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GENETIC STRUCTURE AND PHYLOGEOGRAPHIC OF *TULIPA ILIENSIS*

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Abstract

Biogeographical barriers to gene flow are central to plant phylogeography. *Tulipa iliensis* are mainly distributed in Xinjiang in China. Field survey found that there were differences in plant phenotypes traits among different populations of *Tulipa iliensis* at different altitudes. However, few studies have investigated how altitude affects the genetic diversity of species that are distributed in this area. Due to slow evolution and conserved sequences, cpDNA is widely used in phylogenetic analysis, species identification, and species origin studies. Here we used chloroplast DNA fragments of *rbc*LaF-*rbc*L to examine genetic diversity and distribution patterns of 7 populations of *Tulipa iliensis*. The distribution pattern based on cpDNA data showed that the *rbc*LaF-*rbc*L fragment had 312 variable sites in sampling area, and a total of 6 haplotypes were obtained, among which haplotypes Hap-1 and Hap-2 were ancient haplotypes. The genetic diversity is rich and the variation mainly comes from within populations, but there is a certain gene flow between populations. There is no abvious phylogeographic structure and no recent expansion in the sampling area, and the population size remains stable. The analysis of suitable areas showed that Bio13 (Precipitation of Wettest Month) and Bio14 (Precipitation of Driest Month) were the key factors affecting the distribution of *Tulipa iliensis* in China. From the three paleoclimates, current, and future climate scenarios, *Tulipa iliensis* suitability the distribution range of suitable areas of *Tulipa iliensis* in China showed a shrinking trend and migrated to higher latitudes. This study lays a foundation for the subsequent research on the history of *Tulipa iliensis* pedigree, adaptive traits, and analysis of stress response mechanisms.

Key words: *Tulipa iliensis*, cpDNA-PCR, Genetic structure, Phylogeography, Altitudinal gradients.

Introduction

Tulipa L. of the lily family, Liliaceae is one of the world's most important ornamental plants (Neriya *et al.*, 2020). Wild tulip germplasm resources in China account for more than 10% of global resources (Qu *et al.*, 2016). *Tulipa iliensis* is an early spring short-lived plant with a single flower growing at the top, The outer petals are oblong and have green-purple, purple-green, or yellow-green colors on the back. The flowering stalk is 10-20 centimeters high and the plant has 3-4 leaves (Aysajia, 2013; Mei, 2006). *T. iliensis* has bright colors and strong ecological adaptability and is widely distributed in the deserts, grasslands, foothills, and low mountain slopes of northern Xinjiang, China, and Central Asia, often growing in large areas. Early spring blooms, while dormancy occurs in summer and winter, during which time floral bud differentiation is completed (Nie, 2015; Zhang *et al*, 2023). Xinjiang, China, located in the hinterland of the Eurasian continent, has a climate typical of arid zones. Its fragile ecosystem is highly sensitive to global climate change, it is the region with the widest distribution of deserts in China (Hao *et al.*, 2024; Dong *et al.*,2024). Desert grassland is the most widely distributed in Xinjiang. It is mainly composed of shrubs, perennial herbs and annual herbs, it is an important part of terrestrial ecosystem (Tang *et al.*, 2015; Chen *et al.*, 2021). *T. iliensis* is an important component of desert grassland vegetation in Xinjiang, under long-term natural selection, it has strong resistance (low temperature, drought, heat) and plays a vital role in the stability of regional ecosystems (Jiao *et al.*, 2015). Therefore, exploring the population evolutionary pattern, genetic differentiation, and evolutionary history of the *T. iliensis* is significant for utilizing and protecting its wild germplasm resources, the rational utilization of *T. iliensis* in arid areas, vegetation restoration and reconstruction of desert ecosystem.

Phylogeography, first proposed by Avise, is an emerging interdisciplinary subject that studies the principles and processes of phylogeographic distribution between or within closely related species. Since its emergence in the 1980s, the field of phylogeography has rapidly developed into a comprehensive scientific field linking micro and macro evolutionary processes (Avise *et al.*, 1987; Avise, 2000), it helps to infer processes of substitution, dispersal, speciation, and other population levels (Tsuji *et al.*, 2023). In recent years, with the deepening of molecular phylogeography in the study of the distribution, migration, and endangered mechanisms of endangered and rare plant populations (Lu *et al.*, 2021; Bobo-Pinilla *et al.*, 2022), and the biological control of alien invasive plant (Canavan *et al.*, 2021), this discipline has provided a comprehensive theoretical guidance for the study of plant population evolution, genetic differentiation, and evolutionary history (Zhang *et al.*, 2020; Singh *et al.*, 2021; Liu *et al.*, 2023;).

Chloroplasts (CPs) are the photosynthetic organelles of plants and play a crucial role in photosynthesis (Kim *et al.*, 2021; Yi *et al.*, 2022). The chloroplast genome is independent of the nuclear genome and possesses semi-autonomous genetic traits. Owing to its sluggish evolution and sequence conservation, the chloroplast genome is extensively utilized in phylogenetic analysis, species identification, and species origin studies (Zhai *et al.*, 2021; Li *et al.*, 2022; Zong *et al.*, 2023). DNA barcode sequences are effective tools for promoting rapid and extensive species identification. In chloroplast DNA (cpDNA), regions such as *rbc*L, *atp*F-H, *ndh*F, *mat*K, *trn*H-*psb*A, *rps*16-*trn*Q, *rpl*32-*trn*L, and *trn*L-F are considered as preferred barcodes (Sevindik *et al.*, 2024). Among these, the cpDNA *rbc*L gene sequence is frequently employed for

analyzing the origin, phylogeny, evolution, biogeography, population genetics, and systematics of plants (Chen *et al.*, 2022). cpDNA markers constitute an effective means for researching systematic geography and clarifying the migration and dispersal routes of species during refugia and post-glacial periods (Wang *et al.*, 2023).

In this study, the genetic structure, genetic diversity, and lineage distribution pattern of *T. iliensis* were analyzed based on the chloroplast gene fragments, to lay a foundation for the subsequent research on the history of *T. iliensis* lineage, adaptive traits, and analysis of stress response mechanism, and provide a theoretical reference for the conservation of wild germplasm resources of *T. iliensis*.

Material and Methods

Plant sampling collection: In May 2023, Field sample collection was carried out in Zhaosu County (81°0′11"E, 42°49′31"N) and Gongliu County (82°16′27"E 43°28′7" N) in Xinjiang, China, according to the different altitudes of the sampling site, it was divided into 7 populations (Table 1, Fig. 1), namely Gongliu County (QX1300, QX1400), Zhaosu County (ZS1600), and Kashagar Town of Zhaosu County (HT1700, XT1800), Wuzunbulak Town, Zhaosu County (MC1900, MC2000). The interval between individual in each population was more than 10 m, a total of 100 individuals, The well-growing tender leaves of each individual were numbered and placed in a sealed bag with color-changing silica gel for rapid drying and stored at -80°C for use.

DNA extraction, amplification, and sequencing: The individual genomic DNA was extracted by modified CTAB method (Luo, 2023), and the quality of the DNA was detected by 1.0 % agarose gel electrophoresis. The concentration of DNA was determined using nucleic acid protein analyzer (OD260/OD280 was 1.8 ~ 2.0).

