Population genetic structure and evolutionary history of Psammochloa villosa (Trin.) Bor (Poaceae) revealed by AFLP marker

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Abstract

We sought to generate a preliminary demographic framework for Psammochloa villosa to support of future studies of this ecologically important desert grass species, its conservation, and sustainable utilization. Psammochloa villosa occurs in the Inner Mongolian Plateau where it is frequently the dominant species and is involved in sand stabilization and wind breaking. Here, we characterized the genetic diversity and structure of 210 individuals from 43 natural populations of P. villosa using amplified fragment length polymorphism (AFLP) markers. We obtained 1728 well-defined amplified bands from eight pairs of primers, of which 1654 bands (95.72%) were polymorphic.All these values indicate that there is abundant genetic diversity, but limited gene flow in P. villosa. However, an analysis of molecular variance (AMOVA) showed that genetic variation mainly exists within 43 populations of the species (64.16%), and we found that the most genetically similar populations were often not geographically adjacent. Thus, this suggests that the mechanisms of gene flow are surprisingly complex in the species and may occur over long distances. In addition, we predicted the distribution dynamics of P. villosa based on the spatial distribution modeling and found that its range has contracted continuously since the last inter-glacial period. We speculate that dry, cold climates have been critical in determining the geographic distribution of P. villosa during the Quaternary period. Our study provides new insights into the population genetics and evolutionary history of P. villosa in the Inner Mongolian Plateau, which can be used to design in-situ conservation actions and to prioritize sustainable utilization of germplasm resources.

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Running Head: Population genetic structure of *P. villosa*

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Abstract

We sought to generate a preliminary demographic framework for*Psammochloa villosa* to support of future studies of this ecologically important desert grass species, its conservation, and sustainable utilization. *Psammochloa villosa* occurs in the Inner Mongolian Plateau where it is frequently the dominant species and is involved in sand stabilization and wind breaking. Here, we characterized the genetic diversity and structure of 210 individuals from 43 natural populations of *P. villosa* using amplified fragment length polymorphism (AFLP) markers. We obtained 1728 well-defined amplified bands from eight pairs of primers, of which 1654 bands (95.72%) were polymorphic. Results obtained from the AFLPs estimated a number of effective alleles among populations of 1.3229, a Nei's standard genetic distance value of 0.2056, a Shannon index of 0.3316, a coefficient of gene differentiation (G_{ST}) of 0.4689, and a gene flow parameter (N m) of 0.5761. All these values indicate that there is abundant genetic diversity, but limited gene flow in *P. villosa* . However, an analysis of molecular variance (AMOVA) showed that genetic variation mainly exists within 43 populations of the species (64.16%), and we found that the most genetically similar populations were often not geographically adjacent. Thus, this suggests that the mechanisms of gene flow are surprisingly complex in the species and may occur over long distances. In addition, we predicted the distribution dynamics of *P. villosa*based on the spatial distribution modeling and found that its range has contracted continuously since the last inter-glacial period. We speculate that dry, cold climates have been critical in determining the geographic distribution of *P. villosa* during the Quaternary period. Our study provides new insights into the population genetics and evolutionary history of *P. villosa* in the Inner Mongolian Plateau, which can be used to design in-situ conservation actions and to prioritize sustainable utilization of germplasm resources.

Keywords: desert grasslands; population genetics; SAMOVA; ecological niche modelling; Inner Mongolian Plateau;

Psammochloa villosa

Introduction

The Quaternary period, comprising the Holocene and Pleistocene Epochs, spanned the last ˜2.6 million years (Myr) and has been characterized by distinct climatic oscillations, especially alternating glacial and interglacial cycles in the Northern Hemisphere (Shackleton & Opdyke, 1973; Pillans & Gibbard, 2012; Elias, 2013). The glacial cycles co-varied with, and probably profoundly affected, other aspects of the climate, including the intensity of the Asian monsoon, even in unglaciated regions (An, Kutzbach, Prell, & Porter, 2001; Jiang , Lang, Tian, & Guo, 2011; Liu et al., 2018). Climate fluctuations during the Quaternary glaciations led to dramatic changes in the geographical distribution, genetic structure, and population demography of plant species (Hewitt, 2000; Wang et al., 2009; Jia et al., 2012; Liu et al., 2018).

Quaternary climate change is known to have strongly affected the distributions of plants in Northern China (Tian et al., 2009; Qiu, Fu, & Comes, 2011; Liu, Sun, Ge, Gao, & Qiu, 2012). Northern China is dominated by deserts, which developed in the Quaternary to due to sustained orogenesis of the Qinghai-Tibetan Plateau and surrounding areas that ultimately enhanced aridity within the region (Wu, 1992, 2002; Meng & Zhang, 2011). The climatic processes that gave rise to the deserts also shaped regional plant diversity and yielded highly complex demographic histories of native species (Ge, Yu, Yuan, Huang, & Yan, 2005; Ge et al., 2011; Yang, Fang, Dong, Peng, & Li, 2006). In particular, many plants within the deserts of Northern China underwent adaptive and demographic change in response to cold, arid conditions, which occurred during the intermittent glacial periods and which may be primary mechanisms explaining modern distributions (e.g., Comes & Kadereit, 1998; Hewitt, 2000; Ge et al., 2011; Zhang & Zhang, 2012; Su & Zhang, 2013; Zhang,

Zhang, & Williams, 2014; Xu & Zhang, 2015; Shuyskaya, Toderich, Gismatullina, Rajabov, & Khohlov, 2017; Merklinger et al., 2019; El-Tayeh, Galal, Soliman, & Zaki, 2020). For example, Su & Zhang (2013) proposed that the onset of aridity during Quaternary glacial periods was a primary driver of population processes and structures in *Nitraria sphaerocarpa* Maxim. (Nitrariaceae, Sapindales), and Xu & Zhang (2015) revealed that periods of cold, arid conditions during the Pleistocene glaciations resulted in the genetic differentiation and demographic structuring in *Atraphaxis frutescens* (L.) K.Koch (Polygonaceae).

Nevertheless, these prior studies on population histories of desert plants of Northern China have focused primarily on woody species. Studies on herbs of the region are largely lacking, and to our knowledge, the only such study is on *Delphinium naviculare* W. T. Wang (Ranunculaceae) (Zhang & Zhang, 2012), which is endemic at mid-elevations within the Tianshan Mountains of Xinjiang Province of China (Wang & Warnock, 2001). Herbaceous plants of the deserts of Northern China merit further study because they comprise a vital component of desert plant communities, and studies focusing on dominant grass species are especially warranted (Meng & Zhang, 2011). Herbaceous plants may have been more sensitive to Quaternary climatic oscillations because they differ markedly from woody species in their response to cold, often via death of aboveground biomass as either part of an annulment or perennial life cycle. Moreover, dominant species likely achieved their abundance due to their responses to the glacial cycles.

In modern times, deserts and semi-deserts, such as in Northern China, are extremely fragile ecosystems, the stability of which impacts global environmental conditions and influences climate change (Su, 2013). Although deserts typically have sparse vegetation, plants are critical for maintaining their integrity. In Northern China, the desert grassland ecosystems in particular are becoming rapidly degraded due to long-term overgrazing and desertification and, simultaneously, desert is encroaching on arable land within the region (Li, Liu, & Wang, 2004; Hanafi & Jauffret, 2008; Wang, 2009; Peters, Yao, Sala, & Anderson, 2012; Deng, Zhang, & Shangguan, 2014; State Forestry Administration, 2015; Wang & Zhou, 2015; Wei, Wang, & Niu, 2020). These desert grasslands represent a large area within China and adjacent countries and occur at both low and high elevations. Dominant plant species within the grasslands are often psammophytes, which have special adaptations to resist being buried by sand and to tolerate having periodically exposed roots. Simultaneously, these plants help to anchor sands in place and prevented wind erosion, and are thus critical for promoting environmental stability within their ecosystems (Ma, 1991; Pan, 2006; Zhou, Yuan, & Jing, 2011).

