

**Phylogeography and population genetics of key
steppe species: *Artemisia frigida* Willd. (Anthemideae,
Asteraceae)**

Dissertation
for attaining the PhD degree of
Natural Sciences (Dr. rer. nat.)

submitted to the
Faculty of Biology, School of Science
of the Technische Universität Dresden

by

Oyundelger Khurelpurev (M.Sc.)
born in 15.05.1993 in Ulaanbaatar, Mongolia

Defended on 3rd of November 2022

The thesis was supervised by Prof. Dr. Karsten Wesche and PD
Dr. Christiane M. Ritz between June 2018 and April 2022 and
hosted by Senckenberg Museum für Naturkunde Görlitz, Görlitz.

Artemisia frigida Willd.



C. Beauvois.
2022.

Table of contents

Summary	4
List of Abbreviations	6
Chapter 1. Introduction	8
Chapter 2. Material and methods	10
2.1. Study region: Mongolian steppe	10
2.2. Focus species: <i>Artemisia frigida</i> Willd.	11
2.3. Molecular markers	12
Chapter 3. Phylogeography	13
3.1. Eurasian steppe and its evolutionary history	13
3.2. <i>Artemisia</i> L. (Asteraceae) as model plant for phylogeography	16
3.3. A case study: Phylogeography of <i>Artemisia frigida</i> Willd.....	19
Chapter 4. Plant population genetics under changing climate and grazing	40
4.1. Climate – grazing interactions in Mongolian steppe	41
4.2. Climate – grazing interactions on plant population genetics....	59
4.3. Effect of grazing exclusion on plant populations genetics	83
Chapter 5. Overarching synthesis and discussion	101
5.1. Molecular markers: pros and cons.....	102
5.2. A review of phylogeographical studies on Eurasian steppe plants	104
5.3. A review on plant population genetic studies in Mongolia	109
5.4. Outlook.....	113
Acknowledgements	114
References	115
Curriculum vitae	137
Confirmation	143

Summary

The present thesis elucidates facts about a prominent steppe plant's evolutionary history (i.e., geographic origin, migration route, distribution of genealogical lineages and polyploidization events) and contemporary population divergence (i.e., genetic diversity and differentiation, impacts of abiotic and biotic factors). *Artemisia frigida* has been chosen as the target species, with Mongolia as the focus study region. Because *A. frigida* is widely distributed in the both New and Old Worlds, it was a suitable candidate for the phylogeographic study. Moreover, because of its dominance in many communities in Mongolian steppes and tolerance for cold, drought and mechanical disturbances (grazing), evaluating the effect of environmental factors and grazing pressures on its population genetics was profitable. The overall goal of this thesis was to assess the effects of paleo- and current climate, and land use changes on the distribution of *A. frigida*'s genealogical lineages and genetic variations.

The thesis is divided into two main parts: (i) Chapter 3 focuses on Phylogeography. Within this, section 3.3 depicts a study on the phylogeography of *A. frigida*, covering samples from its distributional range across the northern hemisphere. The study resulted in Asia being the species' main origination and diversification center, and the species spread northwards to the Russian Far East and eventually crossed the Bering Strait to North America. Among four geographical regions sampled, seven genetic lineages were found, with Middle Asia having the most diverse populations. According to our phylogenetic analysis, two populations of Kazakhstan in Middle Asia represented the most likely ancestral diploids, and subsequent polyploidization events have occurred on several occasions independently. The observed phylogeographic patterns of the species showed that paleoclimate, especially glaciation events of the Quaternary has predominantly affected species' current distribution, along with the expansion and contraction of the Eurasian steppe.

The second part (Chapter 4) is dedicated to Population genetics to reveal the effects of the current climate and land use on population genetic variation. Three studies were conducted at local and regional levels, focusing on Mongolia. The first study (Section 4.1- review of local literature) was done to offer background information about Mongolian steppes, and the effects of climate and grazing on the steppe vegetation. As a result, steppe vegetation responded to grazing in different ways, depending on the interplay of local environmental factors. In particular, an overall negative effect of grazing was found in desert, dry and high mountain steppes, but no or even positive effects in meadow and mountain steppes. The study highlighted the importance of the interaction effect of local environmental conditions and grazing in Mongolian steppe vegetation. The second study (Section 4.2) employed large scale climatic gradient and local scale grazing gradients to

assess the effects of grazing and environmental factors on the population genetics of *A. frigida*. Precipitation gradient covered 110 – 300mm difference of mean annual precipitation from central to southern Mongolia. While three levels of grazing gradient, such as heavy, moderate, and least grazed sites were examined. According to the study, grazing in overall, had no substantial effect on the genetic diversity of *A. frigida*, while environmental factors, i.e., summer precipitation and soil phosphorous content, promoted high genetic diversity. Genetic differentiation among populations across large climatic gradients was extremely low, suggesting the existence of considerable gene flow among populations across the steppes of Mongolia. The third study (Section 4.3) employed grazing exclosures to evaluate the genuine effects of grazing. Because Mongolia has a long-term nomadic pastoralism history, and grazing of large herbivores is already an integral part of the steppe vegetation. Thus, we utilized reference site fences along the Trans-Mongolian Railway (TMR), where fences have been built and maintained since 1955, resulting in over 60 years of grazing exclusion. In addition, we supplemented this with data from Hustai National Park (HNP), where three fences were established in 2003. As a result, we found a significant positive impact of grazing on the genetic diversity of *A. frigida*, implying that a certain level of grazing is beneficial for the species. While no grazing effect on the population genetic differentiation was detected, but climatic and soil variables strongly influenced population genetic structure.

In summary, this thesis provided an in-depth investigation of the phylogeography and population genetics of the species *A. frigida*, which can stand as an exemplar for other Eurasian steppe species. Paleoclimate had largely shaped the current distribution pattern of the species, while contemporary climate and environmental heterogeneity promoted species' polyploidization and genetic variation. Grazing by large herbivores showed no detrimental effect, or even a positive impact on the genetic diversity of *A. frigida*. *Artemisia frigida* populations in Mongolia are thus apparently genetically 'healthy', in spite of pervasive grazing in the region. Climate variables and environmental heterogeneity had a substantial impact on the species' both genetic diversity and differentiation, indicating its higher sensibility to climate change than to land use change. The findings of the thesis could be valuable in understanding species genetic variation under global land use and climate changes.

List of Abbreviations

AD	Allele Diversity
AFLPs	Amplified Fragment Length Polymorphisms
AGB	Above Ground Biomass
Al	Aluminum
ANOVA	Analysis of Variance
AMOVA	Analysis of Molecular Variance
BLB	Bering Land Bridge
bp	base pair
C	Carbon
Ca	Calcium
ccmp	Consensus Chloroplast Microsatellite Primers
cpDNA	Chloroplast DNA
CQPrec	Precipitation of Coldest Quarter
CQTemp	Temperature of Coldest Quarter
cvP	Coefficient of Variation of Annual Precipitation
DeS	Desert Steppe
DryS	Dry Steppe
D_{ST}	Genetic differentiation measurement of Jost's D
EfN	Number of Effective Alleles
F_{ST}	Genetic differentiation measurement of Wright
GBS	Genotyping by Sequencing
G_{IS}	Nei's Inbreeding coefficient
GLM	General Linear Model
G_{ST}	Genetic differentiation measurement of Nei
H_E	Expected Heterozygosity
HG	Heavy Grazing
HNP	Hustai National Park
H_O	Observed Heterozygosity
HTP	Himalaya and Tibetan Plateau
HTS	High throughput sequencing
IPK	Leibniz Institute of Plant Genetics and Crop Plant Research
ISA	Indicator Species Analysis
ISSR	Inter Simple Sequence Repeats
K	Cluster
Kya	Thousand Years Ago
LGM	Last Glacial Maximum
LMM	Linear Mixed Model
MAP	Mean Annual Precipitation
MAS	Mongolian Academy of Science
MAT	Mean Annual Temperature
Mg	Magnesium
MULS	Mongolian University of Life Science
MoS	Mountain Steppe
mtDNA	Mitochondrial DNA
Mya	Million Years Ago
N	Nitrogen
NALB	North Atlantic Land Bridge
nDNA	nuclear DNA

Abbreviations

NP	National Park
NUM	National University of Mongolia
p	p-value
P	Phosphorous
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PPL	Percentage of Polymorphic Loci
RAD-seq	Restriction site Associated DNA sequencing
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SBIK-F	Senckenberg Biodiversity and Climate Research Center
SC	Species Composition
SLA	Specific Leaf Area
SNP	Single Nucleotide Polymorphism
SpringP	Spring Precipitation
SpringT	Spring Temperature
SR	Species Richness
SRA	Sequence Read Archive
SSR	Simple Sequence Repeat
SU	Sheep Unit
SummerP	Summer Precipitation
SummerT	Summer Temperature
TMR	Trans-Mongolian Railway
VC	Vegetation Cover
WGS	Whole Genome Sequencing
WQPrec	Warmest Quarter Precipitation
WQTemp	Warmest Quarter Temperature

Chapter 1. Introduction

Steppes are characterized by a continental climate, with substantial seasonal variation in precipitation and high diurnal fluctuations in temperature. The origin and climatic characterization of the steppe biome are predominantly shaped by paleoclimate and orogenic processes of the Cenozoic. Hence, contemporary distribution pattern of a steppe plant could be primarily determined by a paleoclimate, while current climate and environmental heterogeneity may affect species genetic variety across their distribution. Global changes in climate and land use are causing ecological land degradation in terms of quality, productivity, biological diversity and genetic variety, particularly in the water-limited steppe biome (Barabanov *et al.* 2018; Zhang *et al.* 2018b).

The study area of this thesis focused on Mongolia, which harbors nearly 1.3 million km² of steppe, accounting for about 13 % of the Palaearctic steppe biome (Henwood 2010; Wesche *et al.* 2016). Steppes have undergone significant transformations, and large areas of steppes in Central and Eastern Europe have been turned into cultivated landscapes (Hurka *et al.* 2019; Smelansky & Tishkov 2012). While Mongolia's steppes are still remarkably intact due to the country's sparse human population and a continued tradition of nomadic pastoralism (Batsaikhan *et al.* 2014). However, 25 – 40% of the Mongolian steppes were found to be moderately degraded, owing mainly to overgrazing (Jamsranjav *et al.* 2018; Sainnemekh *et al.* 2022). Another prevailing factor that threatens land degradation is climate, especially aridity (Sainnemekh *et al.* 2022). The Mongolian steppes receive the lowest mean annual precipitation compared to other Palaearctic steppes (Wesche *et al.* 2016). During the last 75 years, the country's mean annual precipitation has decreased by 7 % and mean annual temperature has increased by 2.24 °C (i.e., almost twice higher than the global average; Han *et al.* 2021), making the Mongolian steppes potentially sensitive to both overgrazing and climate change.

The main aim of this thesis was to assess the impacts of paleo- and current climate and land use changes on the distribution of genetic lineages and genetic variations in *Artemisia frigida*, a prominent steppe species. First of all, a phylogeographic study was conducted to elucidate the species' spatial arrangements of genealogical lineages in its distributional range. Second, regional and local studies on grazing effects on Mongolian steppe vegetation were reviewed to characterize the impacts of grazing and climate. Third, population genetic studies were done to assess the impacts of the environment (climate, geography, soil condition) and grazing on the genetic variation between and within populations of *A. frigida*. Given the substantial impacts of overgrazing in Mongolian steppes, the impact of grazing was investigated using two different study designs in two studies with: (i) grazing intensity gradients and (ii) grazing exclusion.

An introduction to readers:

The main body of this thesis is divided into two parts: **Phylogeography** (Chapter 3) and **Population genetics** (Chapter 4), with separate discussion and synthesis sections for each topic. Section 3.3. “A Case Study: Phylogeography of *A. frigida*” has been published in the Journal of Evolutionary Biology. Sections 4.2. “Climate – grazing interaction on plant population genetics” has been published in the Journal of Conservation Genetics and 4.3. “Effects of grazing exclusion on plant population genetics” has been submitted to the Journal of Arid Environments. In addition, a local literature review was conducted to characterize effects of grazing and climate on steppe vegetation. Thus section 4.1, “Climate – grazing interactions in Mongolian steppe vegetation”, is included as an additional background, which has published in Frontiers in Ecology and Evolution.

The authors retain copyright to the text and illustrations in published papers and drafts. Reprint of any part of this dissertation requires permission of the copyright holders.

Figures and tables in the thesis are not renumbered, keeping the original publications’ numbering. Supplementary materials and appendices can be found from each publication’s online sources. Supplementary materials for the last paper, which has yet to be published, are available in the electronic version of this thesis.

Thesis comprised following publications:

Oyundelger, Kh., Harpke, D., Herklotz, V., Troeva, E., Zheng, Z., Li, Z., Oyuntsetseg, B., Wagner, V., Wesche, K. and Ritz, C.M. (2021). Phylogeography of *Artemisia frigida* (Anthemideae, Asteraceae) based on genotyping-by-sequencing and plastid DNA data: Migration through Beringia. *Journal of Evolutionary Biology*, 35 (1), 64-80.
<https://doi.org/10.1111/jeb.13960>

Oyundelger, Kh., Herklotz, V., Harpke, D., Oyuntsetseg, B., Wesche, K. and Christiane. R. (2021) Contrasting effects of local environment and grazing pressure on the genetic diversity and structure of *Artemisia frigida*. *Conservation Genetics*, 22, 947-962.
<https://doi.org/10.1007/s10592-021-01375-w>

Munkhzul, O., **Oyundelger, Kh.**, Narantuya, N., Tuvshintogtokh, I., Oyuntsetseg, B., Wesche, K. and Jäschke, Y. (2021) Systematic review of grazing effects on vegetation in Mongolian steppes. *Frontiers in Ecology and Evolution*. 9, 703220.
<https://doi.org/10.3389/fevo.2021.703220>

Oyundelger, Kh., Munkhzul, O., Ritz, C.M. and Wesche, K. (submitted) Long-term grazing exclusion effects populations genetics and functional traits of *Artemisia frigida* in Mongolia. *Journal of Arid Sciences*.

Chapter 2. Material and methods

2.1. Study region: Mongolian steppe

Mongolia covers the largest and still intact parts of the Palearctic steppe biome (Hilbig 2007; Pfeiffer *et al.* 2018). Mongolian steppe differs from other Palearctic steppes (European, Middle Asian, Tibetan and Mediterranean) by having the highest continentality (seasonal differences in mean monthly temperatures; 50.3 K), with low mean annual precipitation (MAP; 180 – 400 mm) and low mean annual temperature (MAT; -0.2 – 2.7 °C; Wesche *et al.* 2016).

Steppes encompass about 66 % of the country's area (Tuvshintogtokh 2014), bordering the Siberian taiga to the north and Gobi Desert to the south. The steppes in Mongolia comprise different ecological-physiognomic vegetation subtypes: high cold mountain steppe, mountain steppe, meadow steppe, dry steppe (including typical steppe), desertified and desert steppe following the Steppe Vegetation of Mongolia (Tuvshintogtokh 2014). Characteristics of the highly continental climatic, especially the availability of precipitation, and its seasonal and geographical distribution drive the development of vegetation types across the region. There are pronounced latitudinal and altitudinal vegetation gradients in Mongolia: precipitation decreases as temperature increases along a north-to-south gradient, determining dominant vegetation subtypes. Mountain systems (Altai in the west, Khangai in the central west, Khuvsgul in the north and Khentii in the northern east) provide an altitudinal differentiation of vegetation that sharpens their contrast (Chuluunkhuyag *et al.* 2021).

There are more than 600 vascular plant species from 227 genera and 54 family distributed in the Mongolian steppes, with meadow steppe (with ca. 300 species) being the most species rich and productive type (Tuvshintogtokh 2014). However, they cover only around 3% of the country's total area, while the most prevalent vegetation types are dry steppe (22%), followed by desert steppe (20%), where a number of characteristic grassland species of *Stipa*, *Agropyron*, *Artemisia*, *Leymus*, *Allium*, and *Caragana*, predominate.

Mongolia has a history of more than 4000 years of nomadic pastoralism, and thus, livestock grazing is an integrate part of the steppe ecosystem. Grazing has substantial impact on vegetation productivity, plant community composition and species richness of the steppe ecosystem, and interacts with abiotic environmental conditions (Ahlborn *et al.* 2020; Bat-Oyun *et al.* 2016; von Wehrden *et al.* 2012a). Unfortunately, due to a drastic increase in livestock numbers over the last three decades, Mongolian steppe is partly under threat of degradation and desertification (Jamsranjav *et al.* 2018; Liang *et al.* 2021; Liu *et al.* 2013b).

2.2. Focus species: *Artemisia frigida* Willd.

The flora of Mongolia listed 103 *Artemisia* species (Baasanmunkh *et al.* 2022), among which dry steppe and forest steppe species are the most numerous. One of the most prevalent species, distributing across the country is *Artemisia frigida* Willd. (хүйтсэг шарилж, агь; Fig.1a). The species most likely evolved during the Pleistocene-Holocene (Yurtsev 1987), and it primarily distributed in the circumpolar arctic-alpine region and continental steppes of the northern hemisphere (Oyundelger *et al.* 2021a). It is an aromatic, perennial and very polymorphic species. Its life form varies from xerophyte semi-dwarf shrub with regularly branching and sympodially growing skeletal axes, vertical or prostrate, to almost herb form (Undarmaa *et al.* 2015; Fig. 1b-f). It grows primarily on fixed and semi-fixed silty-sandy soil. *Artemisia frigida* is a prolific seed producer, with each small inflorescence containing ca. 1,000 seeds (Harvey 1981), which maintain a consistent seed bank in the soil. In addition to sexual reproduction, it also has a clonal growth strategy (Jinhua *et al.* 2005).

The species was considered to be diploid ($2n = 2x = 18$) (Garcia *et al.* 2004; Korobkov & Kotseruba 2015). However, the tetraploid cytotypes ($2n = 4x = 36$) were found from Yakutia, Baikal Siberia, and Inner Mongolia (Korobkov *et al.* 2014; Pellicer *et al.* 2010; Wan *et al.* 2011).

Artemisia frigida is tolerant to coldness, drought and grazing (Bai & Romo 1996; Coupland 1950), and therefore it can expressly predominate in degraded steppes of Mongolia. Nonetheless, it is considered in Mongolia as an excellent rangeland plant with high nutritional value in Mongolia (Damiran 2005).

The species' broad distribution across the northern hemisphere, makes it ideal for phylogeographic study. While, its dominance in Mongolian steppes and tolerance to grazing, made it good candidate for population genetics, particularly in assessing effects of grazing and climate on genetic variation.

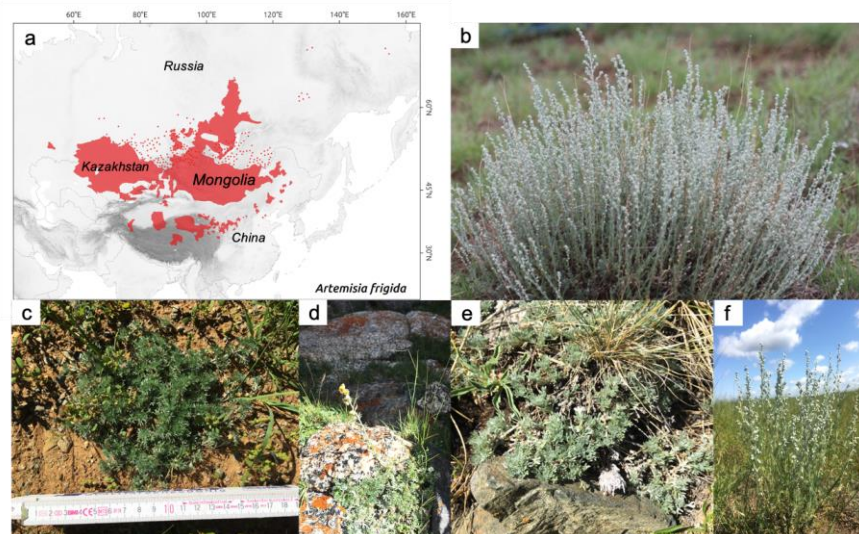


Figure 1. a) Central Asian distribution of *Artemisia frigida* and b-f) species appearances in its typical habitats in Mongolia.

2.3. Molecular markers employed for the phylogeographic and population genetic studies and their characteristics

The phylogeographic study of the thesis is primarily based on a novel approach of high-throughput sequencing (HTS). Although HTS is steadily being utilized in many different research fields (e.g., in disease genetics, metagenomics, and forensics etc.), its adoption in phylogeography has lagged behind others due to non-model organisms usually being targeted, the massive number of samples required per species, and the associated high expenses (McCormack *et al.* 2013). We applied a genotyping-by-sequencing (GBS) approach that uses restriction enzyme to reduce genome complexity, allowing high throughout genotyping of large number of samples with thousands of single nucleotide polymorphisms (SNPs) (Elshire *et al.* 2011). Other advantages of the GBS include the fact that it is applicable even with little or no prior knowledge on genome, and is more cost-efficient than whole genome sequencing (WGS) for large number of individuals.

In addition to that, a traditional method, i.e., a noncoding region of chloroplast DNA (cpDNA) marker was used for the phylogeographical study. The cpDNA markers are initial and still dominant in plant phylogeography, because they are usually uniparentally inherited and permit genealogical traces to be followed between populations, species and higher taxonomic levels (Bermingham & Moritz 1998). Many studies still rely on these markers due to their neutrality, elevated mutation rates, lack of recombination (in most taxa), and ease of isolation due to their high number of copy number per cell (Beheregaray 2008). We employed *rp132 – trnL* intergenic spacer that was tested and proven to contain a high percentage of informative sites compared to other regions (i.e., *psbA – trnH* and *trnL – trnF*), which were frequently applied on the genus *Artemisia* L. (Hussain *et al.* 2019; Malik *et al.* 2017; Riggins & Seigler 2012).

For population genetic studies, co-dominant, neutral and multiallelic markers, i.e., microsatellites (SSRs; Simple Sequence Repeats) were employed. Because SSRs (repeats of two to six nucleotide DNA motifs) are common in genomes, they could be used to investigate a large number of independent and highly variable loci. Its natural codominance and elevated mutation rate result in high allelic diversity that is useful for studying microevolutionary processes, including in population genetics of polyploids. Yet on the down side, SSRs produce frequent null alleles, face difficulty estimating the number of mutations, and has limited applicability outside of the taxon (Albayrak *et al.* 2012; Zink 2010). Due to a lack of species-specific SSR markers for *A. frigida*, we had to develop new markers, which was done utilizing whole genome sequencing (WGS). The WGS of few cytotypes (individuals) could allow mining of hundreds of microsatellite loci, yet to obtain good polymorphic loci requires further polymerase chain reaction (PCR) optimization (Taheri *et al.* 2018).

Chapter 3. Phylogeography

3.1. Eurasian steppe and its evolutionary history

The Eurasian steppe is the largest steppe region worldwide that stretches 8,000 kilometers from the Hungarian basin in the west to the Amur in the east (Lavrenko & Karamysheva 1993). Steppes are defined not only by their physiognomic characteristics, i.e., their vegetation dominated by drought-resistant xeromorphic grasses, perennial herbs, low shrubs, as well as geophytes and therophytes, but also by the geology and climate that sustain them (Wesche *et al.* 2016). Based on the evidences of paleoclimate, paleoenvironment, and paleobotany data, formation of the steppe presumably began in Central Asia in the Early Neogene, with strong influences of the Paleogene cooling trend and the establishment of the Asian monsoon system (Hurka *et al.* 2019; Žerdoner Čalasan *et al.* 2019). Global overall cooling, which interrupted by warming periods, continued in the Late Oligocene and Miocene (see Fig.2 for geological time scale of the Cenozoic), and several spatial and temporal changes had major impacts on the establishment and development of the Eurasian steppe (Hurka *et al.* 2019):

- (i) The Turgai Strait, which connected the Tethys Sea with the Arctic Sea, disappeared in the Early Oligocene, resulting in an extension of terrestrial area and formed land bridges between western and eastern Eurasia.
- (ii) The final disconnection of the Paratethys Sea from the Mediterranean occurred in the Middle Miocene, resulting in isolation and retreat of the Paratethys.
- (iii) The emerging mountain system increasingly separated marine basins in western Eurasia, and uplift of the Himalaya and Tibetan Plateau (HTP), and associated mountain ranges, like Tian Shan, Altai-Sayan, Pamir and Hangai developed in eastern Eurasia in the Middle and Late Miocene.
- (iv) The elevated orography of the HTP influenced atmospheric thermal circulation over Asia, forming the Asian monsoon, with its seasonal reversals in winds and precipitation, resulting in “wet” summers and “dry” winters (Wang 2006). The climate in Central Asia became strongly seasonal and semi-arid. Moreover, Tian Shan and Pamir screened of westerly

Era	Period & Subperiod	Epoch & Subepoch	Age	Age (Ma)		
Cenozoic	Quaternary	Holocene		0.012		
		Pleistocene	L	'Tarantian'	0.126	
			M	'Ionian'	0.781	
			Early	'Calabrian'	1.806	
				Gelasian	2.588	
	Neogene	Pliocene	Piacenzian		3.600	
			Zanclean		5.332	
			Messinian		7.246	
		Miocene	Tortonian		11.608	
			Serravalian		13.65	
			Langhian		15.97	
			Burdigalian		20.43	
			Aquitanian		23.03	
			Oligocene	Chattian		28.4
				Rupelian		33.9
				Priabonian		37.2
				Bartonian		40.4
				Lutetian		48.6
	Eocene	Ypresian		55.8		
		Thanetian		58.7		
		Selandian		61.7		
		Paleocene	Danian		65.5	
Tertiary	Paleogene					

Figure 2. Chronostratigraphic chart of the Cenozoic according to the International Commission on Stratigraphy, 2021/07.

3. Phylogeography

moisture, which certainly affected herb steppe expansion in Central Asia (Barbolini *et al.* 2020).

All the preceding events, global overall cooling, retreat of the Turgai Strait and the Paratethys Sea, uplift of the HTB, together with the Asian monsoon system intensified the aridification of interior Central Asia, and resulted in the expansion of the Eurasian steppe and diversification of steppe plants.

Reflection of Eurasian steppe evolution on steppe plants' distribution

From Paleogene to Neogene, the Earth's climate system shifted from "greenhouse" to "icehouse" and dominant vegetation elements changed from evergreen and thermophilus to deciduous and temperate plants (Ivanov *et al.* 2011). In particular, the Late Miocene and Pliocene were significant periods in the evolution of the Eurasian steppe, where continuous Eurasian steppe belt with a zonal landscape-climatic structure was stabilized and forests were gradually replaced by forest steppe and temperate steppes (Friesen *et al.* 2016). At the end of the Pliocene, true steppes were widespread in Central Asia, whereas forest-steppes prevailed on the West Siberian Plain and in south-eastern Europe (Barbolini *et al.* 2020). Following pollen-based vegetation reconstruction conducted by Feurdean and Vasiliev (2019), a gradual change in dominance of steppe elements (*Artemisia* and *Ephedra*) and herbaceous plants (Poaceae, Asteraceae, Brassicaceae, etc.) occurred in eastern Eurasia, with ~10 % in the Late Miocene (~8.5 Mya), ~20 – 30 %, in the Miocene/Pliocene (~8.5 – 4.3 Mya), and up to ~80% in the Middle Pliocene (~4.3 Mya); at the same time all forest types declined. Yet, with the beginning of Quaternary, the onset of large glaciation started (2.8 – 2.4 Mya) (Hurka *et al.* 2019), and the Northern Hemisphere was largely covered with an ice sheet.

The Quaternary played an important role for shaping the current distribution of steppe plants, because of its numerous cold – warm (glacial and interglacial) macrocycles. There were about 30 glacial events covering different spatial range, with a dominant series of cold and dry glacial periods (80 % of the Quaternary) and interspersed shorter intervals of warmer and moister interglacial climates (Ehlers & Gibbard 2008). The last glacial episode, which had significant changes in landscape in many areas happened between 22 – 19 Kya, and is known as the Last Glacial Maximum (LGM; Hughes & Gibbard 2015). The ice-sheet stretched southwards across Eurasia to about 50°N (Batchelor *et al.* 2019), and strong climatic gradients existed across Eurasia due to the large ice sheet and the changes in ocean currents. Along with these macrocycles, plant geographical distribution in the Northern Hemisphere shifted southward and northward at high latitudes, as well as upward and downward in mountain ranges at low latitudes (Comes & Kadereit 1998). Cold-adapted plants spread in the steppe during the cold periods, while forest plants recolonized the same areas during the warmer interglacial periods, causing expansion and contraction of species' ranges (Taberlet &

3. Phylogeography

Cheddadi 2002). This is known as expansion-contraction biogeographic model as proposed by Provan and Bennett (2008). Furthermore, the Quaternary has also been considered as a crucial period for genetic diversification and speciation of plants. In particular, cycles of fragmentation and re-expansion of species ranges led to extinction of many species that were unable to re-colonize their former range, or to allopatric speciation in which geographical isolation caused genetic differentiation among populations (Willis & Niklas 2004). However, there is scant evidence whether Quaternary isolations were long enough for genetic diversification and speciation to occur. Differences in growth forms (e.g., herbaceous annuals vs. perennials) and generation times are critical, resulting in differential genetic divergence responses during isolation (Jackson & Overpeck 2000).

Nonetheless, steppe origination and steppe species diversification happened largely in Central Asia, and species migration across Eurasia and to North America was substantially influenced by paleoclimate. In addition, formation of some important landscape elements, such as Altai-Sayan Mountains, Tian Shan, or Khangai Mountains in Central Asia, that may have hampered steppe plants' migration during the glacial periods; at the same those mountain systems served as refugium for cold steppe plants during the inter-glacial times (Caves *et al.* 2017).

3.2. *Artemisia* L. (Asteraceae) as model plant for phylogeography

The genus *Artemisia* L. (sagebrush, Beifuß, шарилж) is a large and diverse genus comprising over 500 taxa with trans-continental distributions, including both regional endemics and species found in both the New and Old Worlds (Riggins and Siegler 2012). Species are mainly distributed in temperate areas of the northern hemisphere from sea level to high altitudes, inhabiting arid and semi-arid environments (Sanz *et al.* 2008).

The origin of the genus is dated back to the Late Oligocene (24.6 ± 2.6 Mya) with the onset of diversification taken place in Central Asia between the Early and Middle Miocene (Sanz *et al.* 2011). Based on the most recent comparative analysis of this genus (Hussain *et al.* 2019; and references therein), there are six major divisions, treated as sections: *Absinthium* DC., *Artemisia* L., *Dracunculus* Besser, *Seriphidium* Besser, *Tridantatae* (Rydb.) McArthur and *Pacifica* Hobbs & Baldwin. The phylogenetic analysis based on nuclear and chloroplast DNA sequences of *Artemisia* and their allies by (Sanz *et al.* 2008) indicated that the genus is paraphyletic, excluding several small Asian genera and the North American genus *Sphaeromeria*. One of the important evolutionary traits leading to plant speciation is polyploidy, and it this is presumably be reflected in the genus *Artemisia* with somatic chromosome numbers ($2n$) ranging from 14 to 144 (Vallès *et al.* 2011; Vallès & McArthur 2001; and references therein). Despite the myriad of studies on the genus's relationships between species within the different sections are difficult to interpret because of their taxonomic complexity, large number of species and their often trans-continental distributions, and therefore, a plain explanation of the evolutionary history is challenging.

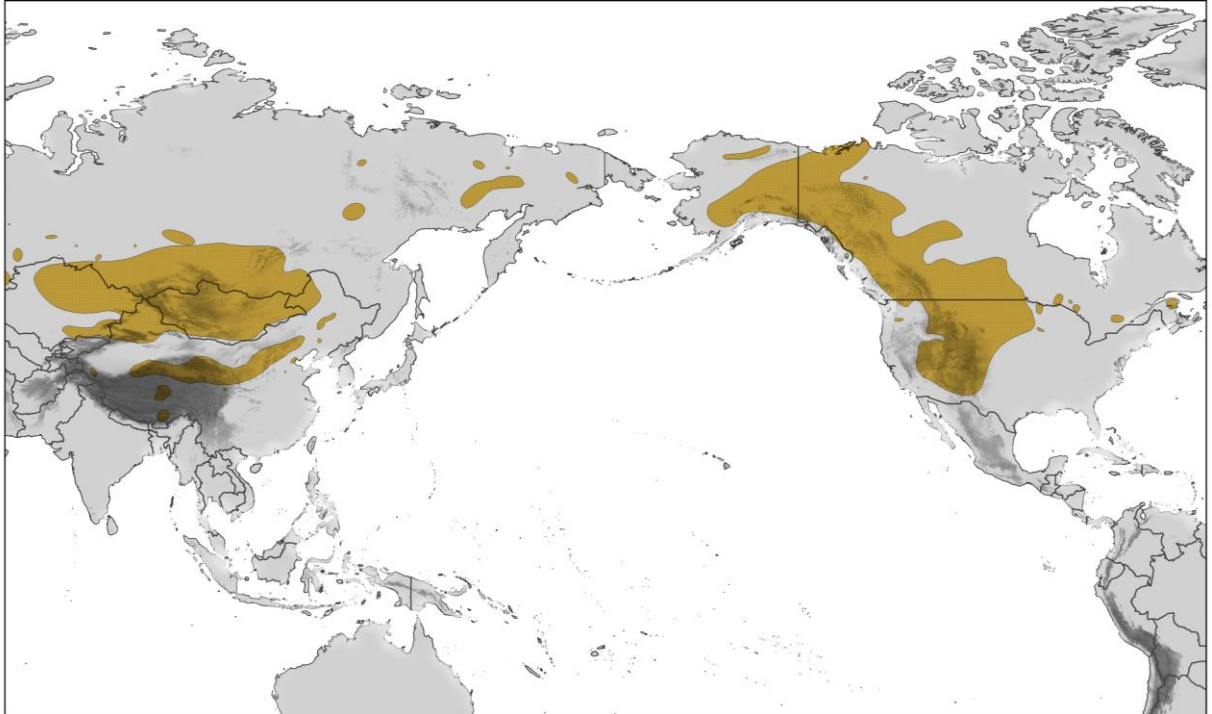
Artemisia's cosmopolitan distribution today and its origin in the Palearctic make it an ideal candidate for phylogeographic studies at various taxonomic levels, contributing to an increasing phylogeographic understanding of the evolutionary history of the northern hemisphere. There are several phylogeographical studies conducted on the genus. In particular, Sanz *et al.*, (2011) studied the diversification process of the genus, and reported that all *Artemisia* lineages (except North American endemics) migrated from Central Asia westward to Europe and colonized the Mediterranean Basin several times at different epochs. There were two arrival occasions to North America, most likely via NALB and BLB: one is the divergence of the North American endemic group in the Mid Miocene (ca. 10 Mya) and the other is the diversification of the American endemic *A. mexicana* Willd. from its Eurasian vicariants of the *A. vulgaris* L. complex (ca. 8 Mya). Moreover, Hobbs and Baldwin (2013) investigated some Hawaiian endemic species (*A. australis* Less., *A. kauaiensis* (Skottsb.) Skottsb. and *A. mauiensis* (A. Gray) Skottsb.) and found that *A. chinensis* L. of southeast Asia is the closest living relative of Hawaiian *Artemisia* taxa. A study on the European high mountain plant *A. eriantha* Ten. was in line with the overall migration

3. Phylogeography

pattern of the genus, i.e., from Central Asia to Europe and there colonizing the European mountains, by long-distance dispersal (Sanz *et al.* 2014). Further investigation on the coastal species of *A. crithmifolia* L. found low inter-population diversity, but well-defined genetic groups along the Atlantic coastline of Europe, suggesting a mixture of punctual founder effects and defined potential refugia during Quaternary glaciations (García-Fernández *et al.* 2017). A recent study on phylogeography of *A. herba-alba* Asso in the Iberian Peninsula and North Africa revealed a complex phylogeographical structure related to the division of the Mediterranean Basin, explaining their recurrent establishment success (Bougoutaia *et al.* 2021).

3. Phylogeography

3.3. A case study: Phylogeography of *Artemisia frigida* Willd.



Artemisia frigida Willd.

Distribution map of *Artemisia frigida* Willd. (Map is drawn by Jan Treiber)

The content of this chapter has published in **Oyundelger, Kh.**, Harpke, D., Herklotz, V., Troeva, E., Zheng, Z., Li, Z., Oyuntsetseg, B., Wagner, V., Wesche, K. and Ritz, C.M. (2021a). Phylogeography of *Artemisia frigida* (Anthemideae, Asteraceae) based on genotyping-by-sequencing and plastid DNA data: Migration through Beringia. *Journal of Evolutionary Biology*. 35: 64-80. <https://doi.org/10.1111/jeb.13960>.

Abstract

Artemisia frigida is a temperate grassland species that has the largest natural range among its genus, with occurrences across the temperate grassland biomes of Eurasia and North America. Despite its wide geographic range, we know little about the species' distribution history. Hence, we conducted a phylogeographical study to test the hypothesis that the species' distribution pattern is related to a potential historical migration over the 'Bering land bridge'. We applied two molecular approaches: Genotyping-by-Sequencing (GBS) and Sanger sequencing of the plastid intergenic spacer region (*rp132* – *trnL*) to investigate genetic differentiation and relatedness among 21 populations from North America, Middle Asia, Central Asia and the Russian Far East. Furthermore, we identified the ploidy level of individuals based on GBS data. Our results indicate that *A. frigida* originated in Asia, spread northwards to the Far East and then to North America across the Bering Strait. We found a pronounced genetic structuring between Middle and Central Asian populations with mixed ploidy levels, tetraploids in the Far East, and nearly exclusively diploids in North America except for one individual. According to phylogenetic analysis, two populations of Kazakhstan (KZ2 and KZ3) represent the most likely ancestral diploids that constitute the basally branching lineages, and subsequent polyploidization has occurred on several occasions independently. Mantel tests revealed weak correlations between genetic distance and geographical distance and climatic conditions, which indicates that paleoclimatic fluctuations may have more profoundly influenced *A. frigida*'s spatial genetic structure and distribution than the current environment.

Keywords: Bering land bridge, phylogeography, GBS, prairie sagewort

Introduction

The topic of phylogeography in the Northern Hemisphere has fascinated scientists since the discovery of remarkable floristic and faunistic similarities between Eurasia and North America (Donoghue *et al.* 2001; Elias & Crocker 2008; Graham 1972; Gray 1840, 1846; Jiang *et al.* 2019b; Tiffney 1985a). Two land connections existed during the Cenozoic that allowed biotic exchange between the New and Old Worlds (Tiffney 1985a; b), such as the North Atlantic land bridge (NALB; from late Paleocene to the late Eocene; ~55 – 35 Mya) and the Bering land bridge (BLB; from early Paleocene to the Pleistocene; ~66 – 2 Mya). The NALB, which connected the northeast of North America with Europe, has been viewed as a principal migration route for thermophilic taxa during the Paleocene (Andrews 1985; Brouillet & Whetstone 2000). During the same period, migration via the BLB occurred between the northwest of North America and East Asia, but it was presumably more limited due to the higher latitude and cold temperatures (Tiffney & Manchester 2001). However, after the disruption of the NALB in the late Eocene (ca. 38 – 34 Mya; Manchester 1999), the BLB region became warmer and served as an important crossing for warm- to cold-temperate taxa (Graham 1993), which played a crucial role in shaping the prevailing species diversity at both the continental and regional levels. Several animal species have apparently crossed the BLB and successfully colonized both sites, including for instance bison, brown bear, several species of voles, tree frogs, and even fleas that found from small mammals (Conroy & Cook 2000; Elias & Crocker 2008; Krasnov *et al.* 2015; Li *et al.* 2015). In contrast, a considerable number of species was restricted to only one side of the BLB, such as the woolly rhinoceros, the North American camel and river otter, and a number of leaf beetle as well as weevil species (Combe *et al.* 2021; Elias & Crocker 2008; Meiri *et al.* 2013; Salis *et al.* 2020). However, plants had a higher intercontinental exchange between the New and Old Worlds compared to taxa of mammals, arthropods, reptiles, amphibians and fungi (Jiang *et al.* 2019a).

The Beringian region (including Alaska, the Yukon Territory and Eastern Siberia) facilitated migration until the Pleistocene (Wolfe 1994). However, due to the significant climatic fluctuations over time (Sanmartin *et al.* 2001), genetic exchange was not uniform in terms of direction or composition (Jiang *et al.* 2019a). There are a number of phylogenetic reconstructions of Beringian woody taxa (Donoghue & Smith 2004; Liu *et al.* 2017), yet studies on herbaceous plants are scarce, and Ickert-Bond *et al.* (2009) revealed a large disparity in taxon sampling. Examples of plant species that crossed the Bering land bridge include *Hordeum* spp. L. (Blattner 2006), *Heracleum lanatum* W. Bartram (Harris 2007), *Carex* L. spp. (Maguilla *et al.* 2018; Yurtsev 2001) and *Artemisia* L. spp. (Riggins & Seigler 2012; Tkach *et al.* 2008b), for which Beringia served as a dispersal route and glacial refugium (Ickert-Bond *et al.* 2009). The most frequent direction of Beringian dispersal

3. Phylogeography

was from Asia to North America (Waltari *et al.* 2007); however, long-distance dispersal between the continents via alternative means has also been reported in a number of studies, particularly for species with high dispersal capacity (Escudero *et al.* 2010; Maguilla *et al.* 2018). In addition, geological and / or climatic shifts have triggered further diversification within continents as well as differentiation between the Asian and North American floras (Donoghue *et al.* 2001).

The uplift of mountains, e.g., the Rocky Mountains in North America, and the Tibetan Plateau, the Tian Shan, the Altai Mountains in Central Asia, had a significant and complex effect on the evolution of the flora on the respective continents (Qian 2002). This uplift increased geographic heterogeneity and altered climatic conditions, including seasonal variations in temperature or precipitation (e.g. rain shadow areas; Broccoli & Manabe 1997). The Altai Mountains constitute a western barrier for the eastern Asiatic summer cyclones and monsoon rains, such that the climate and vegetation between the two sides of the mountain range differ substantially, which results in a pronounced biogeographic separation between Middle and Central Asia (Hurka *et al.* 2019). The climatic conditions between these neighboring regions are mainly differentiated by their seasonal variability, particularly with respect to precipitation (Wesche *et al.* 2016). Most notably, winter precipitation characterizes phytogeographic patterns in Middle Asia, while summer rain during the growing season is the most defining factor for Central Asia. Elsewhere, similar climatic conditions may occur in regions separated by far greater distances, e.g. the prairies of central North America and the steppes of the Russian Far East, where most of the precipitation falls during the growing season (Blair *et al.* 2014) and temperatures range from -60 °C to 45 °C between winter and summer (McGinn 2010).

Artemisia L. (Asteraceae) is a large genus with more than 500 species (Vallès *et al.* 2003), including many Eurasian and North American endemics as well as numerous widespread species (Ling 1982; McArthur 1979; Oberprieler *et al.* 2009; Vallès & McArthur 2001). The genus has served as a model for studying plant systematics and phylogeography, which has broadened the understanding of patterns and processes of diversification in arctic, alpine (Sanz *et al.* 2014; Tkach *et al.* 2008a) and temperate regions (Wang *et al.* 2004; Yunfa *et al.* 2011). Based on paleontological data, *Artemisia* is assumed to have originated in the semi-arid steppes of northwestern Asia during the early Oligocene, about 30 – 32 Mya (Liu *et al.* 2003; Wang 2004; Zhi-chen *et al.* 2004). However, Central Asia is considered to be the genus's main center of speciation (Pellicer *et al.* 2007). According to Tkach *et al.* (2008b), 16 to 22 migration events have occurred from Asia to North America, but only two to five in the reverse direction. Following Ling (1991, 1994), the putative ancestor of the genus *Artemisia* existed in Northern Asia, and the migrations from this place supposedly occurred along three routes: i) westward to Europe, Western Asia, Asia Minor, the Mediterranean

Basin and Africa; ii) eastward to Siberia and to western North America, and iii) further south into Asia.