We successfully sequenced chloroplast molecular markers that showed high levels of intraspecific variability (*rbc*L: GAAACGGTCTCTCCAACGCAT; *rbc*LaF: ATGTCACCACAAACAGAGACTAAAGC) (Hajdari *et al.*, 2021) in 100 individuals. Polymerase chain reaction (PCR) for all cpDNA fragments was performed in 25 μL volumes, containing 1.4 mmol/L primer, 0.3 mmol/L dNTPs, 1.5 mmol/L Mg^{2+}, 50 ng template DNA, 0.75 U *Taq* DNA polymerase. The PCR reaction program consisted of an initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 90 s, and a final extension at 72 ℃ for 7 min, with a final hold at 4 ℃. The PCR products were checked on 1 % agarose gels and then sent to Yangling Tianrun Aoke Biotechnology Co., Ltd. (Shanxi, China) for bidirectional sequencing.

Analysis of haploid distribution and genetic diversity: Individuals of *Tulipa iliensis* from seven populations were subjected to cpDNA-PCR amplification. The sequencing results were compared using Blast on NCBI (https://www.ncbi. nlm.nih.gov/) to determine sequence similarity. Sequence alignment and correction were performed using Chromas software (http://technelysium. com.an/). MEGA 11.0 software (Version 11, https://www. megasoftware. net/) was utilized to analyze sequence composition and detect variable sites. DnaSP5.10 software (Librado and Rozas, 2009) was used to define haplotypes and their quantities, as well as to calculate haplotype diversity (H_d), nucleotide diversity (P_i), average nucleotide differences (K), polymorphic segregating sites (S), and gene flow (N_m) within the populations.

Population genetic structure: The Permut 2.0 software (Pons and Petit, 1996) was used to calculate the genetic differentiation coefficient G_{ST}, N_{ST} values (1000 random permutation tests), overall genetic diversity (H_T), and average genetic diversity within populations (Hs). The MEGA 11.0 software was used to calculate genetic distances between different populations to assess their genetic relationships. The Arlequin 3.5 software (Excoffier and Lischer, 2010) was used for molecular variance analysis (AMOVA) to calculate the distribution of variance within and between populations, as well as genetic differentiation indices (F_{ST}).

Phylogenetic analysis: MEGA 11.0 software used the maximum likelihood method to construct haplotype phylogenetic trees of different populations of *T. iliensis*, and Network 5.0 software (Bandelt *et al.*, 1999) was used to construct haplotype network diagrams to analyze the phylogenetic relationships of *T. iliensis*.

Population historical dynamics: The DnaSP5.10 software was used to calculate Tajima's D, Fu and Li's D*, and Fu and Li's F* values for neutral testing analysis. Combining neutral testing with nucleotide mismatch distribution maps, a population historical dynamic analysis is conducted for the *T. iliensis* population.

Ecological niche simulation: Geographical distribution data: The natural distribution data of *T. iliensis* was sourced from the Chinese Virtual Herbarium (https://www. cvh.ac.cn), National Specimen Information Infrastructure (https://www.nsii. org.cn), Global Biodiversity Information Facility (https://www.gbif.org), as well as field investigation sampling records and relevant literature records. A total of 71 valid distribution points of *T. iliensis* were obtained.

Climate variable data: 19 climate variables were selected for predicting the potential probability distribution of the *T. iliensis* (Table S1). Paleoclimate (Last-inter-Glacial (LIG), Last Glacial Maximum (LGM), Mid-Holocene (MH)) climate variable data were obtained from the WorldClim1.4 global climate database, with spatial resolutions of 30″; recent (1970-2000) and future (2041-2060 (2050s)) SSP585 climate variable data were obtained from the WorldClim2.0 global climate database (https://www.worldclim.org), Spatial resolutions are 2.5′. The global climate model (GCM) used is BCC-CSM2-MR (Wu *et al.*, 2019). The coordinate system, layer boundaries, resolutions, and grid sizes of climate variables for different periods are standardized. Pearson correlation analysis on 19 environmental variables using ENMTools1.0.4 R package was performed (Warren *et al.*, 2021), retaining only a group of environmental variables with correlation coefficients absolute value less than 0.80 and clear ecological significance for subsequent MaxEnt3.4.4 model (Norberto *et al.*, 2023) analysis.

Table 1. Sampling sites of *Tulipa iliensis* in China.

Number	Altitude	Code	Quantity	Sample location	Longitude (E)	Latitude (N)
1	1386m-1395m	QX1300	18	Jilgelang Town, Gongliu County, China	82°36′60″E	43°9′81″N
2	1401m-1409m	QX1400	8		82°37′57″E	43°9′88″N
3	1694m-1700m	ZS1600	31	Kazhagar Town, Zhaosu County, China	81°6′07″E	42°54′21″N
4	1728m-1740m	HT1700	12	Kashagar Town, Zhaosu County, China	81°5′35″E	42°52′75″N
5	1879m-1886m	XT1800	9	Kashagar Town, Zhaosu County, China	81°1′28″E	43°46′32″N
6	1933m-1976m	MC1900	15	Wuzunbulak Town, Zhaosu County, China	81°3′11″E	42°44′17″N
7	2039m-2073m	MC2000	7	Wuzunbulak Town, Zhaosu County, China	81°3′15″E	42°44′16″N

Table S1. Climate variables used for modeling climatic niches.

Variable abbreviation	Variable description	Unit	Variable abbreviation	Variable description	Unit
Bio1	Annual Mean Temperature	℃	Bio11	Mean Temperature of Coldest Quarter	℃
Bio2	Mean Diurnal Range	℃	Bio12	Annual Precipitation	mm
Bio3	Isothermality	-	Bio13	Precipitation of Wettest Month	mm
Bio4	Temperature Seasonality	-	Bio14	Precipitation of Driest Month	mm
Bio5	Max Temperature of Warmest Month	℃	Bio15	Precipitation Seasonality	-
Bio6	Min Temperature of Coldest Month	℃	Bio16	Precipitation of Wettest Quarter	mm
Bio7	Temperature Annual Range	℃	Bio17	Precipitation of Driest Quarter	mm
Bio8	Mean Temperature of Wettest Quarter	℃	Bio18	Precipitation of Warmest Quarter	mm
Bio9	Mean Temperature of Driest Quarter	℃	Bio19	Precipitation of Coldest Quarter	mm
Bio10	Mean Temperature of Warmest Quarter	℃			

Table 2. Genetic parameters of 7 populations of *Tulipa iliensis* based on cpDNA fragments.

Code	Population	Haplotype composition (frequency, %)		H_d	P_i	K	S
1	QX1300	Hap-1 (33.33) Hap-2 (66.67)		0.47059	0.25518	144.94118	308
2	QX1400	Hap-1 (37.5; Hap-2 (50; Hap-3 (12.5)		0.67857	0.29118	165.39286	309
3	ZS1600	Hap-1 (93.5; Hap-2 (3.23; Hap-4 (3.23)		0.12688	0.03399	19.93118	308
4	HT1700	Hap-1 (83.33; Hap-2 (16.64)		0.30303	0.16432	93.33333	308
5	XT1800	Hap-1 (22.22; Hap-2 (66.66; Hap-5 (11.12)		0.05556	0.21176	120.27778	309
6	MC1900	Hap-1 (86.66; Hap-2 (6.66; Hap-4 (6.68)		0.25714	0.0725	41.18095	309
7	MC2000	Hap-1 (71.42; Hap-4 (14.29; Hap-6 (14.29)		0.52381	0.00151	125.54667	308
			Average	0.4735	0.22103	125.547	312