In this study, we focus on one psammophyte, *Psammochloa villosa*(Trin.) Bor, which comprises a monotypic genus in tribe Stipeae of Poaceae. This species, commonly called sand whip, is a perennial rhizomatous herb that is primarily distributed in the desert grasslands of northwestern China, especially the Inner Mongolian Plateau, the Hexi Corridor of Gansu, central and Northern Ningxia, and Northern Shanxi (Liu, 1985; Ma, 1994; Huang, 2003). It also occurs in Mongolia. The flowering and fruiting period of *P. villosa* is from September to November, and the seeds are 5 - 7 mm long with an average weight of 5.507 $\pm$ 0.053 mg (Huang, 2003). Such light seeds are likely dispersed by high winds that occur throughout much of its natural desert habitat. Nevertheless, seedlings are rarely observed (Zhu, 2005).*Psammochloa villosa* is known to have high resistance to drought, cold, alkaline soils, disease, wind, and burial by sand, all of which likely represent evolutionary adaptations and facilitate its survival in grassland and dune areas (Lu, 1987; Huang, 1995, 1997; Wu & Phillips, 2006). Previous research on *P. villosa* has been mainly focused on studying its anatomy, embryology and microbiology (e.g., Dong & Alaten, 1999; Huang, Dong, & Gutterman, 2004; Wang et al., 2011; Zhang et al., 2017; Lv et al., 2018), with few studies so far focused on molecular markers (e.g., Li & Ge, 2001). The species is ecologically widespread at low and high elevations (900-2900 m) (Kuo, 1987; Wu & Phillips, 2006).

In our present study, we investigated the influence of climate aridification and oscillations on the genetic structure and evolutionary processes of *P. villosa*during the Quaternary in north-western China using amplified fragment length polymorphism (AFLP) markers and ENM. We used AFLPs because they remain extremely efficient for investigating genetic diversity, genetic structure, and population demography due to their high levels of polymorphism (Wang, Wang, Liu, Yang, & Chen, 2008), their reproducible, reliable re-

sults that are unaffected by the developmental stage of plant materials, and their universality among plant species. In addition, they have been used to resolve genetic structures and population demography in many diverse grass species such as *Oryza sativa* , *Leymus racemosus* , *Orinus thoroldii* and *O. kokonoricus* (Zhang & Jia, 2002; Sim, 2005; Li, 2015; Cai, 2017; Liu, Harris, Gao, Su, & Ren, 2019). Our main objectives were to (1) analyze the genetic structure from 43 populations of *P. villosa* from Inner Mongolian Plateau using an AFLP dataset, (2) test whether historical genetic divergence occurred among populations in response to Quaternary climate oscillations, and (3) evaluate the abiotic factors that are most influential in driving the distributions of *P. villosa* . Moreover, because no assessment of the conservation needs of *P. villosa* had previously been accomplished, we also performed a preliminary assessment based on Extent of Occurrence (EOO) with interpretation according to guidelines of the International Union for the Conservation of Nature (IUCN). We believe that, taken together, our results can provide a scientific basis for improved protection and sustainable utilization (e.g., as forage) of *P. villosa* within the fragile desert grassland ecosystems of Northern China.

2 Materials and Methods

2.1 Population sampling

We collected a total of 210 individuals from 43 populations of *P. villosa* in the field throughout its natural range in China (Table S1 & (Figure S1) and randomly sampled five to ten individuals from each population. We sampled individuals 20 m apart to avoid sampling a single clone more than once. In the field, we immediately put the fresh leaves into sealed bags filled with silica gel and then stored them in the laboratory in a -20 freezer until processing. We deposited one voucher specimen representing each population in the Herbarium of Qinghai-Tibet Plateau Museum of Biology (QTPMB), Northwest Institute of Plateau Biology, Chinese Academy of Sciences, China.

2.2 DNA extraction and AFLP genotyping

We extracted total DNA from each sample according to a modification of the CTAB procedure (Doyle & Doyle, 1987) and accessed DNA quality using 1.0% agarose gel electrophoresis and A260/A280 ratio determined on a Nanodrop 2000c. Our procedure to obtain AFLPs was based on a modification of the method in Vos et al. (1995). First, we digested the genomic DNA with the restriction enzymes *EcoR* I and *MseI*for 5 h at 37degC, and ligated the adaptors of *EcoR* I and*Mse* I to the digestion products over night at 4degC (Beijing Dingguo Biotechnology Co., Ltd). Using the digested products, we performed a two-stage PCR amplification comprising pre-amplification and selective amplification. The selective amplification was conducted in 25 μl volume of reaction mixture containing of 1.0 μl *EcoR* I/*Mse* I primer combinations (AAC/CAA, AAG/CAC, ACA/CAG, ACT/CAT, ACC/CTA, ACG/CTC, AGC/CTG, AGG/CTT; Table 1). Subsequently, we separated the fluorescently-labeled fragments on an ABI PRISM 377 DNA Calibrator (Applied Biosystems) using GeneScan ROX-500 with an internal size standard, allowing visual inspection of all individual sites (Liu, Harris, Gao, Su, & Ren, 2019). We recorded the presence or absence of AFLP amplification bands (Figure S2) in a binary matrix as 1 or 0, respectively, based on interpretations from GeneScan 3.1 (Applied Biosystems). In total, we assessed 1728 AFLP markers for the 210 individuals, and all interpretations were performed randomly.

2.3 Genetic diversity and population genetic structure

For each population, we evaluated genetic diversity and population genetic structure according to standard metrics in POPGENE 1.32 (Wang, 1996; Yeh, Yang & Boyle, 1999). These metrics included the number of individuals (N), percentage of polymorphic loci (PPL), observed number of alleles (N a), effective number of alleles (Ne), Shannon's information index (I ; Lewontin, 1972), expected heterozygosity (H e; Kimura & Crow, 1964), Nei's genetic diversity (h), total gene diversity (H t), the average gene diversity within populations (H s), and Nei's standard genetic distance (GD). We also calculated the degree of genetic differentiation between populations (G_{ST}) as (H t–H s)/H t (Nei, 1973) and the parameter of gene exchange as N m = 0.5(1- G_{ST})/G_{ST} (McDermott & McDonald, 1993). Similarly, we estimated parameters of genetic diversity, coefficients of gene differentiation and gene flow for eight pairs of AFLP primers.

In order to search for partitions of sampling sites genetically homogenous but maximally differentiated from each other, we conducted a spatial analysis of molecular variance using SAMOVA 1.0 (Dupanloup, Schneider, & Excoffier, 2002) based on AFLP datasets. Within SAMOVA, we used a K -means method to select the best clustering among groups of populations based on genetic variation coefficients (F_{CT}) (Li et al., 2020). For values of Kin the range two to ten, we set simulated annealing processes to 100 with 10,000 steps each. We selected the value of K that maximizedF_{CT} values as the optimal grouping of populations. Using this optimal grouping, we evaluated the genetic variation between populations within groups and between groups in SAMOVA 1.0 via an analysis of molecular variance (AMOVA, Excoffier, Smouse, & Quattro, 1992) in ARLEQUIN v3.01 (Excoffier, Laval, & Schneider, 2005). Neutrality tests, such as Tajima's D and Fu's Fs , were also calculated with this program. Subsequently, we determined the correlation between F_{ST} inferred from the binary matrix of scored AFLPs and geographic distance of the populations via a Mantel test (Mantel, 1967) in GenAlEx 6.5 (Peakall & Smouse, 2012) with 9999 permutations to evaluate significance.

To further investigate the genetic associations among 43 populations of*P. villosa* , we used the SAHN module in NTSYS-pc 2.10e (Rohlf, 1997) to generate a UPGMA tree from the genetic distance matrix derived from the binary AFLP dataset, and also carried out a principal coordinate analysis (PCoA) based on the distance matrix. Moreover, we constructed a similarity-based network in SplitsTree 4.13 (Huson & Bryant, 2006) to infer the relationships between individuals and populations by applying the Neighbor-Net algorithm with the Jaccard's measure of distance.

