African Journal of Biotechnology Vol. 10(36), pp. 6838-6845, 18 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.486
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic structure of *Potentilla acaulis* (Rosaceae) populations based on randomly amplified polymorphic DNA (RAPD) in habitat fragmented grassland of northern China

Jia Mi[1,2], Shuhua Zheng[1], Minyun Xu[1], Ding Huang[1] and Kun Wang[1,3]*

[1]Department of Grassland Science, China Agricultural University, Beijing 100193, China.
[2]Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.
[3]National Grassland Ecosystem Field Station, Guyuan 076550, China.

Accepted 8 April, 2011

***Potentilla acaulis* Linn. (Rosaceae) is an important companion species in central Asia steppe. However, no information was so far detected about genetic diversity of this species. In recent years, effects of habitat fragmentation have become central issues in conservation genetics. In order to evaluate the genetic structure and to measure the effects of isolation caused by habitat fragmentation, the randomly amplified polymorphic DNA (RAPD) data were generated and analyzed from 110 samples collected from seven sites of local populations of *P. acaulis* distributed in northern China. Eleven RAPD primers produced a total of 61 unambiguous bands, of which 51 bands (83.6%) were polymorphic. A high level of genetic diversity was recognized within the populations of *P. acaulis*: 34.4 to 68.9% of polymorphic bands observed in the given population. Analysis of molecular variance (AMOVA) showed that, genetic variability was greater within populations (83.4%) than among populations within regions (12.0%) or among regions (4.6%) investigated in this study. In addition, a low degree of genetic differentiation (Φ_{ST}= 0.17) was detected among all populations, which indicated that isolation had weak effects on genetic structure. The statistical analysis also revealed that, the genetic distances of *P. acaulis* among different populations were not significantly related with their geographic distances. Therefore, *P. acaulis* should be treated as a separate species that needs more attention from a conservation point of view and it should be considered as a conservation strategy to increasing gene exchange among isolated populations.**

Key words: *Potentilla acaulis* Linn. (Rosaceae), steppe, habitat fragmentation, genetic diversity.

INTRODUCTION

Habitat fragmentation associated with land use and grazing activities has long been concerned among ecologists and agriculturists. Some studies have indicated that, land use is the main factor of habitat deterioration and fragmentation, while these degraded tendencies are accelerated in recent years (Du et al., 2004; League and Veblen, 2006; Li et al., 2007; Lioubimtseva and Henebry, 2009). However, in most of the problems involved in habitat fragmentation, the population isolation has become ineluctable and urgent especially for the grassland conservation. Isolated plant populations have a great potential in the loss of genetic diversity and suffer much from related injurious effects on fitness (Reed and Frankham, 2003; Pluess and Stöcklin, 2004). Consequently, genetic effects induced by habitat fragmentation have been widely studied in conservation genetics (Washitani et al., 2005).

*Corresponding author. E-mail: wangkun@cau.edu.cn Tel/Fax: +86 10 62733338.

Abbreviations: AMOVA, Analysis of molecular variance; **NGEFS,** National Grassland Ecosystem Field Station; **RAPD,** random amplified polymorphic DNA; **PCR,** Polymerase chain reaction; **TAE,** tris-acetate-ethylenediaminetetraacetic acid; **UV,** ultraviolet; **PCoA,** principal coordinate analysis; **PIC,** polymorphism information content.

With the increasing pressure of cultivating cropland and grazing livestock in pastoral area, enlarged amounts of endemic species are enlarged and considered to encounter dual menaces from animals and human. Moreover, corresponding strategy often set up priority on the conservation of species which occupy the larger part of the population distribution on their own territory.

In recent years, the research in respect to genetic structure of central Asian steppe species was only focused on the studies about some perennial shrubs from desert lowlands (Xu et al., 2003; Ge et al., 2005; Sheng et al., 2005) or two naturally fragmented endemic herbs of the uplands of Tibet (Chen et al., 2005; Xia et al., 2005). In our study, an explorative research was carried out on the northern China endemic *Potentilla acaulis*, which originally grows in semiarid or sandy land, hilly region and rocky upland habitats. Therefore, it has becomes an important companion species of steppe to soil and water conservation in degraded grassland. There were mainly three objectives in our study on *P. acaulis*: (1) genetic diversity distribution of intra- or inter-populations; (2) influence degree of fragmentation on population genetic traits and the correlation between genetic distance and geographical distance; (3) setting up the basis of conservation strategies.