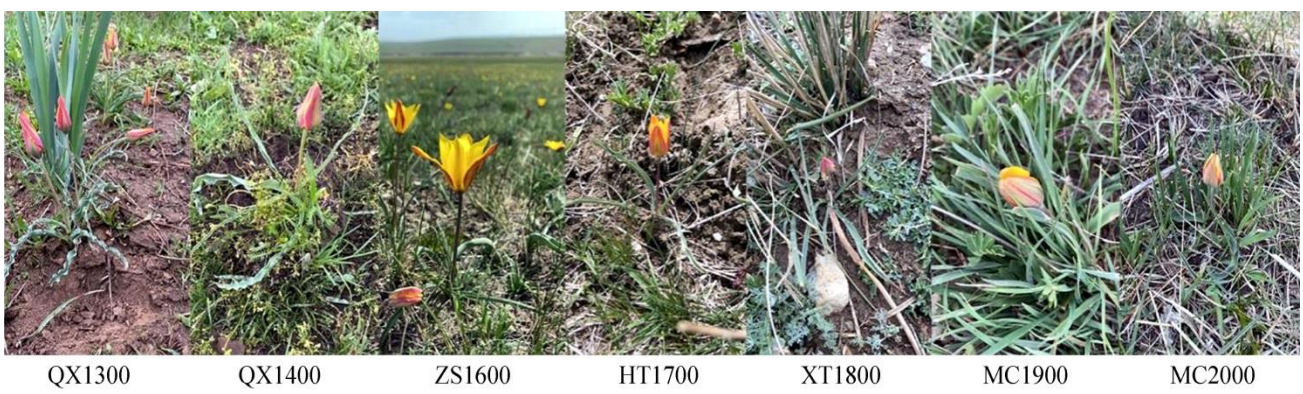

Fig. 1. Habitats of *Tulipa iliensis* in China.

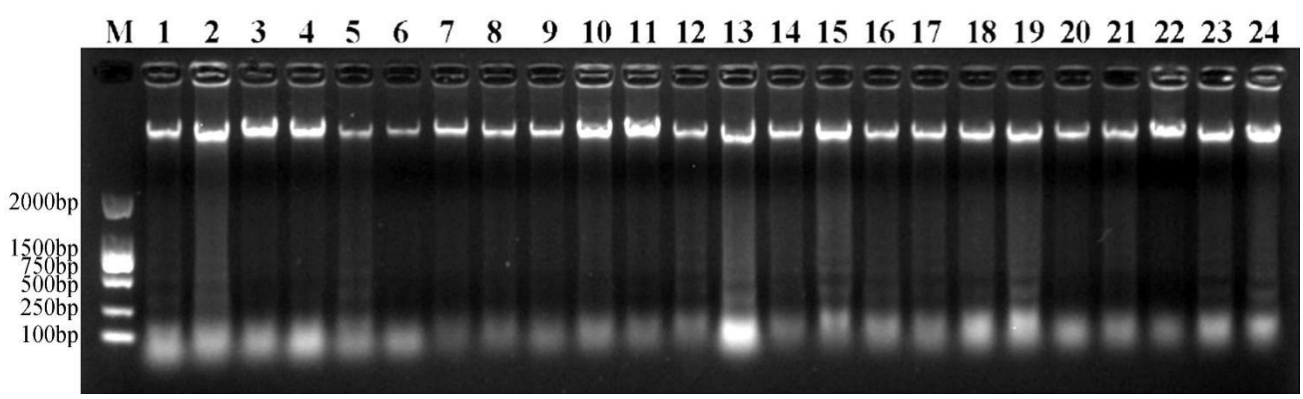

Fig. 2. Quality detection of *Tulipa iliensis* DNA agarose gel electrophoresis. (Note: M: DL2000 DNA marker; 1-24: some individual of *T. iliensis*).

Results and Analysis

DNA quality assessment: The total DNA of *T. iliensis* individuals tested was complete, the electrophoresis bands were correct, clear and bright (Fig. 2), and the extracted DNA purity was detected by OD260/OD280, the values were between 1.8 and 2.0, which could meet the requirements of DNA quality for subsequent cpDNA molecular marker analysis.

Analysis of haploid distribution and genetic diversity: The primer *rbc*LaF-*rbc*L was used to amplify 100 individuals from 7 populations of *T. iliensis*, and the average length of the fragment was 614 bp, including 256 conserved sites, 312 mutation sites (4 single mutation loci) and 308 simple information sites, the results showed that the fragment was well selected and suitable for the study of the genetic structure and genealogical geography of *T. iliensis* populations. In the cpDNA gene sequence, the average content of A+T was 57.4%, and the average content of G+C was 42.6%.

The analysis of cpDNA types in different populations of *T. iliensis* showed that there were 6 haplotypes in cpDNA types and the distribution in each population was different (Table 4). Among them, haplotypes Hap-1, Hap-2, and Hap-4 belonged to the shared haplotypes, accounting for 97.03%. Hap-1 had the highest frequency (68.32%) and was distributed in 7 populations ZS1600 (93.5%), MC1900 (86.66%), HT1700 (83.33%), MC2000 (71.42%), QX1400 (37.5%), QX1300 (33.33%), and XT1800 (22.22%). However, the distribution frequency was different in different populations. The second haplotype Hap-2 was distributed in the other 6 populations except for MC2000, and the highest distribution frequency was in QX1300 (66.67%) and XT1800 (66.66%). Hap-4 appeared in population ZS1600 (3.23%), population MC1900 (3.68%), and population MC2000 (14.29%), but the distribution frequency was not high. Hap-3, Hap-5, and Hap-6 were endemic haplotypes, which were distributed in population QX1400 (12.5%), population MC1900 (6.68%), and population QX1300 (14.29%), respectively. In different populations, the haplotype type is the same, but the distribution frequency is different. For instance, both Hap-1 and Hap-2 were distributed in population QX1300 and population HT1700, but the distribution frequency of Hap-1 in population HT1700 (83.33%) was much higher than that in population QX1300 (33.33%). Hap-1, Hap-2, and Hap-4 were distributed in both ZS1600 and MC1900, but the distribution frequency of Hap-1 was much higher than that of Hap-2 and Hap-4 in the two populations.

The genetic diversity analysis of 7 populations of *T. iliensis* showed that: at the population level, the total haplotype diversity (H_d) of *T. iliensis* is 0.47350, ranging from 0.05556 to 0.67857. Among them, the haplotype diversity of population QX1400 was the highest (0.67857), followed by population MC2000 (0.52381), and the haplotype diversity of population XT1800 was the lowest (0.05556). The total nucleotide diversity (Pi) was 0.22103, ranging from 0.00151 to 0.29118, the nucleotide diversity of QX1400 was the highest (Pi = 0.29118) and the nucleotide diversity of MC2000 was the lowest (Pi = 0.00151). The total average nucleotide difference index K of the population of *T. iliensis* was 125.54700, ranging from 19.93118 to 165.39286, the average nucleotide different index of population QX1400 (K = 165.39286) was the largest, and population of MC1900 (K = 41.18095) was the lowest (Table 2).

Population genetic structure analysis: In the study of phylogeography, Nst and Gst are commonly used to determine whether there is phylogenetic structure in species, G_{ST} represents the frequency of haplotypes, and the Nst represents the similarity between different haplotypes, if the N_{ST} value is greater than the G_{ST} value ($P < 0.05$), it indicates that there is an obvious phylogeographic structure between the populations. The total genetic diversity H_T of *T. iliensis* is 0.640, with an average genetic diversity within populations H_S of 0.473. The genetic differentiation coefficient N_{ST} and G_{ST} were 0.176 and 0.260 ($N_{ST} < G_{ST}$) respectively. The U test showed that $p<0.05$, indicating that there was no obvious phylogeographic structure among the populations of *T. iliensis* in the sampling area.