Artemisia frigida Willd. (section *Absinthium* DC.) is primarily distributed in the circumpolar arctic-alpine region and has a wide distribution across the continental steppes (Fig. 1) of the northern hemisphere (Harvey 1981; Hultén 1968). This perennial subshrub is an important component of the dry steppe flora, as it is resistant to cold temperatures, drought and mechanical disturbance, especially grazing. Therefore, comprehensive studies on its systematics (García *et al.* 2011; Pellicer *et al.* 2011; Riggins & Seigler 2012) and population genetics within its distribution range have already been conducted (Liu *et al.* 2012; Oyundelger *et al.* 2021b; Wan *et al.* 2008; Wang *et al.* 2004). A number of studies have focused on the cytogenetic diversity of *A. frigida* (García *et al.* 2004; Korobkov *et al.* 2014; Korobkov & Kotseruba 2015; Pellicer *et al.* 2007, 2010), which revealed diploid and tetraploid individuals with $2n = 2x = 18$ and $2n = 4x = 36$, respectively. Despite the species' wide distribution range and an increasing understanding of its biology, no extensive phylogeographical study has been conducted on *A. frigida*. There are two opposing and unresolved hypotheses with respect to its migration history: 1) Yurtsev (1987) noted that the species presumably evolved during the Pleistocene as a pre-boreal element of the Altai-Mongolian steppe region before migrating to North America; whereas 2) Riggins and Seigler (2012) stated that *A. frigida* could be one of the tertiary relicts of North America's Rocky Mountains that migrated eastwards.

The main aim of this study was to unravel the phylogeographic history of *A. frigida*, and thus provide more evidence on migration patterns between Asia and North America. Specifically, we investigated the genetic differentiation and relatedness among populations from North America, Middle and Central Asia, and the Russian Far East. We first employed Sanger-sequenced plastid markers, as they are usually maternally inherited and easy to interpret, and they have proven to be valuable tools in numerous phylogeographical studies, including *Artemisia* (e.g., *A. crithmifolia* in Europe by (García-Fernández *et al.* 2017); Hawaiian *Artemisia* species by (Hobbs & Baldwin 2013)). In addition, we applied genome-wide single-nucleotide polymorphisms (SNPs) comparisons, which we obtained through Genotyping-by-Sequencing (GBS). The GBS approach has proven its effectiveness as a tool to resolve relationships within species in a number of recent population genomic studies (Fernández-Mazuecos *et al.* 2018; Nicotra *et al.* 2016; Pérez-Escobar *et al.* 2020). Based on these molecular approaches, we first investigated the origin and possible migration history of *A. frigida*. Secondly, we aimed to reveal the evidence for genetic sub-structuring and ancestry proportions among geographical regions. Third, we explored whether major environmental factors played an important role in the current populations' genetic structure.

Material and methods

Taxon sampling and DNA extraction

Young branches of *A. frigida* were collected during its growing season in July and August in 2018 and 2019. A total of 176 samples from 21 populations (eight to ten individuals per population) were sampled from Canada, Russia, Kazakhstan, Mongolia and China (Fig. 1). Further details about the sampling material are shown in Table 1. Air-dried or silica gel dried leaves were used for DNA isolation using Mag-Bind Plant DNA DS Kit (Omega Bio-Tek, Norcross, GA, United States) following the manufacturer's protocol. Quantification was done on the Qubit Fluorometer 4.0 using Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, United States) and agarose gel electrophoresis to verify high molecular weight molecules.

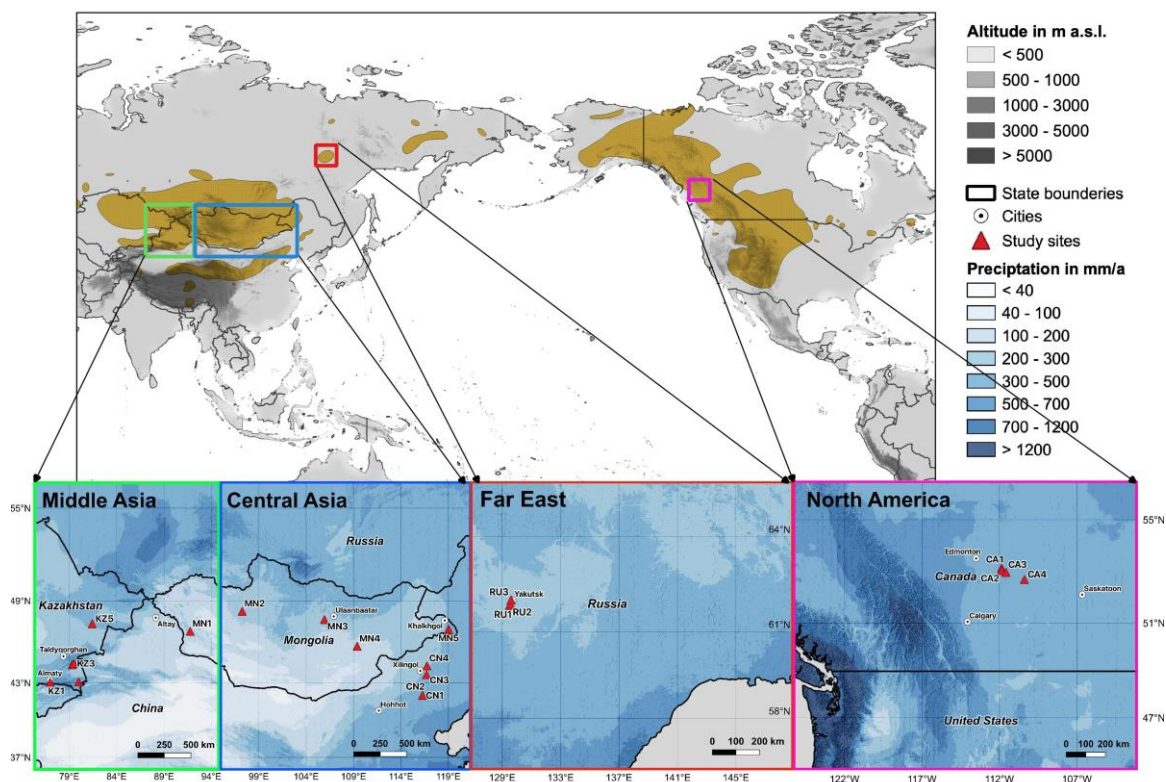


Figure 1. Distribution of *A. frigida* based on different floras (list of the floras is provided in Table S1) and localities of the sampled populations across its distribution range supplemented with data from precipitation.

Sequencing of plastid markers

Based on previous research on the genus *Artemisia* (Hussain *et al.* 2019; Malik *et al.* 2017; Riggins & Seigler 2012), we pre-selected three plastid noncoding regions: *rpl32 – trnL*, *psbA – trnH* and *trnL – trnF* for amplification. The markers were tested on 21 samples (one individual from each population) and were sequenced in both directions. The *rpl32 – trnL* spacer (*rpl32*: 5'-CAGTTCCAAAAACGTA CTT C-3', *trnL*(UAG): 5'-CTGCTTCCTAAGAGCAGCGT-3'; (Shaw *et al.* 2007)) contained the highest percentage of informative sites and was consequently chosen for subsequent

3. Phylogeography

phylogenetic analyses. PCR amplification was performed in a 25 μ L reaction mixture containing 0.25 μ L peqGOLD *Taq* polymerase (Peqlab, VWR International, LLC), 2.5 μ L of 1 \times S-Buffer (Peqlab), 5 μ L of Enhancer (Peqlab), 2.5 μ L of dNTPs (2 mM), 1.5 μ L of MgCl₂ (25 mM), 0.25 μ L each forward and reverse primer (10 μ M) and 1 μ L of genomic DNA (20 – 100 ng). Cycling conditions were carried out for 3 min at 94 °C, followed by 34 cycles of 94 °C for 40 s, 54 °C for 40 s and 72 °C for 1 min, and a final elongation step of 10 min at 72 °C. The PCR products were sequenced at the central laboratory of the Senckenberg Biodiversity and Climate Research Center (SBIK-F, Germany) using an ABI3730 sequencing (Life Technology) machine. We developed an internal sequencing primer to increase the sequencing coverage (*IntraArfi*: 5'-CACGGAATGGATTAGTTTGAAC-3') and used it for the sequencing. Sequence reads of plastid markers were deposited in the NCBI GenBank and made publicly accessible under the accession number BankIt2456346: MZ216749 – MZ216873 (see Table S2 for accession number of each individual).

Library preparation and high-throughput sequencing

In order to obtain genome-wide SNP data, we conducted Genotyping-by-Sequencing (GBS) analyses (Elshire *et al.* 2011). Library preparation and sequencing were performed at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. For each sample, 200 ng of genomic DNA was used and cut with the restriction enzymes *Pst*I-HF (New England Biolabs, R3140S) and *Msp*I (New England Biolabs, R0106S). BluePipin (Sage Science) was used for size-selection of the library consisting of fragments between 400 to 600 base pairs (bp). Fragment size distribution and DNA concentration were evaluated using an Agilent BioAnalyzer High Sensitivity DNA Kit (Agilent Technologies, Inc.) and the Qubit DNA Assay Kit in the Qubit 2.0 Fluorometer. The DNA concentration of the library was checked using a quantitative PCR run. Library preparation, individual barcoding and single-end sequencing (1 \times 110 bp) on the Illumina NovaSeq 6000 followed the manufacturer's protocols (Illumina). Barcoded reads were then de-multiplexed using the CASAVA pipeline 1.8 (Illumina), and quality control was done with FASTQC (Andrews 2010). Cutadapt v1.16 (Martin 2011) was used for adapter and quality trimming of sequence reads (phred score > 25, reads shorter than 65 bp were discarded). Sequence reads for the GBS Illumina runs were deposited in 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>).

Analysis of chloroplast sequencing data

Chloroplast sequences were edited and aligned using the MAFFT algorithm (Kato *et al.* 2002) implemented in Geneious® 10.2.6

3. Phylogeography

(<https://www.geneious.com>). A total of 125 sequences were aligned, with the remaining sequences being removed from the analysis due to poor quality. Plastid haplotypes were determined from nucleotide substitutions and used for building a TCS network (Clement *et al.* 2002) with PopArt (Leigh & Bryant 2015) running under the default settings. The TCS algorithm employs the parsimony criterion (95%) to estimate the maximum number of mutational connections based on all pairwise comparisons of haplotypes (Clement *et al.* 2002). Nucleotide diversity, number of segregating sites, number of parsimony-informative sites and Tajima's D statistics were estimated in PopArt. *Artemisia xerophytica* Krasch. was used as the outgroup in the haplotype network because it was the most closely related to *A. frigida* (Pellicer *et al.* 2011; GenBank accession number JN86112.1). It is a central Asian desert species that does not occur in North America (eFloras 2008; Pellicer *et al.* 2007).

Analysis of Genotyping-by-Sequencing data

A *de-novo* assembly of the GBS data of 176 *A. frigida* individuals was carried out using ipyRAD v0.7.19 (Eaton 2014). The minimal number of samples per locus was set to 100, the clustering threshold was set to 0.85. Despite indications for tetraploidy (Oyundelger *et al.* 2021b), the ploidy level was taken as unproven, and the maximum number of alleles per site parameter was consequently set to two (biallelic SNPs). As a result, 3,575 sites were omitted, accounting for 8% of the total sites detected. For the other parameters, the default settings of parameter files generated by ipyRAD were used. Because of the poor input DNA quality, eight samples from the Mongolian population MN6 (SamplingID-40D) were removed, while eleven samples from Kazakh populations were omitted due to insufficient coverage. After running step7 of the ipyRAD pipeline again, the remaining 40,307 SNPs of the 155 samples (totaling 1,020,646 raw SNPs; 16% missing data) were used in subsequent analyses.

Inferring ploidy levels from GBS data

In the absence of chromosome counts or genome sizes, SNP ratios and heterozygosity were used to infer the ploidy levels. All GBS loci were sequenced multiple times and were therefore represented by several reads. As each allele was sequenced proportional to its frequency ratio, assumptions on ploidy level were possible (e.g., for biallelic SNPs 0.5/0.5 in diploids, 0.33/0.67 in triploids, and a mixture of 0.25/0.75 and 0.5/0.5 in tetraploids). To infer the frequency of SNPs, heterozygous 21 bp k-mer pairs were identified using kmc 3.0 (Deorowicz *et al.* 2013) and smudgeplot (Ranallo-Benavidez *et al.* 2020) for 30 samples comprising at least one representative per population. K-mers of 21 bp with exactly one SNP difference were regarded as a pair. Sequence pairs with a coverage of less than 30× were discarded. Using the output file of smudgeplot, coverage ratios for the heterozygous k-

mer pairs were calculated and plotted as histograms using R-package ggplot2 (Wickham *et al.* 2016).

A second method using Bayesian assignment was additionally employed using observed heterozygosity and the ratio of DNA sequences of ipyrad vcf for the complete dataset to validate the result. The ipyrad vcf output was converted using VCFConverter2.py (<https://github.com/dandewaters/VCF-File-Converter>) and the analysis was conducted using R-package gbs2ploidy (Gompert & Mock 2017). The estimation of SNP proportions was set to 0.25, 0.33, 0.5, 0.66 and 0.75, assuming the possible presence of diploids, triploids and tetraploids. Two independent repetitions were run with each three Monte Carlo Markov chains for 10,000 steps with a 10% burn-in. For uncertain and non-complementary outcomes, to include as much data as possible, gbs2ploidy was run again with the same settings as before, but based on the coverage of heterozygous 21 bp k-mers being obtained with the smudgeplot pipeline rather than the vcf.

Population genetic analyses

Genetic diversity and population differentiation

The number of heterozygous sites of each sample in the vcf output file was estimated, with an assumption that SNPs are biallelic and correspond to the settings in ipyrad. The estimation was done in DnaSP 6 (Rozas *et al.* 2017) using multi-MSA data files. The output was used to calculate the ratio of heterozygous sites to the total number of sites of the sample (hereafter, 'observed heterozygosity (H_O)'). We generated histograms of the H_O to infer whether there were two groups of H_O values. The existence of a second cohort with a higher H_O can indicate the presence of individuals with higher ploidy levels, which are characterized by being more strongly heterozygous, in that they show a higher intra-individual allelic diversity in comparison to diploids.

The vcf file was converted to a genlight object using R-package vcfR (Knaus & Grünwald 2017) to estimate pairwise Nei's genetic distances (Nei 1972) and Fixation Indices between populations (F_{st} ; Weir & Cockerham 1984) using the R-package StAMPP (Pembleton *et al.* 2013). These two distance measures were highly correlated ($r^2 = 0.77$, $p < 0.001$), and thus further analyses were based on the Nei distances only. Population-wise Nei distances were represented on a heatmap using the R-package gplots (Warnes *et al.* 2020).

Population structure and phylogenetic analysis

We explored population genetic structure using model-based Bayesian analysis implemented in the LEA R-package (François 2016). The number of clusters was determined using a cross-entropy criterion, which is based on the prediction of a fraction with masked genotypes (matrix completion), and on the cross-validation approach. An initial run was performed for $K = 1:22$,

3. Phylogeography

and the lowest entropy determining the optimum number of clusters (ancestral populations) was observed for $K = 7$, so $K = 1:7$ was run again with 20 repetitions. In addition, a Q-matrix was obtained by LEA, which includes the ancestral assignment frequencies showing clusters by different color-coding. The clusters were sorted using R-packages tidyverse (Wickham 2017) and plotted with ggplot2 (Wickham *et al.* 2016).

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Maximum Parsimony (MP) approaches. The final alignment was 294,733 bp long, containing 41,332 SNP positions and 17.91 missing sites. As outgroup, two individuals of *A. pontica* L. (sect. *Absinthium*) were used, which were collected from Russia (collected by S. Pfanzelt with collection IDs: SP1077 and SP1078, vouchers deposited in Herbarium Gatersleben (GAT)). The ML analyses were performed using RAxML (Stamatakis, 2014) applying the autoMRE criterion (final bootstraps: 600) with the GTR+G substitution model and search for the best scoring ML tree algorithm. The MP analysis was performed with PAUP* 4a161 (Swofford 2002) using the heuristic search algorithm with TBR branch swapping for the concatenated GBS-derived sequences. Gaps were treated as missing data, and 1,000 random-addition-sequences (RAS) were used to construct the starting trees in the heuristic search for the GBS data to avoid suboptimal tree-islands. Bootstrap support values were obtained by 500 bootstrap re-samples using the same settings as before but without RAS. The splits data was visualized in SplitsTree v5.0.0_alpha (Huson & Bryant 2006) with individual-level Hamming distances as default (Hamming 1950) and the Splits Network algorithm (Dress & Huson 2004), in order to obtain a splits network with 3,344 nodes and 6,182 edges.

Partitioning climate and geographic effect on genetic variation

In order to evaluate the effect of present climatic conditions and geographical location on the observed pattern of genetic structure, we conducted Principal Coordinate Analysis (PCoA) using the ape R-package (Paradis & Schliep 2019) based on Nei distances among populations. Monthly meteorological data covering 35 years (between 1979-2013) were retrieved for each population from the high-resolution time series CHELSA_V1 dataset (Karger *et al.* 2017). Based on the dataset, mean annual temperature (MAT), mean annual precipitation (MAP) and seasonality variables (i.e. summer and winter mean temperature and precipitation) were estimated for each site and used as predictors. A *post hoc* test was used to fit all environmental variables (including latitude, longitude and elevation) on the ordination axes, testing the correlation with 999 permutations. Climographs were built based on the monthly data average of 35 years for each region using the R-package climatol (Guijarro & Guijarro 2019), and typical representatives were chosen for illustration.

To assess the significance of correlation between geographic (Euclidean distance) and genetic distances (Nei), we performed a Mantel test

3. Phylogeography

with 999 permutations. An additional Mantel test was conducted between climatic differences (Euclidean distances based on centered and standardized climate data including MAT, MAP, summer and winter mean temperature and precipitation) and genetic distances (Nei) using the R-package adegenet (Jombart 2008), with two datasets informing each Mantel test: i) among all populations, and ii) among Asian populations. All these analyses were conducted in R v.4.0.3 (R Core Team 2020).

3. Phylogeography

Table 1. Characteristics of the study sites (population code, localities, coordinates (WGS84), altitude, collection date and number of samples) of *Artemisia frigida*. Populations are ordered and coded according to longitude within countries (see Fig. 1).

Pop Code	Country	Region	Location	E	N	Altitude [m a.s.l.]	Date	No. of sample
CA1	Canada	North America	North Viking, Beaver County, Alberta	-111°51'32"	53°10'18"	693	Jul. 2018	10
CA2	Canada	North America	South Viking, Beaver County, Alberta	-111°51'27"	53°05'04"	692	Jul. 2018	8
CA3	Canada	North America	Kinsella, Beaver County, Alberta	-111°34'37"	53°00'35"	702	Jul. 2018	8
CA4	Canada	North America	Ribstone Creek Heritage Rangeland Natural Area, Alberta	-110°23'11"	52°43'53"	644	Jul. 2018	8
KZ1	Kazakhstan	Middle Asia	Region Almaty, Ile-Alatau National Park	76°59'02"	43°03'35"	2506	Jul. 2019	8
KZ2	Kazakhstan	Middle Asia	Region Almaty, Panfilov District	79°18'16"	44°22'54"	1791	Jul. 2019	8
KZ3	Kazakhstan	Middle Asia	Region Almaty, Panfilov District	79°28'45"	44°29'31"	2174	Jul. 2019	8
KZ4	Kazakhstan	Middle Asia	Region Almaty, Raiymbek District	79°59'14"	43°04'36"	2207	Jul. 2019	8
KZ5	Kazakhstan	Middle Asia	East Kazakhstan, Urzhar District	81°25'12"	47°23'25"	1706	Jul. 2019	8
MN1	Mongolia	Middle Asia	Khukh nuur, Khovd Province, western Mongolia	91°45'24"	46°51'07"	2922	Aug. 2018	8
MN2	Mongolia	Central Asia	Zavkhan province, west-central Mongolia	97°14'48"	48°16'43"	1981	Aug. 2018	8
MN3	Mongolia	Central Asia	Hustai National Park, Tuv Province, central Mongolia	105°55'48"	47°41'29"	1325	Aug. 2018	9
MN4	Mongolia	Central Asia	Airag, Dundgovi Province, southern Mongolia	109°22'57"	45°45'52"	1032	Aug. 2018	9
MN5	Mongolia	Central Asia	Khalkh gol, Dornod Province, eastern Mongolia	119°02'17"	47°00'01"	976	Aug. 2018	8
CN1	China	Central Asia	Duolun county, Xilingol League, Inner Mongolia	116°17'03"	42°01'59"	1320	Jul. 2019	9
CN2	China	Central Asia	Duolun county, Xilingol League, Inner Mongolia	116°17'03"	42°02'56"	1320	Jul. 2019	8
CN3	China	Central Asia	Baizin Xile Pasture, Xilingol League, Inner Mongolia	116°42'01"	43°38'00"	1187	Jul. 2019	8
CN4	China	Central Asia	Maoding Pasture, Xilingol League, Inner Mongolia	116°45'00"	44°18'01"	1079	Jul. 2019	8
RU1	Russia	Far East	Yakutsk, Central Yakutia, Sakha Republic	129°30'43"	61°52'02"	117	Jul. 2018	9
RU2	Russia	Far East	Yakutsk, Central Yakutia, Sakha Republic	129°33'19"	61°54'41"	97	Jul. 2018	9
RU3	Russia	Far East	Yakutsk, Central Yakutia, Sakha Republic	129°36'40"	62°02'11"	131	Jul. 2018	9

Results

Variation in the plastid DNA sequences and haplotype network

The alignment of the *rpl32* – *trnL* intergenic spacer sequences was 1,050 bp long, and it showed a considerable level of polymorphism among the 125 samples of *A. frigida*. Specifically, 15 parsimony-informative sites were observed within 27 polymorphic (segregating) sites, and the nucleotide diversity (π) at the species level was 0.0044. The analysis of genetic relatedness of the 21 populations (comprising 125 individuals of *A. frigida*) was based on 27 substitutions from 18 haplotypes (Fig. 2). The most frequent haplotype was found in Central Asian populations, followed by a haplotype detected in populations from the Far East and Middle Asia, then haplotypes sampled in North American populations. The outgroup, *A. xerophytica*, was separated by six substitutions from the Asian haplotypes, indicating the possible origin of *A. frigida* in Asia, and a subsequent migration to the Russian Far East and then on to North America.

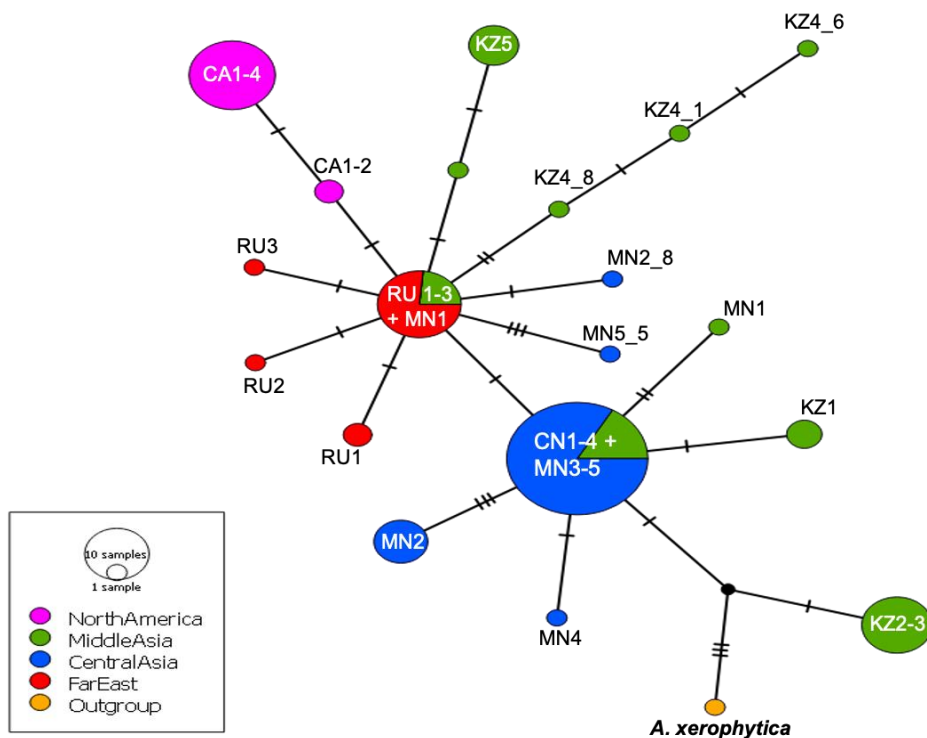


Figure 2. Parsimony network based on plastid marker *rpl32* – *trnL* intergenic spacer covering 21 populations (population code, see Table 1) of *A. frigida* across its distribution range. Size of ellipses reflect the haplotype frequency and hatch marks represent mutational differences.

Population genetic diversity

A total of 40,307 SNPs were identified using the GBS approach within 21 populations of *A. frigida*. The observed number of alleles across populations ranged from 86,456 (CN4) to 99,532 (CN1), with an average of $94,408 \pm 3,144$ alleles. Observed heterozygosity (H_0) among populations ranged from 0.021 (CA1) to 0.067 (KZ5), with an average of 0.044 (Table S3).

3. Phylogeography

The highest genetic diversity was found in the Far East ($H_O = 0.061$), whereas it was lowest in North America ($H_O = 0.023$; Fig. 3).

The frequency distribution of individual levels of heterozygosity showed two groups, one of which had twice as high values of H_O than the other (range: 0.02 to 0.04 vs 0.05 to 0.07; Table S4). The different methods used to infer ploidy levels were in 99% agreement (comparison is given in Table S4), demonstrating their utility for ploidy level estimation from GBS data. Gbs2ploidy assigned tetraploidy to individuals with H_O 0.05 to 0.07 and diploidy for all individuals with H_O lower than 0.04, and mostly with high posterior probabilities (pp) except for three samples (CA1_1 $H_O = 0.019$, CA1_4 $H_O = 0.018$ and CN1_4 $H_O = 0.042$). Consequently, all diploid populations and those of mixed ploidy levels showed lower genetic diversities than tetraploids (Fig. 3). To illustrate this in depth, we chose one to three samples (focusing on uncertain and contrary outcomes) per putative mixed ploidy populations to look closer into the respective SNP ratios (Fig. S1). As a result, all samples with $H_O > 0.05$ had a higher SNPs frequency (0.01 to 0.02) with a percentage proportion of 25/75 in comparison to samples with $H_O < 0.04$. In these cases, the frequency of SNPs with a 50% proportion ranged from 0.025 to 0.04. While samples with $H_O < 0.03$ had a frequency of 25/75 SNPs proportion below 0.01, the 50% proportion of SNPs was higher (0.03 to about 0.05), with the exception of CA1_4, which had a high SNP frequency (25/75 proportion = 0.014; Table S4). Despite its low heterozygosity ($H_O = 0.018$), this individual appeared to be the only putative tetraploid among the North American populations.

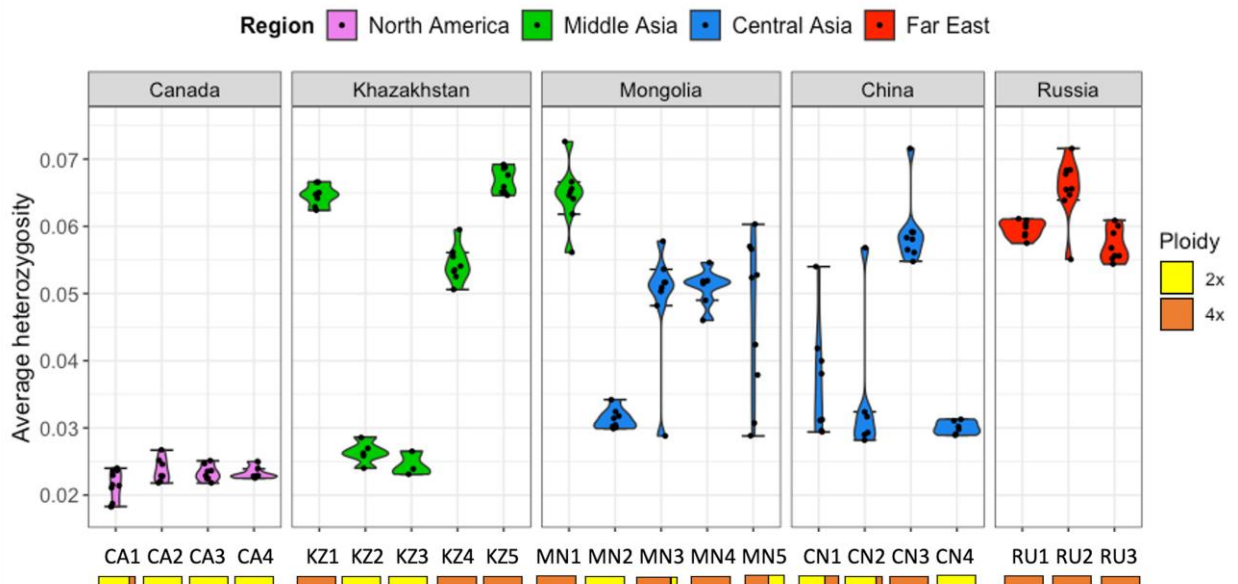


Figure 3. Violin plot of GBS-based observed heterozygosity (H_O) in *A. frigida* samples per population. N – number of samples per population.

Population genetic structure and phylogenetic analysis

3. Phylogeography

Based on minimal cross-entropy with the Bayesian population assignment analysis, a K value of seven was determined as the most likely number of genetic clusters (Fig. S2). As a result, populations from the Far East and North America constitute their own clusters (red and pink, respectively), however, samples from the Far East show a slight pattern of admixture with the North American clusters and one of the Central Asian clusters (light blue; Fig. 4a). Considerable genetic differentiation was found within the Middle Asian populations, as three independent clusters (light green, green and dark green) were observed in Middle Asia. Similarly, Central Asian populations were primarily composed of two genetic lineages (shown as light blue for Mongolia and dark blue for China; the exception of population CN3 from China, which is assigned to the light blue cluster). The highest level of admixture was found within Asian populations, and particularly in two populations of Mongolia from Middle and Central Asia (MN1 and MN2, respectively). Population MN1 was almost equally assigned to clusters from the Far East (red), Central Asia (light blue) and Middle Asia (dark green), while MN2 was assigned mainly to the Central Asian clusters but also to clusters from the Far East and North America. As shown in Fig. 4a, there appears to be no association between ploidy level and population genetic admixture.

Furthermore, genetic differentiation among populations was moderate (global $F_{ST} = 0.33$, $Nei = 0.03$), but very high genetic distances were found between two of the Kazakh populations (KZ2 and KZ3) and the rest (Table S5; see heatmap in Fig. S3). At the regional level, populations from Middle Asia and the Far East showed the lowest genetic distance ($Nei = 0.01$), while the greatest distance was recorded between the North American and Central Asian populations ($Nei = 0.02$).

Phylogenetic analysis with Maximum Likelihood supported our haplotype network analysis based on plastid DNA, showing that diploid populations of Kazakhstan (KZ2 and KZ3) were sister to the rest of the Asian populations, and those are sister to a clade of populations from Russian Far East and North America (Fig. S4 and Fig. 4b). These clades were also complimentary to the Bayesian analysis resulting in seven genetic clusters (Fig. 4a) with populations from Middle and Central Asia harbouring three and two genetic lineages, respectively. In addition, populations from North America are aligned closely with those from Far East as well as with some from Central Asia, indicating genetic exchange (Fig. 4b). According to ploidy estimation this network implies that KZ2 and KZ3 are the most likely ancestral diploids, and subsequent polyploidizations has occurred multiple times independently.

3. Phylogeography

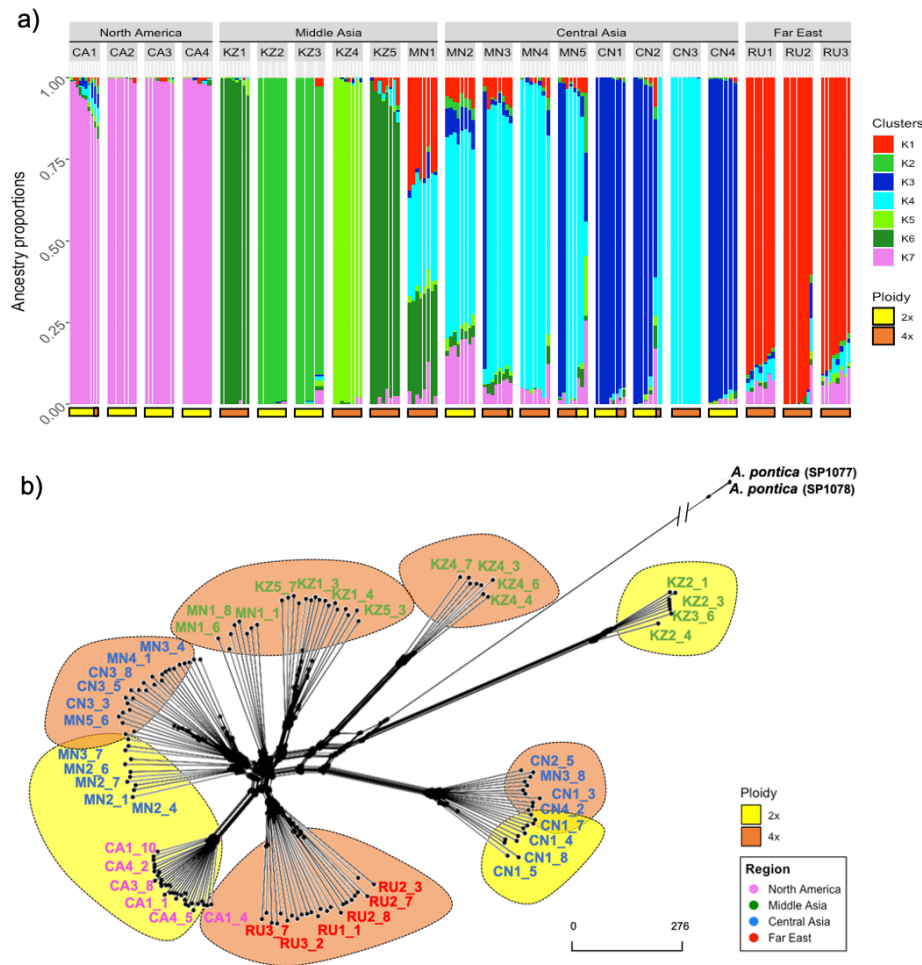


Figure 4 a). Individual ancestry estimation based on Bayesian clustering of GBS data assuming seven ($K = 7$) populations for *A. frigida* (color-coded). On the x-axis, each column represents an individual, while the y-axis shows assigns proportions as posterior probability. The assumed ploidy level of populations is according to Table S4. b). Phylogenetic network based on Hamming distances of GBS data.

Effects of geographic distance and current climate on genetic variation

The Mantel tests revealed an isolation by distance effect; however, it was not strong at either of the scales. In particular, the correlation between geographical and genetic distances among Asian populations was higher ($r^2 = 0.06$, $p = 0.002$) than it was when North American populations were included ($r^2 = 0.02$, $p = 0.03$). The greatest distance between North American and Asian populations was 8,000 km, while a maximum of 4,000 km separated Asian populations, indicating that genetic differentiation was relatively high among Asian populations. A similar pattern was observed for correlations between climatic differences and genetic distances, which showed a higher correlation for Asian populations ($r^2 = 0.05$, $p = 0.006$) than for all sites including North American populations ($r^2 = 0.02$, $p = 0.04$). This indicates that climatic differences only have a minor impact on genetic differentiation across all populations, but the large climatic differences within

3. Phylogeography

Asia were partly associated with the genetic differentiation of *A. frigida* populations in that region.

Principal Coordinate Analyses were conducted on all populations and within Asian populations to reveal significant environmental factors influencing population structure. At the larger scale, spatial effects and seasonal precipitation showed major correlations with population genetic structure (Fig. 5a). Within Asian populations, the amount of seasonal precipitation had the highest impact (Fig. 5b). The level of precipitation and its seasonal variability differed strongly between regions, which is represented in the climographs obtained for the representative localities associated with the regional populations (Fig. 5). Climate patterns in Middle Asian populations were partially consistent with the genetic structure revealed via Bayesian cluster analysis (Fig. 4a).

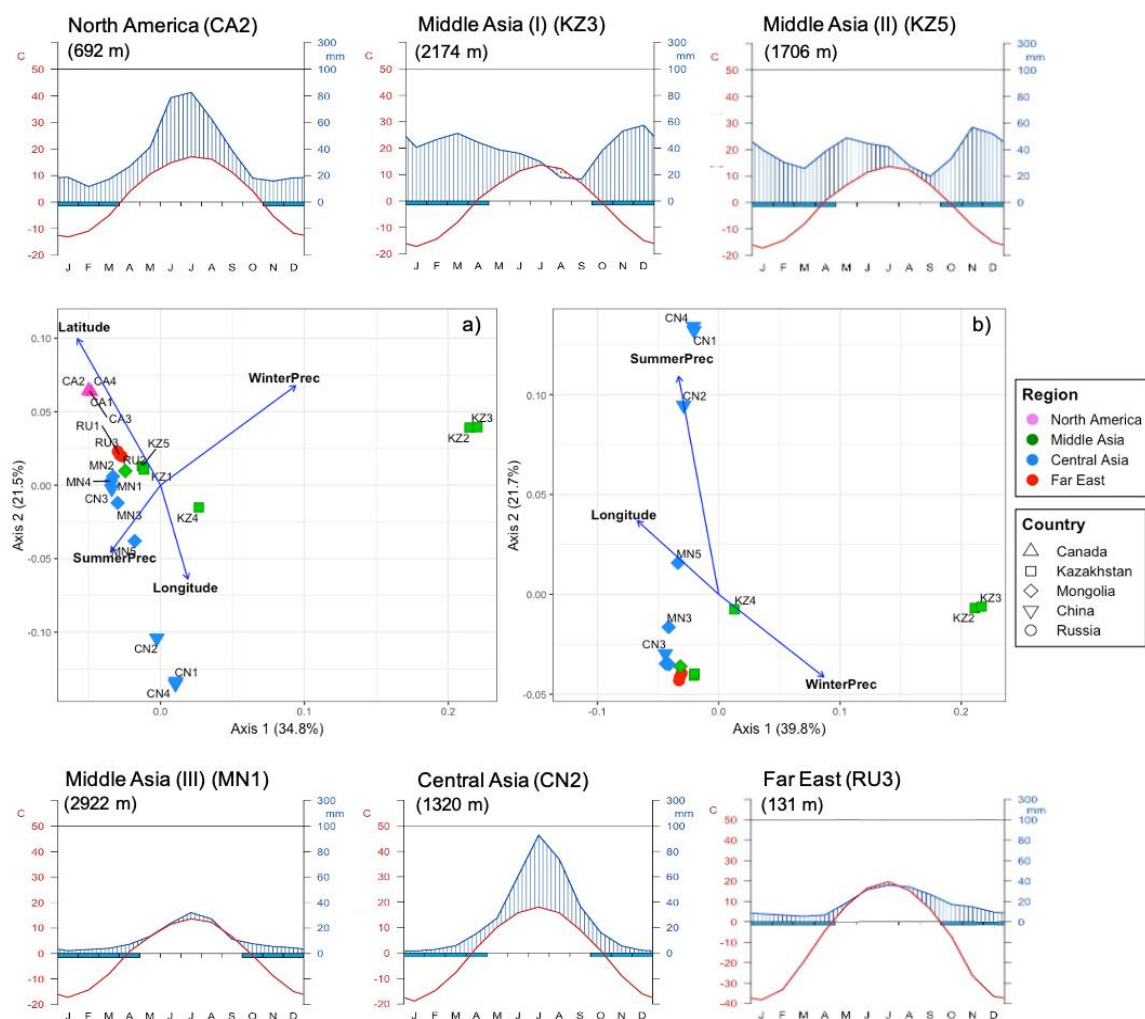


Figure 5. Principal Coordinate Analysis (PcoA) based on population-wise Nei distance: a) including all populations, and b) within Asian populations of *A. frigida*. Environmental predictors were fitted *post hoc* on the ordination plots (only those that passed $p < 0.05$ according to a test with 999 permutations are displayed). WinterPrec – winter mean precipitation, and SummerPrec – summer mean precipitation. Climographs of the regions were illustrated based on the typical representatives: CA2, KZ3, KZ5, MN1, CN2 and RU3, respectively.

Discussion

A number of studies have shown that the genus *Artemisia* presumably originated in northwestern Asia during the early Oligocene (30 – 32 Mya), with major speciation events occurring in Central Asia (Garcia *et al.* 2011; Liu *et al.* 2003; Pellicer *et al.* 2007; Tkach *et al.* 2008b; Wang 2004; Zhi-chen *et al.* 2004). Weber (2003) revealed that some North American *Artemisia* taxa found in the Rocky Mountains may have originated in the Tertiary, and they may consequently represent relics of once widely distributed taxa. Our genetic analyses based on plastid and GBS data suggests that Asia is the ancestral area of *A. frigida* (Fig. 2, Fig. 4b, Fig. S4). However, we cannot specify from plastid data whether they originate from Middle or Central Asia because two haplotypes (Middle Asian, KZ2+KZ3, haplotype and a mixture of Middle and Central Asian, blue and green haplotypes) were separated by the same number of substitutions from the outgroup species (Fig. 2). GBS data, however imply a Middle Asian origin (Fig. 4b, Fig. S4). Nevertheless, as the species' migration appeared to continue to the Far East and then on to North America, it is likely this happened via the Bering Strait, which is consistent with Garcia *et al.* (2011)'s findings that many species of *Artemisia* sect. *Absinthium* (about 80 species within the section) followed this migration route. Furthermore, our findings refute the hypothesis of Riggins and Seigler (2012) that *A. frigida* may represent a tertiary relict in North America.

The dominance of *Artemisia* pollen in many records of the Quaternary indicates the importance of that period for the genus' diversification and expansion (Wang 2004), with the latter process likely having been stimulated by climate oscillations. The Quaternary is characterized by the development of widespread glaciations (onset 2.8 – 2.4 Mya) in temperate mid-northern latitudes, while overall cooling led to the expansion of permafrost, open landscapes and the replacement of forests by various steppe formations (Hurka *et al.* 2019). The greatest diversity and expansion of steppe communities occurred in northern Eurasia during the glacial periods of the Pleistocene, as evidenced by diversification and expansion of typical steppe taxa such as *Capsella* Medik., *Clausia aprica* (Stephan) Korn.-Trotzky, *Goniolimon speciosum* (L.) Boiss., *Sisymbrium* L. and *Krascheninnikovia ceratoides* (L.) Gueldenst. (Franzke *et al.* 2004; Seidl *et al.* 2020, 2021; Volkova *et al.* 2017; Žerdoner Čalasan *et al.* 2021b; a). The same likely holds true for *A. frigida*.

In our study, we found mixed ploidy levels between and within populations of Middle and Central Asia, while exclusively tetraploid cytotypes were found in the Far East and diploid cytotypes in North America, with the exception of one 4x individual in population CA1 (Table 1). Our phylogenetic analyses (Fig. 4b, Fig. S4) suggest that polyploidization happened several times independently and may have arisen by autopolyploidization events within single populations. As represented in Fig. 5, Asia is characterized by highly variable climatic conditions, which may have promoted polyploidization

3. Phylogeography

and colonization of various habitats in the region, as Maguilla et al., (2021) demonstrated in *Centaureum* Hill. The Asian polyploid populations of *A. frigida* were collected from high altitudes, which is in accordance to the general observation that perennial herbs from high elevations in the temperate zones are frequently polyploids, which in turn is interpreted as an adaptation to harsh environments (Rice *et al.* 2019). This is in line with the findings of Mansion et al. (2005) and Maguilla et al. (2021), who discussed that varied climatic conditions promoted polyploidization in the genus *Centaureum*, promoting success in the new or expanded area rather than facilitating the dispersal event *per se*, thus conferring a fitness advantage in heterogeneous areas.

In addition, the presence of tetraploid populations in the Far East is a common characteristic of the arctic flora, which has a high prevalence of polyploids, including many *Artemisia* species (Tkach *et al.* 2008a; b). This has been explained by the fact that more strongly heterozygous and duplicated genomes aid population survival (by avoiding inbreeding and genetic drift) during periods of dramatic climatic changes (Brochmann *et al.* 2004), and that polyploids colonized new ranges more effectively following deglaciation than diploids (Van de Peer *et al.* 2021).