In addition, we inferred groupings and genetic structures of populations of *P. villosa* using STRUCTURE V2.2 (Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, & Pritchard, 2007; Hubisz, Falush, Stephens, & Pritchard, 2009), which differs from SAMOVA by not requiring that groupings be geographically adjacent. In STRUCTURE, we performed the analyses using an admixture model with independent allele frequencies for 90 independent runs for the number of clusters (K) ranging from one to ten. We applied 2×10^5repetitions of the Markov chain Monte Carlo with a burn-in of 25%. To determine the best value of K for the STRUCTURE analyses, we used the ΔK statistical method (Evanno, Regnaut, & Goudet, 2005).

2.4 Distribution modeling of *P. villosa*

In order to predict the impact that Quaternary climatic oscillation might have on the geographic distribution change of *P. villosa* , we employed an ecological niche modelling (ENM) approach to evaluate the potential distribution of *P. villosa* at the Last Inter-Glacial (LIG, ˜ 120,000 - 140,000 years before present), the Last Glacial Maximum (LGM, ˜ 21,000 years before present), the present and future times (2050s and 2070s), respectively. In addition to the distribution records of our field surveys, we also collected GPS data from the Chinese Virtual Herbarium (CVH, http: //www.cvh.ac.cn), Global Biodiversity Information Facility (http: //www. gbif.org), China National Specimen Information Infrastructure (http://www.nsii.org.cn) and Specimen Resources Sharing Platform for Education (http://mnh.scu.edu.cn/main.aspx) for *P. villosa* . In total, after removing duplicate and ambiguous records, we used 155 localities to generate spatial distribution models for *P. villosa*(Table S2). To improve abilities in establishing high-resolution predictions and identifying the critical factors influencing the species' distribution, we obtained 19 bioclimatic variables and three geographic factors, such as altitude, slope and aspect, at 2.5 arc-min resolution from WorldClim database (Hijmans, Cameron , Parra, Jones, & Jarvis, 2005, www.worldclim.org). The future climate data involved in two emission scenarios of representative concentration pathways (RCP8.5 and RCP2.6) with the CCSM4 model (Van et al., 2011). We excluded highly correlated variables according to Spearman's correlation test (Peterson & Nakazawa, 2008). Specifically, we selected the variables with a relative contribution score [?] 0.8 or a correlation of < 0.75 compared to other variables. Based on the outcome of Spearman's, we retained the eleven variables with the lowest correlations to build a maximum entropy model for the habitat of*P. villosa* using. Subsequently, we generated this model representing the potential distribution of *P. villosa* in environmental space in MaxEnt 3.3.3k (Phillips, Anderson, & Schapire, 2006; Phillips & Dudik, 2008). Within MaxEnt, we performed modeling with 75% of localities randomly selected for training and 25% selected for testing 500 times independently to ensure reliable results, and we evaluated model performance using the area under the curve (AUC) of receiver operating characteristic (ROC). The value of AUC ranges

between 0 (randomness) and 1 (exact match), and the value above 0.9 indicated good performance of the model (Swets, 1988). Additionally, we projected the predicted geographic ranges of species based on the ENMs using ArcGIS 10.2. In particular, we divided suitable habitat into four classes: highly suitable habitat (0.5 [?] P [?] 1.0), moderately suitable habitat (0.3 [?] $P < 0.5$), poorly suitable habitat (0.1 [?]$P < 0.3$) and unsuitable habitat (0.0 [?] $P< 0.1$).

In order to measure the niche similarity between populations occurring in groups, we calculated Schoener's D (Schoener, 1968) and standardized Hellinger distance (calculated as I) in ENMTools 1.3 (Warren, Glor, & Turelli, 2008, 2010). We obtained the null distribution of niche models in the identity test based on 1000 pseudo replicates generated by random sampling from the data points pooled for each pair of cluster. We determined measures of niche similarity (D and I) by comparing with null distributions drawn from pooled occurrences retaining original cluster size, and we drew histograms of frequency distributions using R 2.13 (http://www.r-project.org/).

2.5 Conservation assessment of *P. villosa*

We performed a conservation assessment using the Extent of Occurrence (EOO) (Moat, 2007; Velzen & Wieringa, 2014) and following guidelines for their interpretation from IUCN (2001). EOO comprises the minimum convex polygon covering all known or predicted sites for the species. It is frequently used as a preliminary assessment tool, such as when a new species is described or when populations of a species are found in new places or were locally extirpated (e.g., Velzen & Wieringa, 2014; Lachenaud et al., 2013). In the case of *P. villosa* , there had been no prior conservation assessment for the species, and it status is not presently included in the IUCN Red List of Threatened Species (IUCN, 2020). Therefore, we analyzed the EOO using the location data of 155 populations from ecological niche model (Table S2).

3. Results

3.1 Polymorphism of AFLP markers

The eight primer pairs yielded 1728 clearly identifiable amplified bands, of which 1654 (95.72%) were polymorphic (Table 2). Different primers yielded different numbers of bands ranging from 214 (E -ACG/M -CTC) to 199 (E -AGC/M -CTG and$E$ -AAG/M -CAC) with an average of 207. The highest rate of polymorphism for an individual primer was 99.07%, and the lowest was 92.13%. Overall, these eight primers showed high levels of polymorphism among individuals of *P. villosa* .

The indices, N a, N e, h , and I were important indicators of genetic diversity of populations. According to the genetic diversity index of eight primer pairs, the indices of N a,N e, h and I ranged from 1.9950 to 2.0000 with an average of 1.9994, 1.2898 to 1.3445 with an average of 1.3229, 0.1926 to 0.2131 with an average of 0.2056, and 0.3183 to 0.3379 with an average of 0.3316, respectively. Overall, these indices showed the highest genetic diversity for the primer pair of E -AGG/M -CAC, while the lowest was for E -ACT/M -CTT. In general, each of eight primer pairs appeared to facilitate a robust assessment of genetic diversity in *P. villosa* .

3.2 Population Grouping

Based on SAMOVA, we found that the F_{CT} value was the highest when $K = 3$, which resulted in separation of populations into three groups. Among them, both the first group (P1-6) and the second group (P7-12) contained six populations, and the third group consisted of all other populations. However, three populations (P6-8) were highly similar in their geographic distributions and their habitats according to our field surveys, and this was why we did not adopt $K = 4$. When K was > 4, at least one group consisted of a single population (Table S3), indicating over-splitting and loss of geographic structural data. Thus, we thought it was the most reasonable to divide 43 populations of *P. villosa*into two major groups.

The first group, Group 1, identified using SAMOVA, consisted of 12 populations (P1-12), which mainly occurred in the central and eastern regions of the Inner Mongolian Plateau, while the second one, Group 2, was composed all other populations, which distributed throughout the range of the species in China. Notably,

the populations of Group 1 tend to be found at lower elevations compared to those from Group 2 (Table S1 & Figure S1). Groupings of populations can facilitate detection of finer scale geographic structures (i.e., within groups) juxtaposed with broader, regional patterns (i.e., between groups) (Li et al., 2020). Therefore, we used the recovered grouping scheme for downstream data analysis in our present study.

3.3 Genetic diversity of populations

The percentage of polymorphic loci (PPL), observed number of alleles (N a), effective number of alleles (N e), expected heterozygosity (H e) and Shannon information index (I) of 43 populations were from 19.23 to 54.29, 0.5012 to 1.1155, 1.1315 to 1.2908, 0.0791 to 0.1771 and 0.1163 to 0.2709, respectively. The PPL,N a, N e, H e, I of populations from Group 1 were 32.63, 0.7193, 1.1848, 0.1100 and 0.1667, respectively, while those of Group 2 were 32.02, 0.7322, 1.1817, 0.1082 and 0.1640 (Table S1). The genetic diversity indices showed the populations of Group 2 exhibited greater variability than those of Group 1. However, the difference of genetic diversity between two groups was minimal. Overall, the results of genetic diversity analyses from 43 populations of *P. villosa*suggested that there was abundant genetic diversity of this species within the Inner Mongolian Plateau.

