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Genetic Diversity and Geographic Differentiation in Endangered *Ammopiptanthus* (Leguminosae) Populations in Desert Regions of Northwest China as Revealed by ISSR Analysis

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• *Background and Aims* The desert legume genus *Ammopiptanthus* comprises two currently endangered species, *A. mongolicus* and *A. nanus*. Genetic variability and genetic differentiation between the two species and within each species were examined.
• *Methods* Inter-simple sequence repeat (ISSR) marker data were obtained and analysed with respect to genetic diversity, structure and gene flow.
• *Key Results* Despite the morphological similarity between *A. mongolicus* and *A. nanus*, the two species are genetically distinct from each other, indicated by 63 % species-specific bands. Low genetic variability was detected for both population level (Shannon indices of diversity $H_{pop} = 0{\cdot}106$, percentage of polymorphic loci $P = 18{\cdot}55$ % for *A. mongolicus*; $H_{pop} = 0{\cdot}070$, $P = 12{\cdot}24$ % for *A. nanus*) and species level ($H_{sp} = 0{\cdot}1832$, $P = 39{\cdot}39$ % for *A. mongolicus*; $H_{sp} = 0{\cdot}1026$, $P = 25{\cdot}89$ % for *A. nanus*). Moderate genetic differentiation was found based on different measures (AMOVA Φ_{ST} and Hickory θ^{B}) in both *A. mongolicus* (0·3743–0·3744) and *A. nanus* (0·2162–0·2369).
• *Conclusions* The significant genetic difference between the two species might be due to a possible vicariant evolutionary event from a single common ancestor through the fragmentation of their common ancestor's range. Conservation strategies for these two endangered species are proposed.

Key words: *Ammopiptanthus mongolicus*, *Ammopiptanthus nanus*, desert, endangered plants, genetic diversity, ISSR.

INTRODUCTION

Conservation of the genetic resources of endemic desert plants is crucial to worldwide efforts to combat desertification, to prevent further degradation of the fragile ecosystems in arid and semi-arid regions and to sustain biodiversity in deserts. Desert plants play a key role, as the primary producers, in maintaining these ecosystems. Desert ecosystems currently cover about 35 % of the Earth's land surface (Helldén, 1991) and they are expanding. This desertification and ongoing deterioration in arid and semi-arid regions worldwide has recently focused attention amongst the international community on the urgent need to protect the environment of the desert regions (FAO, 1997). The area of desert land in China amounts to approximately 2·080 million km^2. As an adaptation to their dry and extremely cold environments, most desert plants in China have small, deciduous leaves. *Ammopiptanthus* Cheng f. is the only genus of evergreen broadleaf shrubs in the northwestern desert of China. This genus belongs to the Leguminosae, a family consisting of about 690 genera worldwide, and members of the genus have been considered to be some of the most unique plants, and keystone components, of the region's flora.

Ammopiptanthus comprises two diploid species with high morphological similarity: *A. mongolicus* (Maxim.) Cheng f. and *A. nanus* (M. Pop.) Cheng f. (Cheng, 1959; Pan and Huang, 1993). They can be distinguished from each other by the shape of their leaves (trifoliate in *A. mongolicus* compared with simple leaves in *A. nanus*). Both species are narrowly distributed; *A. mongolicus* is endemic to the south Gobi desert (Liu *et al*., 1995; Liu, 1998), and *A. nanus* is restricted to the borders between China and Kyrgyzstan, growing in a narrow altitudinal strip between 1800 and 2800 m.

The evergreen broadleaf habit of *Ammopiptanthus* has been viewed as an ancestral trait that identifies it as a Tertiary relict taxon (Liu *et al*., 1995). During the interval spanning the early Paleocene to mid-Eocene (65–45 Ma; Willis and McElwain, 2002) in the early Tertiary period, the vegetation in north-western China was dominated by evergreen and/or deciduous broadleaf forest (Geng *et al*., 2001), according to fossil evidence. When subsequent changes made the climate colder and drier from the early Miocene (24–16 Ma) in central Asia (Guo *et al*., 2002), the forest was gradually replaced by steppe and then by desert (Yan *et al*., 2000). *Ammopiptanthus* is a relict survivor of the evergreen broadleaf forest of this region from the Tertiary period.

The two species are dominant in the local vegetation (Pan *et al*., 1992; Liu *et al*., 1995). Their habitats are stony and/or sandy deserts where the annual precipitation ranges from 100–160 mm. Plants flower profusely in spring (from early April to late May) with 12–16 and 10–14 flowers on each inflorescence of *A. mongolicus* and *A. nanus*, respectively (Yin and Wang, 1993). Both species are insect-pollinated.

