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Genetic Structure and Eco-Geographical Differentiation of Wild Sheep Fescue (*Festuca ovina* L.) in Xinjiang, Northwest China

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Abstract: Glaciation and mountain orogeny have generated new ecologic opportunities for plants, favoring an increase in the speciation rate. Moreover, they also act as corridors or barriers for plant lineages and populations. High genetic diversity ensures that species are able to survive and adapt. Gene flow is one of the most important determinants of the genetic diversity and structure of out-crossed species, and it is easily affected by biotic and abiotic factors. The aim of this study was to characterize the genetic diversity and structure of an alpine species, *Festuca ovina* L., in Xingjiang, China. A total of 100 individuals from 10 populations were analyzed using six amplified fragment length polymorphism (AFLP) primer pairs. A total of 583 clear bands were generated, of which 392 were polymorphic; thus, the percentage of polymorphic bands (PPB) was 67.24%. The total and average genetic diversities were 0.2722 and 0.2006 (0.1686-0.2225), respectively. The unweighted group method with arithmetic mean (UPGMA) tree, principal coordinates analysis (PCoA) and STRUCTURE analyses revealed that these populations or individuals could be clustered into two groups. The analysis of molecular variance analysis (AMOVA) suggested that most of the genetic variance existed within a population, and the genetic differentiation (Fst) among populations was 20.71%. The Shannon differentiation coefficient (G'st) among populations was 0.2350. Limited gene flow (Nm = 0.9571) was detected across all sampling sites. The Fst and Nm presented at different levels under the genetic barriers due to fragmentation. The population genetic diversity was significant relative to environmental factors such as temperature, altitude and precipitation.

Keywords: *Festuca ovina* L.; AFLP, genetic diversity; genetic barriers; environmental factors

1. Introduction

Glaciation and mountain orogeny are the main factors that shape landscapes and change climate, and they have been linked to recent diversification and speciation events [1,2]. They could create many different environments and microclimates, which provide new habitats for plants and trigger evolutionary processes [3]. Accumulating evidence suggests that historical glacial and orogenic movements, including some recent biotic activities, might cause habitat fragmentation, which seriously increases the vulnerability of many plant species and threatens their survival [4]. These factors could also alter the abundance and behavior of pollinators and restrict seed dispersal [5,6]. The primary changes during habitat fragmentation include reduced population sizes and increased spatial isolation among populations [7]. Consequently, a deleterious erosion of genetic diversity and intensification of inter-population divergence would occur by random genetic drift, elevated inbreeding, and decreased inter-population gene flow [8,9]. As the feedback, fitness, richness and adaptation ability of a population would be weakened, such changes could even cause the local extinction of demes within a meta-population [10,11].

High genetic diversity is fundamental for the ability of a species to survive and develop [12]. It is often associated with traits that enable a species to adapt, such as expanding their distribution range and creating a new niche [13,14]. Fragmentation can affect plant species differently depending on their life history traits. The genetic erosion derived from habitat fragmentation depends on multiple life traits, including population size, distance between populations, time since isolation, seed and pollen dispersal distance, and generation time [15]. For out-crossed species, gene flow via pollen and seed dispersal is one of the most important determinants in the establishment of population genetic diversity and structure [16]. However, gene flow is

often interrupted by isolation and environmental heterogeneity [17-19]. Notably, historical climatic oscillations have greatly influenced the biodiversity of plant species. These effects were normally manifested by population contractions and expansions, long-distance range dispersal, new habitat colonization and *in situ* survival in glacial refugia [20,21]. Consequently, genetic erosion has occurred in some species. Researching the contemporary genetic diversity of species is required to understand ecological adaption processes and historical evolution. Hence, rational utilization and conservation practices are more effectively and efficiently realized for plant germplasms with agricultural importance.

The genus *Festuca* (Poaceae), with its approximately 100 species, is one of the most prevalently used grass genera, presenting a wide cosmopolitan distribution in the world [22]. Among these species, *Festuca ovina* L. is a perennial, cool-season and outbreeding grass aggregate with considerable agricultural and ecological importance [23,24]. Its main ploidy levels are documented: diploid 2n = 4x = 14 and tetraploid 2n = 4x = 28 [25]. Due to its strong tolerance to stress and adversity, *Festuca ovina* L. survives well in various habitats, which range from acid soils, coastal dunes and cliffs to cold deserts and heavy metal soils. Moreover, this species is abundant in Eurasia, ranges from the Arctic to the warm temperate zone, and is even found in outlying areas further south [25,26]. *Festuca ovina* L. is often used as a constructive species, turf grass, hay or grazing grass due to its multi-tillers and nutritional value (i.e., sugar and protein content), and it also presents considerable ecological resistance to sandstorms and soil erosion in some alpine and desert areas [23,26]. Confronting the disturbances of global climate deterioration, overexploitation, overgrazing and pollution, the stability and quantitative characteristics of the *F. ovina* community in Xingjiang, China indicate its high volatility and vulnerability [27,28]. The population size is gradually decreasing, causing genetic erosion that seriously impedes the sustainable development of the region's ecological security and local animal husbandry.