3.4 Genetic differentiation and distance of populations

The genetic differentiation analyses based on 43 populations of *P. villosa* revealed that the total gene diversity (H t) was 0.2052, the average gene diversity within populations (H s) was 0.1088, the genetic differentiation between populations (G_{ST}) was 0.4689, and the gene flow (N m) was 0.5761. The value of N m indicated that a limited level of gene exchange existed within the species (Table 3). With respect to populations of Group 1, the values of H t, H s andG_{ST} were 0.1668, 0.1102, and 0.3402, respectively, while these were 0.1918, 0.1083, and 0.4349 for Group 2. Thus, the genetic diversity of Group 1 was lower than that of Group 2, and the genetic differentiation was smaller. The gene flow among populations within Group 1 and Group 2 was 0.9996 and 0.6605, respectively, which revealed that the gene exchange among populations within Group 1 was more frequent.

3.5 Population genetic structure

Analysis of molecular variance (AMOVA) based on the AFLP dataset from 43 populations of *P. villosa* showed that the proportion of genetic variation among populations was 35.84%, while that within populations was 64.16%, and the value of average pairwiseF_{ST} was 0.35841 ($P < 0.001$) (Table 4), which showed that the genetic variation of *P. villosa*mainly occurred within populations. More interestingly, when aggregating populations into Groups 1 and 2, we found that 22.38% of the genetic variation occurred among populations within groups ($F_{CT} = 0.28501$, $P < 0.001$), while most of genetic variation (56.14%) existed within populations ($F_{ST} = 0.43865$, $P < 0.001$) (Table 4). Overall, the genetic variation at the population- and local geographic-scale was much higher than regionally in *P. villosa* . Additionally, the result of neutrality test suggested the value of Tajima's D and Fu's Fs was positive, but non-significant for all populations of *P. villosa* (Table 3).

The Mantel test revealed that there was a significant positive correlation between geographic distance and $F_{ST}$for 43 populations ($r = 0.282$, $P < 0.05$) (Figure 1). Similarly, we detected a strong, significant, positive correlation between geographic distance and F_{ST} for Group 1 ($r = 0.622$, $P < 0.05$) and a weak but significant positive correlation for Group 2 ($r = 0.372$, $P < 0.05$). Simultaneously, results from UPGMA tree, SplitsTree network, PCoA, and STRUCTURE suggested that 43 populations of *P. villosa*were divided into two groups, which were largely consistent with our assessment using SAMOVA (Figure 2 - 5).

3.6Distributional change of *P. villosa*

ENMs for *P. villosa* yielded relatively high AUC, demonstrating reliable model performance (AUC = 0.969, Figure S3). For the eleven non-biological variables used for modeling, the most significant factor for the spatial distribution pattern of *P. villosa* was altitude (Alt), followed by temperature annual range (bio 7) and precipitation of warmest quarter (bio 18), whose contribution rates were 40.0%, 17.2% and 16.7%, respectively (Table 5). In comparison with the LIG, we observed a contraction in highly suitable habitat

during the LGM based on the MaxEnt models (Table 6 & Figure 6). Similarly, the spatial distribution of the present was continuously shrinking compared to the potential range during the LGM (Figure 7). The simulated distribution based on present climate data was mostly congruent with the actual distribution range of *P. villosa* , which was mainly distributed in the Inner Mongolia Plateau with an area of approximately 111.2450×10^4 km^2 (Table 6 & Figure7). Simultaneously, we estimated the future changes in the potential spatial distribution under the RCP 2.6 and RCP 8.5 scenarios for the 2050 s and 2070 s. According to the future model predictions, the areas of suitable habitat is likely to remain stable under the climatic scenario of RCP 2.6 for the 2050s and 2070s, whereas there was an increase of highly suitable areas based on RCP 8.5 (Table 7 & Figure 8).

When we compared the niches of hypothesis of niche identity was rejected when the empirically observed value for D and/or I was significantly lower than the values expected from the pseudo-replicated data sets. Therefore, identity tests between two groups indicated that there was distinct niche differentiation ($P < 0.01$) (Figure 9). The niche of two groups differs mainly in that it was characterized by high elevation and temperature.

3.7 Conservation status of *P. villosa*

IUCN is a global classification standard system for threatened species. Its main purpose is to provide a clear and objective framework for the classification of species according to their extinction risk. *Psammochloa villosa* qualifies as a species of least concern (LC) under the EOO criterion (EOO = 2,064,370 km^2). However, after observing populations at 43 sampling locations during our field work, we noted that some populations of the species presently grew in severely degraded habitat. Thus, while this species was probably not currently threatened, decreases in its frequency within population and the abundance of populations could jeopardize its vital ecological role. Therefore, we advocate for continued ecological monitoring of this dominant, keystone desert grass species.

4. Discussion

4.1Genetic diversity of *P. villosa*

Genetic diversity refers to intraspecific genetic variation and is closely linked to the evolutionary potential of a species to adapt to adverse environments (Ma & Qian, 1994). The study of genetic diversity can facilitate understanding of spatial genetic patterns of a species, and elucidate its evolutionary processes and adaptive mechanisms (Hao, 2005). In the present study, we observed high genetic diversity at species-level in *P. villosa* (P =95.72%, $I = 0.3316$) and the population-level (P= 32.02%, $I = 0.1640$) (Table 2 & Table S1). Compared to genetic diversity in other species of Poaceae assessed using AFLPs, genetic diversity in *P. villosa* was slightly lower than that of*Chascolytrum bulbosum* ($P = 98.2\%$, $h = 0.1500$; Silva, Essi, Welker, & de, Souza-Chies, 2016), and was higher than that of *Leymus chinensis* ($P = 16.53\%$, $I = 0.0890$),*Leymus racemosus* ($P = 16.53\%$, $I = 0.0890$), and*Dactylis glomerata* ($P = 61.70\%$, $I = 0.2664$) (Gong et al., 2007; Cai, 2016; Zhang, He, Zhao, Zhang, & Xu, 2017).

The underlying drivers of genetic diversity within species are generally a combination of biological properties, such as dispersal abilities and life history, and environmental factors, such as climate and anthropogenic activities (Loveless & Hamrick, 1984; Hamrick & Godt, 1996; Wang & Hu, 1996; Wen, Han, & Wu, 2010). The life history of*P. villosa* frequently involves clonal reproduction via its rhizomes under harsh environmental conditions, although the species reproduces sexually by seed following wind pollination (Wang, Ge, & Dong, 1999; Li & Ge, 2001). In comparison with *L. chinensis* ,*L. racemosus* , and *D. glomerata* , the relatively high genetic diversity of *P. villosa* might be explained by one or more of several factors. Among these, our study design comprised more populations, which might lead to greater accuracy. However, biological explanations are more likely and include possible higher clonal fitness due to *P. villosa* having extremely robust, hardy rhizomes; high rates of seed production; and seedling regeneration, while rare within any one growing season, occurring often over the long lifetime of the species (Eriksson & Bremer, 1993; Helena & Mikko, 1996; Shimizu et al., 1998).

4.2 Genetic differentiation and genetic structure

Genetic structure of a species is effectively the sum of genetic differentiation among and within populations (Hamrick & Godt, 1989). Overall, genetic structure occurring among populations results from the evolutionary history of the species in question; natural selection; genomic factors (e.g., mutations, reorganization, and genetic drift); and biological characteristics, including gene flow, mating system, mode of reproduction, and seed dispersal mechanisms (Slatkin, 1987; Zhen, 2010). Genetic differentiation is primarily controlled by aspects of gene flow, such as its rate and directionality (Hamrick & Godt, 1989). In plants, gene flow occurs primarily via the transmission of pollen and seeds during sexual reproduction. However, for clonal species, such as*P. villosa* , asexual propagules often have limited dispersal distance, and this restricts gene flow among populations (Xia, Li, & Li, 2002).