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TABLE 1. *Populations studied including estimated total population sizes (N) and sample sizes (N_s)*

Species	Population	Population code	Locality	Latitude (N)	Longitude (E)	Altitude (m)	N_s	N
A. mongolicus	West Ningxia							
	Shapotou	1	Zhongwei	37°28′	104°58′	1300	23	100
	East Ningxia							
	Rujigou (1)	2	Pingluo	39°03′	106°07′	1930	23	500
	Rujigou (2)	3	Pingluo	39°05′	106°09′	1920	23	100
	Inner Mongolia							
	Qianlishan	4	Wuhai	39°50′	106°50′	1170	23	>2000
	Xindi	5	Wuhai	39°52′	106°46′	1090	19	500
	Yikebulage	6	Otog Qi	40°05′	106°49′	1070	23	500
	Taositu	7	Otog Qi	40°09′	106°54′	1070	23	500
	Muoshigou	8	Hangjin Qi	40°07′	107°04′	1380	23	100
	Balagong	9	Hangjin Qi	40°16′	107°03′	1100	23	500
	Dengkou (1)	10	Dengkou	40°25′	106°43′	1050	23	100
	Dengkou (2)	11	Dengkou	40°25′	106°45′	1040	23	>1000
A. nanus	Biaoertuokuoyi	1	Wuqia	39°30′	74°51′	2700	24	>2000
	Bacundaban	2		39°39′	75°01′	2120	23	200
	Ohsalur	3		39°40′	74°45′	2250	22	100
	Xiaoerbulake	4		39°42′	75°01′	2200	30	>2000
	Kangsu	5		39°42′	75°04′	2170	22	100
	Baykurt	6		39°50′	75°35′	2100	23	200
	Tielieke	7	do	39°57′	75°39′	2300	24	200

Their heavy seeds are dispersed by gravity within a short distance of the parent plant. Natural regeneration of both species is limited because of low seed germination rates in the harsh environment (Pan *et al*., 1992; Liu, 1998). Few young plants can be found in the wild. There has been no record of accurate data on the specific distribution range and population size for both species. A continuous distribution and large population size were suspected (Liu, 1995). However, in the past two decades, *Ammopiptanthus* have been subject to rapid demographic decline, mainly due to increasing anthropogenic pressures in their natural range (e.g. cutting for fuel wood and pollution). The estimated sizes of the extant populations range from 100 to more than 2000 individuals for both species (Table 1). The two species have been categorized as 'endangered' and given protected status in China (Fu, 1989). Because of the high academic interest in them, and their ecological usefulness in combating further desertification, many studies have been carried out on their anatomy (Liu and Qiu, 1982), drought-resistance mechanisms (Xu *et al*., 2002) and community structure (Liu *et al*., 1995).

Despite the general awareness of the importance of the *Ammopiptanthus* species for fixing moving sands and delaying further desertification, little is known about the distribution of genetic variation across their geographical ranges. Data related to genetic diversity within and between populations are essential for formulating appropriate management strategies for the conservation of rare and endangered species. Several aspects of conservation biology, such as the loss of genetic diversity in conservation programs and the restoration of threatened populations, can only be addressed by detailed population genetic studies (Hamrick and Godt, 1996). Compared with widespread and abundant species, endemic and rare taxa often contain significantly less genetic variability (Gitzendanner and Soltis, 2000). The loss of genetic variability may render populations more vulnerable to extinction in cases of habitat perturbation, reproductive bottlenecks, etc. (Barrett and Kohn, 1991), and such losses would be expected to increase the risk of local extinction in these taxa. In order to help formulate rational strategies to preserve genetic diversity within *Ammopiptanthus*, the levels and patterns of genetic variation in 18 populations of the genus were documented in the study reported here by analysing inter-simple sequence repeats (ISSRs).

ISSR PCR uses a single primer composed of a di- or trinucleotide simple sequence repeat [e.g., $(CA)_8$, $(AGC)_6$] with or without a 5′- or 3′-anchoring sequence of 1–3 nucleotides. ISSR primers target simple sequence repeats (microsatellites) that are abundant and dispersed throughout the genome, and reveal data that reflect the length variation between adjacent microsatellites. This technique has provided a powerful tool for the investigation of genetic variation within species (Wolfe and Liston, 1998). Recent ISSR studies of natural populations have demonstrated the hypervariable nature of these markers and their potential use for population-level studies (Esselman *et al*., 1999; Culley and Wolfe, 2001). Limitations of the ISSR technique, as is the case for Random Amplification of Polymorphic DNA (RAPD; Williams *et al*., 1990), are that bands are scored as dominant markers and that genetic diversity estimates are based on diallelic characters.