The Tianshan Mountains in Xingjiang of China and Central Asia are three parallel fold mountains that present a typical case of glaciation and orogenic movement [29,30]. They have contributed an important influence on plant biodiversity since their uplift during the early Holocene because they have acted as an East-West corridor, allowing lineage exchange, but also as a North-South barrier, promoting vicariance [31]. Moreover, desert expansion, environmental aridification, and river course dynamics have significant roles in providing adequate habitats for persistence of many plant species that could tolerate extreme drought and cold through glacial cycles [32,33]. Investigations of the genetic diversity of many species have been carried out in Xinjiang, but rarely for *F. ovina*. Actually, the heterogeneity of landscapes triggered by orogenic movements has formed many special habitats [34,35]. Many alleles and traits related to adaptation have been filtered under these special eco-geographic habitats due to their independent development at different elevations or under different edaphic characteristics, temperatures or levels of precipitation [18,36]. Replacing morphological assessment and isozyme methods, modern molecular marker techniques such as amplified fragment-length polymorphism (AFLP) analysis provide much more detailed insight into genetic diversity and variation at the DNA level [37,38]. It is possible to evaluate a large number of polymorphic loci for any origin or level of DNA complexity without prior genomic information. Thus, AFLP has been widely used to determine phylogenetic history, answer important ecological questions and assess germplasm resources[39,40].

In this study, AFLP was used to investigate the genetic diversity and structural patterns of 100 wild *F. ovina* individuals from 10 populations found in Xingjiang. The aims were to (1) characterize the level of genetic diversity and the distribution of genetic variation within and among *F. ovina* populations; (2) calculate the gene flow and differentiation among populations at different scale and fragmented patterns; and (3) discuss the influence of environmental factors on population genetic diversity and structure. As a consequence, this study supplies a basis for the collection and protection of germplasm resources.

2. Results

2.1 *AFLP polymorphism and genetic diversity*

In this study, the six selected AFLP primer pairs generated 583 clear bands that corresponded to an average of 97.17 per primer pair (Table 1). Among these bands, 392 were polymorphic. As the polymorphic bands for each primer ranged from 56 to 70, the PPB was 67.24%. The PIC varied from 0.1917 (E85M57) to

0.2221 (E42M85), with an average of 0.2107. The total Hj of the primer pairs was 0.3549 (0.2036-0.3024), which corresponded to an average of 0.25531. The Shannon diversity index (Ho) of primer pairs varied from 0.20462 to 0.4406, and the total and average Ho values were 0.4642 and 0.3164, respectively.

Table 1. Summary of genetic diversity based on AFLP loci amplified by each primer combination. Total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), polymorphism information content (PIC), Nei's gene diversity (Hj), Shannon diversity index (Ho).

Primer	TNB	NPB	PPB (%)	PIC	Hj	Ho
E42M57	109	70	64.22	0.2132	0.2538	0.2136
E42M85	89	56	62.92	0.2221	0.3024	0.3617
E85M57	101	66	65.35	0.1917	0.2628	0.4406
E85M85	103	68	66.02	0.2152	0.2464	0.2261
E86M57	90	67	74.44	0.2095	0.2036	0.2046
E86M85	91	65	71.43	0.2124	0.2499	0.4116
Total	583	392	67.24	0.2107	0.3549	0.4642
Mean	97.17	65.33	67.24	0.2107	0.2531	0.3164

The genetic diversities of 10 *F. ovina* populations are listed in Table 2. The number of polymorphic loci (Np) ranged from 198 (FO-04) to 294 (FO-06), and the total and average Np were 392 and 298.5, respectively. The average percentage of polymorphic loci (PLP) was 67.24% (50.5%-75.0%). The total Hj and total Shannon diversity index (Ho) were 0.2622 and 0.2988, respectively; these values were highest in FO-07 (Hj = 0.2225, Ho = 0.2505) and lowest in FO-04 (Hj = 0.1686, Ho = 0.1966). The average observed number of alleles per locus (Na) was 1.6314 (1.5026-1.7092), and the average effective number of alleles per locus (Ne) was 1.3043 (1.2585-1.3425).

Table 2. Genetic variability in 10 *Festuca ovina* populations. Number of polymorphic loci (Np), percentage of polymorphic loci (PLP), observed number of alleles per locus (Na), effective number of alleles per locus (Ne), Nei's gene diversity index (Hj), Shannon diversity index (Ho).