For *P. villosa* , we inferred that more than 56% of the genetic variation existed within populations, with average pairwiseF_{ST} of 0.35841 for all 43 population and gene flow (N m) of 0.5761 (Table 4). According to Wright's (1978) theory, genetic differentiation among populations might be large when$F_{ST} > 0.25$, but this can be mitigated by gene flow of N m > 1, which can reduce the effects of genetic drift and prevent genetic differentiation among populations. In *P. villosa* , we found highF_{ST} but limited gene flow, which should yield high rates of between population differentiation. However, we found higher rates of within population differentiation for the species. This differs from findings in other studies of *P. villosa* that revealed greater genetic variation among populations, such as in Li & Ge (2001), who studied genetic diversity in *P. villosa* using and seven populations, of which two were from Shihuimiao Ecological Station and five were from the Shilongmiao Ecological Station. Similarly, Wang et al. (1999) assessed the genetic diversity of four populations of*P. villosa* from mobile and fixed sand dunes in the Shihuimiao Ecological Station (two populations) and the Shilongmiao Ecological Station (two populations) and found greater genetic variation among populations. Nevertheless, population genetic structure was a comprehensive result of a variety of factors that were related to the evolutionary origins and modes of dispersal and sexual reproduction, which all might be unique to each population within a species (Wang, Ge, & Dong, 1999). Accordingly, we inferred this inconsistency may be related to the numbers of populations and geographic location. Our sampling, which included a larger number of populations and a greater portion of the geographic range of*P. villosa* , might have yielded results that more robustly detect patterns among unique populations. In addition, the efficiency and accuracy of gene identification are also related to the number of polymorphic bands, and the results obtained by different experimental methods are different.

In this study, our SAMOVA analysis revealed two well-defined groups corresponding to Group1 and Group2 (Table S3). These groups are also consistent with our analyses using UPGMA, STRUCTURE, SplitsTree, and PCoA (Figure 2-5). Within populations of Groups 1 and 2, the average genetic variation was 64% with an average pairwiseF_{ST} value of 0.43865. The gene flow (N m) in Groups 1and 2 was 0.9996, 0.6605, respectively (Table 4). In addition, the UPGMA tree revealed that populations with closer geographic distances did not always cluster together, and we observed a similar pattern in the results from STRUCTURE and SplitsTree. Similarly, the PCoA showed that individuals from the same population did not always group together, further indicating that, while gene flow may be limited in *P. villosa* , it occurs over long distances more often than between adjacent populations. This may suggest that some critical dispersal vector, such as birds, is yet-unknown for the species. Overall, *P. villosa* has undergone considerable genetic divergence and has a high level of genetic structure based on the combined results from our population genetics analyses.

Based on a Mantel test, we found that there was significant correlation between genetic distance and geographic distance, indicating that geographic distance is an important factor affecting the genetic structure of *P. villosa* . Therefore, we inferred that genetic structure might have been resulted mainly from geographic isolation imposed by mountains (e.g., Yin Mountains; Helan Mountains) and large deserts in northwestern China (e.g. Tengger Desert; Mu Us Sandy Land) as well as range contraction and population fragmentation induced by climatic oscillations (e.g., *Gymnocarpos przewalskii* Maxim.;*Helianthemum songaricum* Schrenk) (Liu, 1995; Su, Zhang, & Sanderson, 2011; Ma, Zhang, & Sanderson, 2012; Meng, Gao, Huang, & Zhang, 2014). In addition, founder effects and population bottlenecks might have also contributed to the genetic

structures of the species (Birky, Fuerst, & Maruyama, 1989; Liu et al., 2015).

4.3 Demographic historyof*P. villosa*

The genetic diversity within the Group 1, identified according to SAMOVA, was lower than that of the populations in Group 2 (Table 3). Based on this, extant populations of this species originated from the genetic stock of Group 2, as geographic areas with both high genetic diversity and frequency of dominant genes usually represent centers of origins for source populations (Vavilov, 1926). However, our study design and results cannot discern the exact center of origin for the species nor the main migrational patterns of *P. villosa* , and accomplishing this will require additional molecular data and informatics approaches.

Climate oscillation during the Quaternary has often been hypothesized to be an important factor in influencing the current geographical distribution and demographic history of plant species (Hewitt, 2004; Su & Zhang, 2013). One widely utilized approach to comparing past and future distributions of plant species and determining the primary environment factors driving them is via ENM (e.g., Nabout, Magalhães, Ma, & Da, 2016; Bai et al., 2017; Huang et al., 2017; Noulèkoun, Chude, Zenebe, & Birhane, 2017; Swanti, Kusum, Dhruval, & Rajkanti, 2018; Wei, Wang, Hou, Wang, & Wu, 2018). Based on the neutral test, we found that the species did not expand its range during the Quaternary. Although these results were not statistically significant (Table 3), our ENMs, in general, show that the range of *P. villosa* contracted from the LGM to the present (Figure 6-7). Specifically, our models show that the range of *P. villosa* was the most extensive during the LIG period and included the northeast edge of the Qinghai-Tibet Plateau, Tarim Basin, Tianshan Mountains, Inner Mongolia Plateau, and the western regions of DaXinggan Ling. The range became limited to the Inner Mongolia Plateau, Ordos Plateau, and the Yinshan-Helanshan area during the LGM. The contraction of the range is likely the result of glaciation and climatic shifts within the Tianshan Mountains and Tarim Basin, where temperatures dropped significantly as glaciation developed on a large scale in the Northern Hemisphere during the early-Middle Pleistocene (Williams, Dunkerley, De, Dekker, Kershaw, & Stokes, 1993; Yi et al., 2004; Shi, Cui, & Su, 2005; Lehmkuhl & Owen, 2005; Xu et al., 2010; Meng, Gao, Huang, & Zhang, 2014). Nevertheless, it is surprising that the species range did not rebound as temperatures grew warmer following the LGM. This may be because of the onset of extreme aridity within the region during the Quaternary period, as this is widely-known to have played a significant role in determining the geographic distribution and evolutionary history of many plant species (Meng & Zhang, 2011; Su, Zhang, & Sanderson, 2011; Su & Zhang, 2013). For example, in a previous study of *Helianthemum songaricum* (Cistaceae), which occurs in Northern China and adjacent desert areas of central Asia (Yang & Gilbert, 2007), the worsening of the dry climate restricted the distribution range, and acceptable habitats for the species gradually became reduced and isolated (Su, Zhang, & Sanderson, 2011). Besides, it needs to explain that AFLP dataset used in this manuscript do not reveal too many informative sites, so more markers should be selected to discuss the evolutionary history of *P. villosa* in the future.

4.4 Germplasm conservation of *P. villosa*

Psammochloa villosa is a dominant species in its desert habitat and sometimes the only herbaceous species occurring within its plant community. It helps to maintain a fragile desert ecosystem by preventing wind erosion, development of quicksand, and further desertification (Cai, 2016). While we found that it is a species of least concern (LC) based on EOO, we believe that it can only continue to perform its vital ecosystem services if its populations remain large and abundant.

This species may have great potential for sustainable utilization as a forage plant for livestock. The sand whips have relatively long inflorescences with large spikes that make it suitable for forage. Moreover, its adaptations to drought may make it a valuable source of genetic resources for molecular breeding of other crop and forage species as, presently, it is one of few forage species that can withstand the intensifying long-term drought conditions in northwest China. Developing a sustainable use strategy for *P. villosa* will also help to ensure its continued availability as a keystone species within desert communities of the Inner Mongolian Plateau and adjacent areas.

Conflict of interest

None declared.

Author contributions

Ting Lv: Investigation (equal); Conceptualization (equal); Data curation (equal); Methodology (lead); Formal analysis (equal); Writing-original draft (lead); Writing-review & editing (equal). **AJ Harris:** Conceptualization (equal); Writing-original draft (lead); Writing-review & editing (equal). **Tao Liu:** Investigation (equal); Formal analysis (equal); Methodology (supporting). **Ruifang Liang:** Data curation (equal); Formal analysis (equal); Methodology (supporting).**Zilan Ma:** Investigation (equal); Formal analysis (equal); Methodology (supporting). **Yuping Liu:** Conceptualization (equal); Formal analysis (equal); Methodology (supporting); Funding acquisition (lead); Writing-review & editing (equal). **Xu Su:** Investigation (equal); Conceptualization (equal); Formal analysis (equal); Writing-original draft (supporting); Project administration (supporting); Supervision (lead); Writing-review & editing (equal).

Data accessibility

All tables and figures supporting the results and conclusions were included in the article, except for the binary scoring of AFLP bands, which we have submitted to the Dryad Digital Repository (https://doi.org/10.5061/dryad.dbrv15f0v) and provided an available link for review at https://datadryad.org/stash/share/aepU6ms8Yp7kveC0mQTnYjxptp2GhAr3OUCteVtm5B4.