F_{ST} is the genetic differentiation coefficient, which can reflect the genetic differentiation level of the population, when the value of F_{ST} is 0 ~ 0.05, it indicates that the genetic differentiation is low, when the F_{ST} value is 0.05~0.25, it indicates a moderate degree of genetic differentiation, and when the F_{ST} value is greater than 0.25, it represents a significant level of genetic differentiation. In this study, the F_{ST} was 0.46632 ($F_{ST} > 0.25$, $p<0.01$), indicating that the degree of population differentiation among *T. iliensis* populations was significant. The genetic variation among populations was 46.63%, and the variation within populations was 53.37%, the coefficient of variation within populations was greater than that between populations, indicating that the variation of *T. iliensis* mainly came from the variation within populations (Table 3).

The genetic distances between different populations are different, and the genetic relationship is different. Among them, the genetic distance between ZS1600 and XT1800 was the highest (0.80359), and the genetic relationship was the farthest. The genetic distance between QX1300 and QX1400 was the lowest, at -0.09468, and the genetic relationship was the closest (Table 4).

Phylogenetic analysis: The phylogenetic tree can clearly show the phylogenetic relationship of different haplotypes. In the Neighbour-Joining tree (Fig. 3), the 6 haplotypes of *T. iliensis* and the outgroup *Lilium apertum* were clustered into one group, respectively. Among them, the 6 haplotypes of *T. iliensis* differentiated into two obvious lineages and the support rate was more than 87%, and Hap-2, Hap-3, and Hap-5 are one branch, located at the bottom of the phylogenetic tree, and the genetic backgrounds is similar. Hap-1, Hap-4, and Hap-6 are branches, indicating that they have similar genetic background.

In the constructed central network connection diagram of *T. iliensis*, the 6 haplotypes were mainly divided into two groups, among them, haplotypes Hap-1 and Hap-2 were located at the center of the central network connection diagram, which were ancient haplotypes, haplotypes Hap-3, Hap-4, Hap-5, and Hap-6 evolved from Hap-1 and Hap-2 (Fig. 4), the central network connection diagram was consistent with the results of the above phylogenetic tree.

Table 3. Analysis of AMOVA based on cpDNA fragments of *Tulipa iliensis* populations.

Groups	Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation	Fixation index
All	Among populations	6	2819.435	31.89885	46.63	F_{ST}=0.46632
	Within populations	93	3395.125	36.50672	53.37	
	Total	99	6214.560	68.11891		

Table 4. Genetic distance analysis of different populations.

Population	Among populations						
	QX1300	QX1400	ZS1600	HT1700	XT1800	MC1900	MC2000
QX1300	0.00000						
QX1400	-0.09468	0.00000					
ZS1600	0.65014	0.68752	0.00000				
HT1700	0.35409	0.30010	0.07616	0.00000			
XT1800	-0.05886	-0.07173	0.80359	0.50001	0.00000		
MC1900	0.51658	0.51585	-0.03699	-0.02787	0.68089	0.00000	
MC2000	0.54017	0.54735	-0.07030	0.02732	0.71941	-0.05982	0.00000

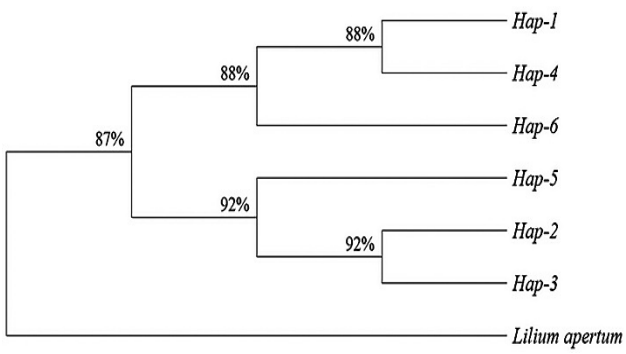

Fig. 3. Neighbour-Joining tree of haplotypes of *T. iliensis* based on cpDNA fragments.

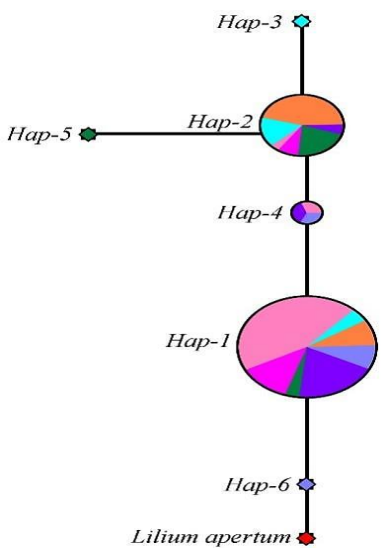

Fig. 4. Median-joining network of haplotypes of *Tulipa iliensis* based on cpDNA fragments.

Population historical dynamics analysis: In order to verify whether the *T. iliensis* has experienced recent expansion in the sampling area, Tajima's D and mismatch analysis were performed on 100 cpDNA sequences of 7 populations of *T. iliensis*.

Tajima's D = 3.58042, (p<0.001), which was positive, indicating that the evolutionary pattern of *T. iliensis* in the sampling area was balanced selection, but there was some haplotype differentiation, Fu and Li's D*=2.38030 (p<0.02), Fu and Li's F*=3.48749 (p<0.02) both of which were positive, which supported that the population of *T. iliensis* was controlled by balanced selection in the sampling area, did not experience recent expansion, and the population size remained stable.

The mismatch analysis of cpDNA fragments in different populations of *T. iliensis* (Fig. 5) showed that the mismatch distribution curves of *T. iliensis* was a bimodal curve, indicating that the populations of *T. iliensis* was in a dynamic equilibrium in the sampling area and did not experience recent expansion events, which was consistent with the results of Tajima's D test.

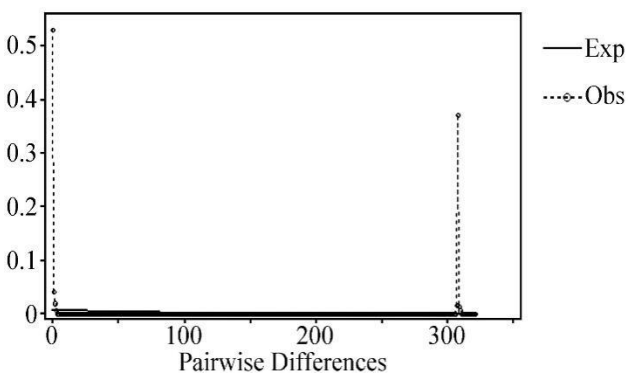

Fig. 5. Mismatch distribution of populations of *Tulipa iliensis* based on cpDNA sequence.

Suitable habitat analysis: Based on the contribution rate of different climatic variables to the suitable distribution area of *T. iliensis* (Table S2) and Pearson correlation analysis (Fig. S1), Bio13 (Precipitation of Wettest Month), Bio14 (Precipitation of Driest Month), Bio15 (Precipitation Seasonality), Bio4 (Temperature Seasonality), Bio16 (Precipitation of Wettest Quarter), and Bio9 (Mean Temperature of Driest Quarter), a total of 6 environmental variables were used for modeling.

The prediction accuracy verification results of MaxEnt3.4.4 modeling (Fig. S2) showed that the value of AUC is 0.979, according to the evaluation criteria, the prediction results of the model were accurate.

The analysis of the contribution rate of different climatic variables to the suitable distribution area of *T. iliensis* showed that Bio13 (precipitation in the wettest month) and Bio14 (precipitation in the driest month), the contribution rates were 33.3% and 26.2, respectively (Table S2).