Population	Np	PLP (%)	Na	Ne	Hj	Ho
FO-01	272	69.4	1.6582	1.2982	0.2042	0.2322
FO-02	271	69.1	1.6709	1.3401	0.2191	0.2471
FO-03	282	71.9	1.6607	1.2920	0.1969	0.2249
FO-04	198	50.5	1.4719	1.2625	0.1686	0.1966
FO-05	274	69.9	1.6786	1.3227	0.2126	0.2406
FO-06	294	75.0	1.7092	1.3006	0.2054	0.2334
FO-07	271	69.1	1.6735	1.3425	0.2225	0.2505
FO-08	256	65.3	1.6352	1.3140	0.2068	0.2348
FO-09	202	51.5	1.5026	1.2585	0.1698	0.1978
FO-10	273	69.6	1.6531	1.3119	0.1998	0.2278
Total	392	100	2.0000	1.3571	0.2622	0.2988
Mean	298.5	67.24	1.6314	1.3043	0.2006	0.2287

### 2.2 *Genetic distance and structure*

A total of 392 fragments from six AFLP loci were used to estimate pairwise Nei's genetic distances (GD) among 10 *F. ovina* populations (Table 3). The genetic distance was relatively low, varying from 0.0802 (FO-03 vs. FO-04) to 0.1508 (FO-05 vs. FO-09) with an average value of 0.1094. Lower genetic distances indicated a closer relationship among the studied populations, although some visible differences obviously

exist between them.

Table 3. Nei's genetic distance matrix of 10 *F. ovina* populations based on AFLP profiles. Genetic distance (GD).

| GD	FO-02	FO-03	FO-04	FO-05	FO-06	FO-07	FO-08	FO-09	FO-10
FO-01	0.0823								
FO-02	0.0853	0.0804							
FO-03	0.1504	0.1418	0.1504						
FO-04	0.0866	0.0802	0.0890	0.1432					
FO-05	0.088	0.0833	0.0811	0.1477	0.0814				
FO-06	0.0867	0.0869	0.0921	0.1486	0.0919	0.0942			
FO-07	0.0934	0.0957	0.0978	0.1473	0.0952	0.0999	0.0889		
FO-08	0.1486	0.1393	0.1499	0.0911	0.1441	0.1462	0.1483	0.1495	
FO-09	0.0897	0.0829	0.0836	0.1508	0.0824	0.0839	0.0962	0.0986	0.1498

To study the genetic structure of *F. ovina*, a UPGMA clustering analysis at the species level was conducted (Figure 1a). The results showed that the 10 populations gathered into two clusters with a bootstrapping value of 100%. Cluster I contained 8 populations (FO-01, FO-02, FO-03, FO-05, FO-06, FO-07, FO-08 and FO-10), whereas Cluster II contained 2 populations (FO-04 and FO-09). We performed a STRUCTURE analysis to further study the genetic structure (Figure 1b&c). This analysis revealed that the 10 populations clustered into two groups, FO-04 and FO-09 clustered into the same group, and the remaining 8 populations were clustered into an alternative group. This result was highly consistent with that of the UPGMA tree.
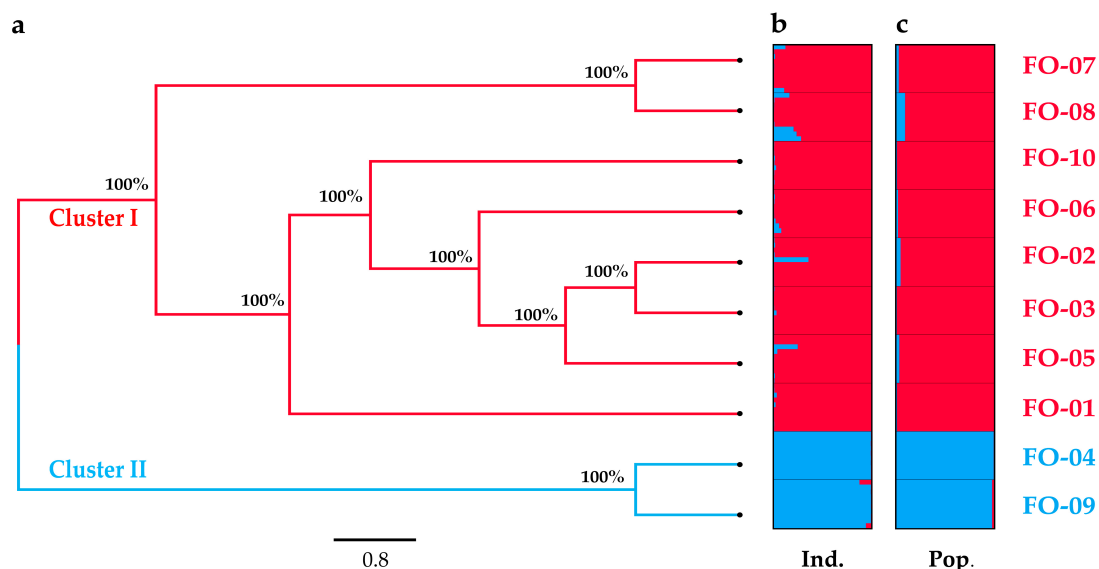
Figure 1. Cluster analysis for *F. ovina* (a: UPGMA tree for 10 populations, b: structure analysis for 10 populations, c: structure analysis for 100 individuals).