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Table 1 Adapters and primer combination sequences used in the present study

Primer	Name	Sequence
Adapters	Adapters	Adapters
E-L	*Eco*R I-adapter	5'-CTCGTAGACTGCGTACC-3'
E-R	*Eco*R I-adapter	5'-AATTGGTACGCAGTCTAC-3'
M-L	*Mse* I-adapter	5'-GACGATGAGTCCTGAG-3'

Primer	Name	Sequence
M-R	*Mse* I-adapter	5'-TACTCAGGACTCAT-3'
Preamplification primer	Preamplification primer	Preamplification primer
P01	*Eco*R I	5'-GACTGCGTACCAATTCA-3'
P02	*Mse* I	5'-GATGAGTCCTGAGTAAC-3'
Selective amplification primer	Selective amplification primer	Selective amplification primer
A-1	*Eco*R I-AAC	5'-GACTGCGTACCAATTCAAC-3'
	Mse I-CAA	5'-GATGAGTCCTGAGTAACAA-3'
A-2	*Eco*R I-AAG	5'-GACTGCGTACCAATTCAAG-3'
	Mse I-CAC	5'-GATGAGTCCTGAGTAACAC-3'
A-3	*Eco*R I-ACA	5'-GACTGCGTACCAATTCACA-3'
	Mse I-CAG	5'-GATGAGTCCTGAGTAACAG-3'
B-1	*Eco*R I-ACT	5'-GACTGCGTACCAATTCACT-3'
	Mse I-CAT	5'-GATGAGTCCTGAGTAACAT-3'
B-2	*Eco*R I-ACC	5'-GACTGCGTACCAATTCACC-3'
	Mse I-CTA	5'-GATGAGTCCTGAGTAACTA-3'
C-1	*Eco*R I-ACG	5'-GACTGCGTACCAATTCACG-3'
	Mse I-CTC	5'-GATGAGTCCTGAGTAACTC-3'
D-1	*Eco*R I-AGC	5'-GACTGCGTACCAATTCAGC-3'
	Mse I-CTG	5'-GATGAGTCCTGAGTAACTG-3'
D-2	*Eco*R I-AGG	5'-GACTGCGTACCAATTCAGG-3'
	Mse I-CTT	5'-GATGAGTCCTGAGTAACTT-3'

Table 2 Summary statistics for eight selective primer combinations of amplified fragment length polymorphism (AFLP) in the present study

Selective nuclear	Polymorphism band	Amplification band	PPL (%)	Size range (bp)	*N*a	*N*e	*h*	*I*
E-AAC/M-CAA	201	216	93.06	69.5-501.5	1.9950	1.3397	0.2118	0.3
E-AAG/M-CAC	199	216	92.13	69.5-501.5	2.0000	1.3261	0.2030	0.3
E-ACA/M-CAG	206	216	95.37	69.5-501.5	2.0000	1.3201	0.2079	0.3
E-ACT/M-CAT	211	216	97.69	69.5-501.5	2.0000	1.3259	0.2084	0.3
E-ACC/M-CTA	211	216	97.69	69.5-501.5	2.0000	1.3060	0.1988	0.3
E-ACG/M-CTC	214	216	99.07	69.5-501.5	2.0000	1.2898	0.1926	0.3
E-AGC/M-CTG	199	216	92.13	69.5-501.5	2.0000	1.3445	0.2131	0.3
E-AGG/M-CTT	213	216	98.61	69.5-501.5	2.0000	1.3311	0.2091	0.3
Total	1654	1728	-	-	-	-		-
Average	207	216	95.72	-	1.9994	1.3229	0.2056	0.3

Note. PPL, percentage of polymorphic loci; N a, observed number of alleles; N e, effective number of alleles; h , Nei's genetic diversity; I , Shannon's information index. H t, total gene diversity; H s, the average gene diversity within populations;G_{ST}, the genetic differentiation between populations; N m, gene flow.

Table 3 Genetic diversity, differentiation parameters, and neutrality test for 43 populations of *P. villosa* in the present study

Population group	*N*a	*N*e	*h*	*I*	*H*t	*H*s	G_{ST}	*N*m	Fu's *Fs* (*P*-Value)
Populations of Group1 (1-12)	1.7645	1.2681	0.1668	0.2656	0.1668	0.1102	0.3402	0.9996	3.2491 (0.5701)
Populations of Group2 (13-43)	1.9710	1.3001	0.1921	0.3116	0.1918	0.1083	0.4349	0.6605	3.3578 (0.5738)

Population group	Na	Ne	h	I	Ht	Hs	G_{ST}	Nm	Fu's Fs (P-Value)
All populations	1.9994	1.3229	0.2056	0.3316	0.2052	0.1088	0.4689	0.5761	3.3274 (0.5702)

N a, observed number of alleles; N e, effective number of alleles; h , Nei's genetic diversity; I , Shannon's information index. H t, total gene diversity; H s, the average gene diversity within populations; G_{ST}, the genetic differentiation between populations; N m, gene flow.

Table 4 Results of analyses of molecular variance (AMOVAs) based on amplified fragment length polymorphism markers for *P. villosa*

Grouping	Source of variation	*df*	SS	VC	Percent variation (%)	Fixation
Total populations	Among populations	42	20586.257	73.45887	35.84%	F_{ST}=0.
	Within populations	167	21960.467	131.49980	64.16%	
	Total	209	42546.724	204.95867		
SAMOVA groups	Among groups	1	4707.100	50.33831 Va	21.49%	F_{CT}=0
	Among populations within groups	41	15879.157	52.41736 Vb	22.38%	F_{SC}=0.
	Within populations	167	21960.467	131.49980 Vc	56.14%	F_{ST}=0.
	Total	209	42546.724	234.25546		

Note. *df* , degrees of freedom; SS, sum of squares; VC, variance components; F_{CT}, variance among groups relative to total variance; F_{SC}, variance among populations within groups; F_{ST}, variance among populations; Significant level:$^{**}p < 0.001$.

Table 5 Environmental variables used for modeling and percent contribution of *P. villosa*

Code	Environment variable	Contribution/%
Alt	Altitude	40
Bio 7	Temperature annual range (bio5-bio6)	17.2
Bio 18	Precipitation of warmest quarter	16.7
Slop	Slope	8.3
Bio 19	Precipitation of coldest quarter	7.2
Bio 15	Precipitation seasonality (coefficient of variation)	4.2
Bio 6	Min temperature of coldest month	2.4
Asp	Aspect	1.7
Bio 5	Max temperature of warmest month	1
Bio 2	Mean diurnal range (mean of monthly (max temp-min temp))	0.9
Bio 3	Isothermality (Bio 2/Bio 7) (×100)	0.3

Table 6 Prediction of potential suitable distribution areas of*P. villosa* in different periods

Period	Prediction area ($\times 10^4$ km^2)	Prediction area ($\times 10^4$ km^2)	Prediction area ($\times 10^4$ km^2)	Prediction area (×10
	Unsuitable habitat	Poorly suitable habitat	Moderately suitable habitat	Highly suitable habi
LIG	532.4994	85.2601	54.3909	282.0408
LGM	724.5804	111.8953	45.7149	74.2954
Present	845.8872	43.8207	30.2673	37.1570
2050s-2.6	804.7547	65.9706	25.4776	60.2943
2050s-8.5	793.9596	72.6263	29.3144	60.5905

Period	Prediction area ($\times 10^4$ km^2)	Prediction area ($\times 10^4$ km^2)	Prediction area ($\times 10^4$ km^2)	Prediction area ($\times 10$
2070s-2.6	804.7547	65.9706	25.4776	60.2943
2070s-8.5	710.9724	112.0121	47.4650	86.0193

Table 7 Dynamic changes in the suitable habitat area for *P. villosa* under different climate scenarios