This investigation had three main purposes. Firstly, to estimate genetic differentiation between *A. mongolicus* and *A. nanus*. Secondly, to assess population genetic diversity and structures in *A. mongolicus* and *A. nanus* in order to obtain basic information for the development of conservation strategies. And thirdly, to contribute to our understanding of the effects of desertification on the genetic diversity of relict species.

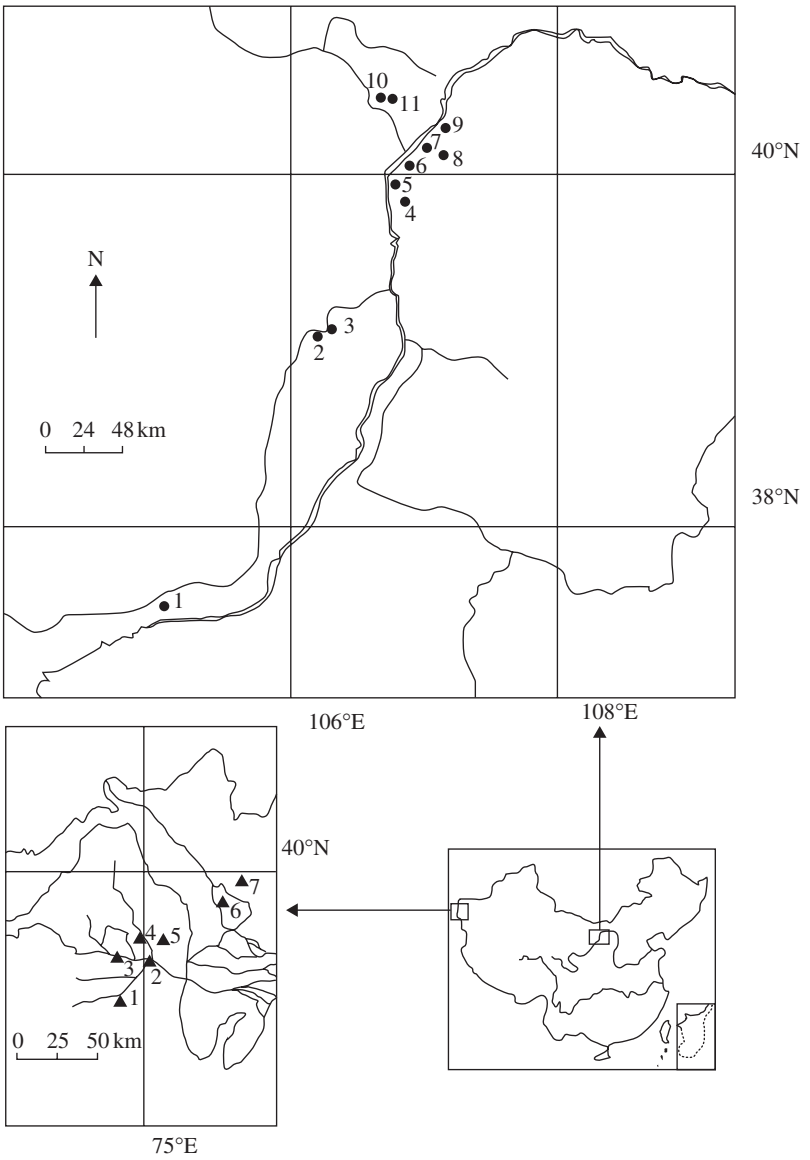

FIG. 1. Locations of sampled populations of *Ammopiptanthus mongolicus* (upper map) and *A. nanus* (bottom left) in China. Population codes correspond to those given in Table 1.

MATERIALS AND METHODS

Plant material

A total of 251 individuals of *Ammopiptanthus mongolicus* representing 11 populations were sampled from three geographically isolated regions near the centre of its distribution: East Ningxia (two populations), West Ningxia (one population) and Inner Mongolia (eight populations). This sampling covers most of the extant *A. mongolicus* populations; however, the populations from Ala-shan region were not available for this study. One hundred and sixty-eighty individuals representing seven populations of *A. nanus* were sampled from Wuqia of Xinjiang Uygur Autonomous Region, the only county hosting *A. nanus* in China (Fig. 1; Table 1). This sampling scheme includes almost all the extant *A. nanus* populations known from China. Nineteen to 30 individuals were randomly collected from each population, regardless of their size and age. Young leaves were collected and dried in silica gel until DNA extraction.