Principal coordinates analysis (PCoA) was performed on the entire dataset of 100 individuals. The results showed that these individuals were divided into two groups (Figure 2). The individuals of FO-01, FO-02, FO-03, FO-05, FO-06, FO-07, FO-08 and FO-10 were gathered into Group I, whereas the individuals of FO-04 and FO-09 were gathered into Group II. The first principal vector explained 16.07% of the genetic variation, whereas the second and third principal vectors explained 4.50% and 3.86% of the genetic variation,

respectively. This result was also consistent with those of the UPGMA tree and STRUCTURE analysis.
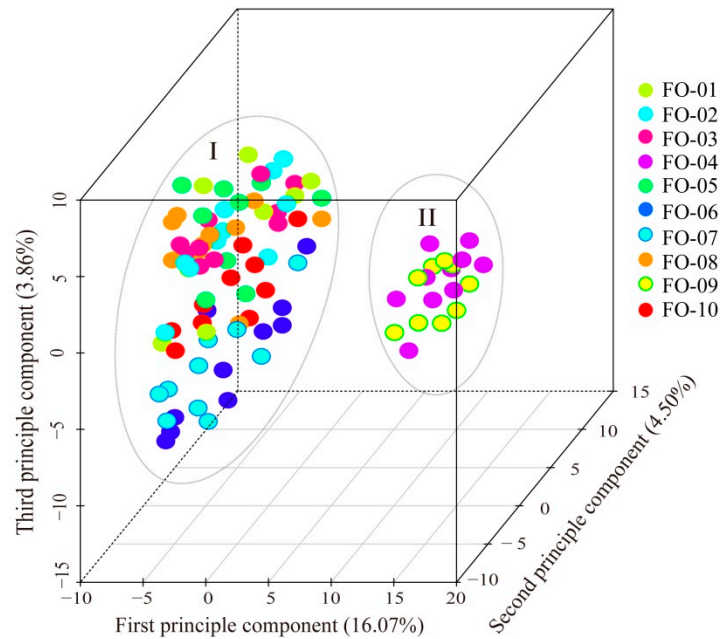
Figure 2. Principal coordinates analysis (PCoA) of 100 *F. ovina* individuals from 10 populations based on genetic distance matrix.

2.3 *Genetic differentiation, gene flow and genetic barrier*

Analysis of molecular variance (AMOVA) was conducted to further evaluate the partitioning of genetic differentiation among and within *F. ovina* populations (Table 4). The results showed that 20.71% of the genetic differentiation occurred among populations, whereas 77.83% was attributable to variability within populations. Furthermore, AMOVA was conducted for the two clusters determined by the UPGMA tree and STRUCTURE analysis. The results suggested that 11.54% of the genetic differentiation occurred between the two clusters. Moreover, the Shannon differentiation coefficient was also calculated as G'st = 0.2350 among all populations. The gene flow was Nm = 0.9571 across all sampling sites.

Table 4. Analysis of molecular variance for *F. ovina* populations.

| Group	Source of variation	D.f.	Sum of squares	Variance components	Percentage of variation	F-statistic	p-value
Two clusters	Among clusters	2	2334.77	11.98	11.54	Fct = 0.1154	< 0.01
	Among pops. within clusters	7	2143.16	9.74	10.54	Fsc = 0.1054	< 0.01
	Within populations	89	4477.93	46.53	77.92	Fst = 0.2208	< 0.01
	Total	98	5647.54	58.68			
All pops.	Among populations	9	1169.60	12.15	20.71%	Fst = 0.2071	< 0.01
	Within populations	89	4477.93	46.53	79.29%		
	Total	98	5647.54	58.68			