Considering the predominantly diploid populations in Canada, we assume that either only diploids migrated to North America through the Russian Far East; or polyploid cytotypes were not captured from the region owing to lack of sampling. We only sampled four populations from the northern prairies in southwestern Canada, where grasslands are scattered due to natural fragmentation and a recent conversion to crop land (Downing & Pettapiece 2006). However, the southern prairies in Canada encompass areas that remained ice-free during the last glacial period (Klassen 1994). Thus, it is important to note that our sampling in Canada may be not representative for the entire area of the species in North America (Fig. 1). However, we found one tetraploid individual in the Canadian population (CA1_4), indicating that autopolyploidization might have occurred there as well (Fig. 4b and Fig. S4).

Polyploidy is usually associated with greater genetic diversity because of the duplicated genomes, which often arise from different lineages (allopolyploidy). Indeed, the tetraploid populations in our study represented an overall higher genetic diversity than the diploids (Fig. 3). The lowest genetic diversity among regions was found in the North American populations ($H_O = 0.021 - 0.024$; Table S3). Isolation by natural and anthropogenic fragmentation can prevent genetic exchange where populations occur in more isolated landscapes shaped by multiple former glaciation events (Jackson Jr and Little, 2004) or by more recent habitat fragmentation caused by extensive transformation of steppes into farmland (Raddatz, 1998). Such fragmentation may have hampered genetic exchange among the North American populations, and have led to genetic drift and inbreeding as was shown for the

3. Phylogeography

prairie forb *Asclepias* L. spp. (Kim et al., 2015). As noted before, our study area only covered a limited range of *A. frigida*'s distribution in North America, which perhaps resulted in low average heterozygosity owing to a local effect. However, within population diversity should not be affected by a smaller samples size. Nevertheless, future research should cover a broader range of landscapes and regions to better understand the evolutionary history of the species across its entire distribution range.

Genetic differentiation between populations within the different regions was not particularly high in our study (global $F_{ST} = 0.33$, $Nei = 0.03$), and the North American populations were more closely related to the Russian Far East populations (average $Nei = 0.018$; Table S5). Meanwhile, two populations from Kazakhstan in Middle Asia (KZ2 and KZ3) showed the greatest genetic distance (average $Nei = 0.068 - 0.071$; Table S5), which may indicate more restricted gene flow among the disjunct populations leading to strong genetic drift and inbreeding. This is also indicated by their low genetic diversity ($H_O = 0.026$ and 0.025 , respectively). Remarkably, these Middle Asian populations were sampled at high altitudes in mountainous regions (1700 – 3000 m; Table 1), where gene flow might be further restricted due to topography. Moreover, extensive glacial complexes occurred in the Asian high mountains (Owen, 2013), including the Altai and the neighboring mountain systems during the Quaternary (Lehmkuhl et al. 2011). Glacial and interglacial periods during the Pleistocene caused plant populations to repeatedly expand and contract, resulting in several events of colonization of new areas (dispersal) and fragmentation of ancestral areas (vicariance), as evidenced by a number of studies on temperate plant phylogeography (Barbolini et al. 2020; Franzke et al. 2004; Hurka et al. 2012; Kirschner et al. 2020; Qiu et al. 2009; Seidl et al. 2021; Wang et al. 2013). This could explain the high genetic differentiation of *A. frigida* populations in Middle Asia, for which structure analysis revealed three genetically distinct clusters, with MN1 having the highest degree of genetic admixture (Fig. 4a). Although speculative at this point, KZ2 and KZ3 may represent remnants of *A. frigida*'s ancestral distribution area.

Based on the Mantel tests, which indicated limited correlation between the current environment and populations' genetic structure, we assume that paleoclimatic fluctuations, and thus ancient migrations, have more profoundly influenced the spatial genetic structure and distribution patterns of *A. frigida* than the current environment. As we discussed above, glacial and interglacial climate oscillations influenced species distribution and population dynamics through an alteration of expansion, contraction and fragment phases (Hewitt 2000; Hurka et al. 2019). These dynamics have shaped the current distribution and structuring of the intra-specific genetic diversity of many plant species (see Richardson & Meyer 2012; Tournebize et al. 2017; Yan et al. 2013a, 2019). In particular, the population structure of Middle Asian populations observed in our study could be a result of small populations being

isolated within refugial habitats during past interglacial phases, where genetic drift was increased and population divergence was favored. Populations of Middle Asia differed both in terms of their genetic structure (three distinct genetic clusters within Kazakhstan were observed; Fig. 4a) and prevailing climate (climographs partly fit to the genetic patterns; Fig. 5), even within smaller spatial space (the average distance among populations is 500 km). In contrast, individuals from North America and the Far East were genetically relatively closely related to each other, despite being approximately 6000 km apart and the ranges of precipitation and temperature being substantially different (Fig. 5). Therefore, the current environment has a rather limited effect on the spatial genetic structure of *A. frigida* populations.

Conclusion

Our study contributes to an increasing phylogeographic understanding of the evolutionary history of one of the most widely distributed temperate grassland species, and provides insights into its migration history by confirming and refining previous findings. Our study species, *Artemisia frigida*, represented a good example of a distribution pattern shaped by former migration over the Bering land bridge (BLB). The species originated presumably in Middle Asia, with its main diversification occurred in Asia likely to be triggered by high climate variability. This may have been aided by several polyploidization events, which could provide a fitness advantage in colonizing and establishing in heterogeneous environments.

Many other arctic and steppe-tundra species used the BLB as a dispersal route and glacial refugium, but the BLB also remained as a biological barrier, as evidenced by a number of animal species, e.g., woolly rhinoceros, musk-oxen, the North American river otter, American marten, and several groups of steppe-tundra beetles, including cold-adapted weevils and leaf beetles that are restricted to only one side of the BLB (Combe *et al.* 2021; Elias & Crocker 2008; Meiri *et al.* 2013). Our study thus contributes to the understanding of the evolutionary history of temperate steppe regions, including their intercontinental disjunctions summarized in comprehensive reviews (Barbolini *et al.* 2020; Hurka *et al.* 2019) and advanced methods in phylogeography (Kirschner *et al.* 2020; Seidl *et al.* 2020, 2021).

Chapter 4. Plant population genetics under changing climate and grazing

Combined pressures of warming climate and increased stocking densities in Mongolia have large impact on vegetation and soil condition. Between 1940 and 2015, MAT in Mongolia increased by 2.24 °C (i.e., twice higher than the global average) and MAP decreased by 7% (Han *et al.* 2021), while livestock number has increased three-fold, reached 56 million heads (National Statistics Office of Mongolia 2020). It is well known that steppe types respond differently to the changing climate and grazing pressure, depending on their interplay (Ahlborn *et al.* 2020; Bat-Oyun *et al.* 2016; Jamiyansharav *et al.* 2018; Khishigbayar *et al.* 2015; Munkhzul *et al.* 2021). Referring a common hypothesis of rangeland systems: southern Mongolia, with dry and desert steppes, characterizes a climate-driven non-equilibrium rangeland system (NEQ), whereas the northern Mongolia, with its moist part with meadow steppes represents a competition-driven an equilibrium rangeland system (EQ; Fernández-Giménez & Allen-Diaz, 1999; von Wehrden *et al.*, 2012; Wesche & Retzer, 2005). However, Ahlborn *et al.*, (2020) demonstrated that there is no border of NEQ and EQ systems in Mongolian steppes, rather a zone, which shifting from year to year. Our study on Climate – grazing interactions in Mongolian steppe (Section 4.1) supported above, resulted with a detrimental effect of grazing on desert steppe vegetation. This indicates the existence of combined pressure of climate and grazing in arid habitats, making them potentially sensitive to overgrazing and climate change.

Repeated monitoring studies in Mongolian steppes have documented declining species diversity and production (Addison *et al.* 2012), as well as vegetation cover and species richness in some steppe types (Khishigbayar *et al.* 2015). In addition, Jamsranjav *et al.*, (2018) demonstrated that under combined climatic and grazing effects, species composition in Mongolian steppes shifts from palatable perennial species to unpalatable or ruderal annual species, particularly in mountain and dry steppes.

However, it is unknown whether and which impacts have a changing climate and increasing grazing pressure on steppe species' genetic diversity in Mongolia. Response of species' genetic diversity and structuring, in form of variation within species, to the abrupt changes in both climate and grazing regimes is important for grassland functioning and thus ecosystem services. My dissertation with sections 4.2. and 4.3 aimed to answer these questions to full the study gap, using one of the most prevalent steppe species.

4.1. Climate – grazing interactions in Mongolian steppe



Typical steppe vegetation of eastern Mongolia. Photo was taken by Oyundelger Kh. in Dornod province in August, 2019.

The content of this chapter has published in Munkhzul, O., **Oyundelger, Kh.**, Narantuya, N., Tuvshintogtokh, I., Oyuntsetseg, B., Wesche, K. and Jäschke, Y. (2021). Grazing effects on Mongolian steppe vegetation — A systematic review of local literature. *Frontiers in Ecology and Evolution*. 9: 703220. <https://doi.org/10.3389/fevo.2021.703220>).

Abstract

The Mongolian steppes cover a large area of the Palaearctic steppe biome and are still relatively intact with a long history of nomadic pastoralism. As livestock number has increased over the last two decades, grazing has been considered as the main reason of pasture degradation. However, the impact of grazing on vegetation dynamics, and its interaction with climate, is still not clear.

We reviewed 44 publications in Mongolian language, covering 109 sites in five main steppe types, i.e., desert, dry, meadow, mountain, and high mountain steppe, with a mean annual precipitation and temperature range from 120 to 370 mm and from -6 to +5 °C, respectively. We calculated relative changes in vegetation cover, species richness, and aboveground biomass from heavily grazed with respect to lightly/non-grazed conditions. Multiple linear regression models were used to test the impact of environmental factors, i.e., mean annual precipitation, coefficient of variation for precipitation, mean annual temperature and elevation.

Grazing had a stronger effect on the vegetation of dry, desert and high mountain steppes, whereas its effect was less pronounced in the meadow and mountain steppes with mesic climate. Vegetation cover, species richness and aboveground biomass were reduced by heavy grazing in the dry, desert and high mountain steppes. In the meadow steppes, grazing reduced vegetation cover, but increased richness and had nearly no effect on biomass. In the mountain steppe, richness and cover were not affected, but biomass was reduced by heavy grazing. Additionally, grazing effects on biomass tended to be more pronounced at sites with higher amounts of annual precipitation, and effects on cover changed from negative to positive as elevation increased.

In conclusion, grazing effects in Mongolian steppes are overall negative in desert, dry and high mountain steppes, but no or even positive effects are found in meadow and mountain steppes. Especially, grazing showed a detrimental effect on all vegetation variables in desert steppes, indicating the existence of combined pressure of climate and grazing in arid habitats, making them potentially sensitive to overgrazing and climate change. Grassland conservation and management should consider characteristics of different steppe types and give importance to local environmental conditions.

Keywords: steppes, grazing, diversity, biomass, climate, human impact

Introduction

Grasslands cover 41% of the World's terrestrial surface (White *et al.* 2000), which sustain livelihoods for nearly 800 million people, and are a crucial source of livestock forage and habitat for diverse wildlife (White *et al.* 2000). Most of the world's grasslands are found in temperate regions, and these temperate grasslands have the distinction of being the most altered terrestrial ecosystems on the planet (Henwood 2010) and the most threatened globally. Of these temperate grasslands, 19.6% (10.3 million km²) belong to the Palaearctic steppe biome, which forms a vast belt across the mid-latitudes of Eurasia, with nearly 1.3 million km² occurring in Mongolia (Henwood 2010; Wesche *et al.* 2016). The Mongolian grasslands are still relatively intact, representing the world's largest grasslands that are still healthy in terms of biodiversity and traditional land use (Batsaikhan *et al.* 2014). Hence in the last two decades, Mongolia has become a key region for studying the effects of land use to devise potentially sustainable land use strategies.

Mongolia has a long tradition of nomadic pastoralism, which has been the main agricultural sector in Mongolia for centuries. Approximately 83% of the territory (1.3 million km²) is rangeland (including grasslands, shrublands, forest steppe, and deserts that are grazed by domestic livestock; Angerer *et al.*, 2008), supporting 71 million heads (118.7 million Sheep Units [SU¹]; (National Statistics Office of Mongolia, 2021)). Until 1990, the number of livestock was 25.8 million heads (54.3 million SU), however, after institutional and societal changes in the 90s, decentralization began and herders were allowed to privatize their herds. Since then, the number of livestock has increased dramatically, especially the number of sheep and goats increased due to the high market value of cashmere. Livestock density has increased more than threefold, i.e., from 32 SU per 100 ha in 1961 to 99 SU in 2017 (National Statistics Office of Mongolia, 2018). According to a national report on the rangeland health of Mongolia, 58% of Mongolian rangeland areas are estimated to be degraded, of which 14%, 21%, 13% and 10% are slightly, moderately, heavily and fully degraded respectively based on field monitoring data (Densambuu *et al.* 2018b).

Several global quantitative reviews (Cingolani *et al.* 2005a; Milchunas & Lauenroth 1993) revealed that grazing effects on vegetation interact with productivity. The latter in turn is controlled by abiotic factors, such as climate (precipitation, temperature) and topographic conditions. Mongolian grasslands often experience very high intra- and interannual rainfall variability, and growing season precipitation has the greatest effect on plant productivity (Liang *et al.* 2002). In wet regions with high productivity, moderate grazing can reduce the dominance of competitively superior species, thus increase plant diversity; while in dry grasslands with relatively low productivity, grazing

¹ horse = 7 SU, cattle = 6 SU, camel = 5 SU, sheep = 1 SU, goat = 0.9 sheep (NSO, 2019).

effects tend to be neutral or moderately negative, with interannual variation of mean annual precipitation having a stronger effect than grazing on plant communities (Olf & Ritchie 1998). Similar results were also found in Mongolian dry steppes, where grazing effects were smaller in drought years (Bat-Oyun *et al.* 2016). However, negative grazing impact on vegetation has been reported both in the forest-steppe in northern Mongolia (Takatsuki *et al.* 2018) and in the desert-steppe in northern China (Deng *et al.* 2013; Zhang *et al.* 2018c). Contradictory results suggest that Mongolian grasslands are a mixture of equilibrium and non-equilibrium systems (Ahlborn *et al.* 2020; Fernandez-Gimenez & Allen-Diaz 1999; von Wehrden *et al.* 2012b; Zemmrich *et al.* 2010b). In areas where mean annual precipitation (MAP) is less than 250 mm and the coefficient of variation of annual precipitation (cvP) is greater than 33% (Ellis & Chuluun 1993), the low and extremely variable annual precipitation has an overriding impact on livestock numbers, while in regions with higher and relatively constant precipitation, grazing should play a greater role in determining plant community structure and composition (Ellis & Swift 1988). Furthermore, Mongolia has a typical continental climate with enormous daily and annual temperature fluctuations, and extreme climate events such as drought and cold harsh winter (*dzud*) also have huge effects on vegetation, animal husbandry, and thus economic situation of households. Local topographical conditions, for example location along a slope, might also interact with grazing effects, because soil moisture might decrease from valley bottom to upper slope (Fujita *et al.*, 2009). And slope direction also affects nutrient availability and plant growth form, therefore interacts with grazing effects (Lkhagva *et al.*, 2013).

Considering such substantial effects of grazing and environment on vegetation in Mongolian grasslands, dozens of studies have been conducted. However, publications on the effects of grazing on vegetation and its interaction with the local climate provide partly contradictory results, and thus, no consensus has arrived in the international literature. Hence, the current study aims to perform the first meta-analysis of publications in Mongolian language, which reported grazing effects on the country's steppe vegetation from 1966 onwards. These publications provide valuable information, but are unfortunately not accessible without knowledge of the language, and many were not published in widely distributed outlets. Based on a thorough review of these publications, we addressed the following hypotheses:

- 1) Heavy grazing by livestock negatively affects vegetation cover, species richness, and aboveground biomass. Species richness and aboveground biomass are supposed to be highest under moderate grazing intensity compared with light and heavy.
- 2) The magnitude of the grazing effects differs among steppe types due to their specific environmental conditions. Grazing effects in meadow steppe and mountain steppe tend to be stronger, in line with classical equilibrium rangeland theory, as they receive usually more summer

rainfall and the productivity of such grasslands is high. In contrast, size of grazing effects on desert steppe and dry steppe is expected to be smaller as a consequence of the overriding impact of precipitation patterns as implied by non-equilibrium dynamics.

Methods

Study region

Mongolia is a landlocked country of Central Asia, situated between 41°35' and 52°08' N, 87°44' to 119°55' E, far from any ocean. Its land area comprises 1.564 Mio. km² (National Statistics Office of Mongolia, 2019), which is roughly equivalent to all countries of western and central Europe. Mongolia experiences an extreme continental climate characterized by long cold winters, short summers, and most precipitation falls during mid-June to August (Liu *et al.* 2013b). The climate is further characterized by high differentiation among four seasons and high diurnal temperature variation. Mongolia can be classified into six ecological zones (Fig. 1), i.e., the high mountain, taiga, forest steppe, steppe, desert steppe and desert zones (Tuvshintogtokh 2014). Among them, the forest steppe, steppe, desert steppe and parts of the desert zone are primarily rangelands. From the north to the south of Mongolia, the climate becomes drier (temperature increases and precipitation decreases).

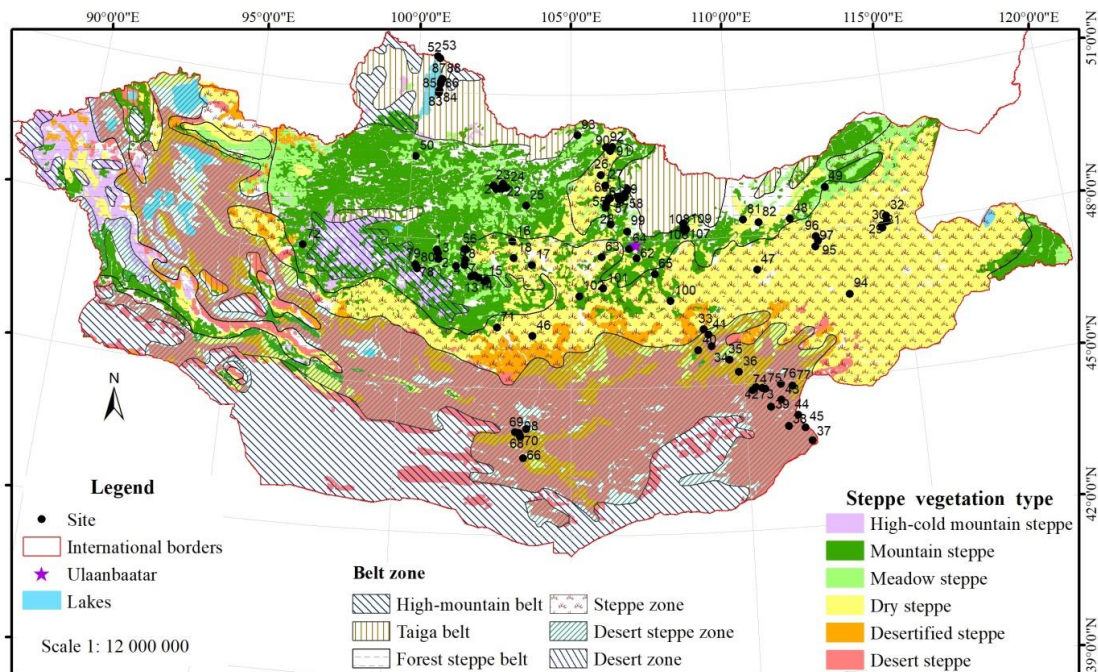


Fig. 1. Ecological zones and steppe vegetation types of Mongolia, with 109 study sites extracted from the reviewed publications. Ecological belt zones are based on the adapted vegetation map of the People's Republic of Mongolia (Ulziikhutag 1985; Yunatov & Dashnyam 1979). Steppe vegetation types follow the Steppe Vegetation of Mongolia (Tuvshintogtokh 2014), and "dry steppe" here includes "typical steppe", as is commonly done in literature. The high-cold mountain steppe is abbreviated as the high mountain steppe in the following. The sites located in the desertified steppe are grouped into the desert steppe in the analyses. The Site ID is the same as in Table A1.

4. Population genetics

Between 1944 and 2014, mean annual temperature (MAT) has increased by 2.1°C on average, with the increase being stronger in winter (3.6°C) than in summer (1.8°C). In particular, MAT increased by 2.0-3.7°C in the forest steppe zone, 0.7-2.0°C in the steppe and desert zones (these two zones were considered together in the analysis; (Dagvadorj et al., 2010; MARCC, 2014). As a result, the aridity index and the number of extreme warm days increased significantly (MARCC 2014). The change in mean annual precipitation (MAP) varied strongly in both spatial and temporal perspective. For instance, MAP decreased by 8.7-12.5 % in the central and southern regions and increased by 3.5-9.3 % in the eastern and western regions of Mongolia (MARCC 2014).

The total number of livestock in Mongolia has increased 2.7 times since 1990, when the democratic revolution took place and livestock was privatized. The magnitude of increment, however, differs among ecological zones: the total number increased 2.0 times in the high mountain belt, 3.2 times in the forest steppe and steppe zone, 2.5 times in the desert steppe zone, and 2.6 times in the desert zone (National Statistics office of Mongolia 2019). As a result, the steppe zone sustains the highest number of livestock, followed by the forest steppe and the desert steppe zone (Fig. 2). The significant drops in livestock numbers in 2000-2002 and 2010 show the effects of *dzud*, i.e., severe winter, which caused the death of 28.5 and 17.6 million SU livestock, respectively, after the winters of 1999 and 2009. Still, and in contrast to the non-equilibrium theory, over the last decade, livestock units have shown a general increase, even in the dry and highly variable desert steppes.

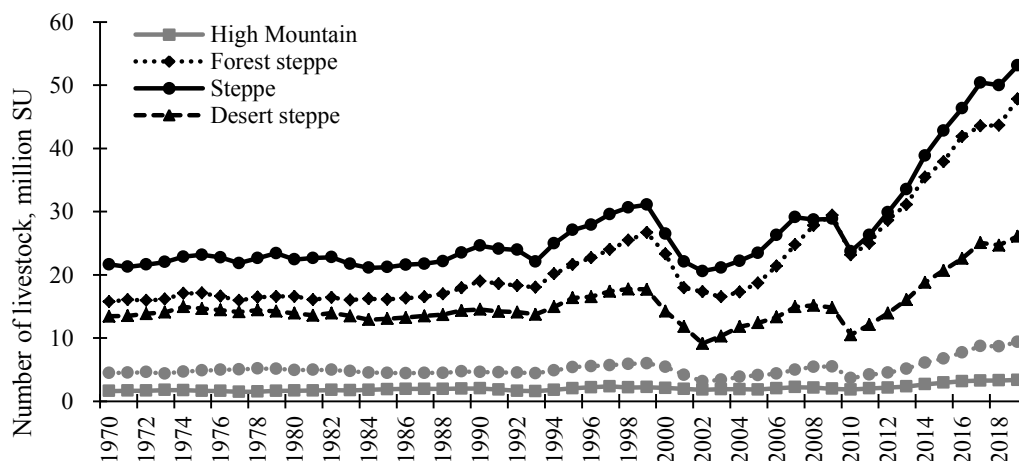


Fig. 2. Change in livestock numbers in different ecological zones of Mongolia. The total number of livestock in a particular zone was calculated from data at the soum (small administrative unit within a province) level (330 soums and 9 districts of the capital city, in total, N=339). A total of 216 soums had only one dominant ecological zone, so the livestock number in this zone is equal to the soum level. When a soum consists of more than one ecological zone, then each of the two (N=111) or three (N=12) most dominant zones were considered to have the same number at soum level, because livestock mobility is high even within a single soum. Therefore, there

is an overestimation of total livestock numbers (the sum of sheep units from ecological zones are 11-20% higher than those from soums for the respective year range). Data at the soum level were provided by the National Statistics Office of Mongolia, 1970-2019. The Taiga zone is not included because this zone is a boreal forest, where hardly any pasture exists.

Data extraction

Considering that our focus was on overcoming language barriers and enhancing the international knowledge base, we searched for all available Mongolian publications on grazing effects on the rangeland vegetation in specialized databases, such as local libraries of the Mongolian Academy of Science, National University of Mongolia, and Mongolian University of Life Science (see detailed list from Table A2). As search criteria, we used the Mongolian terms "*urgamaljilt*", "*belcheer*", "*belcheerleit*", "*belcheerleltiin nolo*" and "*hashsan talba*" which translate to "vegetation", "rangeland," "pastureland," "grassland," "grazing", "grazing effect" and "fenced area". In particular, for literature, books and (conference) proceedings that were published before 1990, we first searched for the title, and checked the context and included the suitable studies. The main vegetation indicators we consider here are species richness (SR), vegetation cover (VC) and aboveground biomass (AGB) as they are the most frequently reported quantitative measurements of rangeland conditions. We included studies published between 1966 and 2019. In total, 44 publications were found (Supplementary material 1), i.e., one dissertation, six master theses, five book sections, and 32 journal articles that together reported data from 109 sites (Table A1). Among them, conditions of different grazing intensity levels (i.e., light, moderate and heavy grazing, indicated by the distance to river or that to winter/summer camp in the original publications) could be compared for 51 sites, by dividing the values of the response variables under heavy grazing to the same variables under light (or zero) grazing at the same site:

relative grazing effect of heavy grazing = (heavy - light) / light grazing, and similarly, relative grazing effect of moderate grazing = (moderate - light) / light grazing.

The dominant grazers in different steppe types are provided in Table 1. Sheep and goats mainly graze on all steppe types, which constitutes ca. 85% (ca. 58 million heads) of the total livestock of Mongolia (National Statistics Office of Mongolia, 2021). Hence, the impact of grazing is largely similar in all steppe types. This way, trends are comparable between main rangeland types, in spite of absolute values for all indicators being much lower in the desert steppes and high-mountain steppes. Multiple-year (≥ 2 years) monitoring data was available from another 32 sites, but grazing intensity was only mentioned at four sites, which had fences to exclude livestock grazing, thus we made a summary of fence effects on those sites only. We did not

analyze the studies which reported either the results of one year at a single grazing level, or studies on seasonal vegetation dynamics within one study year. The workflow chart of the data extraction is summarized in Fig. A1.

We extracted quantitative values either directly from tables, or through the UN-SCAN-IT graph digitizer software (Silk Scientific, Inc.) from figures for the main response variables in the original publications. However, depending on the study design and purpose, surveying methods were inconsistent among studies. In particular, authors surveyed SR in different sizes of plots, which were usually large plots of 10 x 10 m² (3-10 replicates), or occasionally small plots of 0.5 x 0.5 m² or 1 x 1 m². Some were sampled along a 50 m or 100 m transect (3-5 replicates) and recorded the total species number in quadrats of 0.5 x 0.5 m² or 1 x 1 m² along the transect at an interval of 10 m or 20 m for the 50 m and 100 m transect, respectively. Moreover, whether estimates were based on the sum or the mean species number from subplots was not always clear. Reported VC was usually estimated from the same sample areas as for SR, and we calculated mean values of the repeated measures. AGB was estimated based on clipped standing biomass from 0.5 x 0.5 m² or 1 x 1 m² (with 3-10 replicates), and mostly from grazed sites rather than from grazing-exclusion plots; thereby providing only the residual biomass instead of primary productivity. When authors reported AGB values over the whole growing season, we selected the value of the peak biomass (late July or early August). Most biomass data were in units of centner/ha, so we recalculated them to g/m².

Latitude, longitude, and elevation were usually directly reported in the publications. Else, we used Google Earth to estimate elevation based on the specific coordinates. Climate data was not always reported by authors, and even when it was, the timescale for the reported data was often missing. As the reviewed publication ranges between 1966-2000s, we extracted the available long-term climate data from Worldclim 2.1, i.e., monthly precipitation data from 1961-2018 and MAT for time range 1970-2000 (Fick & Hijmans 2017; Harris *et al.* 2014). Data are compared with the data obtained from the National Agency for Meteorological and Environmental Monitoring (<https://namem.gov.mn/eng/>) for quality control. Annual total precipitation from Worldclim 2.1 data are largely realistic compared to station data and are much better than those from Chelsa V2 for our reviewed sites especially for recent years. Although the calculated cvP values based on Worldclim for 40 years seem to underestimate cvP values in this region (Supplementary material 2), the trend that cvP values are higher in desert steppes than other steppe types still hold (Fig. A2), thus we opted Worldclim 2.1 climate data for further analyses.

Data processing

Most of the publications reported the vegetation types for their study sites, which were typically five steppe types, i.e., desert steppe, dry or typical steppe, meadow steppe, mountain steppe, and high mountain steppe. Typical

steppes mostly occur in eastern Mongolia and are dominated by tall feather grasses, while dry steppes, in a strict sense, occur in western and central Mongolia, as well as in the south of the typical steppe. Dry steppes are drier and regularly associated with shorter feather grasses and shrub species (Pfeiffer *et al.* 2020; Tuvshintogtokh 2014). However, here we combine both steppe types into one, because the climatic and vegetation differences are relatively small compared to the differences among other steppe types. Furthermore, in earlier times, researchers often did not differentiate between these two steppe types and even mixed the two terms (Tuvshintogtokh 2014). The main environmental characteristics and dominant plant species and dominant livestock types for five steppe types are summarized in Table 1, and the differences in the major environmental variables are illustrated in Fig. A2.

All measured response variables from the same location (i.e., with the same coordinates) were combined into one dataset. For some studies which were conducted at the same location and focused on the same response variables, but either 1) covering different study years, or 2) by different research groups, we calculated the mean to put them into one dataset.

Statistical analysis

We first calculated means and 95% confidence intervals from absolute values of each vegetation indicator at different grazing intensity levels for each steppe type and did not exclude outliers. Site-based paired t-tests were not performed due to the small number of samples in some cases. We also did not perform a strict formal meta-analysis, because most studies did not report sd / se values. We tested the impact of environmental factors (scaled to zero mean / unit variants) on relative grazing effects using multiple linear regression models. We only tested the main effects of environmental factors (i.e. MAP, cvP, MAT and elevation) and did not include interaction terms because multiple environmental-grazing interactions are difficult to interpret due to the lack of specific ecological hypotheses. We fitted simple models without random factor, because only one value for each site was input for the model. We started with the most complex full model, including all environmental factors, and obtained estimates of effects for each. Then we simplified the initial full model in a stepwise procedure by progressively removing the least significant factor until we arrived at a null model. We compared the initial model with all subsequently reduced models in an ANOVA framework by comparing explained variance. Based on ANOVA results, we selected the final model in which all the remaining predictors were significant, and we reported the estimates for coefficients and standard errors for these predictors. For one of the studies on multiple year fence data, a two-way repeated measure ANOVA was performed to test the main effects of fencing and year on vegetation indicators with repeated subsites under different grazing regimes when such data were available. The significance

level for all tests was $p < 0.05$ and all statistical analyses were conducted in R 3.1.0 (Ripley & Murdoch 2020). R packages “rstatix” (Kassambara, 2020a), “ggplot2” (Wickham, 2016), “tidyverse” (Wickham et al., 2019), “ggpubr” (Kassambara, 2020b) and “cowplot” (Wilke, 2020) were used (R Development Team 2020).

Results

Research history

Our review of Mongolian publications between 1966 and 2019 allowed us to distinguish two major stages of research in Mongolia. The earliest investigations were conducted from 1970 to 1990, as a part of the Mongolian and Russian Joint Biological Expedition (MRJBE). During this expedition period, botanists aimed to study different vegetation types in various ecological zones, and thus established different monitoring stations in (i). mountain and forest steppe (1970-1985 at Tuvshruulekh soum in Arkhangai province), (ii). Meadow steppe (1976-1990 at Shaamar soum in Selenge province), (iii). Flooded meadow steppe (between 1987-1990 and since 2009 at Mungunmorit soum in Tuv province), (iv). Typical steppe (from 1982 to present at Tumentsogt soum in Sukhbaatar province), (v). dry steppe (1973-1976 at Bayan-Unjuul soum in Tuv province), (vi). Desert steppe (1970-1976 at Bulgan soum in Umnugovi province), and (vii). Desert (1977-1990 at Ekhiin gol soum in Bayankhongor province). Studies from this period provided valuable qualitative information on plant community composition and flora. The herbarium collection also began with MRJBE and other expeditions. Moreover, some essential data on SR, VC, AGB, phenology and seasonal growth dynamics etc., have been collected not only at monitoring stations, but also during field trips across the whole range of steppes.

The second stage includes vegetation studies by Mongolian and international researchers in 2000-2019. During this period, researchers have focused on quantitative surveys based on different vegetation sampling methods (e.g., line-point intercept). Both study stages provided essential information about Mongolian vegetation. Although not all vegetation types were equally represented, the most commonly distributed steppe communities have been investigated.

Vegetation conditions in different steppe types

Based on data from 51 sites, where three grazing intensity levels were studied, main indicators were compared among light, moderate and heavy grazing conditions from five steppe types (Table 2). In general, light grazing sites had higher VC in desert steppe and meadow steppe than heavy grazing, and AGB was higher under light grazing compared to heavy grazing across all steppe types, except in meadow steppe. Furthermore, changes in SR were generally not significant except in the desert and high mountain steppe. However, the absolute values of SR might show artifacts due to different sizes of sampling plots among

4. Population genetics

studies as described in the method section, while comparisons within steppe types remain valid.

Relative grazing effects

Relative grazing effects of heavy and moderate grazing differed among vegetation indicators and also among steppe types. Heavy grazing (Fig. 3a) showed 1) negative effects on SR and VC in desert steppes; 2) negative effects on AGB and SR, yet neutral effects on VC in dry steppes; 3) positive effects on SR, yet negative effects on VC in meadow steppes; 4) negative effect on AGB, yet neutral effects on SR and VC in mountain steppes; 5) neutral effects on AGB and SR, yet neutral effects on VC in high mountain steppes. Moderate grazing (Fig. 3b) had relatively weaker negative effects, or neutral and even positive impacts compared to heavy grazing. Particularly, the effect of moderate grazing on SR was neutral except for meadow steppes.

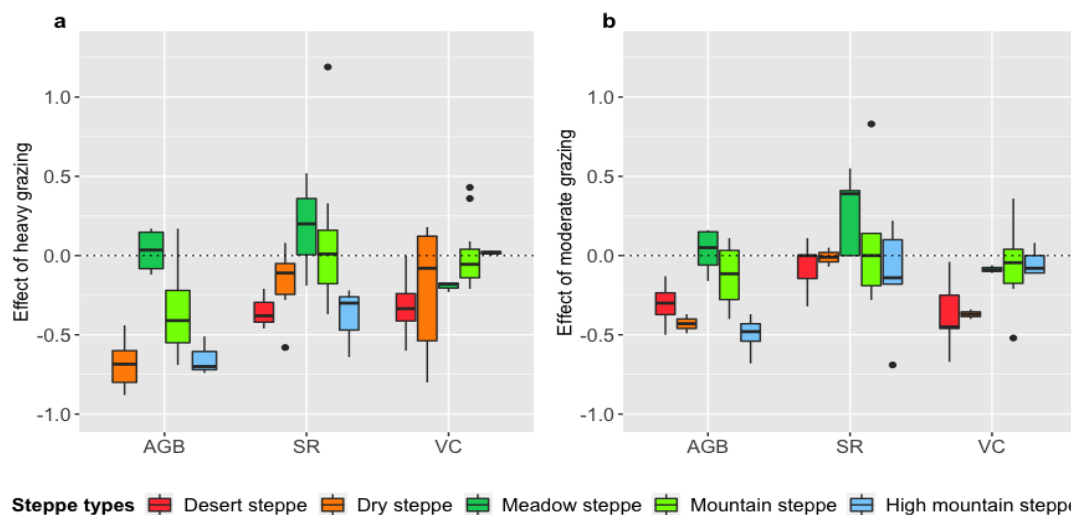


Fig. 3 Relative grazing effects of (a) heavy and (b) moderate grazing on AGB, SR and VC in five different steppe types: Relative grazing effects were calculated as (heavy-light) / light grazing, and (moderate-light) / light grazing, respectively. There was no data on AGB under heavy grazing conditions in the desert steppes, so that box was not drawn. The abbreviations for vegetation indicators are the same as in Table 2.

Environmental interactions on relative grazing effects

Results from linear models showed that MAP and elevation had significant interactions with heavy grazing on AGB and VC, respectively (Table 3). Grazing effects on AGB were more negative when MAP was lower, while the magnitude of effect decreased as MAP increased (Fig. 4a); In addition, effects of heavy grazing on VC were neutral or even slightly positive at higher elevations, but became predominantly negative at lower elevations (Fig. 4b). The relative effects of moderate grazing on VC interacted with MAP, i.e., moderate grazing showed neutral effects on VC when MAP was high (around 320mm), but effects became strongly negative when MAP was lower (Table 3, Fig. A3).

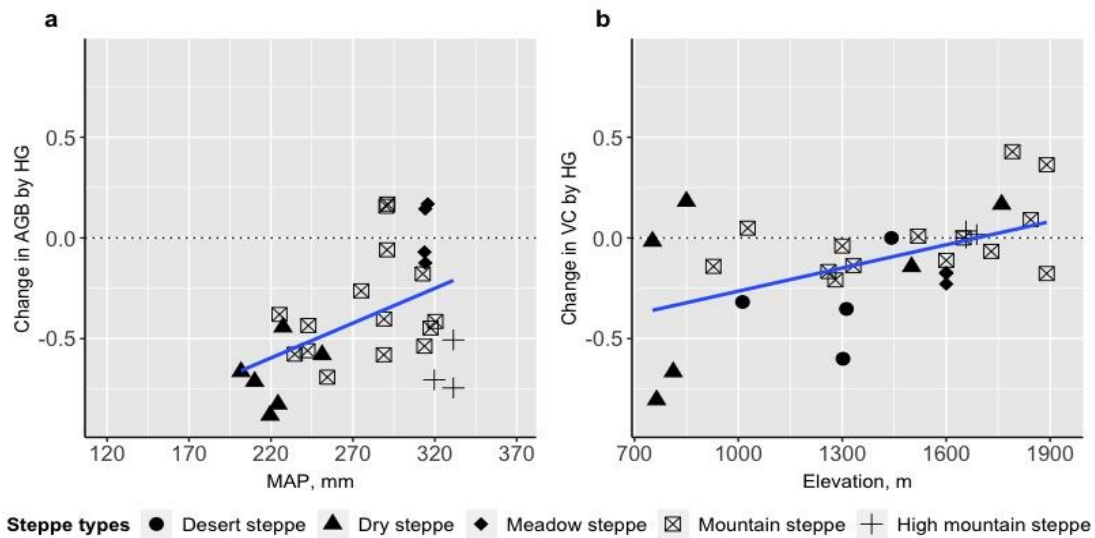


Fig. 4. Change of the relative grazing effects (HG: heavy vs. light grazing) on vegetation indicators along major environmental gradients. (a) change of GE on AGB (N=28; no data on desert steppe) along the MAP gradient, (b) change of GE on VC (N=30) along the elevation gradient. Environmental factors are selected based on the results from Table 3.

Fencing effect on vegetation condition across multiple years

Four studies reported fence effects across multiple years (Table 4). Three of them were carried out in mountain steppes, one in meadow steppe (one site included two steppe types) and one in desert steppe. One of these studies, i.e., the 5-year fence experiment (1970 – 1974) in Arkhangai province in the Khangai mountain taiga range, reported AGB values under fenced vs. grazed conditions (with three subsites of three grazing regimes, i.e., light, moderate and heavy grazing). We considered “subsite” as a repeated measurement because there were no major difference in vegetation conditions among those subsites, fence and year as the two fixed factors. The results of two-way repeated measures of ANOVA showed that year ($F [4, 16] = 9.78, p < 0.001$) and fence ($F [1, 4] = 30.59, p = 0.005$) both had significant effects on AGB. The interannual variation of AGB was evident from both fenced and grazed plots, and the difference between fenced and unfenced plots was larger for the first three years, but became smaller in the fifth year (Fig. 5).

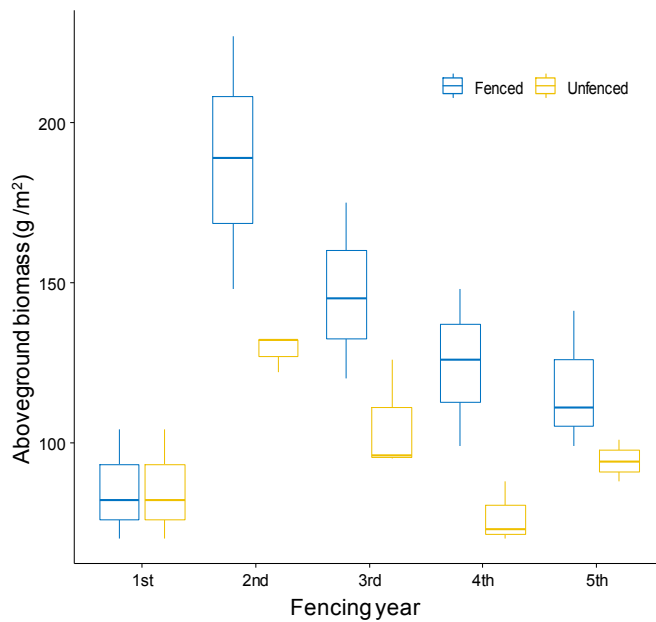


Fig. 5. Fence effects on aboveground biomass across five years in the mountain steppe, Tuvshruulekh monitoring station in Arkhangai province (redrawn based on the data from Banzragch and Chognii, 1975). Three subsites under different grazing intensity levels were considered as repeated measurements here.

Discussion

Our results demonstrated strongly variable responses of vegetation indicators to the intensity of livestock grazing, and the effects of local environmental conditions in the Mongolian steppes on these responses. In line with our first hypothesis, heavy grazing generally had negative effects on AGB, SR and VC, and moderate grazing (compared to no / light grazing) mostly had positive effects on SR in accordance with the global reviews (Gao & Carmel 2020; Milchunas & Lauenroth 1993). Our results also support our second hypothesis that the magnitude of grazing effects differs among steppe types, but this also depends on indicators. Interestingly, grazing had a strongly negative effect on dry steppe, desert steppe and high mountain steppes, whereas the negative effect was less pronounced, and even turned to positive or neutral in the meadow and mountain steppes. Notably, heavy grazing had no effect on 1) AGB in meadow steppes; 2) SR in mountain steppe and 3) VC in dry steppe, mountain steppe and high mountain steppe, and a positive effect on SR in meadow steppe. Different sensitivities of steppe types reflect the interaction of large scale environmental factors with grazing effects. The pattern of moderate grazing effect is largely similar to that of heavy grazing.. Thus, our further discussion will first focus on environmental interactions and then on the specific situations for certain steppe types.

Environmental interactions with relative grazing effects

A recent global meta-analysis on grazing effects on grassland vegetation showed that 41% of the variation in plant richness globally was explained by aridity, vegetation type and stocking rate together (Gao & Carmel 2020). Several meta-

analyses conducted in the grasslands of China showed that heavy grazing significantly decreased plant biomass (above and below ground biomass, also litter), but the extent of these responses was highly dependent on grazing intensity and environmental conditions, especially precipitation (Hao & He 2019; Wang & Wesche 2016; Yan *et al.* 2013b). All of these studies emphasized the importance of the interplay between grazing intensity and environmental conditions. Grazing effects on plant species composition and ecosystem functions, i.e. forage provision, in grasslands largely depend on precipitation and its variability (Sloat *et al.* 2018).

Heavy grazing had mostly negative effects on AGB with exceptions at some sites from meadow steppe and mountain steppe, which is in line with equilibrium theories that emphasize closer feedback between vegetation and herbivores instead of climatic controls. The strong negative effects in dry steppes when MAP is around 220 mm indicate that non-equilibrium theory does not necessarily apply to this steppe type.