DNA extraction and PCR amplification

Genomic DNA was extracted from approximately 0·5 g of leaf tissue using a modified CTAB method (Doyle and Doyle, 1987). The quality and concentration of the DNA were confirmed by electrophoresis on 1 % agarose gels with λ DNA markers. Nuclear DNA was amplified by PCR using ISSR primers from the University of British Columbia primer set 9 (Biotechnology Laboratory, University of British Columbia, primer set # 9: http://www.biotech.ubc.ca/services/naps/primers/Primers.pdf). Following an initial screen of 100 primers, 11 primers (UBC # 808, 809, 811, 813, 834, 840, 842, 880, 881, 886 and 888) that yielded maximum numbers of reliable and reproducible polymorphisms

were then selected to analyse the populations. PCR amplifications were carried out in a total volume of 20 mL consisting of 20 ng of template DNA, 10 mM Tris–HCl (pH 9·0), 50 mM KCl, 0·1 % Triton X-100, 2·5 mM $MgCl_2$, 0·1 mM dNTPs, 2 % formamide, 0·2 mM primer, 1·5 units of *Taq* polymerase and double-distilled water. Initial denaturation was at 94 °C for 5 min, followed by 45 cycles of 30 s at 94 °C, 1 min at 50–54 °C (depending on different primers), 2 min at 72 °C, and a final 7-min extension at 72 °C. PCR reactions were carried out in a PTC-200 thermal cycler (MJ Research, USA). PCR products were separated by gel electrophoresis on 2·0 % agarose gels in 0·5× TBE buffer and visualized using ethidium bromide staining (0·1 mg mL^{-1}). Negative controls (no template DNA) were also included in each PCR. To ensure ISSR reproducibility, most PCR reactions were repeated twice. DNA fragments were visualized by LabWorks Version 3.0 image analysis software for gel documentation (UVP, Upland, CA 91786, USA).

Data analysis

Only bands that could be unambiguously scored across all the sampled populations were used in this study. ISSR profiles were scored for each individual as discrete characters (presence or absence of the amplified products). Genetic diversity was measured by the percentage of polymorphic bands (*P*), which was calculated by dividing the number of polymorphic bands at population and species levels by the total number of bands surveyed. Shannon indices of diversity, namely both the total diversity (H_{sp}) and the intra-population diversity (H_{pop}), were also calculated using the computer program POPGENE 1.31 (Yeh *et al*., 1999). The non-parametric Analysis of Molecular Variance (AMOVA) program v. 1.55 (Excoffier *et al*., 1992) was used to describe the genetic structure between populations. The significance of this *F*-statistic analogue was tested by 1000 random permutations.

In order to overcome potential problems caused by the dominance of ISSR markers, and to obtain an accurate estimate of F_{ST}, a Bayesian program, Hickory (0·8) (Holsinger *et al*., 2002), was also used to estimate parameters related to genetic structure (θ^B). The Bayesian method does not assume that genotypes are in Hardy–Weinberg proportions within populations, and it does not treat multilocus ISSR phenotypes as haplotypes. It takes full advantage of the information provided by dominant markers, allowing us to incorporate uncertainty about the magnitude of the within-population inbreeding coefficient into estimates of F_{ST} (Holsinger *et al*., 2002; Holsinger and Wallace, 2004). We used default values for burn-in (50 000), sampling (250 000) and thinning (50). The '*f*-free' analysis option in Hickory was used because it avoids any potential bias that could be created by unreasonable estimates of the F_{IS} analogue, *f*.

Gene flow was estimated indirectly using the formula: $Nm = 0{\cdot}25(1 - F_{ST})/F_{ST}$, where θ^B is used for the estimator of F_{ST}. In order to test for a correlation between pair-wise genetic distances (Φ_{ST}) and geographical distances (in km) between populations, a Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; Miller, 1997) (computing 999 permutations).