Furthermore, a genetic barrier prediction analysis for 10 *F. ovina* populations was carried out using Monmonier's maximum difference algorithm. This analysis revealed three likely barriers against gene flow (Figure 3). The first barrier (aa) isolated FO-04 from its surrounding populations. The second barrier (bb) separated FO-09 from neighboring populations. The third barrier (cc) was detected between FO-07 and FO-01.
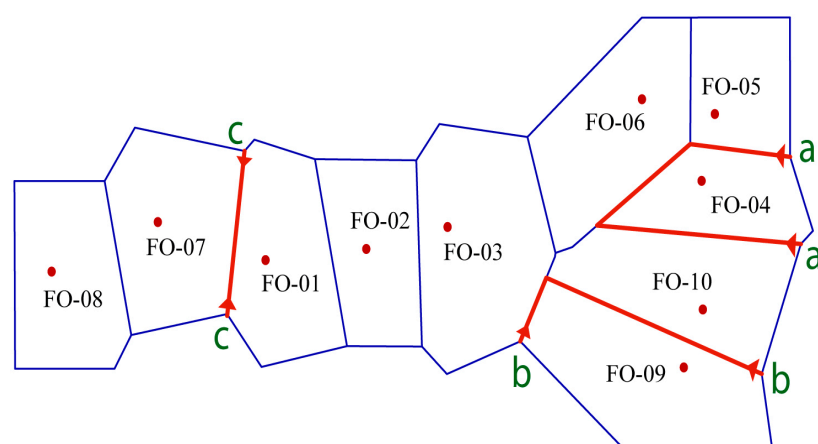
Figure 3. Genetic barriers predicted by BARRIER software. Lines a, b, and c indicate genetic barriers.

2.4 *Genetic diversity associated with environmental factors*

A significantly negative correlation was found between population genetic diversity (Hj) and geographic altitude (r = − 0.85, p < 0.01) (Figure 4a), indicating a pattern of lower genetic diversity at higher altitudes. The population genetic diversity showed a weak positive correlation with mean temperature (r = 0.57, p < 0.01) (Figure 4b), indicating that temperature played a considerable role in changing population genetic diversity. The population genetic diversity showed a weak negative relationship with annual precipitation (r = − 0.60, p < 0.01) (Figure 4c).
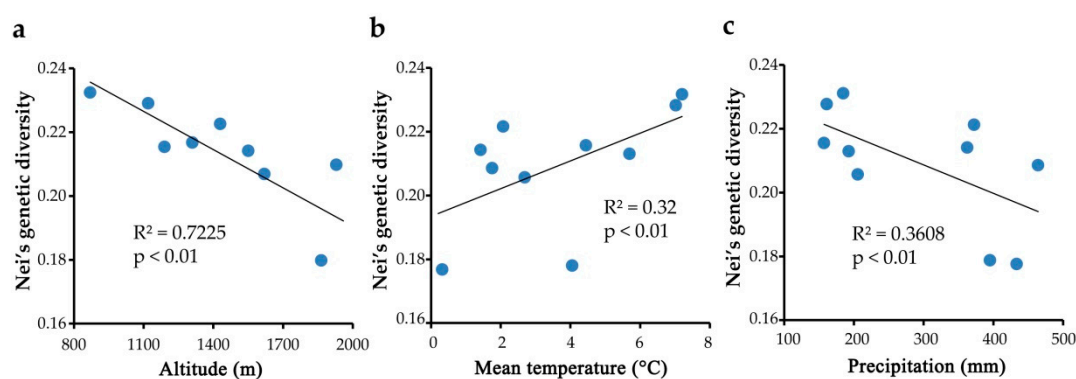
Figure 4. Regression analysis for Hj and environmental factors (a: Hj and altitude; b: Hj and mean temperature; c: Hj and precipitation).

3. Discussion

3.1 *Population genetic diversity and its correlation to environment factors*

Genetic diversity is considered the consequence of long-term evolution and represents the evolutionary potential of a species to survive in various environments[13,41]. Thus, a species must accumulate more genetic diversity in order to adapt itself to diverse environmental pressures [42]. AFLP with high levels of polymorphism represents a powerful tool for assessing genetic diversity in many species [37,43]. In this study, a total of six AFLP primer pairs generated 583 clear bands, of which 392 were polymorphic; thus the PPB was 65.33%. The PIC ranged from 0.1917 to 0.2221, with an average of 0.2107. Previous research by Majidi et al. [44], in which the genetic variation of *Fescue* accessions was assessed using a DNA bulking strategy and AFLP, revealed an average NPB of 41 and a PPB of 85.4%. Higher PIC values, such as 0.2715 (0.1534-0.3842) for genomic SSRs and 0.2224 (0.0760-0.4289) for EST-SSRs, than those of tall fescue were revealed by Tehrain et al. [45]. Generally, the population mean genetic diversity, genetic distance, and population size are positively correlated [46]. In most cases, widespread species tend to possess high genetic