Interaction with precipitation pattern

In semi-arid grassland systems, MAP and cvP play an important role in shaping vegetation dynamics (Vetter 2005; von Wehrden *et al.* 2012b). Our results find that MAP had interactions with grazing effects. This pattern could be partly explained by equilibrium theory, which suggests in humid and productive systems such as the meadow steppe and mountain steppe, grazing effects are more evident, while prediction from non-equilibrium theory is not supported. In systems with low and unstable rainfall, such as dry steppe and desert steppe, abiotic factors have overriding effects on livestock density, thereby alleviating the grazing effects on vegetation condition. In particular, negative grazing effects on AGB (Fig. 4a) were weaker under higher MAP, but became stronger when MAP decreased, which might also indicate interactions with local soil nutrient conditions: AGB could be favored by grazing in nutrient-rich habitats like meadow and mountain steppes. However, reduced AGB only implies livestock removes forage a lot, but does not indicate degradation. were more detrimental in nutrient-poor habitats, such as dry and high mountain steppes, where MAP was low and cvP values were relatively high (Fynn & O'Connor 2000; Proulx & Mazumder 1998). However, we notice that the long-term cvP values based on Worldclim V2 for desert steppes were too low, with median of 22% (Fig. A2). However, in typical non-equilibrium systems, cvP value should usually be large than 33%. Moreover, for recent years (2010-2018), cvP values based on meteorological station data were ca. 37%, indicating stronger variation of precipitation in this steppe type. The use of different cvP values based on different time periods and different data sources might affect the results of the significance tests for cvP values.

Interaction with elevation

Elevation showed significant interactions with grazing, with grazing effects on VC being less pronounced or even slightly positive at the higher elevation. According to our linear models, elevation was more important than MAT and we thus assume that this is not a mere result of temperature declining with elevation. The change of VC was relatively constant between 1500 – 1900 m, but grazing effects were negative at the lower elevations in dry and desert steppes. The difference in plant community properties might explain this pattern. From low to high elevation, species composition changes from grass-sedge-forb to forb-shrub dominated vegetation (Lkhagva *et al.* 2013), and at higher elevation, plants tend to be smaller/ shorter to adapt environmental stress (Halbritter *et al.* 2018), which may lead to less accessible phytomass and VC for livestock. Only a few studies have looked into the relationship between elevation and grazing on Mongolian rangeland vegetation. In particular, Zemrich *et al.* (2010) found that elevation was not an important factor in grazing-induced vegetation change, although they pointed out that their analysis covered a limited altitudinal range. In addition, Lkhagva *et al.* (2013) compared lower and upper steppes on south- and north-facing slopes in northern Mongolia, and found variable responses of VC, AGB, and SR change depending on nutrient and water availability along the elevation gradient.

Negative grazing effects on SR yet neutral effects on VC in high mountain steppes

We found a negative grazing effect throughout on AGB and SR, while there was no effect on VC in the high mountain steppes. Former studies reported that higher elevation mountain steppe pastures in the desert zone were more degraded than lower elevation vegetation receiving less rainfall but similar grazing pressure (Sheehy & Damiran 2012). In our case, the high mountain steppes differ from all other types by their extremely low temperatures (MAT -6°C , Fig. A2a) and lower AGB, thus supporting the lowest number of livestock among all steppe types (Fig. 1). It is surprising that SR decreased under grazing there because most plants in high mountains are well adapted to cold and nutrient-poor environments, which often also is beneficial with respect to grazing avoidance or resistance (Miehe *et al.* 2011). For example, growth forms that stay close to the ground, i.e., rosette or cushion plants, are adapted to strong wind and low temperatures, but are also protected from grazing because livestock can hardly reach and graze them if they are palatable. The average VC in this steppe type is ca. 60 %, in which cold-adapted cushion plants (e.g., *Stellaria pulvinata* Grubov, *Androsace chamaejasme* Wulfen and *Eremogone meyeri* (Fenzl.) Ikonn.) account for nearly 30% of the VC, while common caespitose grass species (e.g., *Festuca lenensis* Drobov, *Koeleria altaica* (Domin.) Krylov and *Poa attenuata* Trin. account for just about 10% of the VC, and the rest are other forbs and semi-shrubs (Tuvshintogtokh 2014). Therefore, heavy grazing might directly reduce the AGB

4. Population genetics

of palatable graminoids and forbs, but grazing-tolerant plants might not be affected. The negative effects on SR might relate to local topographical conditions, such as the steepness of the slope, which lead to sensitivity to trampling and difficulty of seedling establishment.

No grazing effects on VC and SR in mountain steppes

It is also surprising that in the mountain steppes, VC and SR were not affected by heavy grazing, although AGB was reduced. This is contradictory to a previous English publication (Takatsuki *et al.* 2018), which may point to some publication bias that more pronounced results are internationally published while non-significant results remain local. Non-significant grazing effects might be attributed to a shift in community plant shift at species level and plant functional type levels, i.e., balances between decreasing palatable species, and increasing unpalatable species. Several studies noted substantial floristic and edaphic changes caused by grazing in mountain steppes. The proportion of grazing tolerant sedges increased and highly palatable grasses and shrubs decreased under heavy grazing (Jamiyansharav *et al.* 2018; Khishigbayar *et al.* 2015; Zemrich *et al.* 2010b). Lkhagva *et al.* (2013) found that heavy livestock grazing increased SR of wet and nutrient-rich sites in the boreal mountain steppe, which was, however, explained by local colonization of grazing-tolerant species.

Positive effects on SR in meadow steppes

Interestingly, SR and AGB responded positively to heavy grazing, although VC decreased in meadow steppes. Increased SR may be attributed to increases in free patches of bare soil, thus giving new seedlings, especially of annual species, get chances to germinate (Frank 2005). Additionally, plant density is highest in meadow steppes among all steppe types due to high precipitation, high soil moisture and high nutrient levels (Pfeiffer *et al.* 2020), resulting in strong competition among species. Thus, heavy grazing can remove large amounts of AGB of dominant palatable species, and reduce competitive exclusion among species, which then gives less dominant species a chance to survive and grow. However, the increase in SR might not necessarily indicate the improvement of steppe condition in terms of forage quality, because the increase might only involve unpalatable species, as shown in the study by Yuan *et al.*, (2016), where unpalatable annual species (i.e. *Artemisia annua* L. and *A. sieversiana* L.) increased in grazing-disturbed sites. The increase in AGB might also suggest compensatory growth when defoliated plants produce more biomass than non-defoliated plants (Oesterheld & McNaughton 1991). Although no evidence of compensatory growth from meadow steppes in Mongolia is available, the grazing effects on cumulative growth of plants could be positive when the availability of leaf area, meristems, stored nutrients, and soil resources are high, and the frequency and intensity of defoliation are still suitable to stimulate plant regrowth

4. Population genetics

(Noy-Meir 1993). Moreover, the limited effects of grazing on AGB in meadow steppes also imply that the extend of biomass removal was not so large.

Negative effects on AGB, SR, yet neutral effects on VC in dry steppes

We noted negative grazing effects on AGB and SR, and a highly variable response of VC in dry steppes. The reduction of AGB and SR in dry steppes was reported in different studies in Mongolia (Bat-Oyun *et al.* 2016; Jamiyansharav *et al.* 2018), where plant species composition shifted, i.e. a decrease in palatable species due to the increase of grazing intensity. However, the effect was associated with local climate patterns, with grazing having a greater impact at drier sites than on wetter sites (Jamiyansharav *et al.* 2018). The highly variable response of the VC could be attributed to the combination of two steppe types (dry steppes comprise typical steppes as well), in which plant cover might remain invariant in the moister steppes, but in drier sites, variation in VC was pronounced due to variability in onset and total amount of annual precipitation. Thus large inter-annual variations might also lead to large variations in the VC, such as in desert steppes (Table 1). Another possible explanation could be that grazing reduces plant height and sexual reproduction, so the plants increase their vegetative growth by horizontal growth (broadening) (Gao *et al.* 2015). In this way, VC may increase although biomass decreases. Finally, similar to other steppe types, lack of change in VC might be related to potential shifts in species composition (not studied).

Negative effects in desert steppes

In contrast to generally limited or even absent effects on aboveground parts that were found in many other studies in the desert steppes of Mongolia (Fernandez-Gimenez & Allen-Diaz 2001; Jamiyansharav *et al.* 2018; Wesche *et al.* 2010) and Inner Mongolia (Bai *et al.* 2012), we found that SR and VC decreased by nearly 40% under heavy grazing (no data for AGB). The desert steppe vegetation is often considered to show non-equilibrium dynamics, which should be overridingly controlled by rainfall variability rather than grazing. Plants adapted to drought are also often tolerant of grazing, thus grazing effects on vegetation are often insignificant (Jäschke *et al.* 2020). However, soil moisture deficiency is the main driving factor in the desert steppe (Zemrich *et al.* 2010b), thus grazing-reduced litter and plant cover might intensify the water deficiency, make sandy soils more vulnerable to soil erosion (Li *et al.* 2008). Soil degradation then has subsequent negative feedback on vegetation, especially on VC. It is also important to note that the desert steppes of Mongolia and Inner Mongolian are under different ranges of MAP, i.e. 150 - 250 mm in China (Li *et al.* 2019), but 110-170 mm in Mongolia (Pfeiffer *et al.* 2020). The possible reasons for the inconsistency between our results and previous studies might be the differences in 1) moisture availability, which is closely related to soil texture, and 2) dominant plant community, i.e. presence/absence of subshrubs or shrubs, or ruderal

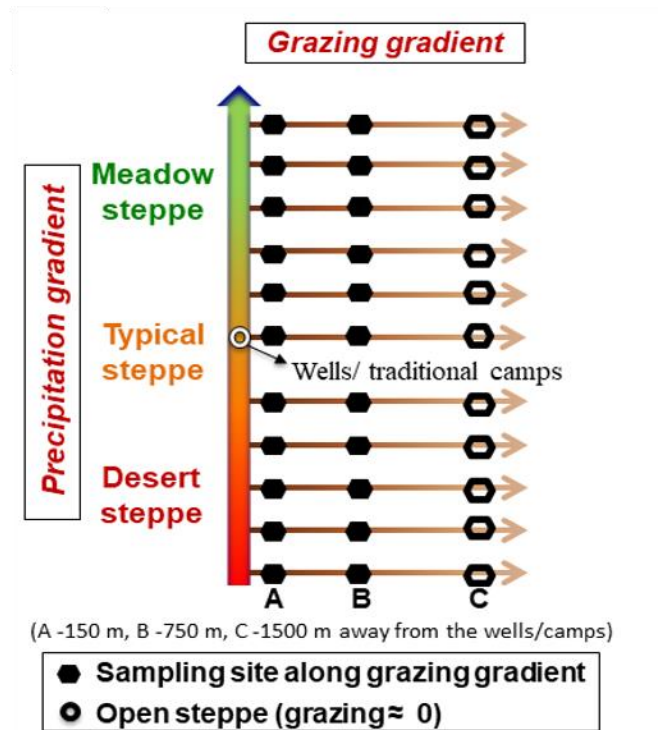
species. For example, when ruderal forbs, particularly *Chenopodium* spp. become prevalent in heavily grazed sites, SR could be much lower compared to no / light grazing (Fernandez-Gimenez & Allen-Diaz 2001). Additionally, when the percentage change of SR caused by grazing was around 10%, it might only involve the loss of 1-2 species compared to 8-19 species under ungrazed conditions in the southern Mongolian desert steppes (Wesche *et al.* 2010) . The desert steppe has limited functional redundancy, thus its ecological functioning is sensitive to species loss (Liu *et al.* 2013a). Perhaps most importantly, although there are still interannual fluctuations in the total livestock number in the desert steppes, the general trend is toward increasing numbers even in desert steppes (Fig. 2), which indicates that non-equilibrium dynamics may not apply anymore.

Conclusion

Our review shows that assessment of grazing effects depends on indicators being selected, and on steppe type under different abiotic environmental conditions. Elevation and precipitation patterns interact with grazing effects. According to our findings, meadow steppes demonstrated equilibrium dynamics, while responses of desert steppes did not support non-equilibrium dynamics. Although desert steppes have the highest cvP among all steppe types, the general increasing trend of livestock numbers (Fig. 2) conclusively demonstrates that non-equilibrium dynamics simply do not hold anymore.

Responses in the mountain steppe might depend on local soil nutrient conditions. As such, further studies focused on vegetation should also include soil conditions. High mountain steppes share some characteristics with alpine steppes in other regions, but further information on topographical conditions might be helpful for clarifying grazing effects there. We also found support that moderate grazing promotes species richness in steppes where resource availability is relatively high and stable, i.e. meadow steppes. Therefore, differences in grazing impact among steppe types should be acknowledged for grassland management and conservation. Moreover, change in VC or AGB alone, without information on species composition change, especially the proportion of forage (palatable) species, may not be the best indicators of grassland condition change, thus grassland assessment on degradation should consider more and more relevant indicators.

4.2. Climate – grazing interactions on plant population genetics



Meadow steppe



Typical steppe



Desert steppe



An illustration of a study design that uses climate and livestock gradients to analyze the impacts of climate and grazing. Study design was drafted by C. Römermann and K. Wesche.

The content of this chapter has published in **Oyundelger, Kh.**, Herklotz, V., Harpke, D., Oyuntsetseg, B., Wesche, K. and Ritz, C.M. (2021b) Contrasting effects of local environment and grazing pressure on the genetic diversity and structure of *Artemisia frigida*. Conservation Genetics. 22: 947-962. <https://doi.org/10.1007/s10592-021-01375-w>

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 the respective interactions have become a major research topic (Grant & Grant 2002; Sork 2018). How populations (and species) react to local selection pressures and perform in long-term survival strongly depend on sufficient levels of genetic diversity and its partitioning between and within populations (Li *et al.* 2018; Pazouki *et al.* 2016). Maintaining population genetic diversity depends on a variety of factors, such as, geographical distribution range, reproductive system, and ecological traits of a species (Nybom 2004). In continuously distributed and outcrossing species, gene flow promotes genetic diversity, and counteracts genetic differentiation among populations. Whereas, in isolated populations and/or self-fertilizing species, gene flow is rather restricted, resulting in erosion of local genetic diversity due to inbreeding and genetic drift, and increases the 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). If gene flow is high between populations from different environments, adaptation to local environment may be stalled; if gene flow occurred among similar environments, it may facilitate local adaptation by increasing population size and introducing new alleles that are beneficial (Sexton *et al.* 2014). Hence, climatic conditions are among the most important natural selective forces. Furthermore, human land use, for example livestock grazing could have a direct effect on population genetic structures as well as genetic diversity, which also interacts with the local environment. It might have positive effects on genetic diversity either by directly accelerating mutation rates due to herbivore damage (Marcotrigiano 2000), or indirectly by diaspore transport across long distances via epizoochory (Bläß *et al.* 2010), and by providing safe sites for germination due to grazing-induced gaps in vegetation (Frank 2005). Negative effects of grazing on genetic diversity could be attributed to decreasing effective population sizes or restricting of gene flow due to direct consumption or damage of the entire plant or its flowers or fruits (Souto & Tadey 2019), or by the spread of selectively favoured genotypes adapted to grazing. Thus, understanding population genetic variability and sub-population structure with respect to both, abiotic constraints and human land use is of high importance for conservation and management of 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 as well as are often intensively grazed. However, population genetics of dryland species remain severely understudied (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 to control plant species occurrence and abundance in this region

4. Population genetics

(von Wehrden *et al.* 2009; von Wehrden & Wesche 2007). Central Asia includes Mongolia, northern and western China and the Tibetan Plateau, and comprises a total of ca. 5 million km² of steppes (Wesche *et al.* 2016), accounting for more than half of the entire Palaearctic steppe biome. Occupying ~80% of Mongolian territory, Mongolian grassland have been subjected to a number of studies dealing with the impact of its extreme continental climate (Bat-Oyun *et al.* 2016; Fernández-Giménez & Allen-Diaz 1999; Sternberg 2008), its >4000 year old history of mobile pastoralism (Herrero-Jáuregui & Oesterheld 2018; Hoshino *et al.* 2009), as well as of interacting effects of both 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.* 2012a).

There are only a few studies investigating grazing effects on population genetics of Central Asian rangeland species and they yielded contrasting results. Wang *et al.* (2004) reported that intensive grazing decreased the genetic diversity in populations of *Artemisia frigida* Willd. in Inner Mongolia because animal's consumption of plants reduced plant sexual reproduction leading to restricted gene flow and to the reduction of the species' 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, because of its small effective population sizes. In contrast, overall genetic diversity of *Stipa glareosa* P.A. Smirn. Was smaller in less-grazed populations (Oyundelger *et al.* 2020). Moderate grazing even promoted the genetic diversity of *Stipa krylovii* Roshev. And *S. grandis* (Peng *et al.* 2015), which was attributed to increased mutation rates due to herbivore damage and/ or higher availability of free soil patches for seed germination. These 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 dealt with the effect of environmental factors on population genetics. For instance in *Caragana* Fabr. From Central Asia, variation in precipitation had stronger negative effects on genetic connectivity than isolation by distance (Yang *et al.* 2013). In addition, total annual as well as total winter precipitation were crucial for shaping population structure in *Dactylis glomerata* L. (Zhang *et al.* 2018a) and *Ammoiptanthus mongolicus* (Komarov) Cheng (Jiang *et al.* 2019b), 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 & Wesche 2011a). Only few studies have demonstrated that soil nutrient availability impacts on genetic variation of plant populations. High-nutrient soil conditions increased growth (i.e., diameter, height and total biomass) of *Populus tremuloides* Michx., and hence increased also genotypic variability in

growth (Stevens *et al.* 2007). 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 the concentration of phosphorous and decreased with the concentration of potassium.

Our study region Mongolia is ideal for testing contrasting effects of environmental conditions and grazing pressure. Mongolia has experienced a strong revival of pastoralism at the beginning of the 1990s, when privatization of livestock has begun reviving discussions on potential degradation of communal rangelands. Since the nineties, the number of livestock has doubled to 71 million (National Statistics Office of Mongolia 2020), which has tremendous effects on both vegetation and soil (Jamsranjav *et al.* 2018). Besides of grazing effects, climatic conditions, such as annual precipitation, its variability and the occurrences of droughts (Dai 2011) exert strong control on vegetation distribution and land degradation (Ahlborn *et al.* 2020; Fatichi & Ivanov 2014). Intensive grazing in mesic steppes results in invasion of more drought-adapted species, a process called xerophytisation (Zemmrlich *et al.* 2010a). In temperate grasslands, intensive grazing results in invasion of more drought-adapted *Artemisia* species, including *A. frigida* (Bai & Romo 1996; Jinhua *et al.* 2005), yet it is not clear if certain ecotypes of this species were more successful than others. *Artemisia frigida* Willd. is a perennial, outbreeding and wind-pollinated species, and one of the most widely distributed rangeland plants (McArthur & Jeffrey 2004). From an ecological point of view, *A. frigida* tolerates a large range of climatic conditions, including coldness and drought. However, seed production and germination of this species are largely dependent on climate, since in years with sufficient precipitation plant growth and seed production are promoted (Bai *et al.* 1995; Ronnenberg *et al.* 2007). Hence, this drought-adapted species is ideal to study the effects of both local environment and grazing pressure on population genetics, with potential high relevance for rangeland management and conservation.

Recent developments in molecular methods have vastly enhanced the understanding of genetic variation and adaptation in many important, yet non-model species. Non-coding DNA regions are highly variable, and thus are widely used for population genetic and phylogeographic studies (Pleines *et al.* 2009). Especially microsatellites, also known as Simple Sequence Repeats (SSRs) are among the most popular and powerful markers for population genetics based on their high polymorphism and co-dominant inheritance (Kalia *et al.* 2011; Vieira *et al.* 2016). Microsatellites still remain an important tool in the genomic era because of their cost-efficiency and possibility of manual screening of loci (reducing error), since SSR analysis relies on a relatively small number of loci compared to high-throughput sequencing fingerprints e.g., RAD-seq (Hodel *et al.* 2016). As a neutral marker, SSR cannot trace positive or negative responses of genotypes to selection; yet

they give an insight in overall distribution of certain genotypes, which could be characterized by favorable suits of traits under unknown (multigenetic) control.

Our first aim was to develop species-specific SSR markers using whole genome sequencing for this wide-spread species and validate them. Hence, for investigating genetic variation from larger to smaller scales (between sites, between populations and within populations) involving a local grazing gradient, we conducted SSR analysis for eleven populations, forming the first genetic study of *A. frigida* in Mongolia. We hypothesize that 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 probably higher in those populations where grazing pressure is more pronounced due to adapted genotypes and/or accelerated genetic exchange compared to non-grazed (least-grazed) populations, and iii) genetic differentiation among populations would increase with increasing distance because of the large geographical distance and strong environmental gradient from north to south.

Materials and methods

Study species: Artemisia frigida Willd. (Asteraceae)

Fringed sagewort is an aromatic perennial sub-shrub of 20 to 60 cm in height (McArthur & Jeffrey 2004) that occurs primarily on fixed and semi-fixed silty-sandy rangelands. The entire plant is covered by dense silvery pubescence (Fig. 1a) and the lower parts of stems are often strongly branched and woody. The species has probably evolved during the Pleistocene-Holocene (Yurtsev 1987), and presently, it has an Amphi-Beringian distribution range growing mainly in the steppe and prairie rangelands of Alaska, western Canada, United States, Siberia, Kazakhstan, Mongolia and northern China (Harvey 1981). Fringed sagewort is a prolific seed producer; each 2.5 cm length of inflorescence contains approximately 1000 seeds (Harvey 1981). In addition to sexual reproduction, it also has a clonal growth strategy (Jinhua *et al.* 2005). The species was first considered to be diploid ($2n=2x=18$; (Garcia *et al.* 2004)), yet 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 becoming 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 is an important forage for livestock (Jigjidsuren *et al.* 2015; Li *et al.* 2012).

4. Population genetics

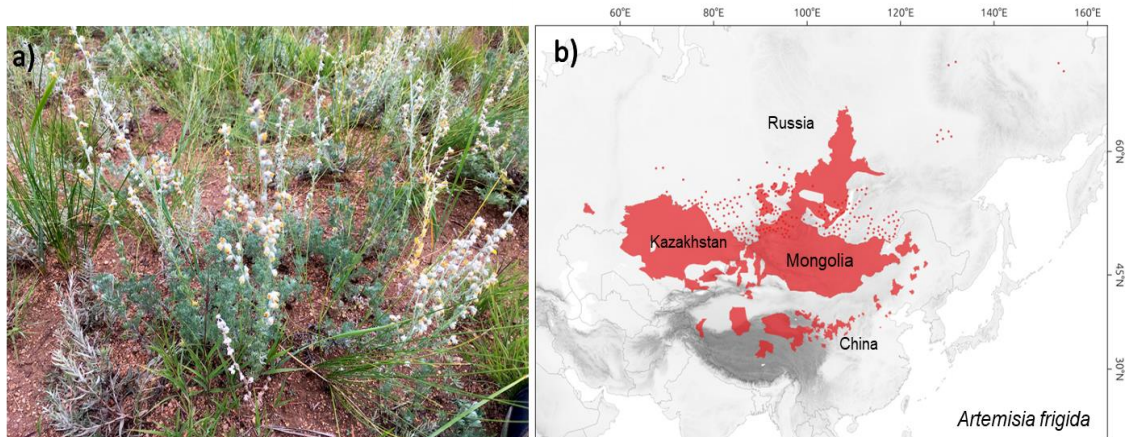


Figure 1a) *Artemisia frigida* in its typical habitat in Mongolia, and b) its distribution in Central Asia (samples were only collected from Mongolia; map drafted by Jan Treiber).

Study design

Our study was conducted in central and southern Mongolia between June and September 2018. Eleven populations were chosen (along a 600 km gradient with 100–300 mm S/N differences in mean annual precipitation) from north to south (Table 1, Fig. 2a). Population 2 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 (150 m – heavily-grazed, 750 m – moderately-grazed and 1500 m – least-grazed) representing different levels of grazing intensity (Fig. 2b). Distances among sub-populations differing in grazing intensity were based on previous successful applications in the region (Oyundelger *et al.* 2020; Sasaki *et al.* 2008) and elsewhere (Besnier & Glover 2013; Peper *et al.* 2011). However, grazing gradients reflect also small-scale spatial gradients, and therefore cannot be directly distinguished from small-scale effects caused by isolation by distance. Hustai NP (Pop2) was different because it was the only population where a fenced plot was available. The fence was built in 2003, and since then grazing by large mammals was prevented. This fenced site has a size of 10 m × 10 m, and represents a permanent sampling site for monitoring of phenology (Tserendulam *et al.* 2018). In Hustai NP, main grazers were Przewalski horse, marmot and red deer, while all other populations were grazed by goat, sheep, cattle or horse.

4. Population genetics

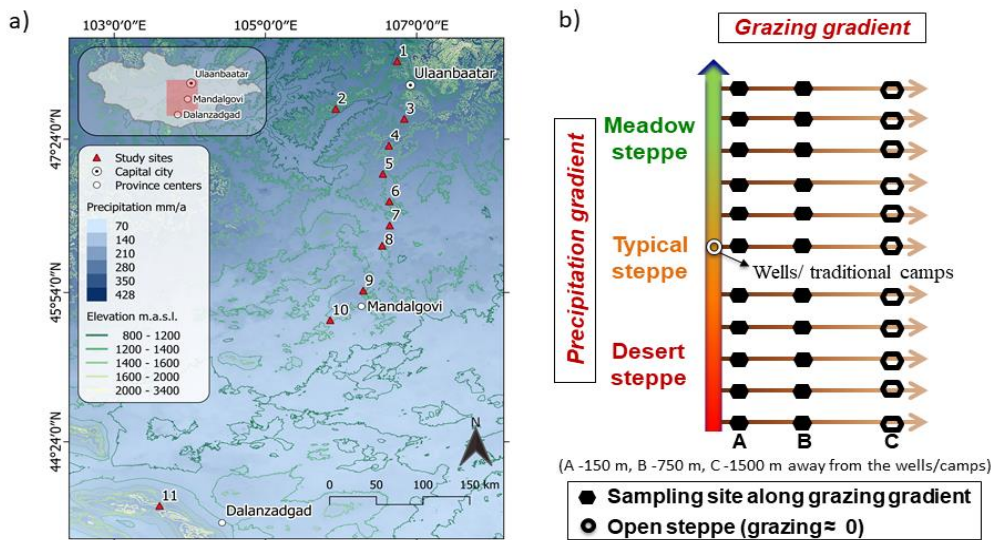


Figure 2a) Map of eleven study sites (corresponding to Table 1) with mean annual precipitation totals and contour lines indicating elevation. Rainfall data were derived from CHELSA and elevation data are from TanDEM-X - Digital Elevation Model (German Aerospace Center (DLR) 2018); map drafted by Jörg Lorenz. b) Experimental design 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 three site sub-populations were sampled along local transect representing different grazing intensities (A–C, initial draft C. Römermann)

4. Population genetics

Table 1. Characteristics of the study sites [localities, population code, coordinates, altitude, steppe habitat type, mean annual temperature (MAT) and 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
Guntiin 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
Khushigtiin 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
Guntiin 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
Guntiin 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

Sample collection and analyses

Field collection

For a total of 361 individuals, fresh leaf material was collected and stored in silica gel. For each population, representative herbarium specimens were deposited at GLM. At each sub-population, 12 individuals of *A. frigida* were sampled within a 10 m × 10 m plot. Within these plots, plant community composition and total vegetation cover (%) of vascular plants were recorded to characterize community properties. Later, plots were classified into different steppe types according to a standard classification of Mongolian steppe vegetation type (Tuvshintogtokh 2014). In addition, a sample of top soil (0±5 cm depth) with fine plant roots and the humic layer of each plot was collected to examine nutrient availability of top soil.

Soil preparation and analyses

Soil samples were initially dried in the laboratory for 18 hours at 75 °C. Samples were then sieved using a 2 mm sieve coarse screen, using the fine material for further analysis. 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). After drying samples at 105°C for 24 hours, rest water was weighed. The carbonate content was first assessed using a simple test with 10% HCl, and samples showing a reaction were further analyzed using a calcimeter following Scheibler's method (ON L 1084-99, 1999). Total N and C concentrations were analysed using a CN analyser (Vario Pyro Cube; Elementar, Langenselbold, DE). The rest water content was used to calibrate the nutrient contents per gram of soil, and the carbonate content to correct C/N measurements.

Climate data extraction

Meteorological data of 20 years, i.e. mean annual temperature (MAT), mean annual precipitation (MAP), and mean spring temperature (SpringT; March-May), mean summer temperature (SummerT) and mean summer precipitation (SummerP; June-August) between 1994 – 2013 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.* 2012a).

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 applying whole genome sequencing (WGS). High molecular weight DNA was extracted with the Mag-Bind® Plant DNA DS kit (Omega Bio-tek, Norcross, USA). Genomic DNA was quantified with 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 (~ 20ng/µl) per sample was used for covarizing (Covaris S220, Duty Factor: 8%, Peak Incident Power: 160, Cycles Per Burst: 200, Time: 60 seconds) and BluePippin (Sage Science) was used to produce fragments with an average size distribution of 450 base pair (bp) length. 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 PCR run allowing a targeted quantification of fragments having adaptor sequences on both ends before cluster generation. Cluster generation on Illumina cBot followed Illumina's recommendation. According to these, paired-end sequencing (2×250 bp) 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); available at <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) 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, <https://www.ncbi.nlm.nih.gov/sra/PRJNA680535>.

Bioinformatics and SSR development

In order to find low coverage contigs, the velvet v1.2.10 (Zerbino 2010) *de novo* genome assembler was utilized with a k-mer size of 45, maximum coverage parameter of 500 and minimum contig length of 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 done with Bowtie2 v2.2.4 (Langmead *et al.* 2009). They were further

4. Population genetics

examined by converting the mapping results and extracting the mapped contigs with SAM tools v1.2 (Li & Wysoker 2009) and visualized by Geneious® v. 10.2.6 (<https://www.geneious.com>). Editing was not necessary since reads were correctly mapped to the contigs. Primers were designed using the ThermoFisher tool with default parameters, i.e., 18 – 22 nucleotides long, with a GC content ca. 40 – 50% (T_m between 58 – 60°C), and preferably G/C-rich at the 3'-end. Heterodimers of the primers were checked, and as a result 40 primers that met these requirements were preferentially selected to produce a PCR product in the range of 100 – 600 bp, allowing multiplex PCR. Primers were synthesized by Metabion International AG. The forward primer of each set was tailed with a M13 sequence (TGT AAA ACG ACG GCC AGT; according to (Schuelke 2000)) to facilitate dye-labeling. The nucleotide sequences of the primer pairs used for each microsatellite are shown in Table 2. The loci named with “*Arfi*” are the markers developed in this study, and the loci names starting with “*Ch*” are from the master thesis of Wang (2011).

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

No	Locus	Repeat motif	Primer sequences (5'–3')	Ta (°C)	Allele size range (bp)	Florescent dye	PCR type	Reference
1	Arfi2	(CA) ₁₉	F: TGT AAA ACG ACG GCC AGT TAGACAATAAGCACACAGAACC R: GAGAGCTAGAGGGATCATTGAG	55	467-552	VIC	multiplex	this study
2	Arfi15	(AAG) ₁₄	F: TGT AAA ACG ACG GCC AGT GTACCGGTGGATACCTTACG R: CCTAATCCAACACACAAGAA	55	207-300	VIC		this study
3	Arfi5	(AC) ₁₁	F: TGT AAA ACG ACG GCC AGT TCCACATGCATGACACTC R: CAGTTGGAACACTATGGGTTTG	55	230-310	6 FAM	multiplex	this study
4	Arfi13	(GAT) ₁₂	F: TGT AAA ACG ACG GCC AGT ACACGCAGAGATGATGAGT R: CACAATACCAGCCTTTGTC	55	406-520	6 FAM		this study
5	Arfi16	(TCA) ₁₀	F: TGT AAA ACG ACG GCC AGT GGTTCTTTCATCAAGTCCA R: ATTGCCAATGAACATCCAG	55	200-275	PET	singleplex	this study
6	Arfi14	(ACA) ₈	F: TGT AAA ACG ACG GCC AGT TGTTTATGTTGCCCTGTG R: GCATGTTAGACCCCTTGA	55	222-335	NED	singleplex	this study
7	Arfi19	(CAA) ₈	F: TGT AAA ACG ACG GCC AGT GTCTAAAATCTGAAGGCGG R: CAACTCATCCCATGGTCAC	55	140-318	VIC	singleplex	this study
8	Ch459	NA	F: TGT AAA ACG ACG GCC AGT TTACCAAATGCTCCATTGAC R: TTGGAAGCACCATGTGAC	42	103-200	6 FAM	singleplex	Wang 2011
9	Ch468	NA	F: TGT AAA ACG ACG GCC AGT TAGGGTTGCAGAAGATAAAC R: CTTTAGTAGGAAGTGAAGAAGC	58	160-226	NED	singleplex	Wang 2011
10	Ch477	NA	F: TGT AAA ACG ACG GCC AGT GGGACCCAATTTACCGAAAG R: CAAAGAATGGGTAAAGCTCAGTG	58	103-140	VIC	singleplex	Wang 2011
11	Ch484	NA	F: TGT AAA ACG ACG GCC AGT TCGTTCAAACGCGTACACAC R: AGCATGCATGAAGAAACAAGC	58	146-240	PET	singleplex	Wang 2011

Application of SSR markers

Genomic DNA was extracted according to the ATMA protocol (Doyle & 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, eight of these loci proved to be reproducible, and those markers have been used again with the same set of samples to receive positive controls (for details about primers see Table 2). Parallel to this, ten SSR markers developed for *A. frigida* published in the master thesis of Wang (2011) were tested with our samples. Potential duplications between both markers' sources were checked beforehand. Based on reproducibility and polymorphisms, 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 mixture 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 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 & Amos 2005). In addition, each plate included two to three positive controls, as a result, all the replications provided identical results. The number of microsatellite alleles per locus was compatible with the proposed tetraploidy, since individuals displayed at maximum four alleles per locus. Due to lacking information on allele dosage in polyploids only “allelic phenotypes” and not genotypes could be assessed. Thus, deviations from Hardy–Weinberg equilibrium could also 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 & Glover 2013). To choose the model with the best fitting number of clusters (K), Evanno's method was employed with Structure Harvester (Earl & VonHoldt 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 & Jasieniuk 2011); Suppl. 2 Table 1). The Bruvo metric has been especially developed for microsatellite data in polyploids and considers allelic dosage in ambiguous polyploid genotypes with a step-wise mutation model (Bruvo *et al.* 2004; Dufresne *et al.* 2014). Therefore, we used the Bruvo distance wherever possible, and estimated mean Bruvo distances for population and sub-population levels using function 'meandist' in R-package vegan ((Oksanen *et al.* 2007); Suppl. 2 Table 2 & 3). To facilitate comparison of genetic differentiation with other studies, we additionally estimated pairwise G_{ST} (Nei & Chesser 1983) in Polysat (Suppl. 2 Table 4 & 5). Principal Coordinate Analysis (PCoA) was conducted for assigning individuals to populations based on i) sub-population level mean Bruvo and ii) individual level Bruvo genetic distances using the R-package vegan (Oksanen *et al.* 2007). 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 intensity that are significantly associated with population 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 analyzed soil nutrient values are provided in Suppl. 1 Table 2.

To evaluate the number of alleles specific for a certain grazing level, 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 & 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 (which share of overall occurrence was found in a given group). Both i) and ii) were multiplied and this number was tested for significance using a permutation test, thereby testing whether alleles were significantly associated with a certain group. By taking the entire data into account, ISA ensured that alleles potentially associated with grazing do not simply reflect small-scale isolation by distance.

Genetic diversity

Genetic diversity among and within populations was assessed with GenoDive v. 3.04 (Meirmans 2020) and the R-package Polysat v. 1.7 (Clark & Jasieniuk 2011; R Core Team 2020), which offer various tools to handle microsatellite data of any ploidy level with allele dosage correction. First of all, diversity and polymorphism of the employed markers were analysed using G-statistics in GenoDive. Further, 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 'mrpp' function in R-package

vegan (Oksanen *et al.* 2007). As a result, we obtained single value for each population (hereinafter Bruvo index), which we used as a representative of genetic diversity measurements. In addition to that, 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 in order to facilitate comparison with other studies (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. In contrast, observed heterozygosity (H_O) was based on allelic phenotypes (e.g. AB), but both full and partial heterozygosity was taken into account (possibilities of genotypes AAAB, AABB or ABBB) to allow direct comparison 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, Bruvo index was used as a response variable, and 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 modeling to make them comparable. Starting from 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 skipped. We plotted residuals of the final parsimonious model to check for possible deviations from normality and reasonable distribution of variances.

Relations between genetic and spatial distances

The relationships between genetic distances and geographical distances (Euclidean distances) were analyzed for 11 populations of *A. frigida* by Mantel test (10,000 randomizations) using 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, whereas sub-population level mean Bruvo 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 Core Team 2020).

Results

Microsatellite marker profile

The 11 SSR markers proved to be highly polymorphic, with an overall 76% of polymorphic information across all primers (Table 3). The locus *Arfi16* was less polymorphic than others, yet did not have negative impact on analyses according to pre-analyses excluding this 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, yet effective number of alleles differed substantially.

Table 3. Characteristics of the employed 11 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).

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, 3% between sub-populations (among different grazing intensity levels), while the largest share was resided between individuals (92%, $p < 0.001$, AMOVA; Table 4). The STRUCTURE analysis suggested the 11 populations form two genetic clusters (best fitting $k = 2$), which were not completely segregated, though Pop2 and Pop10 tended to have some genetic admixture (Suppl. 1 Fig. 1). The PCoA also revealed similar pattern as the STRUCTURE analysis, with the first two axes explaining about 11% of

4. Population genetics

genetic variation (Fig. 3). Furthermore, PCoA ordination with *post hoc* fitted predictor variables revealed significant correlations between genetic structure and environmental variables (MAT, MAP, cvP and latitude) and thus major habitats, yet no main effect of grazing on genetic similarity (Fig. 3 & Suppl. 1 Fig. 2).

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	

However, according to ISA, there were some indicator alleles 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 being 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 is more than a three-fold of those found in the least-grazed sites. In addition, 23 alleles were indicative for moderately-grazed sites, indicating that certain alleles are associated with higher grazing intensity.

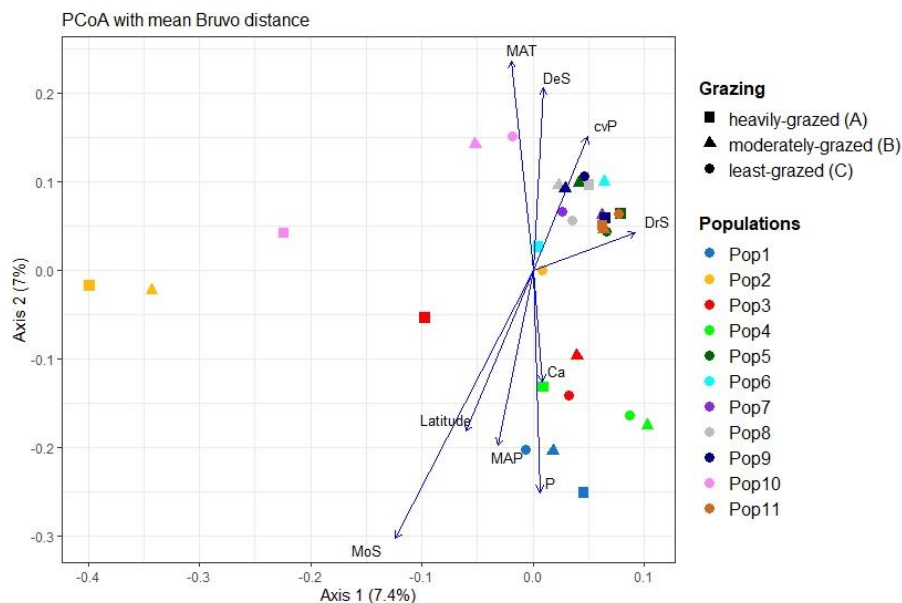


Figure 3 Principal Coordinate Analysis (PCoA) based on sub-population level mean Bruvo distance using 11 SSR markers for the populations of *A. frigida*. Environmental predictors were fitted *post hoc* on ordination plot (shown only those that passed $p < 0.05$ according to test with 1,000 permutations). MoS – mountain steppe, DryS – dry steppe and DeS – desert steppe

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 – 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 were moderate (Suppl. 1 Table 5). The highest genetic diversity was found in the heavily-grazed plot of Pop3 (Bruvo index = 0.67), and 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). Estimators indicate that 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.

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 dryland. The interaction term did not contribute to model quality, and thus was skipped. Grazing was not significant according to model simplification.

	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	*

Pr(>|t|) – significance p-value

Relations between genetic and spatial distances

A Mantel test revealed no correlation of geographical and genetic distances between populations ($r^2 = 0.02$, $p = 0.1$), therefore, no indication of isolation by distance across the 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 & Jasieniuk 2011). Our newly developed SSR markers for the wide-spread polyploid species *A. frigida* proved reliable and provide 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), yet this is often neither known nor easily detected. Since dosage information is missing, complete genotyping is still not feasible with SSR markers, and thus genetic diversity from polyploids might 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 & Jasieniuk 2011) and the software GenoDive (Meirmans 2020) are specifically designed to handle microsatellite data with polysomic inheritance and need for dosage correction, and thus, we decided to choose these two to analyse the at least partly tetraploid samples of *A. frigida*. To keep consistency, one measure of genetic diversity (Bruvo index) was employed through many analyses, yet others were provided for cross comparisons with published studies (Table 1). Further comparisons should, however, be taken with caution because of different markers systems used. Moreover, in SSR analysis the choice of both diversity metrics and software 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 and considered as an excellent rangeland plant with high nutritional value in Mongolia (Undarmaa *et al.* 2015). Our results clearly showed 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 that among different grazing intensity levels was 3% (Table 4). The overall degree of differentiation across populations was low (Global $G_{ST} = 0.034$), which was consistent with another studies on *A. frigida* conducted in Inner

4. Population genetics

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 population 2 and others (Pop2 Hustai NP; $G_{ST} = 0.027 – 0.042$). There, we had our single fenced site (Pop2C), yet this fenced site was not much genetically different from other sites, while both grazed sites from Hustai NP (Pop2A > Pop2B) differed strongly. Given that the sites in the 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 away from the capital city Ulaanbaatar (UB). Every year hundreds of herders with a large number of livestock head to the capital city for purchasing purposes. Due to the close location of Hustai NP to UB, herders from all over Mongolia pass through/ or in the vicinity of the NP, which may results in diaspora input even from rather distant sources. Indeed, it has been shown that epizoochory has a large potential for dispersal over greater distances, and diaspores can be transported in sheep and goat fur of up to 15 km per day in Mongolian dryland (Bläß *et al.* 2010). Genetic diversity was lower in grazed sites compared to the fenced site in Hustai NP (Suppl. 1 Table 5), but grazed sites contained a higher number of indicator alleles (see below). Possibly, genotypes introduced by herder movements might have spread successfully on grazed sites only because the fenced site did not provide free soil patches for germination (Frank 2005).

To reveal the presence of grazing-adapted genotypes, we conducted ISA analysis that detected some indicator alleles characteristic for 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 grazed sites of population 2, as well as in population 6. This might again explain the higher genetical differentiation of the grazed sites of Hustai NP. Given that SSR loci are expected to be evenly scattered throughout the genome and to represent neutral non-coding sections of DNA, this 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 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 between $0.41 – 0.68$ and $0.64 – 0.78$ at sub-population level, respectively (Suppl. 1 Table 5). A further three studies employed RAPD markers (Random Amplification of Polymorphic DNA) on *A. frigida* in Inner Mongolia, China (Liu

4. Population genetics

et al. 2010; Wan *et al.* 2008; Wang *et al.* 2004). All of them detected higher overall shares of polymorphic loci with 83 %, 95 % and 79 % across all populations, compared to ours at PPL= 48 % (Table 1). These data, however, come from profiles of RAPDs as a dominant and completely anonymous marker system. In general, we found high estimates of genetic diversity in *A. frigida* compared to that reported for other widespread, and long-lived perennials (Nybom 2004; Nybom & Bartish 2000).

We further found that higher amounts of summer precipitation and soil phosphorous promoted genetic diversity of *A. frigida* (Table 5), suggesting that environmental conditions have a substantial impact on genetic diversity. In Mongolian rangelands, abiotic constraints exert strong controls on the vegetation, where precipitation and adequate soil moisture in the growing season are critical conditions for seed germination of several species, including *A. frigida* (Ronnenberg *et al.* 2007). It has also been shown that soil carbon and phosphorous had 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, and thus more breeding individuals contribute to the gene pool, leading to an increase of the genetic diversity. Unfortunately, previous population genetic studies on *A. frigida* did not investigate the impact of environmental factors on genetic diversity. However, there are a few other studies on *Artemisia* (shrub and sub-shrub) species from rangelands that have revealed several climatic factors affecting 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 of the sageworts.