TABLE 2. *Parameters of genetic variability*

Population	H_{pop}	*P* (%)
A. mongolicus		
Shapotou	0·123 (±0·243)	22·22
Rujigou (1)	0·112 (±0·235)	19·19
Rujigou (2)	0·117 (±0·246)	19·19
Qianlishan	0·123 (±0·248)	20·20
Xindi	0·110 (±0·237)	18·18
Yikebulage	0·091 (±0·216)	16·16
Taositu	0·110 (±0·237)	18·18
Muoshigou	0·087 (±0·215)	15·15
Balagong	0·081 (±0·199)	15·15
Dengkou (1)	0·104 (±0·214)	21·21
Dengkou (2)	0·110 (±0·238)	19·19
Mean	0·106 (±0·014)	18·55 (±2·32)
A. nanus		
Biaoertuokuoyi	0·113 (±0·239)	18·75
Bacundaban	0·041 (±0·152)	7·14
Ohsalur	0·050 (±0·172)	8·04
Xiaoerbulake	0·063 (±0·179)	11·61
Kangsu	0·061 (±0·179)	10·71
Baykurt	0·058 (±0·178)	9·82
Tielieke	0·106 (±0·229)	18·75
Mean	0·070 (±0·028)	12·12 (±4·78)

H_{pop}, Shannon's index of gene diversity; *P*, percentage of polymorphic loci. Standard deviations are shown in parentheses.

TABLE 3. *Summary of genetic variability and partitioning of diversity*

	A. mongolicus	*A. nanus*
P	39·39 %	25·89 %
H_{SP}	0·1832	0·1026
Φ_{ST}	37·43 %	21·62 %
θ^B	0·3744	0·2369
Gene flow (*Nm*)	0·418	0·805

P, percentage of polymorphic loci; H_{sp}, Shannon index of gene diversity at the species level; Φ_{ST}, genetic differentiation between populations estimated by using AMOVA; θ^B: genetic differentiation between populations estimated by using Hickory analysis; *Nm*, estimated gene flow.

RESULTS

The eleven primers produced 154 bands in the two species studied, among them 99 bands from *A. mongolicus* and 112 from *A. nanus*. The comparison of banding patterns between *A. mongolicus* and *A. nanus* indicated that 63 % of the bands were unique to each species. Forty-two bands were present only in *A. mongolicus*, whereas 55 bands were specific to *A. nanus*.

Ammopiptanthus mongolicus

In *A. mongolicus*, 39 of the 99 clear and reproducible bands (39·39 %) were polymorphic in at least one population. The average percentage of polymorphic loci (*P*) across populations was 18·55 %. The average Shannon's indices were 0·106 at the population level (H_{pop}) and 0·1832 at the species level (H_{sp}) (Tables 2 and 3).

TABLE 4. *Analysis of molecular variance (AMOVA) for 251 individuals in 11 populations of* A. mongolicus *and 168 individuals in seven populations of* A. nanus

Species	Source of variation	d.f.	Sum of squares	Mean squares	Variance components	% total variance	*P*-value
A. mongolicus	Nested analysis						
	Among regions	2	213·66	106·83	1·42	24·93	<0·001
	Among popns within region	8	452·22	29·82	1·17	20·68	<0·001
	Within popns	240	741·10	3·09	3·09	54·89	<0·001
	Analysis among popns						
	Among popns	10	452·22	45·22	1·85	37·43	<0·001
	Within popns	240	741·10	3·089	3·09	62·57	<0·001
	Analysis among regions						
	Among regions	2	213·66	106·83	1·89	32·44	<0·001
	Within regions	248	979·66	3·95	3·95	67·56	<0·001
	Analysis among popns within Inner Mongolia region						
	Among popns	7	206·09	29·44	1·18	28·4	<0·001
	Within popns	172	511·32	2·97	2·97	71·6	<0·001
A. nanus	Among popns	6	71 047	11 841	0 429	21 62	<0 001
	Within popns	161	250·573	1·556	1·556	78·38	<0·001

P-values are the probabilities of having a more extreme variance component than the observed values by chance alone. Probabilities were calculated by 1000 random permutations of individuals across populations.

Results of the Hickory analysis gave a θ^B value of 0·3744 (Table 3). The *Nm* estimate of 0·418 suggests that genetic exchange between populations is limited. The AMOVA analysis provided additional evidence for the genetic structure indicated by these parameters. Highly significant ($P < 0·001$) genetic differences were detected between regions, between populations (within regions), and between individuals (within both populations and regions) (Table 4). Of the total molecular variance, 24·93 % was attributable to regional divergence, 20·68 % to population differences within regions, and 54·39 % to individual differences within populations. When the total variance was partitioned without considering the regional distribution of the populations, 37·43 % was attributable to populations (Φ_{ST}) and 62·57 % to individual differences within populations. Likewise, analysis of the regional distribution of the variance suggested that 32·44 % of the total variance was due to diversity between regions and 67·56 % to individual differences within regions (Table 4). Within Inner Mongolia alone, 28·4 % was attributable to populations (Φ_{ST}).