diversity. Conversely, the mean genetic distance (GD) in this study was 0.1094 (0.0802-0.1508), which corresponded to an average genetic diversity of Hj = 0.2006 (0.1686-0.2225). Majidi et al. reported higher values of GD = 0.55 (0.12-0.81) and Hj = 0.480 (0.273-0.611) [44]. A molecular diversity analysis was conducted by Fjellheim et al. on Norwegian meadow fescue (*Festuca pratensis* Huds.) populations and Nordic cultivars, which revealed a lower genetic diversity (Hj = 0.1412) [47]. Typically, cross-pollinated species maintain high intra-population variability relative to their inter-population variability [48]. The total genetic diversity (Hj = 0.2822) and mean genetic diversity (Hj = 0.2006) indicated the existence of 20.71% genetic differentiation among *F. ovina* populations, which corresponded well to the Shannon differentiation coefficient of G'st = 0.2512. The study by Majidi et al. [44] revealed higher levels of G'st = 0.337 and Fst = 0.355 among populations, whereas Fjellheim et al. [47] showed different differentiation patterns, such as Fst among seed populations (0.308), Fst among leaf populations (0.310) and Fst among cultivars (0.204). Currently, the high level of genetic diversity mirrors the high diversification and variation during long-term natural selection and evolutionary history, and genetic diversity benefits more easily from the broad geographical distribution range and high polyploidy for out-crossing species [24,45]. Conversely, the observations of this study suggested a relatively low genetic diversity of *F. ovina* populations. This low observed value of genetic diversity was possibly caused by different molecular markers or DNA strategies (individual DNAs or bulk DNAs). Alternatively, habitat fragmentation could be the explanation. Since the formerly panmictic population became fragmented, random genetic drift over time has led to both genetic differentiation among, and loss of heterozygosity within the fragmented population [49]. In the alpine area of Xingjiang, human overexploitation, overgrazing and farmland expansion have led to the increasingly patchy distribution and isolation of *F. ovina*, resulting in decreased fitness and reduced population size and thereby causing the observed low genetic diversity [27].

Evidence for lower genetic diversity at higher altitudes was found based on regression analysis between genetic diversity (Hj) and geographic altitude (r = – 0.85, p < 0.01) (Figure 4a). For out-crossing species, wind, insects and animals are essential pollinators that guarantee gene flow within and among populations, these factors also determine the seed distribution of *F. ovina* [28]. Fewer insects and animals are found in alpine areas due to adverse environmental factors, including low temperatures, intense ultraviolet radiation and strong winds [50,51]. As a result, the plant population size gradually decreases, and deleterious erosion of genetic diversity occurs due to elevated levels of inbreeding and increased random genetic draft. In this study, the genetic diversity was positively correlated with the mean temperature (r = 0.57, p < 0.01) (Figure 4b), revealing that low temperatures were harmful to plant genetic diversity. A previous study showed that glacial refugias in Tianshan Mountains and Altay area had obvious effect on alpine plant species, because migration and expansion normally occurred from these refugias [33]. Thus, the low observed value of genetic diversity of *F. ovina* in this study could be partly due to historic climate oscillation. For instance, suffered from the extremely low temperature, extinction occurred in mountain-top populations during glacial periods, whereas lowland populations survived due to the more tolerable temperatures of lowland areas [29,31]. Those alpine populations might have expanded from the few lowland individuals during the interglacial periods; therefore, less genetic diversity was observed. Furthermore, genetic diversity was negative correlated with   annual precipitation (r = – 0.60, p < 0.01) (Figure 4c), indicating that excess rainfall was adverse for population genetic diversity. In Xingjiang, the wet season resulting from a temperate continental climate is concentrated in May and June every year [52,53]. So the continuous heavy rainstorms during the flowering period of *F. ovina* at May would disrupt the fertilization process and reduce the genetic diversity.

3.2 Genetic structure and gene flow among fragmented populations under genetic barriers

The population genetic structure can reveal the interactions of various evolutionary processes, such as habitat fragmentation, population isolation, and gene flow. In the present study, the UPGMA tree, PCoA and STRUCTURE analyses revealed that the 10 *F. ovina* populations comprised two clusters, and FO-04 and

FO-09 were isolated from adjacent populations (Figures. 1&2). Considering the relatively small distribution range of this species, the structural pattern of these populations was not related to their geographical distance, but instead, to their fragmentation. Most of the molecular variation was assigned to within-population variation (Fst = 79.29%) (Table 4), which resulted from the high ploidy level and out-breeding of *F. ovina*. The possession of several copies of the genome and the outcrossing mating system of this species would favor the maintenance of high levels of both intra-individual and intra-population genetic diversity [24]. On the other hand, as discussed above, a relatively recent extinction and replacement could also explain the low genetic divergence among populations, leading to a lack of accumulated differential mutations over a relatively short evolutionary time [54].