Climate scenario	Area ($\times 10^4$ km^2)	Area ($\times 10^4$ km^2)	Area ($\times 10^4$ km^2)	Area
	Loss	Gain	Stable	Tota
Last Inter Glacial (LIG)	1.2193	270.3439	66.2606	269.
Last Glacial Maximum (LGM)	0.0449	52.2913	67.6280	52.2
Representative concentration pathway (RCP) 2.6-2050	0.0905	17.9933	67.5910	17.9
Representative concentration pathway (RCP) 2.6-2070	0.0261	22.0181	67.6961	21.9
Representative concentration pathway (RCP) 8.5-2050	0.0905	17.9933	67.5910	17.9
Representative concentration pathway (RCP) 8.5-2070	0.2209	65.9030	67.5632	65.6

Table S1 Localities and genetic diversity indices for samples of *P.villosa* collected in the present study

Population code	Location	Latitude (N)	Longitude (E)	Altitude (m)
Populations of Group1	Populations of Group1	Populations of Group1	Populations of Group1	Populations of
1	Uxin Banner, NMG	38°52'18.9"	109°10'18.2"	1301
2	Hanggin Banner, NMG	39°20'37.8"	109°00'5.9"	1314
3	Hanggin Banner, NMG	40°01'59.2"	108°28'34.6"	1236
4	Hanggin Banner, NMG	40°12'19.5"	108°29'11.4"	1229
5	Hanggin Banner, NMG	40°26'4.6"	108°37'52.9"	1114
6	Dalad Banner, NMG	40°20'25.8"	109°31'21.5"	1057
7	Dalad Banner, NMG	40°15'39.1"	110°00'43.1"	1126
8	Dalad Banner, NMG	40°11'57.4"	111°07'21.4"	1047
9	Sonid Left Banner, NMG	43°40'36.5"	113°26'57.9"	1010
10	Sonid Left Banner, NMG	43°26'4.1"	114°18'57.8"	1112
11	Sonid Left Banner, NMG	43°16'49.7"	114°26'13.1"	1035
12	Zhengxiangbai Banner, NMG	42°33'20.9"	114°49'0.7"	1211
Average				
Populations of Group2	Populations of Group2	Populations of Group2	Populations of Group2	Populations of
13	Zhenglan Banner, NMG	42°41'0.0"	115°59'0.0"	1321
14	Xilingol League, NMG	43°38'0.0"	116°39'0.0"	1183

Table S1 (Continued)

15	Xilin Hot, NMG	43°39'0.0"	116°10'0.0"	1111	X. Su, 18, 031	5	25.70	0.6360	1.1604	0.1378
16	Abag Banner, NMG	43°23'0.0"	115°00'0.0"	1056	X. Su, 18, 036	5	34.04	0.7757	1.1964	0.1758
17	Abag Banner, NMG	43°18'0.0"	114°46'0.0"	1079	X. Su, 18, 038	5	31.74	0.7328	1.1846	0.1650
18	Sonid Right Banner, NMG	43°07'11.8"	112°54'23.5"	1072	X. Su, 16, 048	5	29.63	0.6892	1.1624	0.1484
19	Zhenglan Banner, NMG	42°42'0.0"	108°56'0.0"	1323	X. Su, 18, 027	5	27.33	0.6663	1.1557	0.1399
20	Sonid Right Banner, NMG	41°41'38.0"	107°00'7.9"	1111	X. Su, 16, 049	3	24.49	0.5979	1.1442	0.1273
21	Alxa Zuoqi, NMG	40°18'39.0"	105°53'35.9"	1211	X. Su, 16, 050	2	21.83	0.5320	1.1315	0.1189
22	Alxa Zuoqi, NMG	40°37'0.0"	104°35'0.0"	1276	X. Su, 18, 046	5	36.70	0.8144	1.2039	0.1860

15	Xilin Hot, NMG	43°39'0.0"	116°10'0.0"	1111	X. Su, 18, 031	5	25.70	0.6360	1.1604	0.1378
23	Alxa Youqi, NMG	39°59'0.0"	104°12'0.0"	1243	X. Su, 18, 011	5	32.47	0.7515	1.1862	0.1677
24	Alxa Youqi, NMG	40°07'49.5"	103°58'20.7"	1430	X. Su, 16, 054	5	32.53	0.7213	1.1709	0.1601
25	Alxa Youqi, NMG	40°01'0.0"	103°53'0.0"	1403	X. Su, 18, 008	5	37.00	0.8295	1.2092	0.1885
26	Alxa Youqi, NMG	39°24'26.3"	102°22'1.8"	1457	X. Su, 16, 057	5	30.53	0.6911	1.1759	0.1567
27	Alxa Youqi, NMG	39°22'11.8"	102°12'52.1"	1570	X. Su, 16, 058	5	28.54	0.6560	1.1751	0.1519
28	Alxa Youqi, NMG	39°21'24.6"	102°06'58.9"	1560	X. Su, 16, 059	5	30.29	0.7104	1.1728	0.1544
29	Liangzhou District, GS	38°06'0.0"	102°59'0.0"	1498	X. Su, 18, 002	5	30.83	0.7195	1.1744	0.1572
30	Liangzhou District, GS	38°13'0.0"	103°18'0"	1459	X. Su, 18, 004	5	38.63	0.8507	1.2138	0.1952
31	Minqin County, GS	39°08'0.0"	103°40'0.0"	1311	X. Su, 18, 006	5	33.62	0.7787	1.1896	0.1719
32	Alxa Zuoqi, NMG	39°16'0.0"	104°57'0.0"	1241	X. Su, 18, 013	5	32.47	0.7557	1.1890	0.1689
33	Alxa Zuoqi, NMG	39°40'1.2"	105°42'12.8"	1025	X. Su, 16, 051	5	19.23	0.5012	1.1359	0.1163

Table S1 (Continued)

34	Alxa Zuoqi, NMG	38°57'43.2"	105°39'16.3"	1458	X. Su, 16, 052	5	25.45	0.6161	1.1523	0.1338
35	Pingluo Xian, NX	38°47'0.0"	105°31'0.0"	1179	X. Su, 18, 015	5	34.40	0.7878	1.1977	0.1774
36	Zhongwei City, NX	37°25'29.1"	104°40'5.0"	1707	X. Su, 16, 001	5	43.35	0.9256	1.2226	0.2102
37	Lingwu County, NX	38°07'9.8"	106°30'57.8"	1227	X. Su, 16, 003	5	37.61	0.8150	1.2046	0.1878
38	Ordos, NMG	37°46'46.0"	108°08'31.4"	1344	X. Su, 16, 005	5	38.15	0.8277	1.1990	0.1860
39	Jingbian County, SX	37°40'52.2"	108°50'23.5"	1328	X. Su, 16, 007	5	35.49	0.7696	1.1984	0.1793
40	Uxin Banner, NMG	37°47'56.7"	108°42'39.3"	1254	X. Su, 16, 008	5	26.78	0.6348	1.1438	0.1326
41	Uxin Banner, NMG	38°22'8.1"	108°40'6.3"	1286	X. Su, 16, 010	5	26.00	0.6288	1.1436	0.1307
42	Otog Banner, NMG	38°53'0.0"	108°18'0.0"	1371	X. Su, 18, 019	5	32.95	0.7545	1.1877	0.1686
43	Otog Banner, NMG	39°01'0.0"	108°00'0.0"	1358	X. Su, 18, 017	5	54.29	1.1155	1.2908	0.2709
Average							32.02	0.7322	1.1817	0.1640

N , number of individuals; polymorphic loci, the percentage of loci that are polymorphic out of total 1654 loci; N a, observed number of alleles; N e, effective number of alleles; I , Shannon's information index; H e, expected heterozygosity.

NX, Ningxia; NMG, Neimenggu; SX, Shaanxi. GS, Gansu.