MATERIALS AND METHODS

Plant and sample sites

P. acaulis, as a perennial, xerophilous, tufted frutescent and stoloniferous herbaceous plant species with digitate ternate compound leaves, is an important species of central Asia steppe and mainly distributes in sandy arid and semiarid steppe. Inflorescences are abundantly produced from April to May and seeds maturation is completed during June, even in drought years, each tuft 0 to 20 bearing branches and each bearing branch grew out 2 to 5 flowers (Liu et al., 2007). The plants are anemophilous or anthophilous but self-compatible and their seeds have no special dispersal adaptations and germinate with slight testa restrictive dormancy (Zhao et al., 2010).

The sample sites are located on the National Grassland Ecosystem Field Station (NGEFS) in northern China (41°46'05.15"N, 115°40'44.45"E), which lies in the 1384 m altitude and has a total annual precipitation of 300 to 400 mm. Detailed information about the vegetation were listed in the study by Huang et al. (2007). In the study sites, we observed that the density of crawling *P. acaulis* on the individuals ranged about 30 to 185 ramets/m^2 and the average height of ramets was below 3 cm.

Collection of plant materials

Seven 1×1 km plots were established within a 14×18 km *P. acaulis* distribution area (Figure 1). In the study area, a total of 110 individuals of *P. acaulis* were randomly selected from the seven plots. The locations of all the individuals of *P. acaulis* were determined by a survey laser instrument (Criterion 300, laser technology). Within the seven plots investigated, 110 individuals of *P. acaulis* were identified. The shapes and dimensions of those measured and *P. acaulis* vegetation within each individual were mapped on graph papers. *P. acaulis* leaf samples were collected from all those selected from the seven plots investigated. Then, the leaf samples were desiccated using silica gel and stored at room temperature before analysis.

DNA extraction and random amplified polymorphic DNA (RAPD) analysis

Total DNA of each sample was extracted from about 20 mg of silica-gel-dried *P. acaulis* leaves with a standard kit (TaKaRa; universal genomic DNA extraction kit, Tokyo, Japan). Sixty primers were screened for polymorphism, readability and reproducibility (Sangon, random primer kit, Shanghai, China). Eleven primers were selected out as the final analysis primers (Table 1). DNA was amplified in reaction volumes of 25 µl containing 1 µl DNA (10 ng/µl), 2 µl of primer (Invitrogen), 2 µl of each dNTP (TaKaRa), 2 µl 10×buffer (TaKaRa), 2 µl Taq polymerase (0.5 U/µl, TaKaRa) and 16 µl H_2O. Polymerase chain reaction (PCR) of all samples was simultaneously carried out in a thermocycler (PTC-0200, Bio-Rad; MJ, California, USA). The thermocycler was programmed for one cycle of 4 min at 94°C, followed by 36 cycles of 30 s at 94°C, 45 s at 38°C and 120 s at 72°C with a final cycle of 7 min at 72°C.

DNA fragments were determined by the electrophoresis in 1% agarose (Sigma, St. Louis, MO) gels with a tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer system at 110 V for 2 h and stained with ethidium bromide. DNA bands were then visualized using ultraviolet (UV) light (Gel Doc 2000, Bio-Rad), only bands in the range between 240 and 2000 bp were scored.

Statistical analysis

Reliable and reproducible random amplified polymorphic DNA (RAPD) bands were converted into a raw data matrix with 1 (present) or 0 (absent). The weak and poorly defined bands with no reproducibility were ignored in the analysis and only polymorphic bands were used in the further statistical analysis. Because RAPDs are dominant markers, the standard measure of genetic diversity can only be estimated when the populations are presumed to abide by Hardy-Weinberg equilibrium. When we did not explicitly test whether the study populations abided by the above equilibrium, we based the analysis on simple measures of the multivariate diversity, which required no assumptions on the genetic structure. Similarities were calculated using Jaccard's coefficient (Jaccard, 1908) as shown below:

$$S_{ij} = a/(a+b+c) \quad (1)$$

Where S_{ij} is the similarity between two individuals; $_i$ and $_j$, refers to shared bands, b refers to bands exclusive to sample; $_i$, and c refers to bands exclusive to sample $_j$.