Gene flow (Nm) has an important impact on population genetic structure, evolutionary biology, conservation biology, and ecology [55]. High levels of gene flow can hinder intraspecific genetic drift and interspecific differentiation [56]. In this study, gene flow was Nm = 0.9571 among populations across all sampling sites, indicating the presence of some genetic barriers against gene flow. Three genetic barriers were directly detected and demonstrated in Fig. 3. The first (aa) and second (bb) barriers isolated FO-04 and FO-09, respectively, from their adjacent populations. This result was congruent with the UPGMA tree, PCoA and STRUCTURE analyses. A further calculation was conducted for Fst and Nm at a smaller scale. The gene flow and genetic differentiation between FO-05, FO-06, FO-10 and FO-04 were Nm = 1.1713 and Fst = 0.1759, respectively. The gene flow and genetic differentiation between FO-03, FO-10 and FO-09 were Nm = 1.3282 and Fst = 0.1584, respectively. FO-04 and FO-09 were distributed in the alpine mountains with higher elevation, and thus, they had difficulty interacting with lowland populations. The explanation for this was the fragmentation induced by deserts and high mountains. Interestingly, FO-10 was distributed in the similar alpine area as FO-04 and FO-09, but low gene flow (Nm = 1.2372) and high genetic differentiation (Fst = 0.1681) were found between these populations. An analogous differential pattern was also detected between FO-07 and FO-01 (Fst = 0.091) in lowland according to the third genetic barrier (cc). The reason for these might be the arid process and fragmentation due to desert expansion and high mountains that promoted the differentiation between adjacent populations [32,33]. Alternatively, FO-10 might be the colonization from lowland populations (as discussed above for historical climate oscillation), thereby explaining its similar genetic lineages and co-clustering in Cluster I (Figure 1).

4. Materials and Methods

4.1 *Plant materials and genomic DNA preparation*

A total of 10 wild *F. ovina* populations (10 individuals per population) were sampled from Xingjiang, China in 2010 (Table 5). Individual spikes over 25 m apart from one another were sampled randomly to assure that they were from different individuals (Figure 5). A single seed from each collected single spike was germinated at Sichuan Agricultural University using an individual pot in an illumination incubator at an approximate temperature of 22°C and under a 16 h photoperiod. Before DNA extraction, chromosome identification was carried out using the squashed root tips technique [57] for each individual 3-day-old germinated seedling to guarantee the DNA purity. The results suggested that all individuals and populations were diploid (2n = 2x = 14).

For each individual, genomic DNA was extracted from an aggregate of 3-5 fresh leaf tissues (approximately 80 mg) using a plant DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The DNA concentration was quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, USA) and diluted to 100 ng/μL for AFLP analysis.
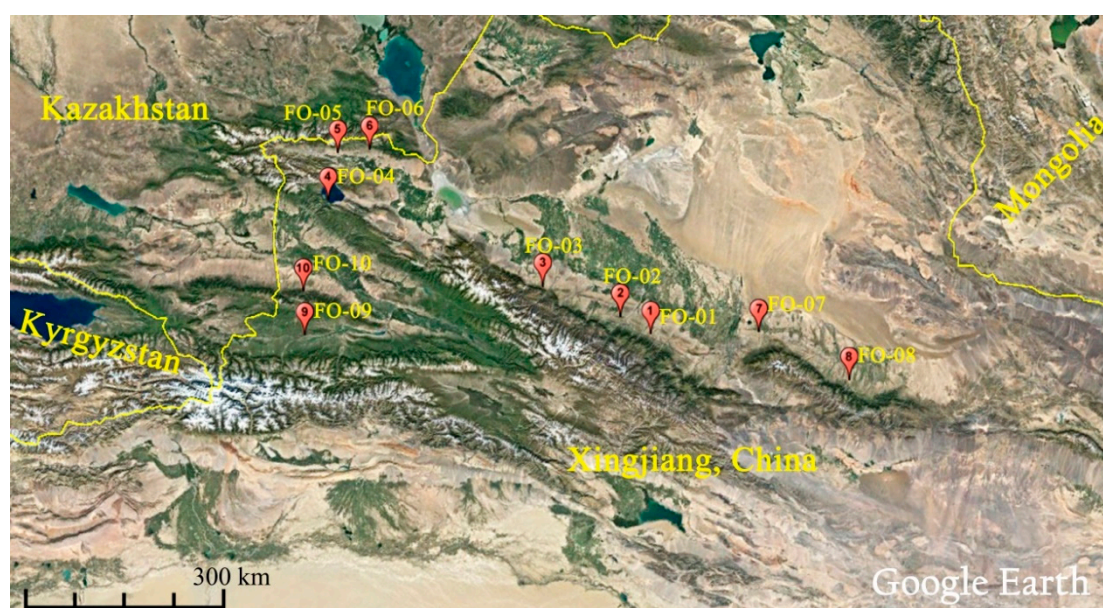
Figure 5. Geographical locations of analyzed populations of *Festuca ovina* in Xinjiang, China.