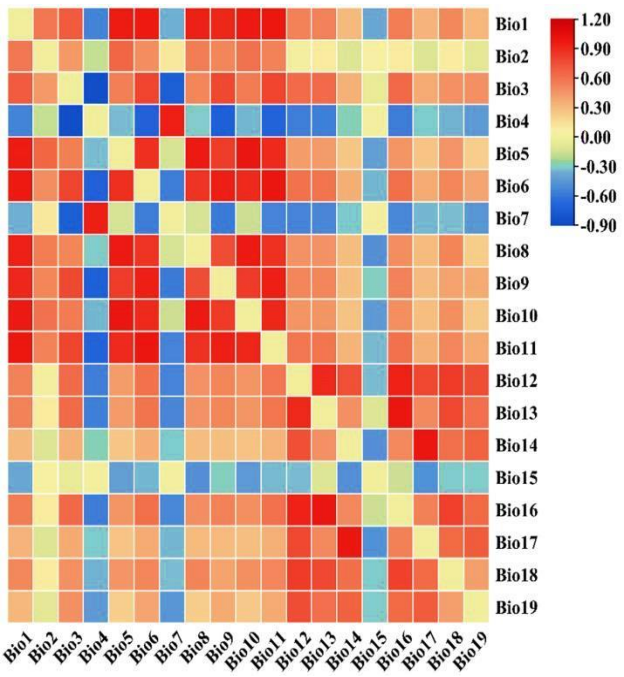

Fig. S1. Correlation analysis of 19 climate factors.

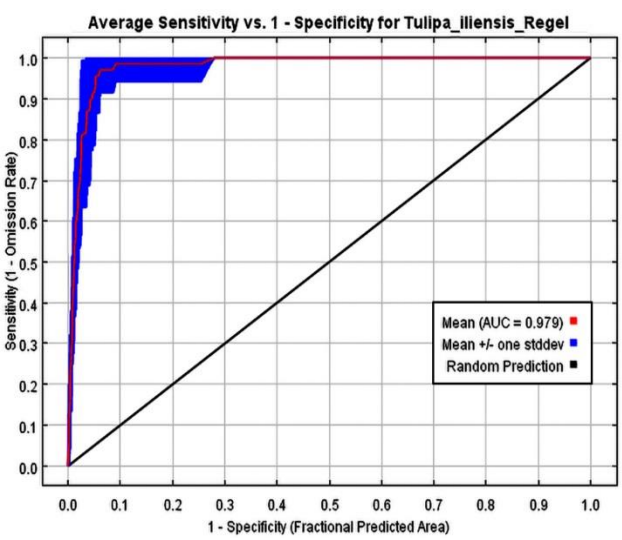

Fig. S2. ROC curve of MaxEnt model.

The suitable areas of *T. iliensis* in China were predicted under five climate scenarios of three paleoclimates, current and future (Fig. 6). Under the three paleoclimates scenarios of LIG (Fig. 6a), LGM (Fig. 6b) and MH (Fig.6c), the suitable area of *T. iliensis* is mainly distributed in the northern region, and the distribution range has no obvious expansion and contraction. Compared with the three paleoclimates, in the current climate scenario, the distribution range of *T. iliensis* showed a significant reduction trend, mainly concentrated in the northern part of Xinjiang, China (north of the Tianshan Mountains in Xinjiang and a small amount in the northern part of the Kunlun Mountains in China). Under the SSP585 climate scenario in the future of 2050, compared with the area of modern suitable areas, there was a small expansion in Inner Mongolia and Heilongjiang Province in China, and the overall distribution range remained stable.

Table S2. The relative contributions (%) of variables to the *Tulipa iliensis* results in the MaxEnt model0.

Variable	Percent contribution (%)	Permutation importance (%)
Bio13	33.3	57.4
Bio14	26.2	8.1
Bio15	13.2	0.8
Bio4	10.1	1.2
Bio16	5.5	0.6
Bio9	4.1	10.3
Bio7	3.2	0.1
Bio3	1.1	6.5
Bio12	0.9	7.1
Bio19	0.7	1.1
Bio2	0.7	0
Bio1	0.6	5.5
Bio5	0.1	0
Bio18	0.1	0
Bio6	0.1	0.9
Bio11	0.1	0.1
Bio8	0	0.1
Bio10	0	0
Bio17	0	0

Discussion

Genetic diversity of *Tulipa iliensis* population: The evaluation of the genetic diversity of wild tulip germplasm resources is of great significance for the development of wild resources for introduction, domestication and the development of tulip hybrid breeding. The Research on wild tulip germplasm resources mainly focuses on environmental adaptation (Bilias *et al.*, 2023), seed germination (Hatzilazarou *et al.*, 2023), interspecific hybridization (Qu *et al.*, 2018, Xing *et al.*, 2020), In the study of genetic diversity of wild tulips, Kiani and his colleagues in 39 wild tulip individuals in Iran considered that the genetic diversity of wild individuals did not necessarily match the morphological differences between tulip species (Kiani *et al.*, 2012, Tang *et al.* 2013) obtained the genetic diversity of different populations in 72 tulip cultivars by SNP molecular markers, among which the genetic diversity of Darwin hybrid type was the highest. Pourkhaloee and colleagues used EST–SSR molecular markers to evaluate the genetic diversity of 36 wild individuals and 244 cultivated individuals from Iran and the Netherlands and concluded that wild tulips had higher genetic diversity (Pourkhaloee *et al.*, 2018). *T. iliensis*, due to its good ornamental value and ecological adaptability has high development potential in China (Xing *et al.*, 2017), the evaluation of genetic diversity can provide accurate genetic background information for the follow-up research of the *T. iliensis*. Khaleghi and colleagues, who conducted research on 6 wild tulip species in Iran, concluded that there is huge variation in wild tulip resources among and within species (Khaleghi *et al.*, 2018), so wild germplasm resources play a very important role in tulip breeding programs.