Values were transformed to a distance measure by subtracting them from one (Legendre and Legendre, 1998). The distance matrix was used to calculate mean distance among individuals, as well as within and among populations. Jaccard's genetic distance was also used in ordination. A principal coordinate analysis (PCoA) of all samples was carried out on square root transformed distances as these were supposed to have metric properties (Legendre and Legendre, 1998). Genetic diversity was estimated by the Shannon index (*H*) (Lewontin, 1972):

$$H = -\sum_{i=1}^{k} p_i \ln p_i \quad (2)$$

Where $_k$ is the number of RAPD bands produced with the respective

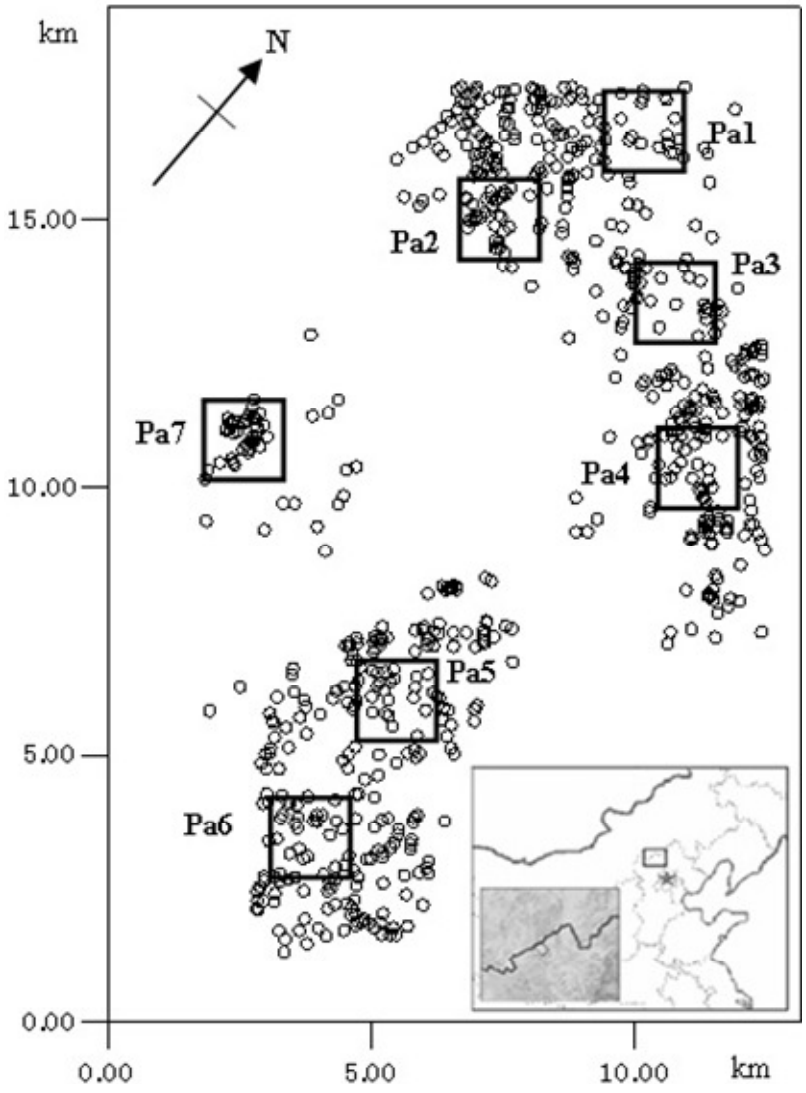

Figure 1. Map of seven study plots (1×1 km). Each circle sign corresponds to an individual colonized by *P. acaulis*.

Table 1. Primers used in this study and statistics for RAPD marks obtained for *P. acaulis* populations.

Primer	Nucleotide sequence(5'-3')	Total number of bands	Number of polymorphic bands	PIC
A-02	TGCCGAGCTG	5	4	0.48
A-03	AGTCAGCCAC	3	2	0.35
A-04	AATCGGGCTG	7	7	0.46
A-10	GTGATCGCAG	5	5	0.47
A-18	AGGTGACCGT	3	2	0.49
B-06	TGCTCTGCCC	5	5	0.47
B-10	CTGCTGGGAC	6	6	0.49
D-02	GGACCCAACC	8	8	0.46
D-07	TTGGCACGGG	6	4	0.33
D-11	AGCGCCATTG	6	2	0.37
D-16	AGGGAGTAAG	7	6	0.50
Total		61	51 (83.61%)	

RAPD, Random amplified polymorphic DNA; PIC, polymorphism information content.