The Mantel test shows that there is a positive correlation between geographical distance and genetic distance in *A. mongolicus* ($r = 0·6478$, $P = 0·001$). The strong genetic differentiation in *A. mongolicus* suggests that the three regions examined are isolated and gene flow between the three regions is limited.

Ammopiptanthus nanus

Twenty-nine of the 112 clear and reproducible bands (25·89 %) were polymorphic in at least one population. The average percentage of polymorphic loci (*P*) across populations was 12·12 %. The Shannon's indices were 0·070 at the population level (H_{pop}) and 0·1026 at the species level (H_{sp}) (Tables 2, 3). Thus, *A. nanus* showed lower levels of genetic diversity than *A. mongolicus*.

The results of the Hickory analysis gave a θ^B value of 0·2369 (Table 3). AMOVA showed that approximately 21·62 % of the genetic variation was found between populations (Φ_{ST}). The gene flow (*Nm*) between populations was 0·805 (Table 3). The Mantel test detected no geographical tendency in the distribution of the genetic distance in *A. nanus* ($r = 0·1544$, $P = 0·3020$).

DISCUSSION

Genetic differentiation between A. mongolicus *and* A. nanus

Fifty-seven of the 154 bands were shared by the two studied species, indicating a common evolutionary history or homoplasy, while the remaining 97 bands reflect divergence between species. If one species was derived from the other, producing a progenitor–derivative pair, and this was associated with a reduction in effective population size, we would expect to observe only a subset of alleles in the derived species (Gemmill *et al*., 1998). The fact that *A. mongolicus* and *A. nanus* harbour 42 and 55 species-specific bands, respectively, does not support the hypothesis of a progenitor–derivative relationship between these two species. An alternative hypothesis is that differentiation of the two species of *Ammopiptanthus* might be due to a possible vicariance evolutionary event from a single common ancestor through the fragmentation of its natural distribution range. About 63 % of bands (97 of 154) that are specific to one of the two species strongly suggest that these two morphologically similar species are genetically very distinct.

The geographical barrier between these two species appears to have arisen as early as the early Miocene, during the aridification and formation of deserts in central Asia induced by the development of the Arctic ice-sheet and uplift of the Himalayan–Tibetan Plateau (Harrison *et al*., 1998; Guo *et al*., 1999). Desert vegetation that was better adapted to the lower temperature and more arid conditions gradually replaced the broadleaf forest that had developed in the warmer and moister global climate of the early Paleocene and mid-Eocene (~65–45 Ma). Evidence from fossil pollen suggests that high percentages of grasses and herbs were present, and that grassy savannah or even desert conditions occurred as early as the mid-Miocene in the Gobi desert region of China (~18–13 Ma) (Willis and McElwain, 2002). As an element of the ancient evergreen broadleaf forest, it has been speculated that the ancestral species of *Ammopiptanthus* was widely and continuously distributed from the eastern border of the Pamir Plateau to the Gobi desert during the Tertiary period (Liu, 1995). Desertification in the Ala-shan Plateau since the Miocene (Guo *et al*., 2002) and in the Tarim Basin since the early Pleistocene (Yan *et al*., 2000) may have been the main factors that caused the fragmentation of the continuous distribution of this ancestral *Ammopiptanthus*. Genetic differentiation of the two species probably occurred after the geographic barrier formed. The significant genetic difference between the two species is likely to be the result of long isolation.

Genetic diversity

Low levels of polymorphism within populations were revealed in both *Ammopiptanthus* species by ISSR markers. Their variation was similar with that of relict *Cycas guizhouensis* (14·21 %) (Xiao *et al*., 2004) and of the clonal plant *Psammochloa villosa* (15 %) (Li and Ge, 2001). The genetic diversity of a species or population is due to the combined effects of genealogical history and evolutionary processes (Comes and Kadereit, 1998). Although low levels of diversity have often been reported for rare and endemic plant species, such as *Dendroseris* spp. (Esselman *et al*., 2000), numerous allozyme studies and increasing numbers of cpDNA and mtDNA studies now provide substantial evidence that refugial plant populations can harbour higher levels of genetic diversity than their likely descendant populations (reviewed in Comes and Kadereit, 1998). Because deserts in the northern hemisphere are mostly located south of latitude 40°N, they have not been dramatically influenced by ice sheets or mountain glaciers (Brown and Gibson, 1983). In the Chinese desert region, no pollen evidence has been found to suggest that glaciation occurred during the Quaternary (Li, 1998). In addition, the western area of Inner Mongolia is characterized by high endemism, possibly because it was a Pleistocene refugium (Zhao, 1997). The extant distribution of *Ammopiptanthus* may indicate refugia for ancient broadleaf forest species and, if so, high ISSR diversity might be expected. Highly polymorphic ISSRs have been found in *Tetraena mongolica* ($P = 48{\cdot}1$ %), which is also endemic to western Inner Mongolia and co-occurs with *A. mongolicus* (Ge *et al*., 2003). In contrast to the expectation of high genetic diversity, *A. mongolicus* and *A. nanus* revealed low levels of genetic variability at both the population and species levels.