In contrast, we found relatively limited grazing effects on genetic diversity of *A. frigida*. This was partly expected, because the species is adapted to drought and disturbance, and/or may even benefit from mechanical disturbance. *Artemisia frigida* is characterized by a highly variable response to disturbance, i.e., propagates by stolons (Li *et al.* 2004) to occupy more space because grazing suppresses its apical dominance (Jinhua *et al.* 2005). Our focus species also produces volatile organic compounds under grazing, inhibiting the growth of grass seedlings (Zhao-Jiang *et al.* 2011), resulting in *Artemisia* dominance stands under severe overgrazing. This might be interpreted as an adaptation of dryland plants to a long history of grazing. However, 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 the geographical distance (600 km distances between the S/N populations), climatic gradient (along 100 – 300 mm S/N differences in MAP), and vegetation differences, we had expected to find greater genetic differentiation with larger distance. However, Mantel tests did not point to a high influence of spatial distance, or simple spatial processes. Analysis on three other *Artemisia* species revealed also no direct genetic relation with geographic distance (Badr *et al.* 2012), and similar findings were reported for *Stipa* species in Central Asia as well (Jing *et al.* 2013; Zhao *et al.* 2006).

Conclusion

Rangeland plant genetic resources have been poorly investigated and conservation genetic parameters are widely neglected in the development of land use policies. Dryland species are, however, crucial for forage and thus as food resource but are often threatened by both intense grazing and moisture deficiency. Our result on *A. frigida* indicated that genetic diversity of this cold, drought and disturbance adapted species was relatively high, and genetic differentiation were low between eleven populations across a north south gradient in Mongolia. Furthermore, we observed no severe consequences of grazing on genetic diversity, yet a high number of grazing-specific indicator alleles were found in grazed sites. This indicates that population differentiation may partly be driven by livestock grazing intensity, but this may have also beneficial effects on the genetic diversity of the species. In addition, sufficient amounts of precipitation and soil nutrients promote the genetic diversity of *A. frigida* in the water- and nutrient-limited arid lands. We conclude that, in spite of the partly degraded and overgrazed landscape in Mongolia, sufficient genetic exchange still exists among populations even across large distances, and that no indication of fragmentation was found. From the perspective of conservation genetics, populations in Mongolia thus are still healthy, which may present a difference to the much more intensively used rangelands in northern China.

4.3. Effect of grazing exclusion on plant populations genetics



Picture showing vegetation difference inside and outside of the Trans -Mongolian Railway. Photo was taken by Oyundeger.Kh in Tuv province, Mongolia in 2019.

The content of this chapter has submitted to the Journal of Arid Environments as **Oyundelger, Kh.**, Munkhzul, O., and Ritz, C.M. and Wesche, K. Long-term grazing exclusion effects populations genetics and functional traits of *Artemisia frigida* in Mongolia. *Journal of Arid Environments*.

Abstract

Mongolian steppes cover the largest still intact parts of the Palearctic steppe biome. They have experienced a long history of nomadic pastoralism, and grazing has a significant impact on steppe vegetation, soil and ecosystem functioning. These effects vary depending on the interplay of local climatic conditions, yet relationships between grazing and plant population genetics have been understudied, and thus remain poorly understood. We investigated influence of grazing on population genetics and functional traits of the most prominent steppe species, *Artemisia frigida*. A total of 187 individuals were sampled from eight locations, comparing fenced and unfenced sites along the Trans-Mongolian Railway and Hustai National Park, and analyzed with ten microsatellite loci.

Grazing had a significant positive impact on the genetic diversity, but had no effect on population genetic differentiation. While, climatic and soil variables strongly influenced population genetic structure, and higher amounts of soil nutrients increased *A. frigida*'s genetic diversity. Grazing significantly influenced functional traits, i.e., plant height, and specific leaf area, yet no relationship was found between genetic diversity and plant functional traits. Overall, given that fenced *A. frigida* populations were genetically less diverse, a moderate level of grazing is beneficial for this species and hence may stabilize ecosystem functioning of steppes.

Introduction

Steppe ecosystems of Eurasia have been heavily influenced by humans, primarily by agriculture, livestock herding, and human settlement. Steppes have undergone significant transformations, and large areas of steppes in Central and Eastern Europe have been turned into cultivated landscapes. In China, 90 % of the grasslands are considered degraded due to overgrazing and plowing, and steppe vegetation in Russia is also severely fragmented today (Hurka *et al.* 2019; Smelansky & Tishkov 2012). In contrast, Mongolia's steppe ecosystems are still remarkably intact due to the country's sparse human population and a continued tradition of nomadic pastoralism. However, as of 2016, 58 % of the Mongolian rangeland area is estimated to be degraded due to strong increases in livestock numbers over the last three decades (Densambuu *et al.* 2018a).

Reviews on the impact of livestock grazing on dryland vegetation have found significant reductions in vegetation cover and species richness, especially in habitats with harsh climate and low productivity (Herrero-Jáuregui & Oesterheld 2018; Jones 2000; Munkhzul *et al.* 2021; Wang & Wesche 2016). Long-term grazing could also cause severe damages on soil structure, and decreases of soil nutrient as well as water contents, which result in increased vulnerability to soil erosion and grassland degradation (Hao & He 2019; Jamsranjav *et al.* 2018; Zhang *et al.* 2017). Moreover, it is well documented that grazing has a significant effect on plant functional traits related to relative growth rate and photosynthetic capacity, e.g., above-ground biomass, plant height, leaf size, leaf dry mass and specific leaf area (SLA; Diaz *et al.* 2007). This reflects the fundamental trade-off between growth and defense against herbivores (Borchardt *et al.* 2013; Díaz *et al.* 2001; Hui & Guoqi 2014; Klimesova *et al.* 2008; Lang *et al.* 2020). Plant functional traits are usually highly plastic, allowing a genotype to adjust to a range of abiotic and biotic stressors (Reich *et al.* 2003). Under extreme climates, grazing effects on plant functional traits may be overruled by climatic controls (Wang & Wesche 2016). However, it still is poorly understood if intraspecific variation in functional traits is under either genetic or environmentally controls (Karbstein *et al.* 2020; Scheepens *et al.* 2010); and different results were obtained depending on the traits and species studied (Caruso *et al.* 2020).

Grazing can also affect population genetic diversity and structure. Effects on plant genetic diversity have, however, not been extensively studied, particularly so in drylands (Greenville *et al.* 2017). Plant genetic diversity can respond negatively or positively to grazing, and can be subject to both direct and indirect grazing effects. Grazing can directly reduce the effective population size and restrict gene flow when whole plants and/or their reproductive parts are consumed (Leimu *et al.* 2006; Souto & Tadey 2019). It can have an indirect negative effect on the frequency of certain genotypes due to the selection of grazing-tolerant phenotypes (Brougham & Harris 1967;

McKinney & Fowler 1991). Herbivory can also result in higher mutation rates due to direct damage, and thus grazing could have a positive impact on genetic variation (Marcotrigiano 2000). Grazers disperse seeds via endozoochory or epizoochory, facilitating gene flow across long distances (Bläß *et al.* 2010; García-Fernández *et al.* 2019). Finally, grazing disturbance can provide safe sites for germination due to gaps in vegetation (Frank 2005; Oesterheld & Sala 1990).

Previous studies revealed that the effect of grazing on population genetics varies with its intensity (Pelliza *et al.* 2020; Peng *et al.* 2015) and this can be strongly modified by environmental conditions, i.e. climatic and soil factors (Lohmann *et al.* 2012; Oyundelger *et al.* 2020, 2021b). Increased grazing has been demonstrated to reduce genetic diversity in common grassland species, like *Stipa* spp. and *Kobresia pygmaea* (C.B. CLARKE) C.B. CLARKE, while moderate grazing has been proposed as a superior strategy (compared to fencing) for grassland management in China (Liu *et al.* 2009; Peng *et al.* 2015; Shan *et al.* 2006). Several studies have assessed the effect of climatic factors on population genetics. In particular, precipitation is an important factor shaping population genetic structures (Jiang *et al.* 2019b; Oyundelger *et al.* 2021b; Zhang *et al.* 2018a), and grazing effects were less pronounced in drier regions (Jamnadass *et al.* 2006). Only few studies reported a relationship between soil nutrient availability and genetic variation in plant populations. Abraham *et al.*, (2018) found that soil moisture and soil nitrogen content were positively correlated with genetic diversity of *Thymus sibthorpii* BENTH., while a higher phosphorous content increased population genetic variation of *Carex nigra* (L.) REICHARD in alpine fens, and of *Artemisia frigida* in drylands (Oyundelger *et al.* 2021b; Reisch *et al.* 2020).

Changes in genetical structures associated with grazing have been investigated primarily with neutral molecular markers (Faville *et al.* 2020; Smith *et al.* 2009). These allow for a general description of grazing-related genetic consequences, although they are not directly related to fitness-related traits such as grazing resistance (Matlaga & Karoly 2004). Hence, to investigate long-term grazing effect (hereinafter referred to as grazing) on genetic diversity and structure, we employed neutral markers, namely microsatellites (SSRs), in *Artemisia frigida* as one of the most widely distributed rangeland species in Mongolian grasslands.

Assessing the impact of grazing in pastoral grazing systems is challenging in the absence of control sites, given that all natural rangelands have typically been utilized for many generations. Yet, in Mongolia, such reference sites could be found along the Trans-Mongolian Railway (TMR), where fences were built and maintained since 1955 resulting in over 60 years of grazing exclusion (Gansukh *et al.* 2018). The fenced railway line effectively works as a large-scale transect across different vegetation zones of Mongolia. Comprehensive vegetation studies along the TMR have been conducted by Bazha *et al.*, (2012), who found that grazed sites were dominated by (dwarf)

shrubs, with notably *A. frigida* becoming dominant in mountain, meadow, typical and dry-steppes under overgrazing. *Artemisia frigida* can tolerate harsh climatic conditions and mechanical disturbance (Bai *et al.* 1995; Li *et al.* 2002), and thus, is one of the first perennials to be established at moderately disturbed sites (Sarvis 1941). Against the backdrop of an apparent positive impact of grazing on *A. frigida*, we studied its genetic and functional diversity under grazing exclusion. Specifically, we aimed to answer the following questions: i). How and to what extent does grazing affect the genetic diversity and structure of *A. frigida*? ii). In contrast to grazing, how do climatic, soil, and vegetation conditions influence genetic diversity and differentiation of *A. frigida* populations? iii). How do functional traits respond to grazing and are those traits related to genetic variation?

Materials and methods

Study area

The study was conducted in the central and southern parts of Mongolia, between Ulaanbaatar (47°52'N, 107°06'E) and Sainshand (45°26' N, 109°39' E; Fig. 1). From north to south, different types of steppe vegetation occurred: mountain steppe, tall grass steppe, typical steppe, and desert steppes (Pfeiffer *et al.* 2019). Mongolia is characterized by a continental dry climate, and our study sites along the NW-SE stretch over a mean annual precipitation (MAP) range between 160 and 300 mm, while mean annual temperature (MAT) lies between -0.8 and 3.5°C, and altitude is on average 1300 m a.s.l (Table 1). Along this aridity gradient, productivity and biomass decrease, while the share of xerophytic species increases (e.g., shrubs and dwarf semi-shrubs increase from ca. 5 % in the meadow steppe to 80 % in the desert steppe; Bazha *et al.* 2012)). Regular burning of the vegetation has taken place inside the fences, while grazing by large mammals has been almost completely restricted since building of the fences (Fig. 2a, b). In order to increase the sample size of our study, three large mammal exclosures established in 2003 (100 m² each; Fig. 2c, d) in Hustai National Park (HNP) have been added as control sites. These sites undergo regular observations and livestock grazing is allowed only in extremely harsh winters (Tserendulam *et al.* 2018).

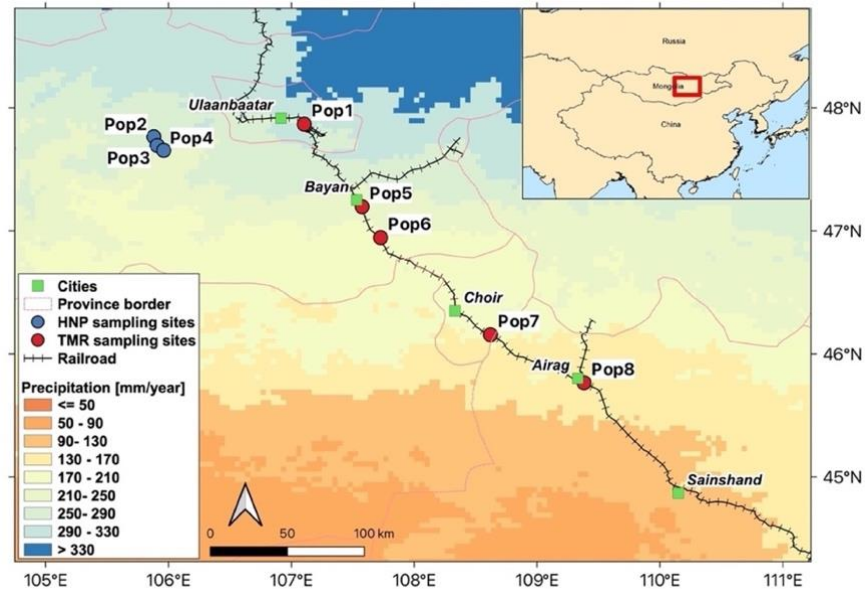


Figure 1. Location of sampled populations of *A. frigida* in Hustai National Park (HNP) and along the Trans-Mongolian Railway (TMR). Precipitation data were derived from Hijmans et al. (2005).

Studied species

We selected *Artemisia frigida* Willd. (Asteraceae) because it is one of the most grazing tolerant key dryland species and it is highly consumed by livestock throughout the year, particularly preferred by sheep, goat and camel (Damiran 2005). The species occurs not only widely in Mongolia, but is distributed across Central and Middle Asia and North America (Oyundelger et al., 2021a). *Artemisia frigida* is a perennial subshrub of 0.2 – 0.6 m height bearing deeply pinnatisect leaves covered with dense silky pubescence. Flowers are arranged in panicles of yellowish capitula, each of which produces around a thousand seeds that contribute to maintaining a persistent seed bank in the soil (Harvey 1981). Diploid ($2n = 2x = 18$) and tetraploid cytotypes ($2n = 4x = 36$) are reported (Garcia et al. 2004; Korobkov et al. 2014; Pellicer et al. 2010; Wan et al. 2011).

Collection and measurement

We sampled five populations along the TMR transect and three in the HNP. At each population, we compared 10 x 10 m plots inside and outside of the fences and thus grazed and ungrazed sub-populations. Distance between grazed and ungrazed sites was a minimum of 20 meters. At each sub-population, 15 individuals of *A. frigida* were sampled. Fresh leaf material was collected for a total of 187 individuals in summer of 2019 (Table 1). In addition, three functional traits were measured on the same individuals: height of inflorescence (if plants were flowering), height of vegetative parts, and specific leaf area (SLA). Height of inflorescence was determined as height from the ground surface to the tip of the highest inflorescence of that plant,

4. Population genetics

and height of vegetative parts as height of a random branch. For leaf area measurements, two fresh leaves from one individual were taken (30 leaves per site) and scanned with a Conrad P-573 handheld document scanner in the field. Scanned pictures were analyzed with ImageJ (Abràmoff *et al.* 2004) to determine leaf area. In the laboratory, leaves were air-dried and weight was measured with Mettler Toledo XP6, and the SLA was calculated by dividing leaf area by dry mass (Perez-Harguindeguy *et al.* 2013).

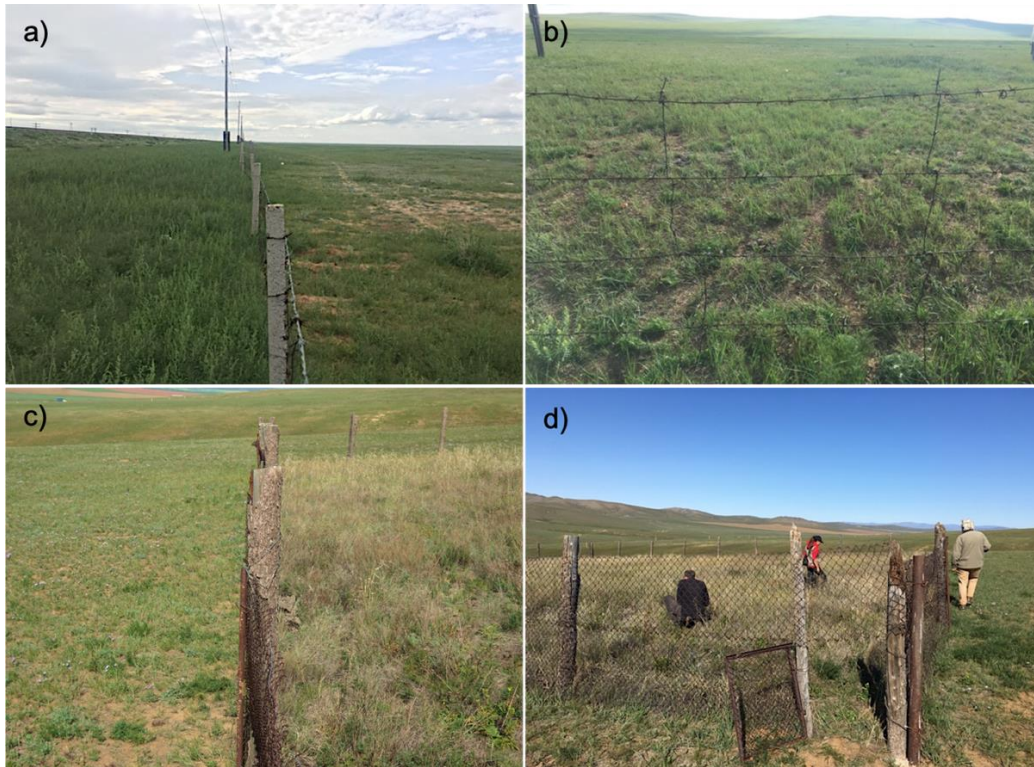


Figure 2. Differences in vegetation structure (a, c) between grazed and ungrazed sites with different fence structures (b, d) along the Trans-Mongolian Railway and in the Hustai National Park, respectively. Picture 'a' is taken along the TMR close to the Choir city.

Vegetation samples and soil analyses

Species composition (SC; cover of each species within a plot), species richness (SR) and total vegetation cover (VC, %) of vascular plants were recorded within a 10 m × 10 m plot to reveal the grazing effect on local vegetation. A sample of top soil (0.05 m depth) was obtained from each site including fine plant roots and the humic layer. Soil samples were first separated from litter and debris, and further measurements were taken in the laboratory after sifting through a 2 mm sieve and incubating at 75 °C for 18 h. To assess the amount of plant available nutrients (P, Mg, Ca, K and Al), we prepared soil extractions following the Olsen method (Sims 2000). Extracts were measured by spectrometry (ICP-OES, Institute of Soil Science, Hannover University). Soil pH was measured by a pH meter (Metler Toledo; water: soil volume = 5:1). Total N and C concentrations of the soil were measured through dry combustion in a CN analyzer (Vario Pyro Cube;

4. Population genetics

Elementar, Langensfeld, DE). Carbonate content was first assessed with a quick test using 10% HCl, and samples showing a reaction were further analyzed using a calcimeter following Scheibler's method (ON L 1084-99, 1999). Soil C was then corrected for inorganic carbon. Rest water was measured after drying of samples at 105 °C for 24h, and was used to calibrate nutrient contents to g dry soil.

Climate data extraction

The meteorological data for 39 years (mean annual temperature (MAT), mean annual precipitation (MAP), coldest quarter temperature and precipitation (December-February; CQTemp and CQPrec), and the warmest quarter temperature and precipitation (June-August; WQTemp and WQPrec) between 1980 – 2018) were retrieved for each locality from the high resolution CHELSA_V2 dataset, which has the advantage of capturing inter-annual precipitation variation (Karger *et al.* 2017). The coefficient of variation of annual precipitation (cvP) was estimated based on the retrieved MAP data and was also used as a predictor.

Molecular analyses

Genomic DNA of 187 individuals was isolated from silica gel dried leaf material of *A. frigida* using the ATMA protocol (Doyle & Doyle 1987), with some modifications (Ziegenhagen 1990). We had developed species-specific SSR markers which were proven to be polymorphic and informative in a former study (see Oyundelger *et al.* 2021b for marker details). Amplifications for a total of ten SSR markers were performed in a volume of 12.5 µl, and customized PCR reaction mixtures and cycling programs were used (see list of the markers and PCR details from Table A.1). Fragment sizes were determined by the central laboratory of the Senckenberg Biodiversity and Climate Research Center (SBiK-F, Germany) using an ABI3730 sequencer (Life Technology) with the LIZ600 size standard. The program Geneious® 10.2.6 (<https://www.geneious.com>) was used to score allele sizes. Individuals exhibited a maximum of four microsatellite alleles per locus, which was consistent with the hypothesized tetraploidy as described in Oyundelger *et al.* (2021b).

Statistical analyses

Analysis of genetic diversity and population structure

To estimate genetic diversity between and within populations, we used two different programs, which allow handling of microsatellite data for polyploid species: 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). Measurements of genetic diversity comprised: allele diversity, effective number of alleles, percentage of polymorphic loci, observed heterozygosity, expected heterozygosity, and inbreeding coefficient, which were all estimated with

4. Population genetics

GenoDive. The Bruvo distances were estimated in the R-package Polysat ; they are specifically designed to estimate distances for microsatellite data with higher and mixed ploidy, while accounting for mutational processes (Bruvo *et al.* 2004). We calculated the mean Bruvo distance among individuals for any given population (hereafter 'Bruvo index'; see detailed from Oyundelger *et al.*, (2021b)) using the R-package vegan (Oksanen *et al.* 2007) and used this as surrogate for genetic diversity. Bruvo index and measures of genetic diversity metrics were highly correlated ($r > 0.7^{***}$; Table A.2). Therefore, we opted to use the Bruvo index as a representative of genetic diversity throughout.

Effect of grazing on genetic diversity and structure in contrast to the local environment

Linear Mixed Models (LMMs) implemented in R-package lme4 (Bates *et al.* 2007) were fitted to assess the effects of grazing pressure and the local environment on genetic diversity. The log-transformed Bruvo distance was used as response variable. Due to multicollinearity of the environmental variables (Table A.3), only selected predictors for climate, soil and community composition were used: MAP, Ca, SR, "fence" was included as a factor. All predictors were scaled to zero mean-unit-variance prior to modeling to make estimates comparable, and population was included as a random factor. The analysis involved stepwise reduction of the full model by progressively removing the least significant factor until the null model. We compared subsequently reduced models in an ANOVA framework, and retained the final model with significant predictors.

We performed a Principal Coordinate Analysis (PCoA) using population level mean Bruvo distances using the R-package vegan (Oksanen *et al.* 2007). Environmental and vegetation variables were fitted *post hoc* on that ordination using vegan (Oksanen *et al.* 2007), and visualized using ggplot2 (Wickham 2011), to reveal environmental variables that are significantly associated with population genetic structure, in particular with fenced and unfenced sites. Environmental variables covered geography (latitude, longitude, and altitude), climate (MAT, WQTemp, CQTemp, MAP, WQPrec, CQPrec and cvP), soil nutrient contents (P, Mg, Ca, K, Al, C, N and pH), and vegetation variables (species richness and vegetation cover). Data on geographic, main climatic and vegetation variables are given in Table 1. Further data on climate, analyzed soil nutrient values and plant species composition of each site (each species' cover within a plot) are provided in Table A.4 & Table A.5.

Effect of grazing on functional traits, and its relationship to the genetic diversity

To investigate grazing effects on plant functional traits, we fitted LMMs using mean and variance of the height of inflorescence, vegetative height, and SLA. In all models, population was included as a random factor. In addition to the environmental variables mentioned above, the Bruvo index was included as a

4. Population genetics

predictor. Height of vegetative parts and inflorescence had normal distributions, and were not log-transformed, while SLA and cover of *A. frigida* were log-transformed to achieve normality. The model simplification procedure was conducted as described above.

Relationship between genetic and spatial distances

To assess the overall relationship between genetic and spatial distances, Mantel tests between genetic distance (population level mean Bruvo) and geographic distances (Euclidean distances) were computed with 10,000 randomizations using the R-package *vegan* (Oksanen *et al.* 2007). Further Mantel tests were conducted between genetic distances and a) climatic differences (Euclidean distance of standardized climatic variables described in 2.5); b) distance of soil indicator variables (Euclidean distance of standardized variables described in 2.4); and c) differences in plant community composition (Bray-Curtis distance based on of log-transformed species' cover).

4. Population genetics

Table 1. Characteristics of the study sites (locality and climate) and respective genetic diversity parameters.

Locality, province	Pop code	Fence	N	E	Altitude, m	MAT, C°	MAP, mm	cvP	SR	VC, %	N	PIC	AD	EfN	H _o	H _E	G _{IS}	Bruvo index
Ulaanbataar, Tuv	Pop1	outside	47.8675	107.1051	1358	-0.65	237.9	28.2	27	75	14	0.78	11.8	6.07	0.62	0.78	0.20	0.62
		inside	47.8678	107.1053	1357	-0.65	237.9	28.2	20	85	8	0.72	7.7	5.62	0.69	0.79	0.12	0.60
Hustai NP, Tuv	Pop2	outside	47.7555	105.8915	1404	-0.31	276.0	29.5	22	40	11	0.66	7.2	3.85	0.44	0.73	0.39	0.56
		inside	47.7648	105.8818	1352	-0.05	278.3	29.5	35	95	11	0.74	8.9	4.66	0.57	0.75	0.24	0.57
Hustai NP, Tuv	Pop3	outside	47.6915	105.9301	1325	-0.05	283.9	29.1	24	76	12	0.71	7.2	3.54	0.53	0.72	0.26	0.54
		inside	47.6944	105.9093	1358	-0.79	304.6	29.1	27	90	12	0.73	8.3	4.58	0.55	0.77	0.29	0.59
Hustai NP, Tuv	Pop4	outside	47.6659	105.9682	1325	0.38	252.8	29.1	25	61	10	0.76	9.9	5.02	0.64	0.78	0.18	0.60
		inside	47.6540	105.9619	1232	0.80	251.0	29.0	22	84	12	0.66	8	3.34	0.52	0.66	0.21	0.51
Bayan, Tuv	Pop5	outside	47.1964	107.5764	1388	0.30	225.6	26.8	25	56	11	0.58	5.8	2.87	0.44	0.70	0.37	0.43
		inside	47.1961	107.5760	1388	0.30	225.6	26.8	21	69	7	0.46	3.7	2.35	0.36	0.68	0.48	0.36
Bayan, Tuv	Pop6	outside	46.9449	107.7278	1324	0.82	204.2	28.4	19	63	10	0.72	7.7	4.31	0.58	0.76	0.24	0.59
		inside	46.9448	107.7270	1331	0.82	204.2	28.4	12	80	9	0.57	5.7	3.36	0.37	0.69	0.47	0.51
Choir, Dundgovi	Pop7	outside	46.1549	108.6206	1221	2.38	179.6	34.1	25	70	15	0.71	9.8	4.75	0.63	0.73	0.15	0.50
		inside	46.1554	108.6208	1222	2.29	180.8	34.0	23	95	15	0.74	10	5.36	0.61	0.78	0.22	0.54
Airag, Dundgovi	Pop8	outside	45.7644	109.3826	1032	3.48	161.2	33.3	23	76	15	0.74	10.3	5.08	0.60	0.77	0.22	0.56
		inside	45.7648	109.3827	1041	3.48	161.2	33.3	22	70	15	0.70	8.9	4.77	0.59	0.73	0.19	0.54
Mean					1291	0.78	229	29.8	23	74	12	0.69	8.18	4.34	0.55	0.74	0.26	0.54

Results

Effect of grazing on genetic diversity and structure, in contrast to the local environment

Genetic diversity of *A. frigida* along the TMR was significantly affected by grazing, which had a significant positive effect on genetic diversity of *A. frigida*. Soil calcium content had a positive impact on genetic diversity, although both effects were modest. In contrast, climatic variables showed no significant effect on *A. frigida* genetic diversity (Table 2; Fig. 3).

Table 2. Summary of Linear Mixed Models assessing the effects of grazing and environmental variables on genetic diversity of the populations of *A. frigida*.

	Estimate	Std. Error	p value	
(Intercept)	-0.29	0.02	<0.001	***
Grazing	0.04	0.01	0.02	*
Soil calcium	0.03	0.01	0.02	*

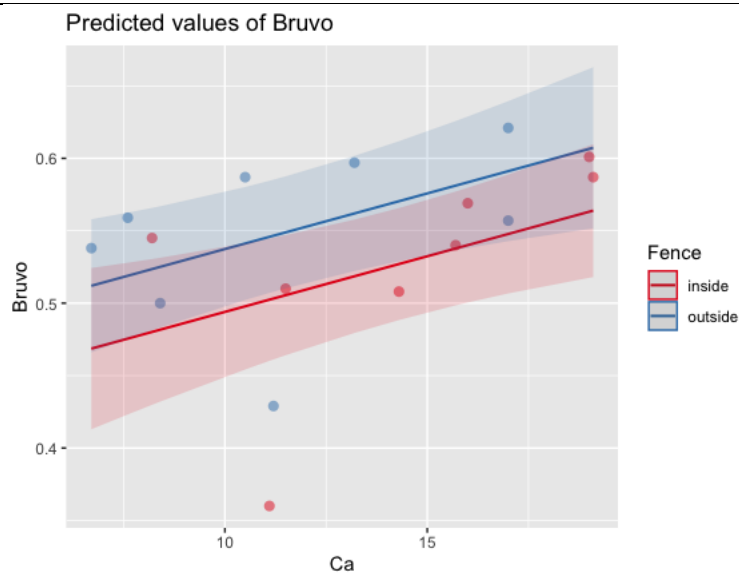


Figure 3. Predicted effects of soil calcium amount (Ca) and grazing based on the best-fitting linear mixed models (LMMs; Table 2). Shaded areas indicate marginal effects.

We found no main effect of grazing on genetic distance between populations (Fig. 4). In the PCoA ordination, populations were segregated along the x-axis, representing three populations from HNP (Pop2 – 4) located on the right side, and populations along the TMR on the left side. Environmental variables and plant species richness showed a more substantial relationship with the genetic distance than grazing according to *post hoc* fitted predictor variables (see Fig. 4).

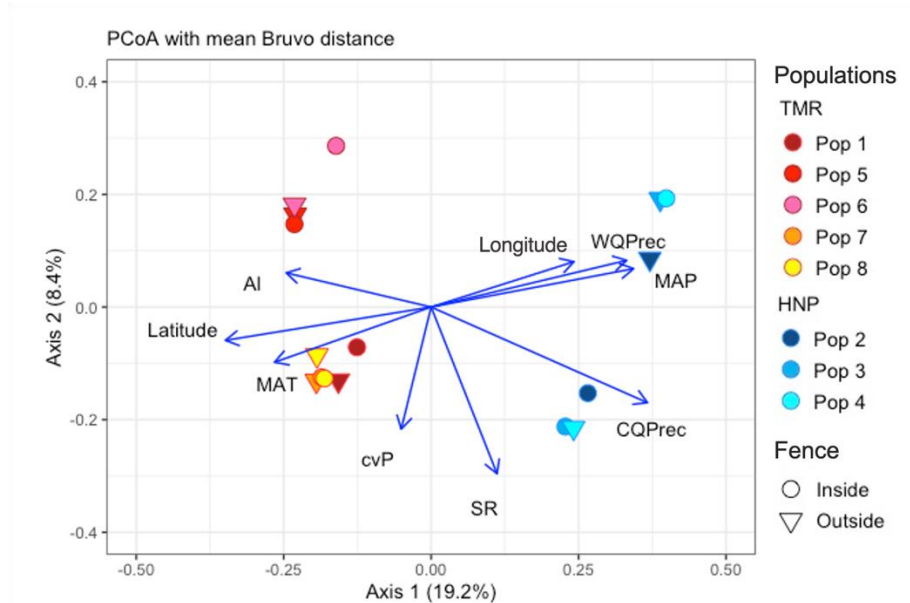


Figure 4. Principle Coordinate Analysis (PCoA) based on mean Bruvo distance between populations obtained from ten SSR markers for fenced (circles) and unfenced (triangles) sub-populations of *A. frigida*. Environmental predictors were fitted *post hoc* on the ordination plot (only those that passed $p < 0.05$ according to a test with 1,000 permutations are shown). MAP – mean annual precipitation, MAT – mean annual temperature, WQPrec – warmest quarter precipitation, CQPrec – coldest quarter precipitation, cvP – coefficient of variation of annual precipitation, AI – soil aluminum content, and SR – plant species richness, TMR – Trans-Mongolian Railway, HNP – Hustai National Park.

We assessed whether effect of grazing on genetic differentiation interacts with climatic variation by calculating mean pair-wise Bruvo distance between pairs of fenced and unfenced sites at a given location. Grazing effect tended to be smaller under higher cvP ($r = -0.67$, $p = 0.007$).

The Mantel tests indicated that genetic distance (Bruvo index) was positively correlated with all environmental distances, i.e., geographic distance ($r^2 = 0.12$, $p < 0.001$), climatic differences ($r^2 = 0.19$, $p < 0.001$), difference in plant species composition ($r^2 = 0.13$, $p < 0.001$), and distance in soil nutrient content ($r^2 = 0.08$, $p = 0.001$). In terms of effect size, the correlations were, however, modest.

Effect of grazing on plant functional traits, and its relationship to genetic diversity

We found that grazing had a significant impact on all functional traits measured, albeit with varying responses. Grazing reduced both height of inflorescence and vegetative parts, but increased SLA of *A. frigida*. Furthermore, a higher MAP resulted in a significant increase in SLA, whereas higher soil pH resulted in lower inflorescence height (Table 3; Fig. 5). We observed no relationship between genetic diversity and functional traits: neither with respect to trait variance (coefficient of variation of the respective

4. Population genetics

variables), nor regarding mean values (mean height of the vegetative and inflorescence, as well as of the SLA).

Table 3. Summary of Linear Mixed Models assessing the effects of grazing and environmental variables on the functional traits of *A. frigida* populations.

Functional traits	Predictor	Estimate	Std. Error	Pr(> t)	
Height inflorescence	(Intercept)	23.72	1.51	<0.001	***
	Grazing	-2.75	0.93	0.024	*
	Soil pH	-8.19	1.52	<0.001	***
Height vegetative parts	(Intercept)	11.51	0.70	<0.001	***
	Grazing	-3.00	0.98	0.008	**
Specific leaf area	(Intercept)	-0.89	0.01	<0.001	***
	Grazing	0.06	0.02	0.010	*
	MAP	0.08	0.01	<0.001	***

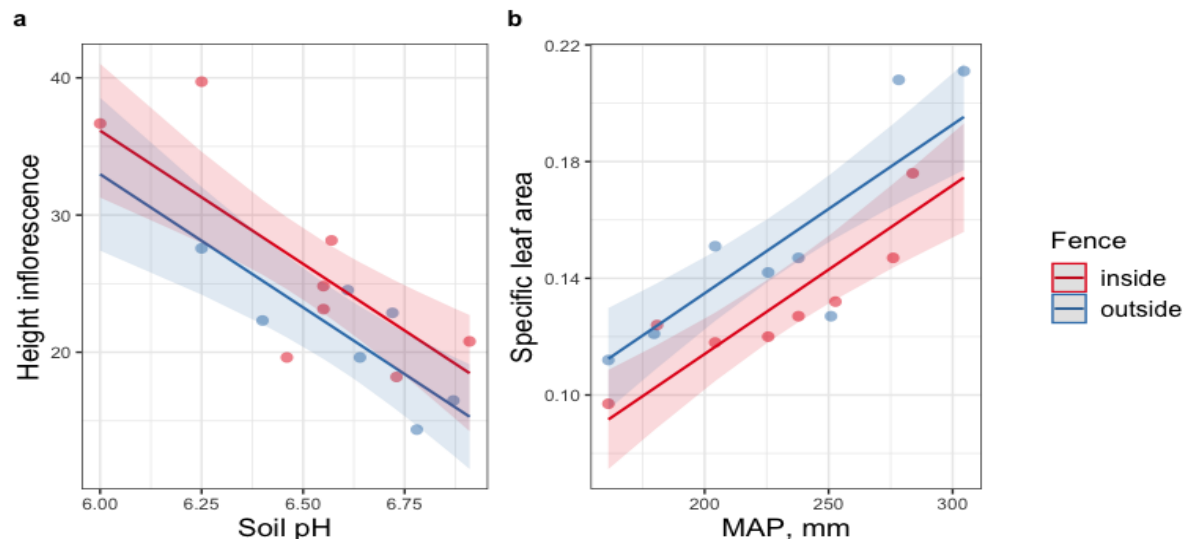


Figure 5. Predicted effects of a) soil pH values on plant inflorescence height and b) MAP on specific leaf area of *A. frigida* between grazed and ungrazed sites based on the best-fitting Linear Mixed Models (LMMs; Table 3). Shaded areas indicate marginal effects of the model.

Discussion

Effect of grazing on genetic diversity and structure, in contrast to the local environment

Population genetic diversity

Long-term grazing disturbance in dryland populations can lead to a loss of genetic diversity across generations (Pelliza *et al.* 2020), while intermediate levels of grazing can facilitate gene flow between populations (Peng *et al.* 2015). We found that the fences along the TMR and HNP had a negative effect on *A. frigida*'s genetic diversity (Table 2), demonstrating that grazing promotes genetic diversity.

4. Population genetics

Large herbivores are important seed dispersal vectors among different pasturelands (Plue *et al.* 2019). In particular, herbivores play an important role in Mongolian steppes, where livestock are herded in order to seek for fresh pastures with frequent movements during the year. Therefore, livestock movement across long distances can promote genetic exchange between populations, given that small diaspores like those of *A. frigida* have high adhesion potential and can be transported in animal fur for up to 15 km per day (Bläß *et al.* 2010). Furthermore, free soil patches are formed for seed germination in the vegetation as a result of browsing and grazing (safe sites; Frank 2005) for seed germination in the vegetation, which would otherwise have a lesser chance of germination, especially in conditions of long-term vegetation succession, such as, inside fences.

Our previous study conducted in rangelands of Mongolia, examining the effect of different grazing intensity levels on *A. frigida* (Oyundelger *et al.* 2021b) revealed no substantial grazing effect on the genetic diversity. There, we compared different levels of grazing intensity from modest to high, but lacked control sites without any grazing. Another study on *A. frigida* conducted in Inner Mongolia, China, found a detrimental grazing effect, with genetic heterozygosity decreasing as grazing intensity increasing from enclosure to heavy grazing (Wang *et al.* 2004), but with high standard deviations of the genetic diversity indices. Mean values of the genetic diversity is decreased as grazing increased according to Wang *et al.*, (2004), however, standard deviations are often as high as the mean values, making differentiation among different grazing intensity levels questionable.

We also found that higher soil calcium amount promotes genetic diversity in *A. frigida*. Soil calcium content was higher inside the fences, possibly due to the construction work of the railway, where different calcium-based stabilizers (e.g., calcium carbonate and calcium chloride) are often used for soil stabilization (Afrin 2017). Calcium is required for plant cell elongation in both shoots and roots (Hepler 2005), and Feagley and Fenn (1998) found that calcium increases ammonium, potassium and phosphorus absorption, promotes photosynthesis, and increases plant size. Furthermore, it has been shown that soil carbon and phosphorous have significant positive effects on the establishment of seedlings and plant growth of *Artemisia* species (Yang *et al.* 2015). our former study (Oyundelger *et al.* 2021b) found that higher levels of soil phosphorus promoted the genetic diversity of *A. frigida*, through facilitating successful seed germination enhancing reproductive success, resulting in more effective sexual reproduction and a consequent increase in genetic diversity. Regrettably, there is a general lack of studies investigating the relationship between soil nutrient content and plant genetic diversity, so it remains speculative if observed effects of Ca and P are direct or just due to covariance with other potential beneficial soil factors.

Population genetic differentiation

Artemisia frigida is a wind-pollinated, wide-spread perennial species, and a prolific seed producer (Harvey 1981). Its life form, geographical distribution, and reproduction system have direct implications for genetic differentiation among populations. Former studies have demonstrated that populations of *A. frigida* sustain adequate gene flow, and therefore, levels of genetic differentiation is low in drylands of China and Mongolia ($D_{ST} = 0.04$; $D_{ST} = 0.05$; $F_{ST} = 0.32$; $G_{ST} = 0.034$; Wang et al., 2004; Liu et al., 2010, 2012; Oyundelger et al., 2021b, respectively). In our study, we found moderate genetic differentiation among populations at $G_{ST} = 0.07$. This is, however twice as high as compared to our former study on different grazing intensity levels conducted in Mongolia. In the present study, the higher genetic differentiation among populations was possibly caused by clear differentiation between regions, i.e., populations of HNP and along the TMR, as illustrated in the PCoA (Fig. 4). This pattern was previously observed in our study as well, where HNP populations differed genetically from other populations, confirming an earlier hypothesis about high diaspore input, i.e. new genotypes transported by moving herds in the national park (see details from Oyundelger *et al.* 2021b). However, we did not find an overall grazing effect on genetic differentiation between grazed and ungrazed sites in both HNP and TMR (mean Bruvo distance inside fence = 0.61, and outside fence = 0.62), as fences permit diaspore and pollen transport through wind (Fig. 2 b, d), and thus genetic exchange. In addition, under drier condition, vegetation is less dense even inside the fences, may limiting differentiation between grazed and ungrazed sites.

In comparison to the grazing effect, environmental variables and plant species richness showed a more substantial relationship with genetic distance. Specifically, the amount of total precipitation, the variability of precipitation, annual temperature and geographical locations showed significant relationships with genetic structure (F. 4). This is consistent with our former study (Oyundelger *et al.* 2021b), where precipitation variability and habitat types were the main drivers of population genetic differentiation in Mongolian drylands. Furthermore, we found a correlation between the coefficient of variation of annual precipitation (cvP) and grazing effect, with stronger grazing impacts on genetic distance in moister habitats with lower cvP ($r = -0.67$, $p = 0.007$). The northern part of Mongolia with forest steppes and mountain- and meadow-steppe habitats receive higher amounts of precipitation, and in moister habitats grazing has a stronger impact due to the direct effect of high stocking rates according to the equilibrium system dynamics (Ahlborn *et al.* 2020).

Effect of grazing on plant functional traits, and its relationship to genetic diversity

We found that grazing had a significant impact on all functional traits measured, which could be interpreted as adaptation to grazing. Specifically, the heights of both plant inflorescence and vegetative parts were smaller, but SLA was larger at grazed sites. This is consistent with the general strategy of grazing resistant species displaying both avoidance traits (high SLA; Díaz *et al.* 2001) Smaller plant height of *A. frigida* under disturbance was also observed by Li., et al (2002). These authors simulated a grazing effect by cutting individuals during their reproductive stage, and reported that severe cutting reduced the height of re-growth and the number of branches, resulting in miniaturized plants. Increased SLA in grazing resistant species under intensified grazing is known from previous studies (Cingolani *et al.* 2005b; Díaz *et al.* 2001; Lang *et al.* 2020); higher SLA should improve the photosynthetic ability and compensate for biomass loss via higher growth rates in response to long-term grazing. Moreover, Lang *et al.*, (2018) found that SLA of *A. frigida* increased with higher water availability, which is concordant with our result of a significant positive correlation between SLA and MAP (Table 3). However, we did not find interactions between climate and grazing on the difference of SLA between populations. Such interactions were found by Lang *et al.*, (2020) in *A. frigida*, yet with weak correlation ($R = 0.04^*$).

We observed no relationship between genetic diversity and mean values of functional traits of *A. frigida*, nor with trait variance. This reflects that many traits exhibit great plasticity in response to local environmental conditions (Mason *et al.* 2020; Stinchcombe *et al.* 2012). Few studies have examined the genetic basis of functional trait variation and its heritability. Geber and Griffen (2003) reviewed the relationship between selection of traits and heritability, finding that the traits under strong selection have the lowest heritability due to a demand for adaptability to high environmental variance. Recently, Ahrens *et al.*, (2020) confirmed that heritability is not consistent among traits, and different levels of variability can be observed in the traits depending on local adaptation to selection pressure. Specifically, SLA in a tree species (*Corymbia calophylla* (LINDL.) K.D. HILL & L.A.S. JOHNSON) was found to be barely heritable confirming that SLA is highly plastic (Shipley 2000). In addition, Scheepens *et al.*, (2010) found a vary plastic response of SLA in the widespread alpine plant *Campanula thyrsoides* L. as well, but with some evidence of genetic constraints on SLA variance.