Table S2 Localities of 155 records used for niche modeling

Population code	Latitude (N)	Longitude (E)	Population code	Latitude (N)	Longitude (E)	Population code	La
P1	49.2126	119.7292	P23	43.3833	115.0000	P45	42
P2	45.5126	116.9779	P24	43.3500	114.8500	P46	42
P3	44.0894	113.9219	P25	43.3000	114.7667	P47	42
P4	43.9390	116.0704	P26	43.2973	113.0510	P48	42
P5	43.8576	113.6554	P27	43.2909	116.6604	P49	42
P6	43.7481	113.7815	P28	43.2805	114.4370	P50	42
P7	43.7000	117.0000	P29	43.2500	113.0000	P51	42
P8	43.6833	116.3167	P30	43.2122	112.9788	P52	42
P9	43.6768	113.4494	P31	43.1523	114.4681	P53	40
P10	43.6749	113.9785	P32	43.1199	112.9065	P54	40
P11	43.6667	113.4500	P33	43.0613	114.5072	P55	42
P12	43.6500	116.1667	P34	43.0119	115.7574	P56	42
P13	43.6460	111.9699	P35	42.9256	114.5646	P57	42

Population code	Latitude (N)	Longitude (E)	Population code	Latitude (N)	Longitude (E)	Population code	La
P14	43.6333	116.6500	P36	42.8632	89.1928	P58	41
P15	43.6281	113.3760	P37	42.8345	114.5676	P59	41
P16	43.6167	115.3333	P38	42.7698	114.6434	P60	41
P17	43.6000	115.6500	P39	42.7329	112.6404	P61	41

Table S2 (Continued)

P18	43.5833	115.7500	P40	42.7293	112.6501	P62	40.8836	107.1434
P19	43.5491	113.2569	P41	42.7167	116.0833	P63	40.7791	107.4050
P20	43.5154	114.1718	P42	42.7028	114.7319	P64	40.7619	107.4222
P21	43.4667	115.0833	P43	42.7000	108.9333	P65	40.7458	104.5031
P22	43.4345	114.3161	P44	42.6833	115.9833	P66	40.7418	107.3821
P67	40.3882	110.0001	P92	39.8000	108.7000	P117	38.8535	105.7036
P68	40.3833	104.7167	P93	39.6670	105.7036	P118	38.8380	105.6981
P69	40.3817	109.3359	P94	39.6630	108.7794	P119	38.8367	99.6144
P70	40.3405	109.5226	P95	39.6281	103.0403	P120	38.8330	105.6476
P71	40.3366	106.9958	P96	39.4333	102.7500	P121	38.7833	105.5167
P72	40.3256	107.0045	P97	39.4073	102.3672	P122	38.7566	110.1787
P73	40.3252	107.0029	P98	39.3771	99.8179	P123	38.7392	109.1013
P74	40.3108	105.8933	P99	39.3616	110.1672	P124	38.6650	105.8017
P75	40.3055	109.9368	P100	39.3476	102.0119	P125	38.6500	108.9333
P76	40.2884	109.9427	P101	39.3438	109.0016	P126	38.6410	108.9262
P77	40.2609	110.0120	P102	39.3333	104.9000	P127	38.6269	106.5652
P78	40.2431	109.9507	P103	39.2667	104.9500	P128	38.6106	108.8343
P79	40.2379	105.9169	P104	39.2167	103.7000	P129	38.6000	108.7667

Table S2 (Continued)

P80	40.2054	108.4865	P105	39.2078	101.6601	P130	38.5304	105.6553
P81	40.1975	110.7404	P106	39.1333	103.6667	P131	38.3667	103.2833
P82	40.1333	110.5000	P107	39.1129	109.0366	P132	38.3282	109.7580
P83	40.1167	104.0500	P108	39.0962	108.0956	P133	38.3000	109.7000
P84	40.0943	109.0196	P109	39.0167	108.0000	P134	38.2167	103.3000
P85	40.0833	103.9500	P110	38.9620	105.6545	P135	38.1799	109.0588
P86	40.0634	103.9141	P111	38.9463	107.8719	P136	38.1194	106.5161
P87	40.0331	108.4763	P112	38.9333	108.1333	P137	38.1167	103.1667
P88	40.0167	103.8833	P113	38.9167	105.5167	P138	38.1000	102.9833
P89	39.9833	104.2000	P114	38.8833	108.3000	P139	38.0333	102.8667
P90	39.8203	109.9558	P115	38.8719	109.1717	P140	38.0306	104.8136
P91	39.8141	109.9729	P116	38.8642	105.7319	P141	37.9761	106.3286
P142	37.9567	108.7709	P147	37.6812	108.8399	P152	37.4248	104.6681
P143	37.9318	102.6068	P148	37.6789	108.3090	P153	37.3636	102.8363
P144	37.9212	107.9943	P149	37.5000	102.9000	P154	36.3000	98.1000
P145	37.7991	108.7109	P150	37.4445	104.9394	P155	35.9944	97.8869
P146	37.7769	107.3913	P151	37.4426	104.9383			

Table S3 F_{CT} values for different numbers of population groups (K) inferred by SAMOVA algorithm based on the AFLP dataset

K	**Population grouping**
K=2	**(1, 2, 3, 4, 5, 6,7, 8, 9, 10, 11, 12) (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30**
K=3	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,**
K=4	**(1, 2, 3, 4, 5, 6) (7, 8) (9, 10, 11, 12) (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29**
K=5	**(1, 2, 3, 4, 5, 6) (7, 8) (9, 10, 11, 12) (13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30**
K=6	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13) (14, 15, 16, 17) (18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,**
K=7	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13) (14, 15, 16, 17) (18, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 3**
K=8	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13) (14, 15, 16, 17, 18, 19, 22) (20, 21, 24, 26, 33, 34) (23, 32,**
K=9	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13) (14, 15, 16, 17) (23, 32, 35) (18, 20,21, 22, 24, 25, 26, 27,**
K=10	**(1, 2, 3, 4, 5, 6) (7, 8, 9, 10, 11, 12) (13) (14, 15, 16, 17) (18, 20, 21, 24, 26, 33) (19, 22) (23, 32, 35,**

Figure legends

Figure 1 Correlation of Mantel test between geographic distance and F_{ST}. **A,** Mantel test from 43 populations of *P. villosa* ; **B,** Mantel test from populations of Group 1; **C,** Mantel test from populations of Group 2

Figure 2 Dendrogram of *P. villosa* generated by unweighted pair group method analysis (UPGMA) cluster analysis from the genetic similarity matrix obtained using amplified fragment length polymorphism genetic distance (see Figure S1 for population codes)

Figure 3 Neighbor-Net split network of *P. villosa* based on amplified fragment length polymorphism dataset using Jaccard's distances. Lines of red and yellow represent Group 1 and Group 2, respectively

Figure 4 A two-dimensional plot of the principal coordinate analysis (PCoA) based on variation of amplified fragment length polymorphism markers for *P. villosa* (see Figure S1 for population codes)

Figure 5 Results of the Bayesian clustering analysis in STRUCTURE of 210 individuals representing *P. villosa.* **A,** $^{K}values from the mean log - likelihood probabilities through STRUCTURE runs wherein ferred cluster(K) ranged from one to ten$; **B,** $Estimated genetic clus 2, where unique colors correspond to assignment at different clusters$; **C,** $Geographic origin from 43 populations of P. villosa and coded grouping according to the structure analysis for the model with K = 2$

Figure 6 Potentially suitable climatic distribution of *P. villosa* under different climate change scenarios in China

Figure 7 Present suitable climatic distribution of *P. villosa* in China

Figure 8 Spatial shifts for *P. villosa* under climate change scenarios

Figure 9 Results of the niche-identity test. **A,**Schoener's D ; **B,** Warren's I . The arrow in each panel represents the observed niche similarity between occurrence points for the corresponding pair of clusters. The histograms represent the distribution of niche similarities obtained from pairs of pseudo niches constructed by random resampling of occurrence points of the two clusters

Figure S1 Localities of Group 1 (red), Group 2 (yellow) sampled of *P. villosa* in the present study

Figure S2 Fluorescently-labeled AFLPs generated using different primer combinations. **A,** E -AAC/M -CAA; B,E -AAG/M -CAC; C, E -ACA/M -CAG; D,E -ACT/M -CAT; E, E -ACC/M -CTA; **F,**E -ACG/M -CTC; **G,** E -AGC/M -CTG;**H,** E -AGG/M -CTT

Figure S3 The mean AUC of the test samples of *P. villosa* based on the MaxEnt model