The low levels of genetic diversity harboured in these two species may be due to the following four possible reasons. Firstly, they may be due to low inherent variability of the ancestral species (see, for instance, Godt *et al*., 1997). Although high levels of genetic diversity are expected for refugial plant populations (as reviewed in Comes and Kadereit, 1998), *A. mongolicus* and *A. nanus* may originate from genetically depauperate populations. Secondly, inbreeding could be one of the major factors responsible for the low genetic variation within the populations of these *Ammopiptanthus* species. *Ammopiptanthus mongolicus* and *A. nanus* have been found to be self-compatible, but pollinator-dependent (unpublished observations, Ge *et al*.). *Ammopiptanthus mongolicus* and *A. nanus* have numerous flowers on a single inflorescence (10–16 flowers per inflorescence), which could facilitate pollinator-mediated self-pollination and geitonogamous pollination. The gravity-disseminated seeds and the insect-dispersed pollen of *Ammopiptanthus* may promote mating between individuals in close proximity within populations. Thirdly, the repeated decrease and increase of temperature in climatic oscillations during the Pleistocene may have caused the repeated enlarging and decreasing of populations, hence causing founder effects. This might partly account for the lower levels of variation within populations of *A. mongolicus* despite its relatively wide distribution, and generally lower levels of variation within *A. nanus* due to the more limited distribution of this species. Finally, the explosive increase in the human population and destructive utilization for firewood have caused a dramatic decline of these two species. These small and isolated populations were probably subjected to genetic drift that may have contributed to the lack of genetic diversity observed today. In *A. nanus*, we observed only two bands (1·7 %) with a frequency lower than 50 %, indicative of such stochastic processes.

The differences in genetic variability between *A. mongolicus* and *A. nanus* could be related to their geographical ranges. Gitzendanner and Soltis (2000) summarized the results of studies in widespread and restricted congeners, and found that genetic variation was significantly lower in the rare species than in the widespread species. Both *Ammopiptanthus* species have similar biological and ecological characteristics, so the higher level of genetic diversity detected in *A. mongolicus* as compared to *A. nanus* could be due to the more restricted geographical distribution of the latter.

Genetic structure

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation and population isolation), genetic drift, mating system, gene flow and selection (Schaal *et al*., 1998). *Ammopiptanthus mongolicus* shows more differentiation between populations than *A. nanus* (Table 3). This is probably due to differences in the geographic fragmentation of populations of these two species, because

the distribution of *A. mongolicus* is more fragmented than that of *A. nanus*. *Ammopiptanthus nanus* populations are generally in closer proximity to each other (5–85 km apart) than are *A. mongolicus* populations (3–362 km apart). It has been demonstrated that the level of genetic heterogeneity among populations is greater in species with geographically disjunct populations than in species with more continuous distributions (Hamrick and Godt, 1996; Premoli *et al*., 2001). Thus, this may account for the high between-region proportion of the total molecular variance found in *A. mongolicus* (24·93 % based on AMOVA). Within the Inner Mongolia region, the genetic differentiation between the populations of *A. mongolicus* was similar to that of *A. nanus* (28·4 % vs. 21·62 % according to AMOVA). Within Inner Mongolia alone, 28·4 % was attributable to populations (Φ_{ST}), indicating significant genetic differentiation.

Generally, the breeding system of flowering plant species greatly affects population genetic differentiation (Hamrick and Godt, 1989). Estimates of genetic differentiation between populations for outcrossing species based on AMOVA derived by analysing RAPD markers have usually been <28 %. For inbred species, estimates of interpopulation genetic variation have usually been >70 % (reviewed in Nybom and Bartish, 2000). The genetic variation between populations of *A. nanus* (AMOVA = 21·62 %) and between populations of *A. mongolicus* within the Inner Mongolia region (AMOVA = 28·4 %) are consistent with those observed in outcrossing species (Hamrick and Godt, 1989). Our preliminary field observations confirm that *A. mongolicus* and *A. nanus* are insect-pollinated species. Wasps (*Vesta* sp.) and honeybees (*Apis* sp.) were often found visiting their flowers (unpublished observations, Ge *et al*.).