Conclusion

Grazing of large herbivores is an integral aspect of Mongolian steppe ecosystems, soil function, and nomadic livelihoods. Our study is one of the few studies, where exclosures were used to investigate grazing effect on population genetic structure of dominant steppe species. We found that fences along the TMR and in the HNP had negative effects on the genetic diversity of *A. frigida*, while soil nutrient contents might promote seedling establishment, thus increasing genetic diversity. In contrast to climate and soil characteristics, grazing showed no significant influence on population genetic structure. Moreover, grazing had a significant impact on all the functional traits measured, in particular plant height decreased and SLA increased as a likely adaptation against grazing. Yet, we found not relationships between genetic differentiation among sub-population and functional traits, pointing to high phenotypic plasticity. Overall, a certain level of grazing may be even beneficial for species genetic diversity and hence functioning of the grassland.

Still, relationships between dryland plant genetic diversity and environmental variables, specifically with soil factors remain not fully understood. This aspect should be considered in future studie

Chapter 5. Overarching synthesis and discussion

This thesis provides an in-depth investigation of the phylogeographic history and genetic structure of contemporary populations of *A. frigida*, which can stand as an exemplar for other typical Eurasian grassland species. Our analyses did not only elucidate many outstanding evolutionary biology issues (i.e., migration route, ancestral area, polyploidization and genetic relatedness of populations) of the species, but also provided a comprehensive understanding of the range dynamics of the species, shown by pronounced genetic structuring of Middle and Central Asia. Furthermore, a considerable portion of intraspecific genetic variation was explained by environmental heterogeneity, which promoted polyploidization in populations, suggesting that local and regional adaptations play an important role in structuring *A. frigida* populations.

Population genetic studies showed that grazing has an overall positive impact on the genetic diversity of *A. frigida* in Mongolia. This could be due to species' tolerance characteristic. *Artemisia frigida* is known as a pioneer species establishing on disturbed sites, and tends to increase in abundance with overgrazing. Thus, its genetic diversity may also benefit from grazing. Population differentiation was only partly driven by grazing intensity, with some grazing-specific indicator alleles found. On the other hand, abiotic factors (geography, climate and soil nutrients), had a greater impact than grazing on population genetic structure. Both population genetic studies found only modest genetic structuring in *A. frigida* populations, suggesting that Mongolia's vast plain steppe supports adequate gene flow among populations of this widely distributed and wind-pollinated species. With regard to conservation genetics, *A. frigida* populations in Mongolia are apparently genetically 'healthy', in spite of the pervasive grazing in the region.

5. Synthesis and discussion: Methods

5.1. Molecular markers: pros and cons

Within this thesis, various molecular marker systems, i.e., from the most traditional to novel NGS methods, were used.

In the phylogeographic study, we employed sequencing of a non-coding region of plastid DNA for the haplotype analysis, and GBS markers for the SNP based population genomic analyses and ploidy inference (see Oyundelger *et al.* 2021a). The Genotyping-by-sequencing (GBS), which offers the potential to harvest large numbers of variable sites (SNPs) from nuclear genome, was primarily used for the analyses of phylogeography. The high marker coverage obtained through GBS enabled us not only to discover polymorphisms and use them to resolve its phylogeography and population relatedness, but also to obtain genotypic information across the whole population of interest. This, together with relative reasonable costs made GBS suitable for applying for hundreds of samples collected from the species' whole distribution. We could even deduce ploidy levels using two different approaches. The first one was based on the sequence reads and corresponding frequency of each allele. All GBS loci were sequenced multiple times and were therefore represented by several reads. As each allele was sequenced proportional to its frequency ratio, assumptions on ploidy level were possible. The second approach applied Bayesian assignment using observed heterozygosity, particularly using frequency distribution of heterozygous sites per individual based on SNP ratio. There were, however, several inconsistency or uncertainties between the outcomes two approaches, that were mainly caused by lesser reads of some individuals and/or lack of training data. But, those accounted for only 2% of the whole samples, indicating that novel approaches are becoming more practicable in large number of samples. Addition to the GBS, plastid DNA (i.e., cpDNA) was employed. The cpDNA is the most frequently used marker in plant phylogeographic studies (69% of all studies according to a review by Morris & Shaw 2018) and its usage has increased over time. Based on the analysis of potentially informative characters in variable regions of the plastome, the haplotypes could be distinguished, as numerous other studies have shown in different taxa (Gao *et al.* 2007; Thórsson *et al.* 2010; e.g., Watano *et al.* 1996; Widmer & Baltisberger 1999). However, there were several limitation of single-locus DNAs: resolution within populations was not adequate and it was not informative enough to build a reliable phylogenetic tree. Moreover, cpDNAs are usually uniparently inherited (maternal in most angiosperms), delivering less information than e.g., nDNA, that is inherited from both parents, causing discrepancy between them. Interestingly, cpDNA sequences are the most utilized marker in plant phylogeography and its use continued to increase even in the light of the perception that they alone do not provide sufficient resolution for most studies according to the review of Morris and Shaw (2018).

5. Synthesis and discussion: Methods

In population genetic studies, neutral markers, such as microsatellite markers (i.e., SSRs), were employed. The 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). Due to their general suitability, including their cost effectiveness on large sample size, we opted to use SSRs. We developed species-specific SSR markers for *A. frigida*, because no specific loci have been published so far. The whole genome sequencing (WGS) of was used to screen and develop species-specific SSR markers. The newly developed eleven markers were proven to be reproducible and polymorphic (PIC= 0.25-0.94; see Oyundelger *et al.* 2021b). Based on its high polymorphism, genetic diversity of the populations could be evaluated and population differentiation was successfully assessed. Another advantage of the SSR was: proper statistical methods and programmes that are specifically developed for the SSRs (e.g., GenoDive and R-package Polysat) are available, even for polyploids. On the down side, SSRs, as neutral markers, it could not trace positive or negative responses of genotypes to selection; but, it provided some insight into the overall distribution of certain genotypes, which can be characterized by favourable suits of traits under unknown (multigenetic) control. Therefore, for analysis of adaptive genotypes (e.g., grazing adaptation, drought adaptation, or phenotypic trait adaptation) other non-neutral genetic markers should be used.

In summary, all the markers employed had certain advantages and limitations. Yet, we were successful to answer all the question raised within this thesis. Neutral genetic markers demonstrated their great potential for investigating processes within landscapes, such as gene flow and dispersal; while traditional marker, like cpDNA have been proven to be still valuable markers in the era of next generation sequencing.

5. Synthesis and discussion: Phylogeography

5.2. A review of phylogeographical studies on Eurasian steppe plants

Understanding of the phylogeographical history of the Eurasian steppe has substantially expanded in recent decades as a result of increasing advances in molecular approaches and extensive field research, as well as international collaboration. In the last decade, advanced molecular techniques allowed us for conducting comprehensive molecular phylogeographic studies, providing dated phylogenies, ancestral area reconstructions, and climate and landscape history correlations at the species and population level to shed light on the evolution of the steppe flora. One of the most recent and comprehensive reviews has been conducted by Hurka et al., (2019), who compiled scattered information on origin and evolutionary history of the Eurasian steppe belt. An another review by Barbolini et al., (2020) looked closer into the Cenozoic evolution of the steppe-desert biome in Central Asia, synthesizing different approaches such as pollen-based reconstruction of vegetation, phylogenetics, paleogeography and climate models. In addition, Wesche et al., (2016) published a synthesis on the Palearctic steppe biome by describing different steppe types and associated macroclimatic niches, as well as giving a synoptic overviews on steppe flora and fauna. Furthermore, Binney et al., (2017) reconstructed the vegetation of Eurasia from the last glacial maximum to the present based on fossil pollen records. Kirschner et al., (2020) dated the isolation time of European steppes from Asian steppes, and highlighted a conservation value of the European steppes that harbor large genetic diversity, a regionally endemic genetic lineages and a mosaic of stable refugia.

To untangle the evolutionary history of steppe species across Eurasia, a number of studies have been recently conducted, using characteristic elements of steppe flora. A summary of steppe species for which phylogeographical studies were conducted is compiled in Table 1, representing main results and type of the molecular approach. Franzke et al (2004) undertook the very first phylogeographical study of an evolution of Eurasian steppe plant, namely *Clausia aprica* (Stephan) Korn.-Trotzky (Brassicaceae). Since then, about 30 more studies (Table 1) have been conducted on different plant families, focusing on a variety of levels from genus through section, species, sub-species, and populations. The minority addressed interspecific relations and distribution patterns, e.g., *Allium* L. sections *Decipientia*, *Rhizirideum* and *Rhizmatosa* (Friesen et al. 2020a, 2021; Sinitzyna et al. 2016) or focusing on one genus, e.g., *Scabiosa* L. (Carlson et al. 2012) and *Potentilla* L. (Dobeš & Paule 2010). However, the majority of the studies concentrate on intraspecific genetic variation sampled from distinct populations. Most of the studies focused on Brassicaceae and Amaryllidaceae, while Poaceae, the most typical element of temperate steppe vegetation, are surprisingly largely missing. Furthermore, only a few studies have focused on other prevalent steppe taxa, such as Asteraceae,

5. Synthesis and discussion: Phylogeography

Chenopodiaceae, and Caryophyllaceae, which are commonly discovered in pollen and plant macrofossil studies of Eurasian temperate steppe vegetation.

The majority of the studied taxa, such as *Allium* L. sections *Decipientia*, *Rhizideum*, *Rhizomatosa*, *Artemisia* L., *A. frigida* Willd., *Camelina* Crantz, *Clausia* Kornuch-Trotzky, *Dontostemon* Andr. Ex C. A. Mey, *Krascheninnikovia ceratoides* (L.) Gueldenst. *Potentilla* L. s. str, *Goniolimon speciosum* (L.) Boiss. and *G. tataricum* (L.) Boiss. are found to be originated in Asia, reflecting the origin and development of Eurasian steppes. Their migration route across Northern Hemisphere followed mainly two routes: (i). westwards to Mediterranean and Europe, and further to North America through North Atlantic Land Bridge (NALB); and (ii). northeastwards to Siberia, and to North America through Bering Land Bridge (BLB). *Artemisia frigida* was found to have migrated through the BLB. The oldest studied taxa dated was *Allium* L. section *Rhizomatosa*, which presumably originated in Mongolia in the late Oligocene (26 ± 9 Mya; detailed information on origination time scale, migration route, time of migration and accessible links of each taxon are provided as Suppl.1 electronically). More than half of the studied taxa were originated in the Pleistocene (2.5 Mya – 12 Kya). Due to repeated glacial periods and orogenic developments of large mountain systems during the Pleistocene, the Eurasian steppe belt was continuously displaced and disrupted, affecting steppe species distributional expansion and contraction. Steppe species large diversification took place during the glacial periods of the Pleistocene as evidenced by diversification and expansion of typical steppe taxa such as *Capsella* Medik., *Clausia aprica* (Stephan) Korn.-Trotzky, *Goniolimon speciosum* (L.) Boiss., *Sisymbrium* L. and *Krascheninnikovia ceratoides* (L.) Gueldenst. The same likely hold true for *A. frigida*.

This thesis focused on one of the less investigated family, corroborating findings of above studies. Even, genus *Artemisia* L., is one of the most prevalent forb genera documented from data on pollen and macrofossils from the LGM to the present (Binney *et al.* 2017; Qin *et al.* 2015; Tarasov *et al.* 2000), phylogeographic study on the genus is found to be scarce. My thesis tackles this gap, presenting a case study on the phylogeography of the steppe plant *Artemisia frigida* (Asteraceae). This study is not only a significant contribution to the evolution of the Eurasian steppe florogenesis, but also has significance for conservational biology. Specifically, assessed levels of genetic diversity shown potential genetic depauperation in some locations, providing information for conservation field in a changing climate and landscapes.

5. Synthesis and discussion: Phylogeography

Table 1. A list of phylogeographical studies conducted on Eurasian steppe plant taxa. Studies are ordered alphabetically according to the family, and within them, the genera and species.

Family	Genus	Species	Study area coverage	Molecular marker	Geographic origin	Publication
Asteraceae	<i>Artemisia</i> L.	45 taxa	Eurasia and North America	nDNA	Central Asia	Sanz et al., (2011)
		<i>frigida</i>	Middle and Central Asia, North America	cpDNA and GBS	Middle Asia	Oyundelger et al., (2021a)
	<i>Carlina</i> L.	<i>Acanthifolia subsp. utzka</i>	Southern and Central Europe	cpDNA, nDNA and AFLPs	Balkan Peninsula	Cieślak and Drobnik., (2019)
	<i>Scorzonera</i> L.	<i>purpurea</i>	Europe	cpDNA and ccmp	Pannonian basin	Meindl et al., (2016)
Amaryllidaceae	<i>Allium</i> section <i>Decipientia</i>	9 taxa	Kazakhstan and adjacent regions of Russia, Ukraine, Iran	cpDNA	Kazakhstan	Friesen et al., (2021)
	<i>Allium</i> L.	<i>tulipifolium</i>	Kazakhstan and adjacent regions of Russia, Ukraine, Iran	cpDNA	Kazakhstan	Friesen et al., (2021)
	<i>Allium</i> section <i>Rhizirideum</i>	19 taxa	Europe, North Kazakhstan, West Siberia and Temperate Asia	cpDNA and nDNA	not studied	Sinitsyna et al., (2016)
	<i>Allium</i> section <i>Rhizomatosa</i>	8 taxa	Russia, Kazakhstan, Mongolia, China	cpDNA and nDNA	Central Asia	Friesen et al., (2020a)
Brassicaceae	<i>Camelina</i> Crantz	genus level	Europe and western and central Asia	cpDNA and nDNA	Western and central Irano-Turanian	Žerdoner Čalasana et al., (2019)
	<i>Capsella</i> Medik.	genus level	Mediterranean to Central Asia	GBS	Middle Asia	Žerdoner Čalasana et al., (2021b)
		<i>bursa-pastoris</i>	Mediterranean to Central Asia	cpDNA GBS	Southeast Europe	Hurka et al., (2012) Žerdoner Čalasana et al., (2021b)

5. Synthesis and discussion: Phylogeography

		<i>grandiflora</i>	Mediterranean to Central Asia	cpDNA; GBS	Mediterranean	
		<i>orientalis</i>	Mediterranean to Central Asia	cpDNA; GBS	Asia (Kazakhstan)	
		<i>rubella</i>	Mediterranean to Central Asia	cpDNA; GBS	Eastern Mediterranean	
		<i>thracica</i>	Mediterranean to Central Asia	cpDNA; GBS	Southwest Europe	
	<i>Clausia Kornuch-Trotzky</i>	genus level	Russia, Kazakhstan, Mongolia	cpDNA and nDNA	Central and eastern Asia	Friesen et al., (2016)
		<i>aprica</i>	Russia, Kazakhstan, Mongolia	cpDNA and nDNA	not studied	Franzke et al., (2004)
	<i>Dontostemon Andr. ex C.A.Mey.</i>	genus level	Middle Asia, Central Asia, and Japan	cpDNA and nDNA	Central Asia	Friesen et al., (2016)
	<i>Schivereckia Andr. Ex DC.</i>	<i>podolica</i>	Volga-Don, Donetsk and Podolia, and Ural regions	cpDNA, nDNA and RAPD	Periglacial steppe	Friesen et al., (2020b)
	<i>Sisymbrium L.</i>	genus level	Eurasia, Mediterranean, South Africa	cpDNA and nDNA	Western Irano-Turanian and Mediterranean	Žerdoner Čalasana et al., (2021a)
Chenopodiceae	<i>Krascheninnikovia Gueldenst.</i>	<i>ceratoides</i>	North America, Eurasia and North Africa	cpDNA and nDNA	Central Asia (Altai mountains)	Seidl et al., (2020)
				cpDNA, nDNA and GBS		Seidl et al., (2021)
Dipsacaceae	<i>Scabiosa L.</i>	24 taxa	Europe and Mediterranean Basin, southern Africa and eastern Asia	cpDNA and nDNA	Europe	Carlson et al., (2012)
Euphorbiaceae	<i>Euphorbia L.</i>	<i>niciana</i>	Southern Balkan Peninsula and western	nDNA and RAD-seq	Transcaucasia	Frajman et al., (2019)

5. Synthesis and discussion: Phylogeography

			and central Anatolia			
		<i>seguieriana</i>	Southern Balkan Peninsula and western and central Anatolia	nDNA and RAD-seq	Transcaucasia	
Fabaceae	<i>Astragalus</i> L.	<i>onobrychis</i>	Pannonian and adjacent (south)eastern regions	RAD-seq	Central Pontic subregion	Záveská et al., (2019)
				cpDNA		Plenk et al., (2020)
Linaceae	<i>Linum</i> L.	<i>flavum</i>	Central Hungary, Pannonian region, and southern Germany	cpDNA and AFLP	not studied	Plenk et al., (2017)
Plantaginaceae	<i>Veronica</i> L.	<i>longifolia</i>	Europe to Central Asia	GBS and RAD-seq	Eastern Asia	Buono et al., (2021)
		<i>spicata</i>	Europe to Central Asia	GBS and RAD-seq	Southern Russia	
Plumbaginaceae	<i>Goniolimon</i> Bioss.	<i>speciosum</i>	European Russia, Middle and Central Asia	cpDNA and nDNA	Central Asia	Volkova et al., (2017)
		<i>tataricum</i>	Southeast Europe (Balkan) and the Apennine Peninsula	cpDNA	Central Asia	Buzurović et al., (2020)
Ranunculaceae	<i>Adonis</i> L.	<i>vernalis</i>	Western part of the Eurasian steppe belt	GBS	Europe	Seidl et al., in prep
Rosaceae	<i>Potentilla</i> L. s.str	98 taxa	North America, Europe to East Asia	cpDNA	Asia	Dobes and Paule (2010)

5. Synthesis and discussion: Population genetics

5.3. A review on plant population genetic studies in Mongolia

Mongolian steppe vegetation has been studied intensively, particularly since 2000, with the collaboration of international researchers (Munkhzul *et al.* 2021). Many studies focused on grazing effect on vegetation, since Mongolia has a long tradition of nomadic grazing (e.g., Fernandez-Gimenez & Allen-Diaz 2001; Liao *et al.* 2020; Na *et al.* 2018; Van Staalduinen *et al.* 2007; Wesche *et al.* 2010). Numerous studies investigated the effects of grazing and climate (Ahlborn *et al.* 2020; Bat-Oyun *et al.* 2016; Cheng *et al.* 2011; Hoshino *et al.* 2009; Khishigbayar *et al.* 2015; Lang *et al.* 2020; Spence *et al.* 2016; von Wehrden *et al.* 2012a), because effects of grazing vary depending on the local climate and they do interact with each other.

Regrettably there are only few studies on steppe plant population genetics in Mongolia (Table 2), and thus remain poorly understood. Population genetics are understudied for dryland species in general, as highlighted by a review of Greenville *et al.*, (2017), where well over 7,000 publications from last 75 years have been reviewed.

On the basis of five key sources, I searched for mongolian publications as well as MSc and PhD theses conducted on plant population genetics in: (i). Proceedings of Biology, and (ii). Proceedings of Botany of the Mongolian Academy of Science (MAS) (<https://biology.ac.mn/>), and (iii). online catalogue of Mongolian Foundation of Science and Technology (<http://stf.mn/>), where dissertations from all universities are archived, (iv). electronic catalogue of National University of Mongolia (NUM) (<https://catalog.num.edu.mn/>) as well as (v). electronic catalogue of Mongolian University of Life Science (MULS) (<https://muls.lib4u.org/>). Additionally, Google Scholar, ScienceDirect, and Scopus are used to search for english-language publications.

The first study of population genetics was conducted by Wesche *et al.*, (2006a) on an endemic species of southern Mongolia, *Potentilla ikonnikovii* Juz., using RAPDs to examine the effects of natural isolation on its genetic structures. Authors found out that the populations of *P. ikonnikovii* were genetically isolated, which could be harmful to the species' fitness. Moreover, Wesche *et al.*, (2006b) studied population genetics of two other rare species, *Galitzkya macrocarpa* (Iconn.-Galitz.) V. Boczantzeva, and *G. potaninii* (Maxim.) V. Boczantzeva, employing RAPD markers. According to the study, *G. macrocarpa* appeared to be able to preserve genetic variety across its range, whereas *G. potaninii* populations showed substantial genetic differentiation, indicating severe fragmentation between them. Another study carried out by Wesche *et al.*, (2011) focused on *Ulmus pumila* L., which is the only tree species occurring in southern and south-eastern Mongolia, and may reproduce by clonal growth. According to the study, populations of *U. pumila* maintained their gene diversity, and no inbreeding depression was found, and differences between northern and southern Mongolia were negligible. Furthermore, Dashzeveg *et al.*, (2017) assessed genetic diversity of rare and endangered species, *Saussurea involucreta* (Kar. et Kir.) Sch. Bip. utilizing

5. Synthesis and discussion: Population genetics

AFLPs. They found low levels of genetic diversity, with the majority of genetic differentiation residing within populations. The above mentioned studies were mainly focused on rare and endangered species with fragmented ranges distributed in mountain ranges and/or Gobi, as they often show small and fragmented distribution patterns. Thus, their genetic variations have been analyzed mainly for the purpose of conservation.

While, Ronnenberg (2011b) undertook the first investigation in a widely distributed species, examining the clonal diversity of the tussock species, *Stipa krylovii* Roshev by RAPD markers. The study reported high clonal diversity (i.e., many different clones) of the species, and relatively weak genetic differentiation between plots and regions. In addition, Wiehle et al., (2016) conducted a study on a widely distributed tree species in western Mongolia, *Populus laurifolia* Ledeb. utilizing SSRs. They found a relatively high level of genetic diversity, but detected overall low genetic differentiation between populations. Another study examining the population genetics of tussock species, *Stipa glareosa* P.A.Smirn was carried out by Oyundelger et al., (2020) employing SSRs. Together with colleagues, I assessed effects of abiotic (geography, climate and soil nutrients) and biotic (grazing) factors on population genetics. According to the study, genetic diversity of *S. glareosa* was relatively low. Environmental constraints affected the genetic structure of *S. glareosa* populations at the regional scale, while grazing disturbance had more effect at local scales differences. In addition, one thesis was found from the library of NUM, that dealt with plant population genetics of *Gentiana algida* Pall. (Nomin-Erdene 2021). She used the ISSR markers on five populations from northern Mongolia and observed a modest level of genetic diversity of the species, yet with only 9 % genetic variation partitioned between populations. Furthermore, a hypothesis that levels of genetic diversity are related to population stability in inter-specific comparisons, especially among species in a local community, is examined by Ishii et al., (2021) using ISSR markers on nine common grassland species of Mongolia: *Stipa krylovii* Roshev., *Convolvulus ammannii* Desr., *Allium bidentatum* Fisch. Ex Prokh. & Ikonn.-Gal., *Cymbaria dahurica* L., *Carex duriuscula* C.A. Mey., *Agropyron cristatum* (L.) Gaertn, *Kochia prostrata* (L.) Schrad., *Artemisia frigida* Willd., and *Caragana leucophloea* Pojark. Level of genetii diversity varied among species. However, they observed no correlation between genetic diversity and population size or population stability at the local-sale in grassland over the four-year experiment.

The first study focused on cultivated species was carried out by Ruan et al., (2004) on sea buckthorn, *Hippophae rhamnoides* L. employing RAPDs. They compared cultivars of Mongolia, China and Russia, and as a result, the highest genetic distance were found between Mongolian and Chinese cultivars, as they represent different subspecies. A study by Narantsetseg et al., (2017) addressed a cultivated crop species, namely the Mongolian wheat species, *Triticum aestivum* L., *T. compactum* Host, and *T. durum* Desf. with

5. Synthesis and discussion: Population genetics

a help of SSRs. Their result revealed a high level of genetic diversity in tested Mongolian germplasm, which has high importance for conservation of wheat genetic resources.

The above shows that investigations on plant population genetics in Mongolia are rather scarce, with only eleven studies to date. However, it's probable that more studies (e.g., thesis and dissertations) were undertaken and published in Mongolian language, which are not archived in the electronic catalogues. Nevertheless, the number of studies has been increasing recently, as in line with developments in molecular markers. In the beginning, RAPDs were the most commonly used marker, but more recently, SSRs or ISSRs have been utilized frequently in the reviewed studies (Table 2). Considering taxa, several rare and endangered species, as well as common species, and their inter-specific comparisons were examined. In addition, common crop species germplasms were studied, which is of great importance for crop genetic resource.

All the studies, except Oyundelger et al., (2020), were solely focused on evaluating the levels of genetic diversity and structure of certain taxa. Population genetic studies of this dissertation, additionally aimed to assess the effects of grazing and climatic factors, which are crucial for understanding population genetic variation in a changing climate and steadily increasing grazing pressure in Mongolia. In particular, both studies resulted that grazing impact is not detrimental in *A. frigida*, in contrast to *Stipa glareosa* (Oyundelger et al., 2020). While, species' genetic structure was largely driven by local environment, providing insights into their main driving factors, and magnitude of influence. Grazing impacts tested with two different study designs, i.e., grazing intensity gradient and grazing exclusion, offered knowledge on potential direct and indirect influences of grazing on genetic diversity at local and regional scales (Fig. 3), as well as level of species tolerance to grazing, such as genetical adaptation and/or phenotypic adaptation.

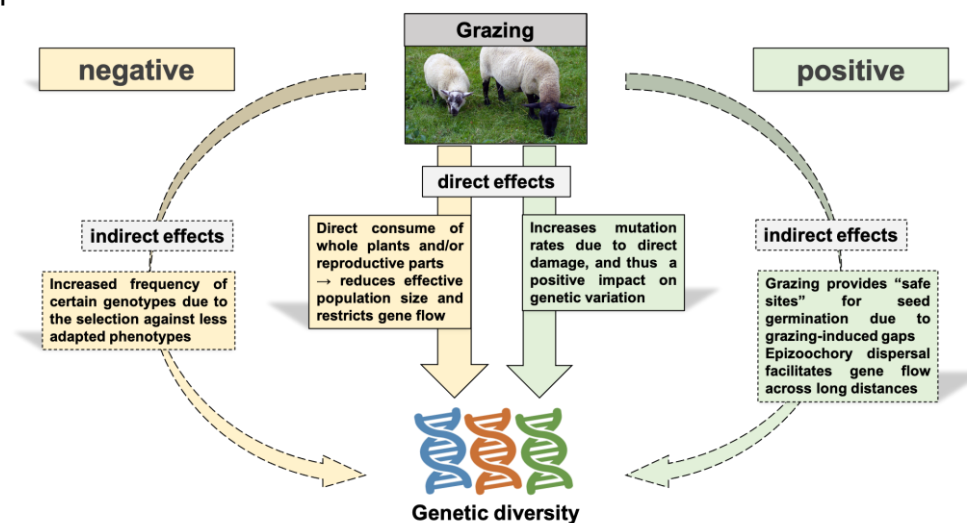


Figure 3. Illustration of potential effects of grazing on plant genetic diversity.

5. Synthesis and discussion: Population genetics

Table 2. A list of population genetic studies conducted in Mongolia. Studies are ordered according to the molecular markers used.

	Taxa	Markers	Publication	Link/ DOI
1	<i>Saussurea involucreta</i>	AFLPs	Dashzeveg et al., (2017)	https://doi.org/10.1007/s10722-016-0412-1
2	<i>Agropyron cristatum</i>	ISSRs	Ishii et al., (2021)	https://doi.org/10.1016/j.jaridenv.2021.104607
3	<i>Allium bidentatum</i>	ISSRs	Ishii et al., (2021)	
4	<i>Artemisia frigida</i>	ISSRs	Ishii et al., (2021)	
5	<i>Caragana leucophloea</i>	ISSRs	Ishii et al., (2021)	
6	<i>Carex duriuscula</i>	ISSRs	Ishii et al., (2021)	
7	<i>Convolvulus ammanii</i>	ISSRs	Ishii et al., (2021)	
8	<i>Cymbaria dahurica</i>	ISSRs	Ishii et al., (2021)	
9	<i>Kochia prostrata</i>	ISSRs	Ishii et al., (2021)	
10	<i>Stipa krylovii</i>	ISSRs	Ishii et al., (2021)	
11	<i>Gentiana algida</i>	ISSRs	Nomin-Erdene (2021)	
12	<i>Galitzkya potaninii</i>	RAPDs	Wesche et al., (2006b)	https://doi.org/10.1093/aob/mcl182
13	<i>Galitzkya macrocarpa</i>	RAPDs	Wesche et al., (2006b)	https://doi.org/10.1093/aob/mcl182
14	<i>Hippophae rhamnoides</i>	RAPDs	Ruan et al., (2004)	https://doi.org/10.1016/j.scienta.2003.11.013
15	<i>Potentilla ikonnikovii</i>	RAPDs	Wesche et al., (2006a)	https://doi.org/10.1111/j.1442-1984.2006.00161.x
16	<i>Stipa krylovii</i>	RAPDs	Ronnenberg (2011b)	PhD dissertation, http://dx.doi.org/10.25673/673
17	<i>Ulmus pumila</i>	RAPDs	Wesche et al., (2011)	https://doi.org/10.1016/j.flora.2010.01.012
18	<i>Artemisia frigida</i>	SSRS	Oyundelger et al., (2021)	https://doi.org/10.1007/s10592-021-01375-w
19	<i>Populus laurifolia</i>	SSRs	Wiehle et al., (2016)	https://doi.org/10.1016/j.flora.2016.07.004
20	<i>Stipa glareosa</i>	SSRs	Oyundelger et al., (2020)	https://doi.org/10.1016/j.flora.2020.151572
21	<i>Triticum aestivum</i>	SSRs	Narantsetseg et al., (2017)	https://doi.org/10.9787/PBB.2017.5.3.213
22	<i>Triticum compactum</i>	SSRs	Narantsetseg et al., (2017)	
23	<i>Triticum durum</i>	SSRs	Narantsetseg et al., (2017)	

5.4. Outlook

This thesis is one of the few investigations conducted in Mongolia that comprehends both species' evolutionary history (migration history, ancestral area genetical lineages, polyploidization events and populations' relatedness) at its distributional range, and the development status of current populations' genetics (genetic diversity and structure) at local and regional scales.

The study on phylogeography provides detailed insights into the evolutionary history of *A. frigida* and its connection to the history of the Eurasian steppe belt. However, we could not conduct molecular dating analysis due to a lack of fossil sample information for calibration; and the use of a single-locus cpDNA marker which did not provide good sequences to estimate mutation rate. Therefore, further study employing multiple-locus markers on molecular dating would provide specific information on age of the species, and time scale of migration as well as diversification. Furthermore, additional samples from the species' distributional range, particularly from the North American southern prairies, which remained ice-free during the last glacial period, could provide broader and interesting pictures of the genetic relatedness of populations and polyploidization events of *A. frigida* in the New World.

Regarding population genetics, a general lack of studies was observed in Mongolia. Hence, such studies focusing on any taxa, regardless of conservation status would be highly valuable. In particular, study on various species, i.e., differing by pollination methods (wind vs. insect pollinated) and differing by life history strategy (sensitivity vs. tolerance to stress and competition) at an inter-specific level would be of high interest, and important for ecosystem functioning as well as for grassland sustainable management. Referring to the marker system, we faced a challenge in inferring the link between functional traits and their relationships to genetic controls. Because neutral markers, such as SSRs, cannot detect adaptive genes (genes that influence species fitness), a definite conclusion on the relationship between plant functional traits and genetic diversity was not possible. Hence, for a study aiming at revealing adaptive genes (e.g., disease causing genes, genes tolerant to drought, disturbance or saline soil conditions etc.), the use of non-neutral markers would be more practical.

Acknowledgements

For the past four years of my doctorate, the path has not always been straight; it has gone up and down. Many people have supported and encouraged me along this challenging path, and thus, I must express my deep gratitude to all of them.

First of all, I would like to thank Prof. Dr. Karsten Wesche and PD Dr. Christiana M. Ritz, two of my supervisors who offered me a doctorate suggestion and helped me write a project proposal and receive a promotion grant. Not only did they help guide my doctoral study, but they were also very supportive psychologically and socially. Of course, my research would not have been possible without the scholarship program, so thank you to the Graduate Academy of Technische Universität Dresden. In addition, I would like to thank all my friends and colleagues in the Department of Botany, Senckenberg Museum of Natural History Görlitz, especially to Yun Jäschke, Jens Wesenberg, Jörg Lorenz, Veit Herklotz, Sebastian Buschmann, Zakieh Zakeri and Petra Gebauer, who always helped me, supported me when I fell, opened my brain when I was stuck, and made me feel like a family. Especially, Yun Jäschke, my big sister and best friend, who taught me how to survive in Germany and always helps me for all my private issues, wherever I was in for her cares and hugs at my sad and sick times, who makes bouze to comfort me as just like my family member. She is also my dear colleague and co-author, who always has some answer for any scientific question and statistics. Besides, without the help of Michaela Schwager and Bettina Schlitt, my lab work wouldn't have been complete. Special thanks to Herman Ansorge and Margit Hanelt for taking me to the summer school in Mongolia, which allowed my sampling and keeping me always connected to my homeland, by doing long-term "soft releasing". I am also appreciated to PD Dr. Dr. h.c. Martin Pfeiffer, who led my way to Germany. I am happy to get many international friends, whom I studied together and shared apartment with, which gave me valuable experience in intercultural fluency, teamwork, and overcoming language / cultural barriers. Furthermore, many thanks to my dear counselor friends and brothers and sisters, Sh.Baasanmunkh, O.Munkhzul, D.Usukhjargal and Ts.Tserendulam. I also thank my esteemed teacher Prof. B. Oyuntsetseg for her supports.

I would like to thank Dr. Dörte Harpke and laboratory employees of the IPK as well as BiK-F laboratory center in Frankfurt. I am also grateful to Jan Treiber and Julian Ahlborn for their support with beautiful maps. I have to thank Prof. Irene Ring for her kind supports, employment since my master study and valuable suggestions she always gave me.

Last but not the least, I own most and forever to my mother and two brothers, for their unconditional love and trust, which helped me to be strong and independent. Special to thank my partner Steffen, who is always there for me when I am down and sick, and encourages me with his great suggestions.

References

- Abraham, E.M., Aftzalanidou, A., Ganopoulos, I., Osathanukul, M., Xanthopoulou, A., Avramidou, E., Sarrou, E., Aravanopoulos, F. & Madesis, P. (2018) Genetic diversity of *Thymus sibthorpii* Bentham in mountainous natural grasslands of Northern Greece as related to local factors and plant community structure. *Industrial Crops and Products* 111: 651–659.
- Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International* 11: 36–42.
- Addison, J., Friedel, M., Brown, C., Davies, J. & Waldron, S. (2012) A critical review of degradation assumptions applied to Mongolia's Gobi Desert. *The Rangeland Journal* 34: 125–137.
- Afrin, H. (2017) A review on different types soil stabilization techniques. *International Journal of Transportation Engineering and Technology* 3: 19–24.
- Ahlborn, J., von Wehrden, H., Lang, B., Römermann, C., Oyunbileg, M., Oyuntsetseg, B. & Wesche, K. (2020) Climate–grazing interactions in Mongolian rangelands: Effects of grazing change along a large-scale environmental gradient. *Journal of Arid Environments* 173: 104043. <https://doi.org/10.1016/j.jaridenv.2019.104043>
- Ahrens, C.W., Andrew, M.E., Mazanec, R.A., Ruthrof, K.X., Challis, A., Hardy, G., Byrne, M., Tissue, D.T. & Rymer, P.D. (2020) Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. *Ecology and Evolution* 10: 232–248.
- Albayrak, T., Gonzalez, J., Drovetski, S. V & Wink, M. (2012) Phylogeography and population structure of Krüper's Nuthatch *Sitta krueperi* from Turkey based on microsatellites and mitochondrial DNA. *Journal of Ornithology* 153: 405–411.
- Andrews, J.A. (1985) True polar wander: an analysis of Cenozoic and Mesozoic paleomagnetic poles. *Journal of Geophysical Research: Solid Earth* 90: 7737–7750.
- Andrews, S. (2010) FastQC: a quality control tool for high throughput sequence data.
- Angerer, J., Han, G., Fujisaki, I. & Havstad, K. (2008) Climate change and ecosystems of Asia with emphasis on Inner Mongolia and Mongolia. *Rangelands* 30: 46–51.
- Baasanmunkh, S., Urgamal, M., Oyuntsetseg, B., Sukhorukov, A.P., Tsegmed, Z., Son, D.C., Erst, A., Oyundelger, K., Kechaykin, A.A. & Norris, J. (2022) Flora of Mongolia: annotated checklist of native vascular plants. *PhytoKeys* 192: 63–169.
- Badr, A., El-Shazly, H.H., Helail, N.S. & El Ghanim, W. (2012) Genetic diversity of *Artemisia* populations in central and north Saudi Arabia based on morphological variation and RAPD polymorphism. *Plant Systematics and Evolution* 298: 871–886.
- Bai, Y., Wu, J., Clark, C.M., Pan, Q., Zhang, L., Chen, S., Wang, Q. & Han, X. (2012) Grazing alters ecosystem functioning and C: N: P stoichiometry of grasslands along a regional precipitation gradient. *Journal of Applied Ecology* 49: 1204–1215.
- Bai, Y.G. & Romo, J.T. (1996) Fringed sagebrush response to sward disturbances: Seedling dynamics and plant growth. *Journal of Range Management* 49: 228–233.
- Bai, Y.G., Romo, J.T. & Young, J.A. (1995) Influence of temperature, light and water stress on germination of fringed sage (*Artemisia frigida*). *Weed Science* 43: 219–225.
- Banzragch, D. & Chognii, O. (1975) Productivity change of herbs feathergrass steppe in preservation regime. *Proceeding of Institute of Botany* 1: 63–69.
- Barabanov, A.T., Dolgov, S. V & Koronkevich, N.I. (2018) Effect of present-day climate changes and agricultural activities on spring overland runoff in forest-

-
- steppe and steppe Regions of the Russian plain. *Water Resources* 45: 447–454.
- Baranova, A.N. (2018) Grazing impacts on vegetation patterns in the Qilian Mountains, HeiHe River Basin, NW China. Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky
- Barbolini, N., Woutersen, A., Dupont-Nivet, G., Silvestro, D., Tardif, D., Meijer, N., Chang, C., Zhang, H.-X., Licht, A. & Rydin, C. (2020) Cenozoic evolution of the steppe-desert biome in Central Asia. *Science Advances* 6: eabb8227.
- Bat-Oyun, T., Shinoda, M., Cheng, Y. & Purevdorj, Y. (2016) Effects of grazing and precipitation variability on vegetation dynamics in a Mongolian dry steppe. *Journal of Plant Ecology* 9: 508–519.
- Batchelor, C.L., Margold, M., Krapp, M., Murton, D.K., Dalton, A.S., Gibbard, P.L., Stokes, C.R., Murton, J.B. & Manica, A. (2019) The configuration of Northern Hemisphere ice sheets through the Quaternary. *Nature Communications* 10: 1–10.
- Bates, D., Sarkar, D., Bates, M.D. & Matrix, L. (2007) Fitting Linear Mixed-Effects Models Using “lme4.” *Journal of Statistical Software* 2: 74.
- Batsaikhan, N., Buuveibaatar, B., Chimed, B., Enkhtuya, O., Galbrakh, D., Ganbaatar, O., Lkhagvasuren, B., Nandintsetseg, D., Berger, J., Calabrese, J.M., Edwards, A.E., Fagan, W.F., Fuller, T.K., Heiner, M., Ito, T.Y., Kaczensky, P., Leimgruber, P., Lushchekina, A., Milner-Gulland, E.J., Mueller, T., Murray, M.G., Olson, K.A., Reading, R., Schaller, G.B., Stubbe, A., Stubbe, M., Walzer, C., Von Wehrden, H. & Whitten, T. (2014) Conserving the World’s finest grassland amidst ambitious national development. *Conservation Biology* 28: 1736–1739. <https://doi.org/10.1111/cobi.12297>
- Bazha, S.N., Gunin, P.D., Danzhalova, E. V, Drobyshev, Y.I. & Prishcepa, A. V (2012) Pastoral degradation of steppe ecosystems in Central Mongolia. In: M. J. A. Werger & M. A. van Staalduinen (eds.) *Eurasian Steppes. Ecological problems and livelihoods in a changing world*. Springer, Dordrecht, pp. 289–319.
- Beheregaray, L.B. (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology* 17: 3754–3774.
- Benham, J.J. (2001) Genographer. <http://www.genographer.com/>
- Bermingham, E. & Moritz, C. (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology* 7: 367–369.
- Besnier, F. & Glover, K.A. (2013) ParallelStructure: A R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. *PLoS One* 8: e70651.
- Binney, H., Edwards, M., Macias-Fauria, M., Lozhkin, A., Anderson, P., Kaplan, J.O., Andreev, A., Bezrukova, E., Blyakharchuk, T. & Jankovska, V. (2017) Vegetation of Eurasia from the last glacial maximum to present: Key biogeographic patterns. *Quaternary Science Reviews* 157: 80–97.
- Blair, J., Nippert, J. & Briggs, J. (2014) Grassland Ecology. In: R. K. Monson (ed.) *Ecology and the Environment*. Springer, New York, pp. 389–423.
- Bläß, C., Ronnenberg, K., Tackenberg, O., Hensen, I. & Wesche, K. (2010) The relative importance of different seed dispersal modes in dry Mongolian rangelands. *Journal of arid environments* 74: 991–997.
- Blattner, F.R. (2006) Multiple intercontinental dispersals shaped the distribution area of *Hordeum* (Poaceae). *New Phytologist* 169: 603–614.
- Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
- Borchardt, P., Oldeland, J., Ponsens, J. & Schickhoff, U. (2013) Plant functional traits match grazing gradient and vegetation patterns on mountain pastures in SW Kyrgyzstan. *Phytocoenologia* 43: 171–181.
- Bougoutaia, Y., Garnatje, T., Vallès, J., Kaid-Harche, M., Ouhammou, A., Dahia, M.,

-
- Tlili, A. & Vitales, D. (2021) Phylogeographical and cytogeographical history of *Artemisia herba-alba* (Asteraceae) in the Iberian Peninsula and North Africa: mirrored intricate patterns on both sides of the Mediterranean Sea. *Botanical Journal of the Linnean Society* 195: 588–605.
- Broccoli, A.J. & Manabe, S. (1997) Mountains and midaltitude aridity. In: W. F. Ruddiman (ed.) *Tectonic uplift and climate change*. Springer, Boston, MA, pp. 89–121.
- Brochmann, C., Brysting, A.K., Alsos, I.G., Borgen, L., Grundt, H.H., Scheen, A.C. & Elven, R. (2004) Polyploidy in arctic plants. *Biological journal of the Linnean Society* 82: 521–536.
- Brougham, R.W. & Harris, W. (1967) Rapidity and extent of changes in genotypic structure induced by grazing in a ryegrass population. *New Zealand Journal of Agricultural Research* 10: 56–65.
- Brouillet, L. & Whetstone, R.D. (2000) Climate and physiography of North America. *Flora of North American Editorial Committee. Flora of North America*. Oxford Univ. Press, Oxford, U. K, pp. 1–23.
- Bruvo, R., Michiels, N.K., D'Souza, T.G. & Schulenburg, H. (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13: 2101–2106.
- Buono, D., Khan, G., Von Hagen, K.B., Kosachev, P.A., Mayland-Quellhorst, E., Mosyakin, S.L. & Albach, D.C. (2021) Comparative phylogeography of *Veronica spicata* and *V. longifolia* (Plantaginaceae) across Europe: Integrating hybridization and polyploidy in phylogeography. *Frontiers in Plant Science*: 1993.
- Buzurović, U., Tomović, G., Niketić, M., Bogdanović, S. & Aleksić, J.M. (2020) Phylogeographic and taxonomic considerations on *Goniolimon tataricum* (Plumbaginaceae) and its relatives from south-eastern Europe and the Apennine Peninsula. *Plant Systematics and Evolution* 306: 1–22.
- De Caceres, M. & Jansen, F. (2014) Package “indicspecies”: Studying the statistical relationship between species and group of sites. 2019. Available from: <https://cran.r-project.org/web/packages/indicspecies/indicspecies.pdf>
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J. & Bealer, K. (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10: 1–9.
- Carlson, S.E., Linder, H.P. & Donoghue, M.J. (2012) The historical biogeography of *Scabiosa* (Dipsacaceae): implications for Old World plant disjunctions. *Journal of Biogeography* 39: 1086–1100.
- Caruso, C.M., Mason, C.M. & Medeiros, J.S. (2020) The evolution of functional traits in plants: is the giant still sleeping? *International Journal of Plant Sciences* 181: 1–8.
- Caves, J.K., Bayshashov, B.U., Zhamangara, A., Ritch, A.J., Ibarra, D.E., Sjostrom, D.J., Mix, H.T., Winnick, M.J. & Chamberlain, C.P. (2017) Late Miocene uplift of the Tian Shan and Altai and reorganization of Central Asia climate. *GSA Today* 27: 19–26.
- Chaney, L., Richardson, B.A. & Germino, M.J. (2017) Climate drives adaptive genetic responses associated with survival in big sagebrush (*Artemisia tridentata*). *Evolutionary Applications* 10: 313–322.
- Cheng, Y., Tsubo, M., Ito, T.Y., Nishihara, E. & Shinoda, M. (2011) Impact of rainfall variability and grazing pressure on plant diversity in Mongolian grasslands. *Journal of Arid Environments* 75: 471–476.
- Chuluunkhuyag, O., Chaoyan, L., Treiber, J., Batlai, O., von Wehrden, H., Feller, R. & Wesche, K. (2021) Influence of altitude and longitude on vegetation in the Dzungarian Gobi and the south-western Mongolian Altai. *Phytocoenologia* 50: 339–369.
- Cieślak, E. & Drobniak, S. (2019) Phylogeography of xerothermic *Carlina acanthifolia* subsp. *utzka* in Central Europe. *Flora* 253: 76–86.