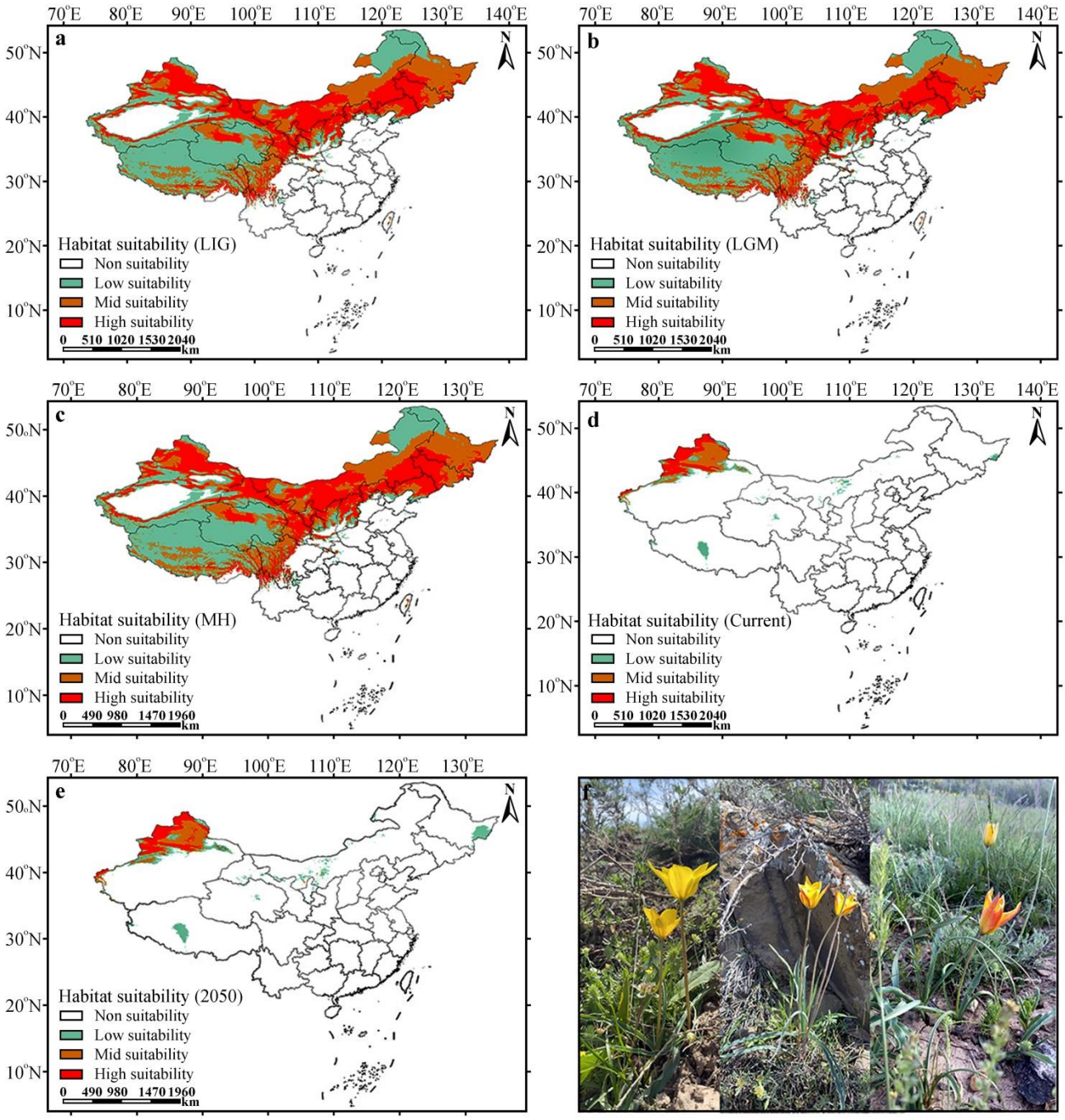

Fig. 6. Distribution of suitable areas for *Tulipa iliensis* under different climate scenarios: a, last-inter-glacial; b, last-glacial-maximum; c, mid-holocene; d, current (1970-2000); e, future (2050s); f, photos of *T. iliensis*

In this study, the population of *T. iliensis* distributed in China were sampled at natural distribution sites. The evaluation of genetic diversity of different populations also showed that *T. iliensis* had rich genetic diversity in the distribution area, and the genetic diversity of populations was closely related to their living environment, many factors can directly or indirectly affect the genetic diversity of species. Xinjiang, China, has a vast territory and different geographical environment types, while *T. iliensis* has a wide distribution range in Xinjiang. In the process of field investigation, it was found that there were differences in plant phenotype, bulb size and other morphological aspects of *T. iliensis* under different habitat conditions, indicating that there were genetic and morphological variations among populations in different geographical environments. The total genetic diversity $H_T = 0.640$, and the total haplotype diversity $H_d = 0.473$, the higher genetic diversity proves that the widely distributed taxa have higher genetic diversity than the narrow-range taxa.

Population genetic structure: Genetic diversity is closely related to population genetic structure, population adaptability, evolution, and survival viability, protecting genetic diversity and population genetic structure is very important for maintaining biodiversity and ecosystem stability (Yan *et al.*, 2016), and different species have differences genetic structures. For example, the variation within populations (68.91%) of *Lilium pumilum* distributed

in the southeastern Tibetan Plateau was greater than that among populations (31.09%), indicating that there was a certain gene exchange between different wild populations of *L. pumilum*, but the gene exchange was not frequent (Jiang, 2017). However, the genetic variation among populations (68.98%) was greater than that within populations (31.02%) of *Xanthopappus subacaulis*, which was also distributed in the northeast of Tibetan Plateau, the frequent gene exchange among populations indicated that the genetic variation of *X. subacaulis* mainly comes from among populations (Zhang *et al.*, 2022). *T. ilienisis*, the genetic variation within populations (53.03%) was greater than that among populations (46.97%), and the gene flow among populations was Nm = 0.33, indicating that there was a certain but not frequent gene exchange among different populations, which was speculated to be related to the original habitat of *T. ilienisis* in China. *T. ilienisis* is mainly distributed in the piedmont plain and low mountain slopes of Xinjiang, because there is no obvious geographical barrier to its large-scale distribution, and the flowers of *T. ilienisis* are terminal, the bright flower color attracts insect to pollinate, considering that pollen diffusion may be the main source of gene flow, if gene flow is blocked, resulting in less gene exchange between populations. At the same time, as a perennial bulbous plant, the *T. ilienisis* mainly propagates through seeds and bulbs in the underground part, which is also one of the reasons for the increase of genetic variation within the population.

Suitable habitat analysis: Climate fluctuations is the main factors affecting the geographical distribution patterns of species (Hewitt *et al.*, 2004, Qiu *et al.*, 2011), species respond to continuous climate change by changing their characteristics and physiological activities, which in turn leads to changes in their geographical distribution range, community composition and pattern (Parmesan and Yohe, 2003). The results showed that climate warming would lead to the migration of plant species to higher altitudes and latitudes (Li, 2022), compared with the distribution area of *T. iliensis* suitable areas under the three paleoclimate scenarios, the distribution area of *T. iliensis* suitable areas decreased sharply under the current climate scenarios, it is speculated that great changes in the current climatic scenario may have led to changes in the living environment of the original suitable distribution area of *T. iliensis*, resulting in a reduction in the distribution area. With global warming, most plants have predicted that the distribution of suitable areas will migrate to higher latitudes in the future, such as *Pinus yunnanensis*, *Cryptomeria fortunei* (Ouyang, 2022), *Acer cordatum* Pax, etc. (Liu *et al.*, 2022).

In this study, with the continuous occurrence of climate change, the distribution range of *T. iliensis* also showed a decrease from low latitudes to high latitudes in the prediction of future suitable areas. As an important part of desert vegetation, *T. iliensis* population may choose areas with more suitable temperature and precipitation under the condition of continuous global warming. Tulips belong to the long-day flowers, especially the vegetative growth period requires adequate water (Wang, 2021), However, the increasing high temperature will aggravate the occurrence of drought, desertification and soil erosion, aggravate environmental degradation, make the habitat no longer suitable, thus reducing its suitable area. Global warming has led to the melting of glaciers providing a large amount of water for plants and form new habitats (You, 2018). Studies have shown that global warming will promote the migration of species to cooler areas and expand to higher latitudes and altitudes (Yang *et al.*, 2024). Tulipa L. prefers warm and humid climate in winter and cool and slightly dry climate in summer. The suitable temperature for growth period is 8 ~ 20°C (Wang, 2020), at higher latitudes, the temperature is lower, so the *T. iliensis* show a tendency to migrate from low latitudes to high latitudes, it shows that there are still great challenges in the survival and reproduction of *T. iliensis* under the background of climate change. Therefore, combined with the distribution dynamics of *T. iliensis*, it is necessary to carry out long-term climate monitoring in the key distribution areas, so as to timely assess the impact of climate change on the growth of *T. iliensis*, and take corresponding protection measures in advance for the reasonable protection of *T. iliensis*.

References

Avise, J.C. 2000. Phylogeography: The history and formation of species. Harvard University Press.