The fact that estimates of *Nm* were <1 for both species suggests that gene flow between populations is insufficient to counter the effects of random drift (Real, 1994). In this study, the relatively high genetic differentiation and low levels of gene flow detected (*Nm* = 0·418 and 0·805 for *A. mongolicus* and *A. nanus*, respectively) strongly indicate that genetic drift has greatly affected the genetic composition of individual populations. Although there is a significant relationship between geographic and genetic distance for the 11 populations of *A. mongolicus*, some pairs of populations with low genetic distances are not geographic neighbours. This may be partly due to the effects of genetic drift on small populations. The lack of correlation between genetic distances and geographic distances in *A. nanus* suggests that there is low genetic flux between populations of this species, and that stochastic differentiation due to genetic drift has occurred. Between-population gene flow is limited by pollen and seed dispersal. Being an insect-pollinated plant, pollen dispersal is limited by the short flight ranges of the insects. Moreover, seed dispersal is not likely to be very efficient, given the weight of seeds typical for these two species (approx. 50 mg and 30 mg for *A. mongolicus* and *A. nanus*, respectively, effectively excluding wind transport) and their lack of dispersal structures. The limited seed dispersal contributes to the restricted gene flow and increases the probability that individuals in close physical proximity mated with one another. Both effects will promote inter-population differentiation.

The effects of desertification on genetic diversity

This study represents a unique opportunity to examine the effects of desertification on genetic diversity. Desertification has dramatic and comprehensive affects on the genetic diversity of plants living in this region, both at the specific and intraspecific levels. Lack of water and extreme temperature fluctuations make the desert a very harsh environment where few plant species can survive. Following the desertification in central Asia, most ancient Tertiary plant species in this region became extinct and only a few relict species survived in a limited number of refugia. The genus *Ammopiptanthus* represents a typical example. As mentioned above, desertification in central Asia and subsequent isolation led to the differentiation of *A. mongolicus* and *A. nanus*. At the intraspecific level, the desertification dramatically diminished the distribution and fragmented the once continuous populations. The harsh, and also homogeneous, environment imposes an extremely strong selection pressure on the plants. Any maladapted genotype could be eliminated rapidly. The genetic structure of extant populations therefore might reflect the cumulative results of this effect. As discussed above, the observed low genetic diversity in the two *Ammopiptanthus* species was suggested to be the result of low inherent variability, increased inbreeding, founder effects and genetic drift. Desertification might have played a fundamental role in shaping and linking these effects.

Conclusions and implications for conservation

The results reported here reveal distinct genetic differentiation between *A. mongolicus* and *A. nanus*. This differentiation might be due to a possible vicariant evolutionary event from a single common ancestor through the fragmentation of its natural distribution range. Both species displayed low levels of ISSR genetic variation and moderate genetic differentiation, but *A. mongolicus* showed higher genetic variation and differentiation than *A. nanus*.

The ultimate goal of conservation is to ensure the continuous survival of populations and to maintain their evolutionary potential. Information on current levels of genetic diversity of threatened and endangered species is essential for designing appropriate strategies for conservation (Falk and Holsinger, 1991). According to the results of this study, different strategies should be adopted for both the *in situ* and *ex situ* conservation of genetic diversity in the two *Ammopiptanthus* species. For *A. mongolicus*, the moderate genetic differentiation found among the regions, and the similarity between populations within each region, indicate that the most effective strategy for preserving its genetic variation would be to conserve a large number of individuals in a large population within each of as many regions as possible. For *A. nanus*, because of its highly uniform genetic make-up, any of the populations surveyed could represent a large proportion of the genetic variation within the species. Therefore, the most effective strategy for *ex situ*

conservation of this species would be to sample a larger number of plants from one or two populations rather than to collect smaller samples from many different sites. The Biaoertuokuoyi and Tielieke populations harbour relatively high amounts of the genetic diversity within *A. nanus* and these two populations should therefore be a priority for *in situ* conservation action. Finally, because the level of genetic variation in selectively neutral marker loci is mainly determined by mutation and genetic drift (Kimura, 1983), the level of variation detected for marker loci, such as ISSR, will not necessarily be a direct reflection of the level of variation that determines adaptability or individual fitness (Booy *et al*., 2000). Therefore, samples from different habitats, such as from different mountain slopes and sandy deserts, should be considered in conservation.

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