-
- Cingolani, A.M., Noy-Meir, I. manuel & Díaz, S. (2005a) Grazing effects on rangeland diversity: a synthesis of contemporary models. *Ecological Applications* 15: 757–773. <https://doi.org/10.1890/03-5272>
- Cingolani, A.M., Posse, G. & Collantes, M.B. (2005b) Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *Journal of Applied Ecology* 42: 50–59.
- Clark, L. V & Jasieniuk, M. (2011) POLYSAT: an R package for polyploid microsatellite analysis. *Molecular Ecology Resources* 11: 562–566.
- Clement, M., Snell, Q., Walker, P., Posada, D. & Crandall, K. (2002) TCS: estimating gene genealogies. *Parallel and Distributed Processing Symposium, International*. IEEE Computer Society, pp. 184–190.
- Combe, F.J., Sikes, D.S., Tkach, V. V & Hope, A.G. (2021) Origins and diversity of the Bering Sea Island fauna: shifting linkages across the northern continents. *Biodiversity and Conservation* 30: 1205–1232.
- Comes, H.P. & Kadereit, J.W. (1998) The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3: 432–438.
- Conroy, C.J. & Cook, J.A. (2000) Molecular systematics of a Holarctic rodent (Microtus: Muridae). *Journal of Mammalogy* 81: 344–359.
- Coupland, R.T. (1950) Ecology of mixed prairie in Canada. *Ecological Monographs* 20: 271–315.
- Dagvadorj, D., Dorjpurev, J. & Namkhainyam, B. (2010) *Climate change mitigation (Requirements and opportunities to reduce greenhouse gas emissions)*. Ulaanbaatar. 87 pp.
- Dai, A. (2011) Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* 2: 45–65.
- Damiran, D. (2005) *Palatability of mongolian rangeland plants*. Eastern Oregon Agricultural Research Center, Oregon State University., Union, OR, USA. 91 pp.
- Dashzeveg, N., Buerkert, A. & Wiehle, M. (2017) Morphological and genetic diversity and seed germination behavior of a snow lotus (*Saussurea involucreta*, Asteraceae) from the Mongolian Altay Mountains, Western Mongolia. *Genetic Resources and Crop Evolution* 64: 927–934.
- Deng, L., Sweeney, S. & Shangguan, Z.P. (2013) Grassland responses to grazing disturbance: plant diversity changes with grazing intensity in a desert steppe. *Grass and Forage Science* 69: 524–533.
- Densambuu, B., Sainnemekh, S., Bestelmeyer, B. & Budbaatar, U. (2018a) *National report on the rangeland health of Mongolia: Second Assessment. Green Gold-Animal health project*. Ulaanbaatar
- Densambuu, B., Sainnemekh, S., Bestelmeyer, B. & Budbaatar, U. (2018b) *National Report on the Rangeland Health on Mongolia*. Ulaanbaatar
- Deorowicz, S., Debudaj-Grabysz, A. & Grabowski, S. (2013) Disk-based k-mer counting on a PC. *BMC bioinformatics* 14: 1–12.
- Diaz, S., Lavorel, S., McIntyre, S.U.E., Falczuk, V., Casanoves, F., Milchunas, D.G., Skarpe, C., Rusch, G., Sternberg, M. & NOY-MEIR, I. (2007) Plant trait responses to grazing—a global synthesis. *Global Change Biology* 13: 313–341.
- Díaz, S., Noy-Meir, I. & Cabido, M. (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *Journal of Applied Ecology* 38: 497–508.
- Dobeš, C. & Paule, J. (2010) A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): implications for its geographic origin, phylogeography and generic circumscription. *Molecular Phylogenetics and Evolution* 56: 156–175.
- Donoghue, M.J., Bell, C.D. & Li, J. (2001) Phylogenetic patterns in Northern Hemisphere plant geography. *International Journal of Plant Sciences* 162: 41–52.
- Donoghue, M.J. & Smith, S.A. (2004) Patterns in the assembly of temperate forests

-
- around the Northern Hemisphere. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 1633–1644.
- Downing, D.J. & Pettapiece, W.W. (2006) Natural regions and subregions of Alberta, Natural Regions Committee. *Alberta: Government of Alberta*.
- Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
- Dress, A.W.M. & Huson, D.H. (2004) Constructing splits graphs. *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 1: 109–115.
- Dufresne, F., Stift, M., Vergilino, R. & Mable, B.K. (2014) Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. *Molecular Ecology* 23: 40–69.
- Earl, D.A. & VonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.
- Eaton, D.A.R. (2014) Pyrad: assembly of *de novo* RADseq loci for phylogenetic analyses. *Bioinformatics* 30: 1844–1849.
- eFloras (2008) eFloras., 2008. *Flora of China*. Available from: http://www.efloras.org/flora_page.aspx?flora_id=2 (October 1, 2021)
- Ehlers, J. & Gibbard, P. (2008) Extent and chronology of Quaternary glaciation. *Episodes* 31: 211–218.
- Elias, S.A. & Crocker, B. (2008) The Bering Land Bridge: a moisture barrier to the dispersal of steppe–tundra biota? *Quaternary Science Reviews* 27: 2473–2483.
- Ellis, J.E. & Chuluun, T. (1993) Cross-country survey of climate, ecology, and land use among Mongolian pastoralists. *Paper prepared for the Conference on Grassland Ecosystems of the Mongolian Steppe*.
- Ellis, J.E. & Swift, D.M. (1988) Stability of African pastoral ecosystems: Alternate paradigms and implications for development. *Journal of Range Management* 41: 450–459.
- Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. & Mitchell, S.E. (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6: e19379.
- Escudero, M., Valcárcel, V., Vargas, P. & Luceño, M. (2010) Bipolar disjunctions in *Carex*: long-distance dispersal, vicariance, or parallel evolution? *Flora* 205: 118–127.
- Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620.
- Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- Fatichi, S. & Ivanov, V.Y. (2014) Interannual variability of evapotranspiration and vegetation productivity. *Water Resources Research* 50: 3275–3294.
- Faville, M.J., Crush, J.R., Hong, W., Phillips, H., Lee, J.M. & Chapman, D. (2020) Effects of pasture age on the genotype and phenotype of perennial ryegrass. *Grass and Forage Science* 75: 135–144.
- Feagley, S.E. & Fenn, L.B. (1998) Using soluble calcium to stimulate plant growth. *Texas Farmer Collection L-5212*: 1–4.
- Fernandez-Gimenez, M. & Allen-Diaz, B. (2001) Vegetation change along gradients from water sources in three grazed Mongolian ecosystems. *Plant Ecology* 157: 101–118.
- Fernandez-Gimenez, M.E. & Allen-Diaz, B. (1999) Testing a non-equilibrium model of rangeland vegetation dynamics in Mongolia. *Journal of Applied Ecology* 36: 871–885.
- Fernández-Mazuecos, M., Mellers, G., Vigalondo, B., Sáez, L., Vargas, P. & Glover, B.J. (2018) Resolving recent plant radiations: power and robustness of

-
- genotyping-by-sequencing. *Systematic Biology* 67: 250–268.
- Fernández-Giménez, M.E. & Allen-Díaz, B. (1999) Testing a non-equilibrium model of rangeland vegetation dynamics in Mongolia. *Journal of Applied Ecology* 36: 871–885.
- Feurdean, A. & Vasiliev, I. (2019) The contribution of fire to the late Miocene spread of grasslands in eastern Eurasia (Black Sea region). *Scientific Reports* 9: 1–7.
- Fick, S.E. & Hijmans, R.J. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37: 4302–4315.
- Frajman, B., Závěská, E., Gamisch, A., Moser, T., Schönswetter, P. & Consortium, S. (2019) Integrating phylogenomics, phylogenetics, morphometrics, relative genome size and ecological niche modelling disentangles the diversification of Eurasian *Euphorbia seguieriana* s.l. (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 134: 238–252.
- François, O. (2016) Running structure-like population genetic analyses with R. *R tutorials in population genetics*: 1–9.
- Frank, D.A. (2005) The interactive effects of grazing ungulates and aboveground production on grassland diversity. *Oecologia* 143: 629–634.
- Franzke, A., Hurka, H., Janssen, D., Neuffer, B., Friesen, N., Markov, M. & Mummenhoff, K. (2004) Molecular signals for Late Tertiary/Early Quaternary range splits of an Eurasian steppe plant: *Clausia aprica* (Brassicaceae). *Molecular Ecology* 13: 2789–2795.
- Friesen, N., German, D.A., Hurka, H., Herden, T., Oyuntsetseg, B. & Neuffer, B. (2016) Dated phylogenies and historical biogeography of *Dontostemon* and *Clausia* (Brassicaceae) mirror the palaeogeographical history of the Eurasian steppe. *Journal of Biogeography* 43: 738–749.
- Friesen, N., Smirnov, S. V., Leweke, M., Seregin, A.P. & Fritsch, R.M. (2021) Taxonomy and phylogenetics of *Allium* section *Decipientia* (Amaryllidaceae): morphological characters do not reflect the evolutionary history revealed by molecular markers. *Botanical Journal of the Linnean Society* 197: 190–228.
- Friesen, N., Smirnov, S. V., Shmakov, A.I., Herden, T., Oyuntsetseg, B. & Hurka, H. (2020a) *Allium* species of section *Rhizomatosa*, early members of the Central Asian steppe vegetation. *Flora* 263: 151536.
- Friesen, N., Žerđoner Čalasan, A., Neuffer, B., German, D.A., Markov, M. & Hurka, H. (2020b) Evolutionary history of the Eurasian steppe plant *Schivereckia podolica* (Brassicaceae) and its close relatives. *Flora* 268: 151602.
- Fujita, N., Amartuvshin, N., Yamada, Y., Matsui, K., Sakai, S. & Yamamura, N. (2009) Positive and negative effects of livestock grazing on plant diversity of Mongolian nomadic pasturelands along a slope with soil moisture gradient. *Grassland Science*. <https://doi.org/10.1111/j.1744-697X.2009.00147.x>
- Fynn, R.W.S. & O'Connor, T.G. (2000) Effect of stocking rate and rainfall on rangeland dynamics and cattle performance in a semi-arid savanna, South Africa. *Journal of Applied Ecology* 37: 491–507.
- Gansukh, U., Ming, X. & Ali, S.A. (2018) Analysis of the current situation of Mongolian railway and its future development. *International Business Research* 11: 119–128.
- Gao, J. & Carmel, Y. (2020) A global meta-analysis of grazing effects on plant richness. *Agriculture, Ecosystems and Environment* 302: 107072.
- Gao, L.M., Möller, M., Zhang, X., Hollingsworth, M.L., Liu, J., Mill, R.R., Gibby, M. & Li, D. (2007) High variation and strong phylogeographic pattern among cpDNA haplotypes in *Taxus wallichiana* (Taxaceae) in China and North Vietnam. *Molecular Ecology* 16: 4684–4698.
- Gao, W., Angerer, J.P., Fernandez-Gimenez, M.E. & Reid, R.S. (2015) Is overgrazing a pervasive problem across Mongolia? An examination of livestock forage demand and forage availability from 2000 to 2014. *Proceedings of the*

-
- Trans-disciplinary Research Conference: Building Resilience of Mongolian Rangelands*, Ulaanbaatar Mongolia, pp. 35–41.
- García-Fernández, A., Manzano, P., Seoane, J., Azcárate, F.M., Iriondo, J.M. & Peco, B. (2019) Herbivore corridors sustain genetic footprint in plant populations: a case for Spanish drove roads. *PeerJ* 7: e7311.
- García-Fernández, A., Vitales, D., Pellicer, J., Garnatje, T. & Vallès, J. (2017) Phylogeographic insights into *Artemisiacanthifolia* (Asteraceae) reveal several areas of the Iberian Atlantic coast as refugia for genetic diversity. *Plant Systematics and Evolution* 303: 509–519.
- García, S., McArthur, E.D., Pellicer, J., Sanderson, S.C., Vallès, J. & Garnatje, T. (2011) A molecular phylogenetic approach to western North America endemic *Artemisia* and allies (Asteraceae): Untangling the sagebrushes. *American Journal of Botany* 98: 638–653.
- García, S., Sanz, M., Garnatje, T., Kreitschitz, A., McArthur, E.D. & Vallès, J. (2004) Variation of DNA amount in 47 populations of the subtribe Artemisiinae and related taxa (Asteraceae, Anthemideae): karyological, ecological, and systematic implications. *Genome* 47: 1004–1014.
- Geber, M.A. & Griffen, L.R. (2003) Inheritance and natural selection on functional traits. *International Journal of Plant Sciences* 164: S21–S42.
- German Aerospace Center (DLR) (2018) TanDEM-X - Digital Elevation Model (DEM) - Global, 90m.
- Gompert, Z. & Mock, K.E. (2017) Detection of individual ploidy levels with genotyping-by-sequencing (GBS) analysis. *Molecular Ecology Resources* 17: 1156–1167.
- Graham, A. (1972) Outline of the origin and historical recognition of floristic affinities between Asia and eastern North America. In: A. Graham (ed.) *Floristics and Paleoflorists of Asia and Eastern North America*. Elsevier, Amsterdam, pp. 1–18.
- Graham, A. (1993) History of the vegetation: Cretaceous (Maastrichtian)–Tertiary. *North America Editorial Committee, Flora of North America*. Oxford University Press, New York, pp. 55–70.
- Grant, P.R. & Grant, B.R. (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296: 707–711.
- Gray, A. (1840) Dr. Siebold, flora japonica (review). *American Journal of Science and Arts* 39: 175–176.
- Gray, A. (1846) Analogy between the flora of Japan and that of the United States. *American Journal of Science and Arts* 2: 175–176.
- Greenville, A.C., Dickman, C.R. & Wardle, G.M. (2017) 75 years of dryland science: Trends and gaps in arid ecology literature. *PLoS ONE* 12: e0175014.
- Guijarro, J.A. & Guijarro, M.J.A. (2019) An R package “climatol.”
- Halbritter, A.H., Fior, S., Keller, I., Billeter, R., Edwards, P.J., Holderegger, R., Karrenberg, S., Pluess, A.R., Widmer, A. & Alexander, J.M. (2018) Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology* 31: 784–800.
- Hamming, R.W. (1950) Error detecting and error correcting codes. *The Bell System Technical Journal* 29: 147–160.
- Han, J., Dai, H. & Gu, Z. (2021) Sandstorms and desertification in Mongolia, an example of future climate events: A review. *Environmental Chemistry Letters* 19: 4063–4073.
- Hao, Y. & He, Z. (2019) Effects of grazing patterns on grassland biomass and soil environments in China: A meta-analysis. *PLoS one* 14: e0215223.
- Harris, I., Jones, P.D., Osborn, T.J. & Lister, D.H. (2014) Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology* 34: 623–642.
- Harris, S.A. (2007) Biodiversity of the Alpine Vascular Flora of the NW North

-
- American Cordillera: The Evidence from Phyto-Geography. *Erdkunde* 61: 344–356.
- Harvey, S.J. (1981) Life history and reproductive strategies in *Artemisia*. Montana State University
- Henwood, W.D. (2010) Toward a strategy for the conservation and protection of the world's temperate grasslands. *Great Plains Research* 20: 121–134.
- Hepler, P.K. (2005) Calcium: a central regulator of plant growth and development. *The Plant Cell* 17: 2142–2155.
- Herrero-Jáuregui, C. & Oesterheld, M. (2018) Effects of grazing intensity on plant richness and diversity: a meta-analysis. *Oikos* 127: 757–766.
- Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.
- Hilbig, W. (2007) The vegetation zones of Mongolia and their key plant communities. *Verhandlungen der Zoologisch Botanischen Gesellschaft in Oesterreich* 144: 119–163.
- Hobbs, C.R. & Baldwin, B.G. (2013) Asian origin and upslope migration of Hawaiian *Artemisia* (Compositae–Anthemideae). *Journal of Biogeography* 40: 442–454.
- Hodel, R.G.J., Segovia-Salcedo, M.C., Landis, J.B., Crowl, A.A., Sun, M., Liu, X., Gitzendanner, M.A., Douglas, N.A., Germain-Aubrey, C.C. & Chen, S. (2016) The report of my death was an exaggeration: a review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* 4: 1600025.
- Hoffman, J.I. & Amos, W. (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Molecular Ecology* 14: 599–612.
- Hoshino, A., Yoshihara, Y., Sasaki, T., Okayasu, T., Jamsran, U., Okuro, T. & Takeuchi, K. (2009) Comparison of vegetation changes along grazing gradients with different numbers of livestock. *Journal of Arid Environments* 73: 687–690.
- Huang, W., Zhao, X., Zhao, X., Li, Y., Lian, J. & Yun, J. (2014) Relationship between the genetic diversity of *Artemisia halodendron* and climatic factors. *Acta Oecologica* 55: 97–103.
- Hughes, P.D. & Gibbard, P.L. (2015) A stratigraphical basis for the Last Glacial Maximum (LGM). *Quaternary International* 383: 174–185.
- Hui, A.N. & Guoqi, L.I. (2014) Differential effects of grazing on plant functional traits in the desert grassland. *Polish Journal of Ecology* 62: 239–251.
- Hultén, E. (1968) *Flora of Alaska and neighboring territories: a manual of the vascular plants*. Stanford University Press, Stanford
- Hurka, H., Friesen, N., Bernhardt, K.-G., Neuffer, B., Smirnov, S., Shmakov, A. & Blattner, F. (2019) The Eurasian steppe belt: Status quo, origin and evolutionary history. *Turczaninowia* 22: 5–71.
- Hurka, H., Friesen, N., German, D.A., Franzke, A. & Neuffer, B. (2012) 'Missing link' species *Capsella orientalis* and *Capsella thracica* elucidate evolution of model plant genus *Capsella* (Brassicaceae). *Molecular Ecology* 21: 1223–1238.
- Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.
- Hussain, A., Potter, D., Kim, S., Hayat, M.Q. & Bokhari, S.A.I. (2019) Molecular phylogeny of *Artemisia* (Asteraceae–Anthemideae) with emphasis on undescribed taxa from Gilgit-Baltistan (Pakistan) based on nrDNA (ITS and ETS) and cpDNA (psbA-trnH) sequences. *Plant Ecology and Evolution* 152: 507–520.
- Ickert-Bond, S.M., Murray, D.F. & DeChaine, E. (2009) Contrasting patterns of plant distribution in Beringia. *Alaska Park Science* 8: 26–32.
- Ishii, N.I., Takahashi, M., Yoshihara, Y., Dashzeveg, N., Ayush, T., Suyama, Y. &

-
- Sasaki, T. (2021) Genetic diversity, population size, and population stability of common plant species in a Mongolian grassland. *Journal of Arid Environments* 194: 104607.
- Ivanov, D., Utescher, T., Mosbrugger, V., Syabryaj, S., Djordjević-Milutinović, D. & Molchanoff, S. (2011) Miocene vegetation and climate dynamics in Eastern and Central Paratethys (Southeastern Europe). *Palaeogeography, Palaeoclimatology, Palaeoecology* 304: 262–275.
- Jackson, S.T. & Overpeck, J.T. (2000) Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology* 26: 194–220.
- Jamiyansharav, K., Fernández-Giménez, M.E., Angerer, J.P., Yadamsuren, B. & Dash, Z. (2018) Plant community change in three Mongolian steppe ecosystems 1994-2013: Applications to state-and-transition models. *Ecosphere* 9: e02145.
- Jamnadas, R., Mace, E.S., Hiernaux, P., Muchugi, A. & Hanson, J. (2006) Population genetic responses of wild forage species to grazing along a rainfall gradient in the Sahel: A study combining phenotypic and molecular analyses. *Euphytica* 151: 431–445.
- Jamsranjav, C., Reid, R.S., Fernández-Giménez, M.E., Tsevelee, A., Yadamsuren, B. & Heiner, M. (2018) Applying a dryland degradation framework for rangelands: the case of Mongolia. *Ecological Applications* 28: 622–642.
- Jäschke, Y., Heberling, G. & Wesche, K. (2020) Environmental controls override grazing effects on plant functional traits in Tibetan rangelands. *Functional Ecology* 34: 747–760.
- Jiang, D., Klaus, S., Zhang, Y.-P., Hillis, D.M. & Li, J.-T. (2019a) Asymmetric biotic interchange across the Bering land bridge between Eurasia and North America. *National Science Review* 6: 739–745.
- Jiang, S., Luo, M.-X., Gao, R.-H., Zhang, W., Yang, Y.-Z., Li, Y.-J. & Liao, P.-C. (2019b) Isolation-by-environment as a driver of genetic differentiation among populations of the only broad-leaved evergreen shrub *Ammopiptanthus mongolicus* in Asian temperate deserts. *Scientific Reports* 9: 1–14.
- Jigjidsuren, S., Lkhagvajav, N., Bulgamaa, D., Otgontuya, L., Bolormaa, B., Budbaatar, U., Burmaa, D., Sumjidmaa, D. & Ankhtsetseg, B. (2015) *Handbook of key functional species of pastureland in Mongolia*. S. Jigjidsuren & D. Bulgamaa (eds.). TP Publisher, Ulaanbaatar, Mongolia. 148 pp.
- Jing, Z., Yu, J. & Cheng, J. (2013) Genetic diversity of a dominant species *Stipa bungeana* and its conservation strategy in the Loess Plateau of China. *Biochemical Systematics and Ecology* 47: 126–131.
- Jinhua, L., Zhenqing, L. & Jizhou, R. (2005) Effect of grazing intensity on clonal morphological plasticity and biomass allocation patterns of *Artemisia frigida* and *Potentilla acaulis* in the Inner Mongolia steppe. *New Zealand Journal of Agricultural Research* 48: 57–61.
- Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.
- Jones, A. (2000) Effects of cattle grazing on North American arid ecosystems: a quantitative review. *Western North American Naturalist* 60: 155–164.
- Kalia, R.K., Rai, M.K., Kalia, S., Singh, R. & Dhawan, A.K. (2011) Microsatellite markers: an overview of the recent progress in plants. *Euphytica* 177: 309–334.
- Kamvar, Z.N., Tabima, J.F. & Grünwald, N.J. (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2: e281.
- Karbstein, K., Prinz, K., Hellwig, F. & Römermann, C. (2020) Plant intraspecific functional trait variation is related to within-habitat heterogeneity and genetic diversity in *Trifolium montanum* L. *Ecology and Evolution* 10: 5015–5033.
- Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P. & Kessler, M. (2017) Climatologies at high

-
- resolution for the earth's land surface areas. *Scientific Data* 4: 170122.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.
- Khishigbayar, J., Fernández-Giménez, M.E., Angerer, J.P., Reid, R.S., Chantsalkham, J., Baasandorj, Y. & Zumberelmaa, D. (2015) Mongolian rangelands at a tipping point? Biomass and cover are stable but composition shifts and richness declines after 20 years of grazing and increasing temperatures. *Journal of Arid Environments* 115: 100–112.
- Kimura, M. (2020) Introduction to population genetics. *My Thoughts on Biological Evolution*. Springer, Singapore, pp. 85–101.
- Kirschner, P., Závieská, E., Gamisch, A., Hilpold, A., Trucchi, E., Paun, O., Sanmartín, I., Schlick-Steiner, B.C., Frajman, B. & Arthofer, W. (2020) Long-term isolation of European steppe outposts boosts the biome's conservation value. *Nature Communications* 11: 1–10.
- Klassen, R.W. (1994) Late Wisconsinan and Holocene history of southwestern Saskatchewan. *Canadian Journal of Earth Sciences* 31: 1822–1837.
- Klimesova, J., Latzel, V., de Bello, F. & van Groenendael, J.M. (2008) Plant functional traits in studies of vegetation changes in response to grazing and mowing: towards a use of more specific traits. *Preslia* 80: 245–253.
- Knaus, B.J. & Grünwald, N.J. (2017) vcfr: a package to manipulate and visualize variant call format data in R. *Molecular Ecology Resources* 17: 44–53.
- Korobkov, A.A. & Kotseruba, V. V (2015) Chromosome numbers of some *Artemisia* species (Asteraceae) in European Russia – Chisla chromosom nekotorykh vidov roda *Artemisia* (Asteraceae) Evropeyskoy Rossii. [in Russian with abstr. in English]. *Botanicheskii zhurnal* 100: 1189–1217.
- Korobkov, A.A., Kotseruba, V. V & Probatova, N.S. (2014) Chromosome numbers of some species of *Artemisia* L. from Altai region, South Siberia. *Botanica Pacifica: Journal of Plant Science and Conservation* 3: 61–66.
- Krasnov, B.R., Shenbrot, G.I. & Khokhlova, I.S. (2015) Historical biogeography of fleas: the former Bering Land Bridge and phylogenetic dissimilarity between the Nearctic and Palearctic assemblages. *Parasitology Research* 114: 1677–1686.
- Lang, B., Ahlborn, J., Oyunbileg, M., Geiger, A., von Wehrden, H., Wesche, K., Oyuntsetseg, B. & Römermann, C. (2020) Grazing effects on intraspecific trait variability vary with changing precipitation patterns in Mongolian rangelands. *Ecology and Evolution* 10: 678–691.
- Lang, B., Geiger, A., Oyunbileg, M., Ahlborn, J., von Wehrden, H., Wesche, K., Oyuntsetseg, B. & Römermann, C. (2018) Intraspecific trait variation patterns along a precipitation gradient in Mongolian rangelands. *Flora* 254: 135–146.
- Langmead, B., Trapnell, C., Pop, M. & Salzberg, S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 10: R25.
- Lavrenko, E.M. & Karamysheva, Z. V (1993) Steppes of the former Soviet Union and Mongolia. In: R. T. Coupland (ed.) *Natural grasslands. Ecosystems of the world*. Elsevier, Amsterdam.
- Lehmkuhl, F., Klinge, M. & Stauch, G. (2011) The extent and timing of Late Pleistocene Glaciations in the Altai and neighbouring mountain systems. *Developments in Quaternary Sciences*. Elsevier, pp. 967–979.
- Leigh, J.W. & Bryant, D. (2015) popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116.
- Leimu, R., Mutikainen, P.I.A., Koricheva, J. & Fischer, M. (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94: 942–952.
- Li, C., Hao, X., Zhao, M., Han, G. & Willms, W.D. (2008) Influence of historic sheep grazing on vegetation and soil properties of a desert steppe in Inner Mongolia.

-
- Agriculture, Ecosystems & Environment* 128: 109–116.
- Li, F.R., Zhao, A.F., Zhou, H.Y., Zhang, T.H. & Zhao, X. (2002) Effects of simulated grazing on growth and persistence of *Artemisia frigida* in a semiarid sandy rangeland. *Grass and Forage Science* 57: 239–246.
- Li, F.Y., Jäschke, Y., Guo, K., Wesche, K., (EDGG), E.D.G.G., Wang, Y., Guo, K. & Wesche, K. (2019) Grasslands of China. J. Dengler & P. Török (eds.). *Encyclopedia of the World's Biomes*.
- Li, H. & Wysoker, A. (2009) Durbin R; 1000 Genome project data processing subgroup. The sequence alignment/Map format and SAMtools. *Bioinformatics* 25: 2078–2079.
- Li, J.-T., Wang, J.-S., Nian, H.-H., Litvinchuk, S.N., Wang, J., Li, Y., Rao, D.-Q. & Klaus, S. (2015) Amphibians crossing the bering land bridge: evidence from holarctic treefrogs (*Hyla*, Hylidae, Anura). *Molecular Phylogenetics and Evolution* 87: 80–90.
- Li, J., Li, Z. & Liu, Z. (2004) Growth and resource allocation pattern of *Artemisia frigida* under different grazing and clipping intensities. *Chinese Journal of Applied Ecology* 15: 408–412.
- Li, S., Gan, X., Han, H., Zhang, X. & Tian, Z. (2018) Low within-population genetic diversity and high genetic differentiation among populations of the endangered plant *Tetracentron sinense* Oliver revealed by inter-simple sequence repeat analysis. *Annals of Forest Science* 75: 1–11.
- Li, X.H., Jiang, D.M., Alannusa, Zhou, Q.L. & Oshida, T. (2012) Comparison of seed germination of four *Artemisia* species (Asteraceae) in northeastern Inner Mongolia, China. *Journal of Arid Land* 4: 36–42.
- Liang, C., Michalk, D.L. & Millar, G.D. (2002) The ecology and growth patterns of *Cleistogenes* species in degraded grasslands of eastern Inner Mongolia, China. *Journal of Applied Ecology*.
- Liang, X., Li, P., Wang, J., Shun Chan, F.K., Togtokh, C., Ochir, A. & Davaasuren, D. (2021) Research progress of desertification and its prevention in Mongolia. *Sustainability* 13: 6861.
- Liao, H., Tuvshintogtokh, I., Guo, T. & Zhao, J. (2020) Effects of grazing exclusion on the vegetation community composition and the community stability of dry steppe and mountain steppe ecosystems in Mongolia. *Beijing Da Xue Xue Bao* 56: 471–478.
- Ling, Y.R. (1982) System of the genus *Artemisia* Linn. and the relationship with its allies. *Bulletin of Botanical Research* 2:1–60.
- Ling, Y.R. (1991) The Old World *Artemisia* (Compositae). *Bulletin of Botanical Research, Harbin* 12: 1–108.
- Ling, Y.R. (1994) The genera *Artemisia* L. and *Seriphidium* (Bess.) Poljak. in the world. *Compositae Newsletter* 25: 39–45.
- Liu, G., Xie, X., Ye, D., Ye, X., Tuvshintogtokh, I., Mandakh, B., Huang, Z. & Dong, M. (2013a) Plant functional diversity and species diversity in the Mongolian steppe. *PLoS ONE* 8: e77565.
- Liu, P., Wen, J. & Yi, T. (2017) Evolution of biogeographic disjunction between eastern Asia and North America in *Chamaecyparis*: Insights from ecological niche models. *Plant Diversity* 39: 111–116.
- Liu, W., Wang, X., Gan, Y., Huang, L., Xie, W. & Miao, J. (2009) Genetic diversity of *Kobresia pygmaea* populations along a grazing gradient. *Chinese Journal of Plant Ecology* 33: 966–973.
- Liu, Y., Pan, N., Huang, Y., Wen, J. & Zhou, L. (2010) Study on genetic diversity of *Artemisia frigida* by RAPD. *Lishizhen Medicine and Materia Medica Research* 21: 1577–1579.
- Liu, Y., Wang, Z., Feng, J. & Gu, Y.Q. (2012) Analysis of genetic diversity in different *Artemisia frigida* Willd. populations by SSR method. *International Plant and Animal Genome Conference XX*.

- Liu, Y.Y., Evans, J.P., McCabe, M.F., De Jeu, R.A.M., van Dijk, A.I.J.M., Dolman, A.J. & Saizen, I. (2013b) Changing climate and overgrazing are decimating Mongolian steppes. *PLoS ONE* 8: e57599.
- Liu, Z., Zhao, X., Wang, C., Liu, S. & Yi, H. (2003) Magnetostratigraphy of Tertiary sediments from the Hoh Xil Basin: implications for the Cenozoic tectonic history of the Tibetan Plateau. *Geophysical Journal International* 154: 233–252.
- Lkhagva, A., Boldgiv, B., Goulden, C.E., Yadamsuren, O. & Lauenroth, W.K. (2013) Effects of grazing on plant community structure and aboveground net primary production of semiarid boreal steppe of northern Mongolia. *Grassland Science* 59: 135–145.
- Lohmann, D., Tietjen, B., Blaum, N., Joubert, D.F. & Jeltsch, F. (2012) Shifting thresholds and changing degradation patterns: climate change effects on the simulated long-term response of a semi-arid savanna to grazing. *Journal of Applied Ecology* 49: 814–823.
- Maguilla, E., Escudero, M., Jiménez-Lobato, V., Díaz-Lifante, Z., Andrés-Camacho, C. & Arroyo, J. (2021) Polyploidy expands the range of *Centaurium* (Gentianaceae). *Frontiers in Plant Science* 12: 373.
- Maguilla, E., Escudero, M. & Luceño, M. (2018) Vicariance versus dispersal across Beringian land bridges to explain circumpolar distribution: A case study in plants with high dispersal potential. *Journal of Biogeography* 45: 771–783.
- Malik, S., Vitales, D., Qasim Hayat, M., Korobkov, A.A., Garnatje, T. & Vallès, J. (2017) Phylogeny and biogeography of *Artemisia* subgenus *Seriphidium* (Asteraceae: Anthemideae). *Taxon* 66: 934–952.
- Manchester, S.R. (1999) Biogeographical relationships of North American tertiary floras. *Annals of the Missouri Botanical Garden*: 472–522.
- Mansion, G., Zeltner, L. & Bretagnolle, F. (2005) Phylogenetic patterns and polyploid evolution within the Mediterranean genus *Centaurium* (Gentianaceae-Chironieae). *Taxon* 54: 931–950.
- MARCC (2014) *Mongolia second assessment report on climate change-2014*. D. Dagvadorj, Z. Batjargal, & L. Natsagdorj (eds.). Ulaanbaatar. 302 pp.
- Marcotrigiano, M. (2000) Herbivory could unlock mutations sequestered in stratified shoot apices of genetic mosaics. *American Journal of Botany* 87: 355–361.
- Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* 17: 10–12.
- Mason, C.M., LaScaleia, M.C., De La Pascua, D.R., Monroe, J.G. & Goolsby, E.W. (2020) Learning from dynamic traits: Seasonal shifts yield insights into ecophysiological trade-offs across scales from macroevolutionary to intraindividual. *International Journal of Plant Sciences* 181: 88–102.
- Matlaga, D. & Karoly, K. (2004) Long-term grazing effects on genetic variation in Idaho fescue. *Rangeland Ecology and Management* 57: 275–279.
- McArthur, E. & Jeffrey, R. (2004) *Artemisia frigida* Willd. In: J. K. Francis (ed.) *Wildland shrubs of the United States and its territories: Thamnisc descriptions*. US Department of Agriculture, Forest Service, International Institute of Tropical Forestry, San Juan, Puerto Rico, p. 63.
- McArthur, E.D. (1979) Sagebrush systematics and evolution. 14–22.
- McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C. & Brumfield, R.T. (2013) Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution* 66: 526–538.
- McGinn, S.M. (2010) Weather and climate patterns in Canada's prairie grasslands. In: J. D. Shorthouse & K. D. Floate (eds.) *Arthropods of Canadian Grasslands: Ecology and Interactions in Grassland Habitats*. Biological Survey of Canada, Alberta, Canada, pp. 105–119.
- McKinney, K.K. & Fowler, N.L. (1991) Genetic adaptations to grazing and mowing in the unpalatable grass *Cenchrus incertus*. *Oecologia* 88: 238–242.
- Meindl, C., Brune, V., Listl, D., Poschlod, P. & Reisch, C. (2016) Survival and

-
- postglacial immigration of the steppe plant *Scorzonera purpurea* to Central Europe. *Plant Systematics and Evolution* 302: 971–984.
- Meiri, M., Lister, A.M., Collins, M.J., Tuross, N., Goebel, T., Blockley, S., Zazula, G.D., Doorn, N., van Guthrie, R.D. & Boeskorov, G.G. (2013) Faunal record identifies Bering Isthmus conditions as constraint to end-pleistocene migration to the New World. *Proceedings of the Royal Society B* 281: 20132167.
- Meirmans, P.G. (2020) Genodive version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. *Molecular Ecology Resources* 20: 1126–1131.
- Meyer, M. & Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*: 5448.
- Miehe, G., Bach, K., Miehe, S., Kluge, J., Yang, Y.P., Laduo, Sonam, C., Wesche, K., Yongping, Y., Duo, L., Co, S. & Wesche, K. (2011) Alpine steppe plant communities of the Tibetan highlands. *Applied Vegetation Science* 14: 547–560.
- Milchunas, D.G. & Lauenroth, W.K. (1993) Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs* 63: 327–366.
- Morris, A.B. & Shaw, J. (2018) Markers in time and space: A review of the last decade of plant phylogeographic approaches. *Molecular Ecology* 27: 2317–2333.
- Munkhzul, O., Oyundelger, K., Narantuya, N., Tuvshintogtokh, I., Oyuntsetseg, B., Wesche, K. & Jäschke, Y. (2021) Grazing effects on Mongolian steppe vegetation – a systematic review of local literature. *Frontiers in Ecology and Evolution* 9: 703220.
- Na, Y., Li, J., Hoshino, B., Bao, S., Qin, F. & Myagmartseren, P. (2018) Effects of different grazing systems on aboveground biomass and plant species dominance in typical Chinese and Mongolian steppes. *Sustainability* 10: 4753.
- Narantsetseg, Y., Raveendar, S., Bayarsukh, N., Ya, M., Lee, J.R., Lee, K.J., Shin, M.J., Cho, G.T., Ma, K.H. & Lee, G.A. (2017) Genetic diversity and population structure of Mongolian wheat based on SSR markers: implications for conservation and management. *Plant Breeding and Biotechnology* 5: 213–220.
- National Statistics office of Mongolia (2018) *Determine the number of livestock in accordance with pasture capacity and conditions*.
- National Statistics office of Mongolia (2019) *Mongolian statistical yearbook-2019*. A. Ariunzaya, C. Bayanchimeg, A. Demberel, B. Batbayar, N. Munkhbayar, & T. Zaya (eds.). Ulaanbaatar. 823 pp.
- National Statistics office of Mongolia (2021) *Mongolian statistical yearbook-2020*. A. Ariunzaya, C. Bayanchimeg, A. Demberel, B. Batbayar, N. Munkhbayar, & T. Zaya (eds.). Ulaanbaatar
- National Statistics Office of Mongolia (2020) *Mongolian statistical yearbook webpage*. Accessed 01 December 2020
- National Statistics Office of Mongolia (2019) Determine the number of livestock in accordance with pasture capacity and conditions. 12–13.
- Nei, M. (1972) Genetic distance between populations. *The American Naturalist* 106: 283–292.
- Nei, M. & Chesser, R.K. (1983) Estimation of fixation indices and gene diversities. *Annals of Human Genetics* 47: 253–259.
- Nicotra, A.B., Chong, C., Bragg, J.G., Ong, C.R., Aitken, N.C., Chuah, A., Lepschi, B. & Borevitz, J.O. (2016) Population and phylogenomic decomposition via genotyping-by-sequencing in Australian pelargonium. *Molecular Ecology* 25: 2000–2014.
- Nomin-Erdene, T. (2021) Population genetics of regionally endangered species, *Gentiana algida* Pall. National University of Mongolia. School of Arts and Sciences. Department of Biology. Master thesis
- Noy-Meir, I. (1993) Compensating growth of grazed plants and its relevance to the

-
- use of rangelands. *Ecological Applications* 3: 32–34.
- Nyblom, H. (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13: 1143–1155.
- Nyblom, H. & Bartish, I. V (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3: 93–114.
- Oberprieler, C., Himmelreich, S., Källersjö, M., Valle`s, J., Watson, L.E. & Vogt, R. (2009) Anthemideae. In: V. A. Funk, A. Susanna, T. F. Stuessy, & R. J. Bayer (eds.) *Systematics, Evolution and Biogeography of the Compositae*. International Association Plant Taxonomy, Vienna, pp. 632–666.
- Oesterheld, M. & McNaughton, S.J. (1991) Effect of stress and time for recovery on the amount of compensatory growth after grazing. *Oecologia* 85: 305–313.
- Oesterheld, M. & Sala, O.E. (1990) Effects of grazing on seedling establishment: the role of seed and safe-site availability. *Journal of Vegetation Science* 1: 353–358.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J. & Suggests, M. (2007) The vegan package. *Community Ecology Package* 10: 631–637.
- Olf, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution* 13: 261–265.
- Oyundelger, K., Harpke, D., Herklotz, V., Troeva, E., Zheng, Z., Li, Z., Oyuntsetseg, B., Wagner, V., Wesche, K. & Ritz, C.M. (2021a) Phylogeography of *Artemisia frigida* (Anthemideae, Asteraceae) based on Genotyping-by-Sequencing and Plastid DNA Data: Migration through Beringia. *Journal of Evolutionary Biology* 35: 64–80.
- Oyundelger, K., Herklotz, V., Harpke, D., Oyuntsetseg, B., Wesche, K. & Ritz, C. (2021b) Contrasting effects of local environment and grazing pressure on genetic diversity and structure of *Artemisia frigida*. *Conservation Genetics* 22: 947–962. <https://doi.org/10.1007/s10592-021-01375-w>
- Oyundelger, K., Ritz, C.M., Munkhzul, O., Lang, B., Ahlborn, J., Oyuntsetseg, B., Römermann, C. & Wesche, K. (2020) Climate and land use affect genetic structure of *Stipa glareosa* PA Smirn. in Mongolia. *Flora* 266: 151572.
- Paradis, E. & Schliep, K. (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528.
- Pazouki, L., Shanjani, P.S., Fields, P.D., Martins, K., Suhhorutshenko, M., Viinalass, H. & Niinemets, Ü. (2016) Large within-population genetic diversity of the widespread conifer *Pinus sylvestris* at its soil fertility limit characterized by nuclear and chloroplast microsatellite markers. *European Journal of Forest Research* 135: 161–177.
- Van de Peer, Y., Ashman, T.-L., Soltis, P.S. & Soltis, D.E. (2021) Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell* 33: 11–26.
- Pellicer, J., Garcia, S., Canela, M.A., Garnatje, T., Korobkov, A.A., Twibell, J.D. & Vallès, J. (2010) Genome size dynamics in *Artemisia* L. (Asteraceae): following the track of polyploidy. *Plant Biology* 12: 820–830.
- Pellicer, J., Garcia, S., Garnatje, T., Hidalgo, O., Korobkov, A.A., Dariimaa, S. & Vallès, J. (2007) Chromosome counts in Asian *Artemisia* L.(Asteraceae) species: from diploids to the first report of the highest polyploid in the genus. *Botanical Journal of the Linnean Society* 153: 301–310.
- Pellicer, J., Vallès, J., Korobkov, A.A. & Garnatje, T. (2011) Phylogenetic relationships of *Artemisia* subg. *Dracunculus* (Asteraceae) based on ribosomal and chloroplast DNA sequences. *Taxon* 60: 691–704.
- Pelliza, Y.I., Souto, C.P. & Tadey, M. (2020) Unravelling effects of grazing intensity on genetic diversity and fitness of desert vegetation. *Perspectives in Ecology and Conservation* 18: 178–189.
- Pembleton, L.W., Cogan, N.O.I. & Forster, J.W. (2013) StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level

-
- populations. *Molecular Ecology Resources* 13: 946–952.
- Peng, J., Liang, C., Niu, Y., Jiang, W., Wang, W. & Wang, L. (2015) Moderate grazing promotes genetic diversity of *Stipa* species in the Inner Mongolian steppe. *Landscape ecology* 30: 1783–1794.
- Peper, J., Jansen, F., Pietzsch, D. & Manthey, M. (2011) Patterns of plant species turnover along grazing gradients. *Journal of Vegetation Science* 22: 457–466.
- Pérez-Escobar, O.A., Bogarín, D., Schley, R., Bateman, R.M., Gerlach, G., Harpke, D., Brassac, J., Fernández-Mazuecos, M., Dodsworth, S. & Hagsater, E. (2020) Resolving relationships in an exceedingly young Neotropical orchid lineage using Genotyping-by-sequencing data. *Molecular Phylogenetics and Evolution* 144: 106672.
- Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M. & Gurvich, D.E. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167–234.
- Pfeiffer, M., Dulamsuren, C., Jäschke, Y. & Wesche, K. (2018) Grasslands of China and Mongolia: Spatial extent, land use and conservation. In: H. Squires, Victor R.; Dengler, Jürgen; Hua, Limin; Feng (ed.) *Grasslands of the World: Diversity, Management and Conservation*. CRC Press, Boca Raton, pp. 167–196.
- Pfeiffer, M., Dulamsuren, C. & Wesche, K. (2019) Grasslands and shrublands of Mongolia. In: J. Dengler & P. Török (eds.) *Encyclopedia of the World's Biomes*. Elsevier, Amsterdam.
- Pfeiffer, M., Dulamsuren, C. & Wesche, K. (2020) Grasslands and shrublands of Mongolia. In: M. I. Goldstein & D. A. B. T.-E. of the W. B. Della Sala (eds.) *Encyclopedia of the World's Biomes*. Elsevier, Oxford, pp. 759–772.
- Pleines, T., Jakob, S.S. & Blattner, F.R. (2009) Application of non-coding DNA regions in intraspecific analyses. *Plant Systematics and Evolution* 282: 281–294.
- Plenk, K., Bardy, K., Höhn, M., Thiv, M. & Kropf, M. (2017) No obvious genetic erosion, but evident relict status at the westernmost range edge of the Pontic-Pannonian steppe plant *Linum flavum* L.(Linaceae) in Central Europe. *Ecology and Evolution* 7: 6527–6539.
- Plenk, K., Willner, W., Demina, O.N., Höhn, M., Kuzemko, A., Vassilev, K. & Kropf, M. (2020) Phylogeographic evidence for long-term persistence of the Eurasian steppe plant *Astragalus onobrychis* in the Pannonian region (eastern Central Europe). *Flora* 264: 151555.
- Plue, J., Aavik, T. & Cousins, S.A.O. (2019) Grazing networks promote plant functional connectivity among isolated grassland communities. *Diversity and Distributions* 25: 102–115.
- Proulx, M. & Mazumder, A. (1998) Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. *Ecology* 79: 2581–2592.
- Provan, J. & Bennett, K.D. (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution* 23: 564–571.
- Qian, H. (2002) Floristic relationships between eastern Asia and North America: test of Gray's hypothesis. *The American Naturalist* 160: 317–332.
- Qin, F., Wang, Y.-F., Ferguson, D.K., Chen, W.-L., Li, Y.-M., Cai, Z., Wang, Q., Ma, H.-Z. & Li, C.-S. (2015) Utility of surface pollen assemblages to delimit eastern Eurasian steppe types. *PLoS ONE* 10: e0119412.
- Qiu, Y.-X., Guan, B.-C., Fu, C.-X. & Comes, H.P. (2009) Did glacials and/or interglacials promote allopatric incipient speciation in East Asian temperate plants? Phylogeographic and coalescent analyses on refugial isolation and divergence in *Dysosma versipellis*. *Molecular Phylogenetics and Evolution* 51: 281–293.
- R Core Team (2020) R: A language and environment for statistical computing.

