than the perennial wild rice species (52%). While Balfourier *et al*. (1998) and Heeleman *et al*. (2015) reported that most of the total genetic variation was accounted for within populations in perennial (91%) and annual ryegrass (90%); and wild rosemary species (perennial: 89% and annual: 87%), respectively. Our result was in line with the latter, as within population variations were as high as 96% in both species. Furthermore, genetic variation between populations of the perennial was only marginally higher than that of annual species; yet both were comparably low. The low level of genetic variation between populations and regions, as well as weak correlations between genetic differences with environmental distances, indicate considerable historical and current gene flow between populations, supporting our former studies (Oyundelger *et al.* 2021, 2023).

4.2. **Associations of functional traits with genetic and environmental variations**

Mean values as well as variations of morphology- (IH and VH) and (eco)physiology-(SLA) related traits were predominantly associated with environmental variables rather than with genetic variation (Table 4). This indicates that the traits showed substantial plasticity in response to environmental differences, as demonstrated by a number of other studies (see Gratani 2014; Chevin & Hoffmann 2017; Matesanz & Ramírez-Valiente 2019). Specifically, climate (MAP, MAT, and cvP) was found to be the most important factor influencing the morphological trait variations of the perennial *Artemisia*. This, of course, indicates the importance of climatic conditions for plant growth, as has been previously shown for plant species occurrence and abundance in the Mongolian steppe (von Wehrden & Wesche 2007; von Wehrden *et al.* 2010). In *A. frigida*, morphological differentiation is probably promoted by site-dependent microhabitat

differences, primarily in temperature and water availability. Morphological differences become even more pronounced, particularly due to the harsh climate in steppes (MAT: min (-6.1) to max +3.8 C°) with overall limited water availability (MAP: min 117 mm to max 300 mm), as demonstrated by our linear model (Table 4). Phenotypic differences were pronounced between sites/populations, whereas genetic differentiation was less evident (Global F_{ST}= 0.064). This is in line with a large body of literature showing plant phenotypic trait responses and genetic differentiation patterns varying highly in abiotic and biotic environmental conditions (Odat *et al.* 2004; Bucher *et al.* 2016; König *et al.* 2018), and plant trait differentiations being even enhanced in extreme environments (Chevin & Hoffmann 2017; Karbstein *et al.* 2019).

Specific leaf area (SLA) relates to photosynthesis, relative growth rate, and stress tolerance (Perez-Harguindeguy *et al.* 2013), and is known to be subject to substantial plasticity (Pan *et al.* 2013; Stotz *et al.* 2022) as well as being partly under genetic control (Knight & Ackerly 2003; Scheepens *et al.* 2010). In our study, mean SLA was significantly associated with altitude in *A. frigida* and with genetic diversity in *A. scoparia*. Soil nutrient availability also had a significant impact on the variation of the SLA in *A. scoparia*, supporting the common observations, as we detected the effect of both environment and genetics on SLA (Table 4). Significant relationships of the mean and cvSLA with environmental variables were observed in other studies. For instance, Woodward (1983) noted a negative association between altitude and SLA in *Festuca* L. and *Carex* L. species, which was explained by an underlying relationship between altitude and temperature. Yulin *et al* (2005) detected an increasing SLA in habitats with higher amounts of soil nutrients (total nitrogen and organic carbon) in *Artemisia halodendron* Turcz. ex Besser, as soil nutrient stress is a major limiting factor for plant growth. A global study has shown a positive association between soil fertility and SLA, whereas negative relationships exist between soil C/N ratio and SLA (Ordoñez *et al.* 2009), supporting our findings. Furthermore, genetic effects on SLA variance were observed in *Campanula* L. (Scheepens *et al.* 2010), which were attributed to selection-induced adaptations. The same may hold true for our observation that genetically less diverse populations represented a larger mean SLA, as a result of local adaptation. Yet, this negative association might be rather an artifact attributed to the (natural outlier) population 5 (Hustai NP), where the lowest population level diversity (H_E = 0.78) and the largest mean specific leaf area (SLA = 0.24 mm/mg) were detected (see relationship in Suppl. Table 10).

Conclusion

Understanding plant adaptation — both in terms of morphological and genetic aspects — to environmental heterogeneity has been a focal point of many studies. However, steppe plants have rarely been investigated, and no comparative studies of species with different life-history traits have been conducted to date. Our findings demonstrated that genetic diversity in both species was relatively high (*A.f*: H_E = 0.79 and *A.s*: H_E = 0.86), and their genetic variation and functional trait characteristics were significantly affected by geographical factors and soil nutrient contents. Surprisingly, climatic factors exhibited a relatively limited impact, and when there was an effect, it was primarily associated with the amount and variation of precipitation. This aligns with the overarching observation in Mongolia that precipitation serves as the primary limiting factor for

plant growth, occurrence, and abundance. Thus, plants in these areas require significant adaptations to thrive in the water-limiting habitats while retaining sufficient genetic diversity.

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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 LG and 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.

Data accessibility

WGS raw sequencing data is available in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA680535. Further dataset generated and analyzed during the current study are provided in the Supplement material tables.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Khurelpurev Oyundelger reports financial support was provided by TU Dresden. Karsten Wesche reports travel was provided by German Federal Ministry of Education and Research. Christiane Ritz reports travel was provided by German Academic Exchange Service.

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