Avise, J.C., J. Arnold, R. Ball, E. Bermingham, T. Lamb, J. Neigel, C. Reeb and N.C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.*, 18: 489-522.

Aysajia, A. 2013. Reproductive biology of *Tulipa iliensis* and its adaptive strategies to the early spring environment. Xinjiang Agricultural University, Urumqi, China.

Bandelt, H.J., P. Forster and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, 16(1): 37-48.

Bilias, F., A.G. Karagianni, I. Ipsilantis, I. Samartza, N. Krigas, G. Tsoktouridis and T. Matsi. 2023. Adaptability of wild-growing tulips of Greece: Uncovering relationships between soil properties, rhizosphere fungal morphotypes and nutrient content profiles. *Biol.*, 12: 605.

Bobo-Pinilla, J., E. Salmerón-Sánchez, A.J. Mendoza-Fernández, J.F. Mota and J. Peñas. 2022. Conservation and phylogeography of plants: from the Mediterranean to the rest of the world. *Diversity*, 14: 78.

Canavan, K., N.L. Magengelele, I.D. Paterson, D.A. Williams and G.D. Martin. 2021. Uncovering the phylogeography of *Schinus terebinthifolia* in South Africa to guide biological control. *AoB Pl.*, 14(1): plab078. http://www.doi.org/10.1093/aobpla/plab078.

Chen, C., C.Q. Jing, W.Y. Xing, X.J. Deng, H.Y. Fu and W.Z. Guo. 2021. Desert grassland dynamics in the last 20 years and its response to climate change in Xinjiang. *Acta Prataculturae Sinica.*, 30(03): 1-14.

Chen, X., L. Tian, J. Tian, G. Wang, X. Gong, S. Feng and A. Wei. 2022. Extensive sampling provides new insights into phylogenetic relationships between wild and domesticated *Zanthoxylum* species in China. *Horticult.*, 8: 440.

Dong, D.W., H. Tao, Z.X. Zhang and S.K. Mondal. 2024. Projected heatwaves in Xinjiang Uygur autonomous region, China. *Front. Earth Sci.*, 12: 1286012.

Excoffier, L. and H.E. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.*, 10(3): 564-7.

Hajdari, A., B. Pulaj, C. Schmiderer, X. Mala, B. Wilson, K. Lluga-Rizani and B.E. Mustafa. 2021. A phylogenetic analysis of the wild *Tulipa* species (liliaceae) of Kosovo based on plastid and nuclear DNA sequence. *Adv. Genet.*, 2(3): e202100016. https://www.doi.org/10.1002/ ggn2. 202100016.

Hao, H.C., J.Q. Yao, Y.N. Chen, J.H. Xu, Z. Li, W.L. Duan, S. Ismail and G.L. Wang. 2024. Ecological transitions in Xinjiang, China: Unraveling the impact of climate change on vegetation dynamics (1990-2020). *J. Geogr. Sci.*, 34: 1039-1064.

Hatzilazarou, S., E. Pipinis, S. Kostas, R. Stagiopoulou, K. Gitsa, E. Dariotis, M. Avramakis, I. Samartza, I. Plastiras, E. Kriemadi, P. Bareka, C. Lykas, G. Tsoktouridis and N. Krigas. 2023. Influence of temperature on seed germination of five wild-growing *Tulipa* species of Greece associated with their ecological profiles: implications for conservation and cultivation. *Plant.*, 12: 1574.

Hewitt, G.M. 2004. Genetic consequences of climatic oscillations in the quaternary. philosophical transactions of the Royal Society of London. Series B. *Biol. Sci.*, 359(1442): 183-195.

Jiang, F.J. 2017. Phylogeography of *Lilium Pumilum Redouté* in Southeast of Qinghai-Tibetan Plateau. Qinghai University, Xining, China.

Jiao, F., Q. Liu, G.F. Sun, Q.W. Lin, X.D. Li and J.Z. Zhang. 2015. Study on the germination traits of two kinds of wild tulip seeds in Sinkiang. *North Hort.*, 2: 55-60.

Khaleghi, A., A. Khadivi and B.J.M. Zonneveld. 2018. Morphological variations among and within species of wild tulip (*Tulipa* L.) from Iran. *Genet. Resour. Crop Evol.*, 65: 2241-2266.

Kiani, M., F. Memariani and H. Zarghami. 2012. Molecular analysis of species of *Tulipa* L. from Iran based on ISSR markers. *Pl. Syst. Evol.*, 298: 1515-1522.

Kim, S.C., J.M. Lee and B.K. Choi. 2021. Seven complete chloroplast genomes from *Symplocos*: genome organization and comparative analysis. *Forest.*, 12(5): 608.

Li, Y., L. Zhang, T. Wang, C. Zhang, R. Wang, D. Zhang, Y. Xie, N. Zhou, W. Wang, H. Zhang, B. Hu, W. Li, Q. Zhao, L. Wang and X. Wu. 2022. The complete chloroplast genome sequences of three lilies: genome structure, comparative genomic and phylogenetic analyses. *J. Pl. Res.*, 135: 723-737.

Li, Y., W. Shao, S. Huang, Y.Z. Zhang, H.F. Fang and J.G. Jiang. 2022. Prediction of Suitable Habitats for *Sapindus delavayi* based on the MaxEnt Model. *Forest.*, 13(10): 1611.

Librado, P and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformat.*, 25(11): 1451-1452.

Liu M.L., H.Y. Sun X. Jiang T. Zhou, Q.J. Zhang, Z.D. Su, Y.N. Zhang, J.N. Liu and Z.H. Li. 2022. Simulation and prediction of the potential geographical distribution of *Acer cordatum* Pax in different climate scenarios. *Forest.*, 13(9): 1380.

Liu, G., G. Xue, T. Zhao, Y. Li L.G. Yue, H.X. Song and Q.L. Liu. 2023. Population structure and phylogeography of three closely related tree peonies. *Ecol. Evol.*, 13(6): e10073.

Lu, M., H.S. Zhang and H.M. An. 2021 Chloroplast DNA-based genetic variation of *Rosa roxburghii* in Southwest China: Phylogeography and conservation implications. *Hort. Pl. J.*, 7(4): 9.

Luo, Z., Z. Yao, Y. Yang, Z. Wang, H. Zou, X. Zhang, J. Chen, B. Fang and L. Huang. 2023. Genetic fingerprint construction and genetic diversity analysis of sweet potato (*Ipomoea batatas*) germplasm resources. *BMC Pl. Biol.*, 23: 355.

Mei, L.J. 2006. Phenological characteristic of *Tulipa* from China and its morphological differentiation of populations. Xinjiang Agricultural University, Urumqi, China.

Neriya, Y., T. Morikawa, K. Hamamoto, K. Noguchi, T. Kobayashi, T. Suzuki, H. Nishigawa and T. Natsuaki. 2020. Characterization of tulip streak virus, a novel virus associated with the family Phenuiviridae. *J. Gen. Virol.*, 102(2): 001525.

Nie, X.X. 2015. Studies on biological characteristics of *Tulipa iliensis* and introduction experiment of *Tulipa gesneriana*. Xinjiang Agricultural University, Urumqi, China.

Norberto, M., N. Sillero, J. Coimbra and M. Cunha. 2023. Filling the maize yield gap based on precision agriculture-a MaxEnt approach. *Elect. Agric.*, 211: 107970.

Ouyang, X., H. Lin, S. Bai, J. Chen and A. Chen. 2022. Simulation the potential distribution of *Dendrolimus houi* and its hosts, *Pinus yunnanensis* and *Cryptomeria fortunei*, under climate change in China. *Front. Pl. Sci.*, 13: 1054710.