-
- Ranallo-Benavidez, T.R., Jaron, K.S. & Schatz, M.C. (2020) GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nature Communications* 11: 1–10.
- Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M. & Walters, M.B. (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164: S143–S164.
- Reisch, C., Meier, S., Schmid, C. & Bartelheimer, M. (2020) Clonal diversity and genetic variation of the sedge *Carex nigra* in an alpine fen depend on soil nutrients. *PeerJ* 8: e8887.
- Rice, A., Šmarda, P., Novosolov, M., Drori, M., Glick, L., Sabath, N., Meiri, S., Belmaker, J. & Mayrose, I. (2019) The global biogeography of polyploid plants. *Nature Ecology & Evolution* 3: 265–273.
- Richardson, B.A. & Meyer, S.E. (2012) Paleoclimate effects and geographic barriers shape regional population genetic structure of blackbrush (*Coleogyne ramosissima*: Rosaceae). *Botany* 90: 293–299.
- Riggins, C.W. & Seigler, D.S. (2012) The genus *Artemisia* (Asteraceae: Anthemideae) at a continental crossroads: Molecular insights into migrations, disjunctions, and reticulations among Old and New World species from a Beringian perspective. *Molecular Phylogenetics and Evolution* 64: 471–490.
- Ripley, B. & Murdoch, D. (2020) R for Windows. Version for R-4.0.2.
- Ronnenberg, K. & Wesche, K. (2011a) Effects of fertilization and irrigation on productivity, plant nutrient contents and soil nutrients in southern Mongolia. *Plant and Soil* 340: 239–251.
- Ronnenberg, K., Wesche, K., Pietsch, M. & Hensen, I. (2007) Seed germination of five mountain steppe species of Central Asia. *Journal of Arid Environments* 71: 404–410.
- Ronnenberg, K. & Wesche, P.D.D.K. (2011b) Doctor Naturwissenschaftlichen Fakultät I Biowissenschaften Population ecology of *Stipa* species in Mongolian drylands. Ch6: Baseline data on *Stipa krylovii*: Plasticity in pollination traits and vegetative persistence. Martin Luther University Halle-Wittenberg
- Rosenberg, N.A. (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4: 137–138.
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. & Sánchez-Gracia, A. (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34: 3299–3302.
- Ruan, C., Qin, P., Zheng, J. & He, Z. (2004) Genetic relationships among some cultivars of sea buckthorn from China, Russia and Mongolia based on RAPD analysis. *Scientia Horticulturae* 101: 417–426.
- Sainnemekh, S., Barrio, I.C., Densambuu, B., Bestelmeyer, B. & Aradóttir, Á.L. (2022) Rangeland degradation in Mongolia: A systematic review of the evidence. *Journal of Arid Environments* 196: 104654.
- Salis, A.T., Bray, S.C.E., Lee, M.S.Y., Heiniger, H., Barnett, R., Burns, J.A., Doronichev, V., Fedje, D., Golovanova, L. & Harington, C.R. (2020) Lions and brown bears colonized North America in multiple synchronous waves of dispersal across the Bering Land Bridge. *bioRxiv*. <https://doi.org/https://doi.org/10.1101/2020.09.03.279117>
- Sanmartin, I., Enghoff, H. & Ronquist, F. (2001) Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biological journal of the Linnean Society* 73: 345–390.
- Sanz, M., Schneeweiss, G., Vilatersana Lluçh, R. & Vallès Xirau, J. (2011) Temporal origins and diversification of *Artemisia* and allies (Anthemideae, Asteraceae). *Collectanea Botanica* 30: 7–15.
- Sanz, M., Schönswetter, P., Vallès, J., Schneeweiss, G.M. & Vilatersana, R. (2014) Southern isolation and northern long-distance dispersal shaped the

-
- phylogeography of the widespread, but highly disjunct, European high mountain plant *Artemisia eriantha* (Asteraceae). *Botanical Journal of the Linnean Society* 174: 214–226.
- Sanz, M., Vilatersana, R., Hidalgo, O., Garcia-Jacas, N., Susanna, A., Schneeweiss, G.M. & Vallès, J. (2008) Molecular phylogeny and evolution of floral characters of *Artemisia* and allies (Anthemideae, Asteraceae): Evidence from nrDNA ETS and ITS sequences. *Taxon* 57: 66–78.
- Sarvis, J.T. (1941) *Grazing investigations on the northern Great Plains*. North Dakota
- Sasaki, T., Okayasu, T., Jamsran, U. & Takeuchi, K. (2008) Threshold changes in vegetation along a grazing gradient in Mongolian rangelands. *Journal of Ecology* 96: 145–154.
- Scheepens, J.F., Frei, E.S. & Stöcklin, J. (2010) Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* 164: 141–150.
- Schuelke, M. (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.
- Seidl, A., Pérez-Collazos, E., Tremetsberger, K., Carine, M., Catalán, P. & Bernhardt, K.-G. (2020) Phylogeny and biogeography of the Pleistocene Holarctic steppe and semi-desert goosefoot plant *Krascheninnikovia ceratoides*. *Flora* 262: 151504.
- Seidl, A., Tremetsberger, K., Pfanzelt, S., Blattner, F.R., Neuffer, B., Friesen, N., Hurka, H., Shmakov, A., Batlai, O. & Žerdoner Čalasan, A. (2021) The phylogeographic history of *Krascheninnikovia* reflects the development of dry steppes and semi-deserts in Eurasia. *Scientific Reports* 11: 1–15.
- Sexton, J.P., Hangartner, S.B. & Hoffmann, A.A. (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* 68: 1–15.
- Shan, D., Zhao, M., Han, B., Han, G., Dan, S., Mengli, Z., Bing, H. & Guodong, H. (2006) Examining the genetic diversity of *Stipa grandis* under various grazing pressures. *Acta Ecologica Sinica* 26: 3175–3182.
- Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94: 275–288.
- Sheehy, D.P. & Damiran, D. (2012) *Assessment of Mongolian Rangeland Condition and Trend (1997-2009)*: 00–47.
- Shipley, B. (2000) Plasticity in relative growth rate and its components following a change in irradiance. *Plant, Cell & Environment* 23: 1207–1216.
- Sims, J. (2000) Soil test phosphorus: Olsen P. In: G. Pierzynski (ed.) *Methods of phosphorus analysis for soils, sediments, residuals, and waters*. North Carolina State University, Raleigh, pp. 20–21.
- Sinitsyna, T.A., Herden, T. & Friesen, N. (2016) Dated phylogeny and biogeography of the Eurasian *Allium* section Rhizirideum (Amaryllidaceae). *Plant Systematics and Evolution* 302: 1311–1328.
- Sloat, L.L., Gerber, J.S., Samberg, L.H., Smith, W.K., Herrero, M., Ferreira, L.G., Godde, C.M. & West, P.C. (2018) Increasing importance of precipitation variability on global livestock grazing lands. *Nature Climate Change* 8: 214–218.
- Smelansky, I.E. & Tishkov, A.A. (2012) The steppe biome in Russia: Ecosystem services, conservation status, and actual challenges. In: M. J. A. Werger & M. van Staalduinen (eds.) *Eurasian steppes. Ecological problems and livelihoods in a changing world*. Springer, Heidelberg, pp. 45–101.
- Smith, S.E., Arredondo, T., Aguiar, M., Huber-Sannwald, E., Alpuche, A., Aguado, A., Grageda, O.A., Halbrook, K. & Bottini, C. (2009) Fine-scale spatial genetic structure in perennial grasses in three environments. *Rangeland Ecology & Management* 62: 356–363.

-
- Sork, V.L. (2018) Genomic studies of local adaptation in natural plant populations. *Journal of Heredity* 109: 3–15.
- Souto, C.P. & Tadey, M. (2019) Livestock effects on genetic variation of creosote bushes in patagonian rangelands. *Environmental Conservation* 46: 59–66.
- Spence, L.A., Liancourt, P., Boldgiv, B., Petraitis, P.S. & Casper, B.B. (2016) Short-term manipulation of precipitation in Mongolian steppe shows vegetation influenced more by timing than amount of rainfall. *Journal of Vegetation Science* 27: 249–258.
- Van Staalduinen, M.A., During, H. & Werger, M.J.A. (2007) Impact of grazing regime on a Mongolian forest steppe. *Applied Vegetation Science* 10: 299–306.
- Sternberg, T. (2008) Environmental challenges in Mongolia's dryland pastoral landscape. *Journal of Arid Environments* 72: 1294–1304.
- Stevens, M.T., Waller, D.M. & Lindroth, R.L. (2007) Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. *Evolutionary Ecology* 21: 829–847.
- Stinchcombe, J.R., Kirkpatrick, M. & Group, F.T.W. (2012) Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends in Ecology & Evolution* 27: 637–647.
- Swofford, D.L. (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.
- Taberlet, P. & Cheddadi, R. (2002) Quaternary refugia and persistence of biodiversity. *Science* 297: 2009–2010.
- Taheri, S., Lee Abdullah, T., Yusop, M.R., Hanafi, M.M., Sahebi, M., Azizi, P. & Shamshiri, R.R. (2018) Mining and development of novel SSR markers using next generation sequencing (NGS) data in plants. *Molecules* 23: 399.
- Takatsuki, S., Sato, M. & Morinaga, Y. (2018) Effects of grazing on grassland communities of the forest-steppe of northern Mongolia: A comparison of grazed versus ungrazed places. *Grassland Science* 64: 167–174.
- Tarasov, P.E., Volkova, V.S., Webb III, T., Guiot, J., Andreev, A.A., Bezusko, L.G., Bezusko, T. V, Bykova, G. V, Dorofeyuk, N.I. & Kvavadze, E. V (2000) Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. *Journal of Biogeography* 27: 609–620.
- Thiel, T., Michalek, W., Varshney, R. & Graner, A. (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106: 411–422.
- Thórsson, Æ.T., Pálsson, S., Lascoux, M. & Anamthawat-Jónsson, K. (2010) Introgression and phylogeography of *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland inferred from cpDNA haplotype variation. *Journal of Biogeography* 37: 2098–2110.
- Tiffney, B.H. (1985a) Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. *Journal of the Arnold Arboretum* 66: 73–94.
- Tiffney, B.H. (1985b) The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretum* 66: 243–273.
- Tiffney, B.H. & Manchester, S.R. (2001) The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* 162: 3–17.
- Tkach, N. V, Hoffmann, M.H., Röser, M. & von Hagen, K.B. (2008a) Temporal patterns of evolution in the Arctic explored in *Artemisia* L. (Asteraceae) lineages of different age. *Plant Ecology & Diversity* 1: 161–169.
- Tkach, N. V, Hoffmann, M.H., Röser, M., Korobkov, A.A. & Von Hagen, K.B. (2008b) Parallel evolutionary patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). *Evolution* 62: 184–198.
- Tournebize, R., Manel, S., Vigouroux, Y., Munoz, F., de Kochko, A. & Poncet, V.

-
- (2017) Two disjunct Pleistocene populations and anisotropic postglacial expansion shaped the current genetic structure of the relict plant *Amborella trichopoda*. *PLoS ONE* 12: e0183412.
- Tserendulam, T., Bayarsaikhan, U., Oyuntsetseg, B. & Wesche, K. (2018) The vascular plant flora of Hustai National Park, Mongolia: Composition, life forms, ecological groups and geographical elements. *Feddes Repertorium* 129: 137–160.
- Tuvshintogtokh, I. (2014) *The steppe vegetation of Mongolia*. C. Sanchir (ed.). Bembisan, Ulaanbaatar. 610 pp.
- Ulziikhutag, N. (1985) *Identification card of plants in pastures and hayfields of the People's Republic of Mongolia*. C. Sanchir (ed.). Ulsiin hevleliin gazar, Ulaanbaatar. 556 pp.
- Undarmaa, J., Toshiya, O., Manibazar, N. & Yamanaka, N. (2015) *Rangeland Plants of Mongolia: High mountain belt, Mountain forest-steppe belt, Steppe zone*. Arid Land Research Center in Tottori University and Center for Ecosystem Study in Mongolian State University of Agriculture, Ulaanbaatar, Mongolia. 404–405 pp.
- Vallès, J., Garcia, S., Hidalgo, O., Martín, J., Pellicer, J., Sanz, M. & Garnatje, T. (2011) Biology, genome evolution, biotechnological issues and research including applied perspectives in *Artemisia* (Asteraceae). In: J.C. Kader & M. Delseny (eds.) *Advances in Botanical Research*. Academic Press, pp. 349–419.
- Vallès, J. & McArthur, E.D. (2001) *Artemisia* systematics and phylogeny: cytogenetic and molecular insights. *USDA Forest Service Proceedings* 21: 67–74.
- Vallès, J., Torrell, M., Garnatje, T., Garcia-Jacas, N., Vilatersana, R. & Susanna, A. (2003) The genus *Artemisia* and its allies: phylogeny of the subtribe Artemisiinae (Asteraceae, Anthemideae) based on nucleotide sequences of nuclear ribosomal DNA internal transcribed spacers (ITS). *Plant Biology* 5: 274–284.
- Vetter, S. (2005) Rangelands at equilibrium and non-equilibrium: recent developments in the debate. *Journal of Arid Environments* 62: 321–341.
- Vieira, M.L.C., Santini, L., Diniz, A.L. & Munhoz, C. de F. (2016) Microsatellite markers: what they mean and why they are so useful. *Genetics and Molecular Biology* 39: 312–328.
- Volkova, P.A., Herden, T. & Friesen, N. (2017) Genetic variation in *Goniolimon speciosum* (Plumbaginaceae) reveals a complex history of steppe vegetation. *Botanical Journal of the Linnean Society* 184: 113–121.
- Waltari, E., Hoberg, E.P., Lessa, E.P. & Cook, J.A. (2007) Eastward Ho: phylogeographical perspectives on colonization of hosts and parasites across the Beringian nexus. *Journal of Biogeography* 34: 561–574.
- Wan, T., Cai, P., Zhang, C.B., Yi, W., Li, F. & Zhang, H. (2008) Analysis of genetic diversity of *Artemisia frigida* populations by RAPD in different synusiological area of Inner Mongolia. *Journal of Northwest A&F University (Natural Science Edition)* 36: 166–169.
- Wan, T., Sun, Q., Cai, P., Meng, X., Yi, W. & Wang, W. (2011) Observation of chromosome karyotypes of *Artemisia frigida* in different ecological areas of Inner Mongolia. *Acta Botanica Boreali-Occidentalia Sinica* 31: 456–461.
- Wang, B. (2006) *The Asian monsoon*. Springer Science & Business Media, Chichester, UK. 842 pp.
- Wang, J., Gong, X., Chiang, Y. & Kuroda, C. (2013) Phylogenetic patterns and disjunct distribution in *Ligularia hodgsonii* Hook. (Asteraceae). *Journal of Biogeography* 40: 1741–1754.
- Wang, J., Yang, C., Yin, J., Wang, T.-J. & Liu, P.-T. (2004) Changes of the genetic diversity of *Artemisia frigida* population under the disturbance of grazing. *Acta Ecologica Sinica* 24: 2465–2471.
- Wang, J.L., Zhao, N.X., Gai, Y.B., Lin, F., Ren, A.Z., Ruan, W.B. & Chen, L. (2006) RAPD analysis of genetic diversity and population genetic structure of *Stipa*

-
- krylovii* Reshov. in inner Mongolia steppe. *Russian Journal of Genetics* 42: 468–475.
- Wang, W.M. (2004) On the origin and development of *Artemisia* (Asteraceae) in the geological past. *Botanical Journal of the Linnean Society* 145: 331–336.
- Wang, Y. & Wesche, K. (2016) Vegetation and soil responses to livestock grazing in Central Asian grasslands: a review of Chinese literature. *Biodiversity and Conservation* 25: 2401–2420.
- Wang, Z. (2011) Study on genetic diversity of traditional Mongolian medicine *Artemisia frigida*. Minzu University of China Master thesis.
- Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Wolfgang, Huber, Liaw, A., Lumley, T., Maechler, M., Magnusson, A., Steffen, Moeller, Schwartz, M. & Venables, B. (2020) gplots: Various R programming tools for plotting data.
- Watano, Y., Imazu, M. & Shimizu, T. (1996) Spatial distribution of cpDNA and mtDNA haplotypes in a hybrid zone between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae). *Journal of Plant Research* 109: 403–408.
- Weber, W.A. (2003) The Middle Asian element in the southern Rocky Mountain flora of the western United States: a critical biogeographical review. *Journal of Biogeography* 30: 649–685.
- von Wehrden, H., Hanspach, J., Kaczensky, P., Fischer, J. & Wesche, K. (2012a) Global assessment of the non-equilibrium concept in rangelands. *Ecological Applications* 22: 393–399.
- von Wehrden, H., Hanspach, J., Kaczensky, P., Fischer, J., Wesche, K., von Wehrden, H., Hanspach, J., Kaczensky, P., Fischer, J. & Wesche, K. (2012b) Global assessment of the non-equilibrium concept in rangelands. *Ecological Applications* 22: 393–399.
- von Wehrden, H. & Wesche, K. (2007) Relationships between climate, productivity and vegetation in southern Mongolian drylands. *Basic and Applied Dryland Research* 1: 100.
- von Wehrden, H., Wesche, K. & Miede, G. (2009) Plant communities of the southern Mongolian Gobi. *Phytocoenologia* 39: 331–376.
- Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution*: 1358–1370.
- Wesche, K., Ambarlı, D., Kamp, J., Török, P., Treiber, J. & Dengler, J. (2016) The Palearctic steppe biome: a new synthesis. *Biodiversity and Conservation* 25: 2197–2231.
- Wesche, K., Hensen, I. & Undrakh, R. (2006a) Range-wide genetic analysis provides evidence of natural isolation among populations of the Mongolian endemic *Potentilla ikonnikovii* Juz. (Rosaceae). *Plant species biology* 21: 155–163.
- Wesche, K., Hensen, I. & Undrakh, R. (2006b) Genetic structure of *Galitzkya macrocarpa* and *G. potaninii*, two closely related endemics of central Asian mountain ranges. *Annals of Botany* 98: 1025–1034.
- Wesche, K., Ronnenberg, K., Retzer, V. & Miede, G. (2010) Effects of large herbivore exclusion on southern Mongolian desert steppes. *Acta Oecologica* 36: 234–241.
- Wesche, K., Walther, D., Von Wehrden, H. & Hensen, I. (2011) Trees in the desert: reproduction and genetic structure of fragmented *Ulmus pumila* forests in Mongolian drylands. *Flora-Morphology, Distribution, Functional Ecology of Plants* 206: 91–99.
- White, R.P., Murray, S. & Rohweder, M. (2000) *Pilot Analysis of Global Ecosystems: Grassland Ecosystems*. World Resource Institute, Washington DC
- Wickham, H. (2011) ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics* 3: 180–185.
- Wickham, H. (2017) The tidyverse. An R package. Available from: <https://www.tidyverse.org/>
- Wickham, H., Chang, W. & Wickham, M.H. (2016) ggplot2: *Elegant Graphics for*

-
- Data Analysis*. Springer Verlag, New York. 1–189 pp.
- Widmer, A. & Baltisberger, M. (1999) Extensive intraspecific chloroplast DNA (cpDNA) variation in the alpine *Draba aizoides* L. (Brassicaceae): haplotype relationships and population structure. *Molecular Ecology* 8: 1405–1415.
- Wiehle, M., Vornam, B., Wesche, K., Goenster, S. & Buerkert, A. (2016) Population structure and genetic diversity of *Populus laurifolia* in fragmented riparian gallery forests of the Mongolian Altai Mountains. *Flora* 224: 112–122.
- Willis, K.J. & Niklas, K.J. (2004) The role of Quaternary environmental change in plant macroevolution: the exception or the rule? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 159–172.
- Wolfe, J.A. (1994) An analysis of Neogene climates in Beringia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 108: 207–216.
- Wu, J., Gao, Y., Bao, X., Gao, H., Jia, M., Li, J. & Zhao, N. (2010) Genetic diversity of *Stipa grandis* P. Smirn populations across the species' range in the Inner Mongolia Plateau of China. *Biochemical Systematics and Ecology* 38: 471–477.
- Yan, F., Zhou, W., Zhao, H., Yuan, Z., Wang, Y., Jiang, K., Jin, J., Murphy, R.W., Che, J. & Zhang, Y. (2013a) Geological events play a larger role than Pleistocene climatic fluctuations in driving the genetic structure of *Quasipaa boulengeri* (Anura: Dicoglossidae). *Molecular Ecology* 22: 1120–1133.
- Yan, L., Zhou, G. & Zhang, F. (2013b) Effects of Different Grazing Intensities on Grassland Production in China: A Meta-Analysis. F.-H. Yu (ed.). *PLoS ONE* 8: e81466.
- Yan, M., Liu, R., Li, Y., Hipp, A.L., Deng, M. & Xiong, Y. (2019) Ancient events and climate adaptive capacity shaped distinct chloroplast genetic structure in the oak lineages. *BMC Evolutionary Biology* 19: 1–14.
- Yang, J., Cushman, S.A., Yang, J., Yang, M. & Bao, T. (2013) Effects of climatic gradients on genetic differentiation of *Caragana* on the Ordos Plateau, China. *Landscape Ecology* 28: 1729–1741.
- Yang, X., Huang, Z., Zhang, K. & Cornelissen, J.H.C. (2015) C: N: P stoichiometry of *Artemisia* species and close relatives across northern China: unravelling effects of climate, soil and taxonomy. *Journal of Ecology* 103: 1020–1031.
- Yuan, Z.Y., Jiao, F., Li, Y.H. & Kallenbach, R.L. (2016) Anthropogenic disturbances are key to maintaining the biodiversity of grasslands. *Scientific Reports* 6: 22132.
- Yunatov, A.A. & Dashnyam, B. (1979) The vegetation map of People's Republic of Mongolia (1:1 500 000). E. M. Lavrenko (ed.). *Mongolian and Russian Joint Biological Expedition*.
- Yunfa, M., Qingquan, M., Xiaomin, F., Xiaoli, Y., Fuli, W. & Chunhui, S. (2011) Origin and development of *Artemisia* (Asteraceae) in Asia and its implications for the uplift history of the Tibetan Plateau: a review. *Quaternary International* 236: 3–12.
- Yurtsev, B.A. (1987) *The Arctic Flora of the USSR [Arkticheskaya flora SSSR. Semeystva Rubiaceae – Compositae]*. Nayuka, Leningrad. 411 pp.
- Yurtsev, B.A. (2001) The Pleistocene “Tundra-Steppe” and the productivity paradox: the landscape approach. *Quaternary Science Reviews* 20: 165–174.
- Záveská, E., Maylandt, C., Paun, O., Bertel, C., Frajman, B., Schönswetter, P. & Consortium, S. (2019) Multiple auto- and allopolyploidisations marked the Pleistocene history of the widespread Eurasian steppe plant *Astragalus onobrychis* (Fabaceae). *Molecular Phylogenetics and Evolution* 139: 106572.
- Zemrich, A., Hilbig, W. & Oyuunchimeg, D. (2010a) Plant communities along an elevation gradient under special consideration of grazing in Western Mongolia. *Phytocoenologia* 40: 91–115.
- Zemrich, A., Manthey, M., Zerbe, S. & Oyuunchimeg, D. (2010b) Driving environmental factors and the role of grazing in grassland communities: A comparative study along an altitudinal gradient in Western Mongolia. *Journal of*

-
- Arid Environments* 74: 1271–1280.
- Zerbino, D.R. (2010) Using the velvet *de novo* assembler for short-read sequencing technologies. *Current Protocols in Bioinformatics* 31: 11.5.1-11.5.12.
- Žerdoner Čalasan, A., German, D.A., Hurka, H. & Neuffer, B. (2021a) A story from the Miocene: Clock-dated phylogeny of *Sisymbrium* L. (Sisymbrieae, Brassicaceae). *Ecology and Evolution* 11: 2573–2595.
- Žerdoner Čalasan, A., Hurka, H., German, D.A., Pfanzelt, S., Blattner, F.R., Seidl, A. & Neuffer, B. (2021b) Pleistocene dynamics of the Eurasian steppe as a driving force of evolution: Phylogenetic history of the genus *Capsella* (Brassicaceae). *Ecology and Evolution* 11: 12697–12713.
- Žerdoner Čalasan, A., Seregin, A.P., Hurka, H., Hofford, N.P. & Neuffer, B. (2019) The Eurasian steppe belt in time and space: phylogeny and historical biogeography of the false flax (*Camelina* Crantz, Camelinaeae, Brassicaceae). *Flora* 260: 151477.
- Zhang, C., Sun, M., Zhang, X., Chen, S., Nie, G., Peng, Y., Huang, L. & Ma, X. (2018a) AFLP-based genetic diversity of wild orchardgrass germplasm collections from Central Asia and Western China, and the relation to environmental factors. *PLoS ONE* 13: e0195273.
- Zhang, J., Zuo, X., Zhou, X., Lv, P., Lian, J. & Yue, X. (2017) Long-term grazing effects on vegetation characteristics and soil properties in a semiarid grassland, northern China. *Environmental Monitoring and Assessment* 189: 216.
- Zhang, R., Liang, T., Guo, J., Xie, H., Feng, Q. & Aimaiti, Y. (2018b) Grassland dynamics in response to climate change and human activities in Xinjiang from 2000 to 2014. *Scientific Reports* 8: 2888.
- Zhang, R., Wang, Z., Han, G., Schellenberg, M.P., Wu, Q. & Gu, C. (2018c) Grazing induced changes in plant diversity is a critical factor controlling grassland productivity in the desert steppe, Northern China. *Agriculture, Ecosystems and Environment* 265: 73–83.
- Zhao-Jiang, Z., Ru-Min, Z., Pei-Jun, G., Guo-Sheng, W., Ping, H. & Yan, G. (2011) Allelopathic effects of *Artemisia frigida* Willd. on growth of pasture grasses in Inner Mongolia, China. *Biochemical Systematics and Ecology* 39: 377–383.
- Zhao, N.X., Gao, Y.B., Wang, J.L. & Ren, A.Z. (2006) Genetic diversity and population differentiation of the dominant species *Stipa krylovii* in the Inner Mongolia Steppe. *Biochemical Genetics* 44: 504–517.
- Zhi-chen, S., Wei-ming, W. & Fei, H. (2004) Fossil pollen records of extant angiosperms in China. *The Botanical Review* 70: 425–458.
- Ziegenhagen, B. (1990) ATMAB DNA Extraction. Method Birgit Ziegenhagen. unpublished manuscript.
- Zink, R.M. (2010) Drawbacks with the use of microsatellites in phylogeography: the song sparrow *Melospiza melodia* as a case study. *Journal of Avian Biology* 41: 1–7.

Curriculum vitae

Personal information:

Family name: Khurelpurev
Surname: Oyundelger
Nationality: Mongolian
Date of birth: 15.05.1993
Place of birth: Ulaanbaatar, Mongolia
Marital status: Not married, no children
Mother tongue: Mongolian
Current address: Emmerich Str. 46, 02826 Görlitz, Germany
Phone number: + 49 152 57429805
E-mail address: oyundelger.kh@gmail.com;
oyundelger.khurelpurev@senckenberg.de

Academic history:

July 2018 – *Doctoral study: Biology*

present Chair of Biodiversity of Higher Plants, Technische Universität Dresden | Dresden and Senckenberg Museum of Natural History Görlitz | Görlitz, Germany.

Dissertation: Evolutionary biology and population genetics of key dryland species: *Artemisia frigida* Willd. (Anthemideae, Asteraceae)

Oct. 2015 – *Master of Science (MSc): Biodiversity and Collection Management*

March 2018 International Institute (IHI) Zittau / TU Dresden | Dresden, Germany

Master thesis titled 'Impact of changing grazing and precipitation on genetic structure of key-stone feather grasses of Central Asian drylands'

Final grade: 1,7

Sept. 2010 – *Bachelor of Science (BSc): Ecology and Nature Conservation*

June 2014 Department of Biology, School of Arts and Sciences, National University of Mongolia | Ulaanbaatar, Mongolia

Bachelor thesis titled 'Species richness and community pattern of Coleoptera in Altai Mountains'

Final grade: 3.7 (equivalent to German grade of 1,2)

Sept. 2000 – *High School Education:*

June 2010 10-year study, Secondary school number 2 | Ulaanbaatar, Mongolia.

Final grade: 99.7%

Work experiences:

- March 2017 – present** *Scientific assistant* at the Chair of Ecosystem Service, International Institute Zittau, TU Dresden | Zittau, Germany
- Assisting with lecture material preparation
 - Data gathering and literature review for the MORESTEP project report, and translation from Mongolian to English as well as statistical analyses
- June 2014 – Sept. 2015** *Technical assistant and labor manager* at the Molecular Ecology Laboratory, National University of Mongolia | Ulaanbaatar, Mongolia
- Established new molecular laboratory in the department of Ecology, funded by DAAD - Project of Genetic Database
- Sept. 2013 – Sept. 2015** *Teaching assistant* at the department of Ecology, School of Arts and Sciences, National University of Mongolia | Ulaanbaatar, Mongolia
- Simultaneous lecture translation from English to Mongolian.

Research methodology, computer skills and language knowledge:

Research methodology:

- Experienced in field works of botanical, small mammal and entomological studies; conducted camera trapping, pitfall trapping survey technique and different biological data collections methods.
- Well-practiced in a lab work at molecular laboratory; proficient in DNA isolation from plant and insect, PCR, microsatellite, and sequencing analyzes.
- Bioinformatics expertise at a medium level
- Well-demonstrated experience in project manager in fieldwork, as well as laboratory management, good communication and team player
- Experience with the evaluation of molecular and ecological data as well as their presentation in written and oral form

Computer and software:

- Experienced with Windows and Mac Operating systems
- Demonstrated computer skills in all Microsoft office programs inc., Publisher, and Adobe Photo Editor; EndNote, Mendeley
- Experienced in professional analysis programs such as, R, EstimateS, Statistica, Past, BioEdit, Genographer, Peakscanner, GenAlex, PopArt, Mega and Geneious.

Language knowledge:

	Mongolian (native)	English (C2)	German (C1)
Speaking	● ● ● ● ●	● ● ● ● ●	● ● ● ● ●
Writing	● ● ● ● ●	● ● ● ● ●	● ● ● ● ●

Awards and scholarships:

- April 2022 – June 2022** – Grant for the funding of young scientists during the completion and wrap-up phase of the doctorate, TU Dresden | Germany
- July 2018 – Sept. 2021** – Scholarship program for the promotion of early-career female scientists of TU Dresden | Germany
- Jan. 2018 – June 2018** – Grant of Senckenberg Society for Nature Research (SNG) for pre-promotion | Germany
- Oct. 2017** – DAAD Scholarship for particularly committed international students (STIBET I) | Germany
- Oct. 2015 – Sept. 2017** – SNG Grant of “Dietmar-Schmid-Education Fonds” for master study of International Institute Zittau / TU Dresden | Germany
- Feb. 2014** – Grant for academic trip to Japan, conducted by Jenesys 2.0 Program, International Cooperation Center | Japan
- 2014** – Internal Full National Scholarship 2013/2014 from Oyu Tolgoi - Rio Tinto | Mongolia
- 2014** – Outstanding graduate of School of Biological Science, School of Arts and Sciences, National University of Mongolia | Mongolia
- 2013** – Outstanding student of academics of School of Biology and Biotechnology of the National University of Mongolia | Mongolia
- 2012** – Winner of National 10th Ecological Olympiad | Mongolia

Conference, international visiting experience and field research

- Nov. 2021** Senckenberg Young Scientists Retreat and workshop “Effective Data Visualization – Convert Information into visual forms, and tell a story” | Germany
- May 2021** 33th Conference of the Plant Population Biology of the Ecological Society of Germany, Austria and Switzerland (GfÖ) | Prague, Czech Republic (27-29 May)
- Sept. 2019** Workshop on Next Generation Sequencing (NGS), LOEWE - Research Centre for Translational Biodiversity | Frankfurt, Germany (2 weeks)
- May 2019** 7th Repeat Explorer Workshop on the Application of Next Generation Sequencing to Repetitive DNA Analysis, Institute of Plant Molecular Biology | České Budějovice, Czech Republic (21-24 May)
- July 2018** “Gobi University – Summer School Mongolia” in the Great Gobi B Strictly protected area | southern Mongolia (4 weeks)
- June 2018** Senckenberg Young Scientists Retreat | Dresden, Germany (8-10 June)
- May 2018** 31st Conference of the Plant Population Biology Section of the Ecological Society of Germany, Austria and Switzerland (GfÖ) | Innsbruck, Austria (3-5 May)
- Sept. 2017** International Conference in Biodiversity Research of Mongolia (ICBRM) | Ulaanbaatar, Mongolia (20-23 Sept.)
- July 2016** Botanical expedition to Munkhkhairkhan National Park under the scientific partnership project among the Andong National University, Changwon National University, Korea and NUM | western Mongolia (4 weeks)
- June 2016** Free-field workshop of monitoring of water quality in a research station at the University of Wroclaw | Sněžka in Giant Mountains, Poland (1 week)
- June 2014** International summer school in Conservation biology organized by Zoological Society of London | Hustai National Park, Mongolia (3 weeks)
- Feb. 2014** Academic trip in science and culture of Japan | Tokyo and Tottori, Japan (2 weeks)
- July 2013** Field work for entomological expedition in the region of Altai Mountain | western Mongolia (1 month)
- June 2013** Practical course of “DNA technology in ecology, evolution and systematics” at NUM | Ulaanbaatar, Mongolia (1 week)
- July 2012** Ecological methodology field trip | Tuv province, central Mongolia (1 month)
- June 2011** Zoological and botanical field excursion in Batsumber research station | Tuv province, central Mongolia (1 month)

Publication list:

- Khurelpurev. O** and Pfeiffer, M. (2017). Coleoptera in the Altai Mountains (Mongolia): species richness and community patterns along an ecological gradient. *Journal of Asia-Pacific Biodiversity*. 10 (3): 362-370. <https://doi.org/10.1016/j.japb.2017.06.007>.
- Oyuntsetseg, B., Baasanmunkh, Sh., **Oyundelger, Kh.**, Oyunbileg, M., Kim, J.Y., Cho, H.J, Batkhuu, N., Chung, G.Y. and Choi, H.J. (2017). Contribution to the knowledge on the flora of Munkhkairkhan mountain area, Mongolia. *Journal of Asia-Pacific Biodiversity*. 10 (4): 573-582. <https://doi.org/10.1016/j.japb.2017.05.005>.
- Baasanmunkh, Sh., Oyuntsetseg, B., **Oyundelger, Kh.**, Khaliunaa, Kh., Urgamal, M., Batkhuu, N., Shiga, T., Chung. G.Y. and Choi, H.J. (2019). Contribution to the knowledge on the flora of northern Mongolia. *Journal of Asia-Pacific Biodiversity*. 12(4): 643-660. <https://doi.org/10.1016/j.japb.2019.08.009>.
- Oyundelger. Kh.**, Ritz, C., Oyunbileg, M., Lang, B., Ahlborn, J., Oyuntsetseg, B., Römermann, C. and Wesche, K. (2020). Climate and land use affect genetic structure of *Stipa glareosa* P. A. Smirn. in Mongolia. *Flora – Special Issue on Steppe Ecology*. 266: 151572. <https://doi.org/10.1016/j.flora.2020.151572>
- Baasanmunkh, Sh., Nyamgerel, N., Bayarmaa, G., Oyuntsetseg, B., **Oyundelger, Kh.** and Choi, H.J. (2020). A new record of critically endangered *Saussurea bogedaensis* (Asteraceae) from Dzungarian Gobi, Mongolia. *PhytoKeys*. 160: 109-121. <https://doi.org/10.3897/phytokeys.160.55603>
- Baasanmunkh, Sh., Oyuntsetseg, B., Oyundari, Ch., **Oyundelger, Kh.**, Urgamal, M., Darikhand, D., Soninkhishig. N., et al. (2021) The vascular plant diversity of Dzungarian Gobi in western Mongolia, with an annotated checklist. *Phytotaxa*. 501(1): 1-55. <https://doi.org/10.11646/phytotaxa.501.1.1>
- Oyundelger, Kh.**, Herklotz, V., Harpke, D., Oyuntsetseg, B., Wesche, K. and Christiane. R. (2021) Contrasting effects of local environment and grazing pressure on the genetic diversity and structure of *Artemisia frigida*. *Conservation Genetics*, 22: 947-962. <https://doi.org/10.1007/s10592-021-01375-w>
- Baasanmunkh, Sh., Oyuntsetseg, B., Efimov, P., Tsegmed, Z., Vandandorj, S., **Oyundelger, Kh.**, Urgamal, M., Undruul, A., Khaliunaa, Kh., Namuulin, T. and Choi, H.J. (2021) Orchids of Mongolia: Taxonomy, species richness and conservation status. *Diversity*. 13, 302. <https://doi.org/10.3390/d13070302>
- Munkhzul, O., **Oyundelger, Kh.**, Narantuya, N., Tuvshintogtokh, I., Oyuntsetseg, B., Wesche, K. and Jäschke, Y. (2021) Systematic review of grazing effects on vegetation in Mongolian steppes. *Frontiers*

- in *Ecology and Evolution*. 9, 703220.
<https://doi.org/10.3389/fevo.2021.703220>
- Dashpurev, B., Wesche, K., Jäschke, Y., **Oyundelger, Kh.**, Phan, T.N., Bendix, J. and Lehnert, L.W. (2021). A cost-effective method to monitor vegetation changes in steppes ecosystems: A case study on remote sensing of fire and infrastructure effects in eastern Mongolia. *Ecological Indicators*. 132, 108331.
<https://doi.org/10.1016/j.ecolind.2021.108331>
- Baasanmunkh, Sh., Urgamal, M., Oyuntsetseg, B., Gundegmaa, V., Tsegmed, Z., **Oyundelger, Kh.**, Kechaykin, A.A., Pyak, A.I. and Choi, H.J. (2021). Updated checklist of vascular plants endemic to Mongolia. *Diversity*. 13, 619. <https://doi.org/10.3390/d13120619>
- Oyundelger, Kh.**, Harpke, D., Herklotz, V., Troeva, E., Zheng, Z., Li, Z., Oyuntsetseg, B., Wagner, V., Wesche, K. and Ritz, C.M. (2021). Phylogeography of *Artemisia frigida* (Anthemideae, Asteraceae) based on genotyping-by-sequencing and plastid DNA data: Migration through Beringia. *Journal of Evolutionary Biology*. 35 (1), 64-80.
<https://doi.org/10.1111/jeb.13960>
- Baasanmunkh, Sh., Oyuntsetseg, B., Tsegmed, Z., **Oyundelger, Kh.**, Urgamal, M., Gantuya, B., Javzandolgor, Ch., Nyambayar, N., Petr., K. and Choi, H.J. (2022). Distribution of vascular plants in Mongolia - I Part 1. *Mongolian Journal of Biological Sciences*. 20 (1), 3-28.
<https://doi.org/10.22353/mjbs.2022.20.01>
- Baasanmunkh, Sh., Urgamal, M., Oyuntsetseg, B., Sukhorukov, A.P., Tsegmed, Z., Son, D.C., Erst, A., **Oyundelger, Kh.**, Kechaykin, A.A., Norris, J., Kosachev, P., Ma, J-Sh., Chang, K.S. and Choi, H.J. 2022.. Flora of Mongolia: Annotated checklist of native vascular plants. *PhytoKeys*. 192, 63-169.
<https://doi.org/10.3897/phytokeys.192.79702>.
- Baasanmunkh, Sh., Oyuntsetseg, B., Tsegmed, Z., Urgamal, M., **Oyundelger, Kh.**, Nyambayar, N., Naymgerel, N., Andrey, E., Undruul, A. and Choi, H.J. (submitted). Distribution of vascular plants in Mongolia – II Part. *Mongolian Journal of Biological Sciences*.
- Oyundelger, Kh.**, Munkhzul, O., Ritz, C.M. and Wesche, K. (submitted) Long-term grazing exclusion effects populations genetics and functional traits of *Artemisia frigida* in Mongolia. *Journal of Arid Sciences*.

Confirmation

I herewith declare that I have produced this thesis, entitled with “Phylogeography and population genetics of key steppe species: *Artemisia frigida* Willd. (Anthemideae, Asteraceae)” without the prohibited assistance of third parties and without making use of aids other than those specified; notions taken over directly or indirectly from other sources have been identified as such. This thesis has not previously been presented in identical or similar form to any other German or foreign examination board.

The thesis/doctorate was supervised by Prof. Dr. Karsten Wesche and PD Dr. Christiane M. Ritz between June 2018 and April 2022 and hosted by Senckenberg Museum für Naturkunde Görlitz, Görlitz.

Place, date:

Signature: Oyundelger Khurelpurev