4.2 *AFLP procedure*

The AFLP procedure was performed according to Sorkheh et al. [43]. A preliminary amplification using a set of 100 primer pairs identified six selective primer pairs as the most informative and reliable; these six primers were used for amplification. The PCR amplification reaction was performed in a 20 mL system: 1 PCR buffer, 2 mM MgCl2, 2 mM dNTP, 40 ng of each of EcoRI primer and MseI primer, 1 U Taq polymerase, and 5.0 ml pre-amplified template DNA. The fragments amplified in the latter step were subjected to capillary electrophoresis using an ABI 3500 (Applied Biosystems, USA). GeneMarker v2.6 was used to treat the fluorescent AFLP fragments for each individual sample.

4.3 *Data analysis*

4.3.1 *Genetic diversity*

Each band in the AFLP fingerprint pattern was considered a separate putative locus, and the bands clearly indicating AFLP amplification as 1 (presence) or 0 (absence) in a readable range (between 50 and 400 bp) were used to generated the binary matrices. The following parameters were calculated using EXCEL 2013 software: total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB) and polymorphism information content (PIC, according to Zhang et al. [58]). The Shannon information index (Ho), observed number of alleles per locus (Na), and effective number of alleles per locus (Ne) were calculated using POPGENE v3.0 [59] under Hardy-Weinberg equilibrium (HWE). Nei's genetic diversity (Hj) was calculated using AFLP-SURV v1.0 [60].

4.3.2 *Genetic structure*

First, Nei's genetic distance (GD) of the population was calculated using AFLP-SURV v1.0 [60] with 10,000 bootstraps, and the results were used as inputs for computing the unweighted group method with arithmetic mean (UPGMA) tree using the CONSENSE module in PHYLIP v3.69 [61]. Second, principal coordinates analysis (PCoA) for 100 individuals was conducted using the R packages "stats" [62] and "scatterplot3d" [63] to evaluate their spatial distribution pattern. Third, Bayesian model-based cluster analysis was performed to infer the number of clusters using the software STRUCTURE v2.3.4 [64] with correlated allele frequencies and an admixed model with a burn-in period of 50,000 and 200,000 MCMC replicates after burn-in. The predefined cluster (K) was 1-10 with 10 runs per K. The optimum K was decided by determining L(K) and ΔK (the subtraction of two continuous L(K) values) using STRUCTURE HARVESTER [65]. Those values usually reached a plateau or increased slightly after reaching the "optimum K".

4.3.3 *Genetic variation, gene flow and genetic barrier*

To further study the genetic differentiation (Fst) within and among populations, analysis of molecular variance (AMOVA) was conducted at different levels using the R package "vegan" [66]. The Shannon differentiation coefficient (G'st) was calculated according to the following formula: G'st = (Hsp – Hpop) / Hsp (Hsp, total Shannon information index; Hpop, average Shannon information index within the population). Gene flow (Nm) was calculated as Fst (Nm = (1 – Fst) / 4Fst). Moreover, a genetic barrier analysis was devised to suggest historical barriers to gene flow among or between collection sites by using BARRIER v2.2 [67] according to Monmonier's maximum difference algorithm, which treated geographical coordinates and genetic distance (GD) of each population as inputs.

4.3.4 *Correlation analysis*

To examine the level of significance, the relationship between genetic diversity and environmental factors (average temperature, annual precipitation and elevation) was estimated using the R package "ade4" [68] with 1000 random permutations.

Table 5. List of the 10 wild *F. ovina* populations in this study.

Population	Altitude (masl)	Annual mean temperature (AMT, °C)	Annual precipitation (AP, mm)	Longitude	Latitude
FO-01	1550	5.7125	207	43°53′1″	86°22′0″
FO-02	1120	7.0583	175	44°0′23″	85°52′13″
FO-03	1620	2.6625	220	44°9′12″	84°38′5″
FO-04	2065	0.1375	451	44°31′0″	81°8′24″
FO-05	1430	2.0333	389	45°3′7″	81°5′8″
FO-06	1190	1.3750	379	45°10′0″	81°33′9″
FO-07	870	7.2417	199	44°7′22″	87°58′17″
FO-08	1310	4.4417	171	43°46′0″	89°27′32″
FO-09	1880	4.0458	412	43°1′42″	81°18′23″
FO-10	1960	1.7125	482	43°29′26″	81°7′24″

5. Conclusion

The results of this study demonstrate that environmental conditions in different habitats are major factors influencing the hereditary characteristic of species. The genetic diversity of *F. ovina* was relatively low. Population genetic diversity was decreased with increasing elevation, and it was also related to temperature and precipitation. Restricted gene flow was observed at different spatial scales, whereas genetic differentiation due to fragmentation was also detected between adjacent populations.

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