Parmesan, C and G.A. Yohe. 2003. Globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421: 37-42.

Pons, O. and R.J. Petit. 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genet.*, 144(3): 1237-1245.

Pourkhaloee, A., M. Khosh-Khui, P. Arens, H. Salehi, H. Razi, A. Niazi, A. Afsharifar and J.V. Tuyl. 2018. Molecular analysis of genetic diversity, population structure, and phylogeny of wild and cultivated tulips (*Tulipa* L.) by genic microsatellites. *Hort. Environ. Biotechnol.*, 59: 875-888.

Qiu, Y.X, C.X Fu and H.P. Comes. 2011. Plant molecular phylogeography in China and adjacent regions: Tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. *Mol. Phylogen. Evol.*, 59(1): 225-244.

Qu, L.W., J.J. Lei, Y.Q. Zhang, G.M. Xing and J.W. Su. 2016. The present situation, existing problems and development strategies of Chinese tulip research. *North Hort.*, (11): 188-194.

Qu, L.W., L. Xue, G.M. Xing, Y.Q. Zhang, J.J. Chen, W. Zhang and J.J. Lei. 2018. Karyotype analysis of eight wild *Tulipa* species native to China and the interspecific hybridization with tulip cultivars. *Euphytica.*, 214: 65.

Sevindik, E., Y. Korkom and Z.T. Murathan. 2024. Evaluating DNA barcoding using cpDNA *matK* and *rbc*L for species identification and phylogenetic analysis of *Prunus armeniaca* L. (Rosaceae) genotypes. *Genet. Resour. Crop Evol.*, 71: 1825-1835.

Singh, B., A. Kumar, V.P. Uniyal and K.S. Gupta. 2021. Phylogeography and population genetic structure of red muntjacs: evidence of enigmatic Himalayan red muntjac from India. *BMC Ecol. Evol.*, 21: 49.

Tang, N., A. Shahin, P.J.J.J. Bijman, J.M. Liu, V. Tuyl and P. Arens. 2013. Genetic diversity and structure in a collection of tulip cultivars assessed by SNP markers. *Sci. Hort.*, 161: 286-292.

Tang, Z.H., Y.F. Ji, F.B. An, Y.H. Zhang and J.N. Tang. 2015. Relationship between change of plant community and precipitation in desert grassland of Minqin county in recent 10 years. *Bull. Soil Water Conser.*, 35(01): 47-53.

Tsuji, S., N. Shibata, R. Inui, R. Nakao, Y. Akamatsu and K. Watanabe. 2023. Environmental DNA phylogeography: Successful reconstruction of phylogeographic patterns of multiple fish species from cups of water. *Mol. Ecol. Resour.*, 23(5): 1050-1065.

Wang, B.K. 2021. Tulip cultivation techniques. *Mod. Agri. Sci. Technol.*, (12): 151-152.

Wang, D., Y. Huang, L. Rui, H. Du, J. Qi, M. Ma and N. Zhou. 2023. Population genetic analysis of *Paris polyphylla* var. yunnanensis based on cpDNA fragments. *Genes*., 14: 1754.

Wang, Z.L. 2020. Tulip and its cultivation techniques. *Contemp. Hort*., 43(19): 90-91.

Warren, D.L., N.J. Matzke, M. Cardillo, J.B. Baumgartner, L.J. Beaumont, M. Turelli R.E. Glor, N.A. Huron, M. Simões, T.L. Iglesias, J.C. Piquet and R. Dinnage. 2021. ENMTools 1.0: an R package for comparative ecological biogeography. *Ecography.*, 44: 504-511.

Wu, T.W., Y.X. Lu, Y.G. Fang, X.G. Xin, L. Li, W.P. Li, W.H. Jie, J. Zhang, Y.M. Liu, L. Zhang, F. Zhang, Y.M. Zhang, F.H. Wu, J.G. Li, M. Chu, Z.Z. Wang, X.L. Shi, X.G. Liu, M. Wei, A. Anning Huang, Y.C. Zhang and X.H. Liu. 2019.The Beijing Climate Center Climate System Model (BCC-CSM): The main progress from CMIP5 to CMIP6. *Geosci. Model Dev.*, 12: 1573-1600.

Xing, G., L.W. Qu, W. Zhang, Y.Q. Zhang, X.F. Yuan and J.J. Lei. 2020. Study on interspecific hybridization between tulip cultivars and wild species native to China. *Euphytica.*, 216: 66.

Xing, G.M., L.W. Qu, Y.Q. Zhang, L. Xue, J.W. Su and J.J. Lei. 2017. Collection and evaluation of wild tulip (*Tulipa* spp.) resources in China. *Genet. Resour. Crop Evol.*, 64: 641-652.

Yan, L.X., J.M. Li, T. Yuan, A.P. Zhou, D. Zong, D. Li, P.Y. Xin and C.Z. He. 2016. Genetic analysis of *populus yunnanensis* by SRAP markers. *Biotechnol. Bull.*, 32(04): 159-167.

Yang, G., N. Liu, X. Zhang, H. Zhou, Y. Hou, P. Wu and X. Zhang. 2024. Prediction of the potential distribution of *Chimonobambusa utilis* (Poaceae, Bambusoideae) in China, based on the MaxEnt model. *Biodivers. Data J.*, 12: e126620.

Yi, S., H. Lu, W. Wang, G. Wang, T. Xu, M. Li, F. Gu, C. Chen, B. Han and D. Liu. 2022. The chloroplast genome of wild *Saposhnikovia divaricata*: genomic features, comparative analysis and phylogenetic relationships. *Genes.*, 13: 931.

You, J., X. Qin, S. Ranjitkar, S.C. Lougheed, M. Wang, W. Zhou, D. Ouyang, Y. Zhou, J. Xu, W. Zhang, Y. Wang, J. Yang and Z. Song. 2018. Response to climate change of montane herbaceous plants in the genus *Rhodiola* predicted by ecological niche modelling. *Sci. Rep.*, 8(1): 5879.

Zhai, Y.F., X.Q. Yu, J.G. Zhou, J. Li, Z. Tian, P.Q. Wang, Y. Meng, Q.Z. Zhao, Q.F. Lou, S.G. Du and J.F. Chen. 2021. Complete chloroplast genome sequencing and comparative analysis reveals changes to the chloroplast genome after allopolyploidization in *Cucumis*. *Genome.*, 64(6): 627-638.

Zhang, G.L., Z.Y. Wang, H. Wu and M.Z. Sun. 2020. Chloroplast phylogeography of *Iris dichotoma* (Iridaceae), a widespread herbaceous species in East Asia. *Nord. J. Bot.*, 38(11). https://www.doi.org/10.1111/njb.02888.

Zhang, W., J. Zhao, L. Xue, H. Dai and J. Lei. 2023. Seed morphology and germination of native *Tulipa* species. *Agricult.*, 13(2): 466.

Zhang, Y., Z.L. Ma, S.S. Xu, X. Su and M.Y. Li. 2022. Phylogeography of *Xanthopappus subacaulis* (Asteraceae), an endemic species from the northeastern of the Qinghai-Tibet Plateau. *Bull. Bot. Res.*, 42(04): 565-573.

Zong, D., Z.S. Qiao, J.T. Zhou, P.L. Li, P.H. Gan, M.R. Ren and C.Z. He. 2023. Chloroplast genome sequence of triploid *Toxicodendron vernicifluum* and comparative analyses with other lacquer chloroplast genomes. *BMC Genom.*, 24: 56. https://www.doi.org/ 10.1186/s12864-023-09154-2

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