Table 2. Genetic variation among populations of *P. acaulis* estimated with 11 RAPD primers.

Population	Number of samples	Polymorphic bands (%)	Jaccard's distance mean	Shannon's index (*H*)
Pa1	11	63.93	0.075	0.365
Pa2	25	68.85	0.042	0.328
Pa3	13	57.38	0.052	0.284
Pa4	18	54.10	0.044	0.263
Pa5	14	45.90	0.062	0.227
Pa6	12	34.43	0.074	0.177
Pa7	11	49.18	0.045	0.256
Total	104*			
Mean (SE)		53.40 (4.36)		0.271

RAPD, Random amplified polymorphic DNA;*6 clonal individuals represented in 110 samples (not analyzed).

primer and p_i is the frequency of the *i*-th fragment.

The polymorphism information content (PIC) for each RAPD marker was calculated with the formula described by Roldán-Ruiz et al (2000):

$$PIC_i = 2f_i(1-f_i) \quad (3)$$

Where PIC_i is the polymorphic information content of marker; $_i$, f_i the frequency of the marker bands which were present; $1-f_i$ represents the frequency of marker bands which were absent.

Polymorphism information content (PIC) values for dominant marker bands such as RAPD markers have a maximum of 0.5 for f_i= 0.5 (De-Riek et al., 2001).

The genetic diversities among populations were quantified with the pairwise Φ_{ST}, calculated using ARLEQUIN version 2.0 (Excoffier et al., 2005). A Φ_{ST} value was calculated for each population pair, which is an analogous measure to the fixation index (F_{ST}) (Excoffier et al., 1992) and its significance is determined based on 1000 permutations. Φ_{ST} values were also used for indirect determination of the effective number of migrants among populations (Excoffier et al., 1992). Analysis of molecular variance (AMOVA) was based on the nonparametric permutational approach (Excoffier et al., 1992) and on pairwise squared Euclidean distances between RAPD phenotypes. Relationship between genetic distance and geographical distance was tested by Mantel's test (Mantel, 1967). Simple bivariate correlations were calculated with SPSS ver.13.0 (SPSS, 2005).

RESULTS

Intra-population genetic structure

Random amplified polymorphic DNA (RAPD) analysis of *P. acaulis* was performed with 11 selected primers, which yielded 61 reliable bands, of which 51 bands were polymorphic (83.61%) (Table 1). The 110 samples represented 104 RAPD phenotypes and 6 clonal samples were found. Among the given populations, 34.43% to 68.85% of all bands present were polymorphic, in which the low figures were found in the south-eastern (Pa6) and high in the north-western (Pa2). The difference in genetic distance among samples from given populations showed a small range to that found by the number of polymorphic bands (0.042 to 0.075) and the Shannon index showed more scattered values between 0.177 (Pa6) and 0.365 (Pa1) (Table 2).

Inter-population genetic differentiation

The principal coordinate analysis provided evidence of an inter-population genetic differentiation of *P. acaulis* (Figure 2). The first and second axes were equally important and respectively explained 23% and 19% of the total variation among populations. Remote samples from the Pa1, Pa3 and Pa6 were comparatively separated in the ordination space, while samples from the other region overlapped, although Pa7 was isolated from the other populations by cropland (Figure 1).

All values in the AMOVA, including Φ statistics for each pairwise comparison, were significant (Table 3). The highest variance was observed within populations (83%, P= 0.0001) and the variances among regions and among populations within given regions are respectively, 5% (P= 0.0002) and 12% (P= 0.0001) (Table 3).

Correlation of geographical and genetic distances

Geographical distance alone could not explain the genetic distance between populations, which was showed by the pairwise values of genetic distances and geographical distances (Table 4). Among all seven populations, correlations between genetic and geographical distances were nearly significant (R= 0.415, P= 0.0598) by the means of Mantel tests. There was no correlation between the pairwise Φ_{ST} values and the geographical distances (R= 0.273, P= 0.0762), however, the correlation between Φ_{ST} values and genetic distances was highly significant R= 0.884 (P= 0.0006).

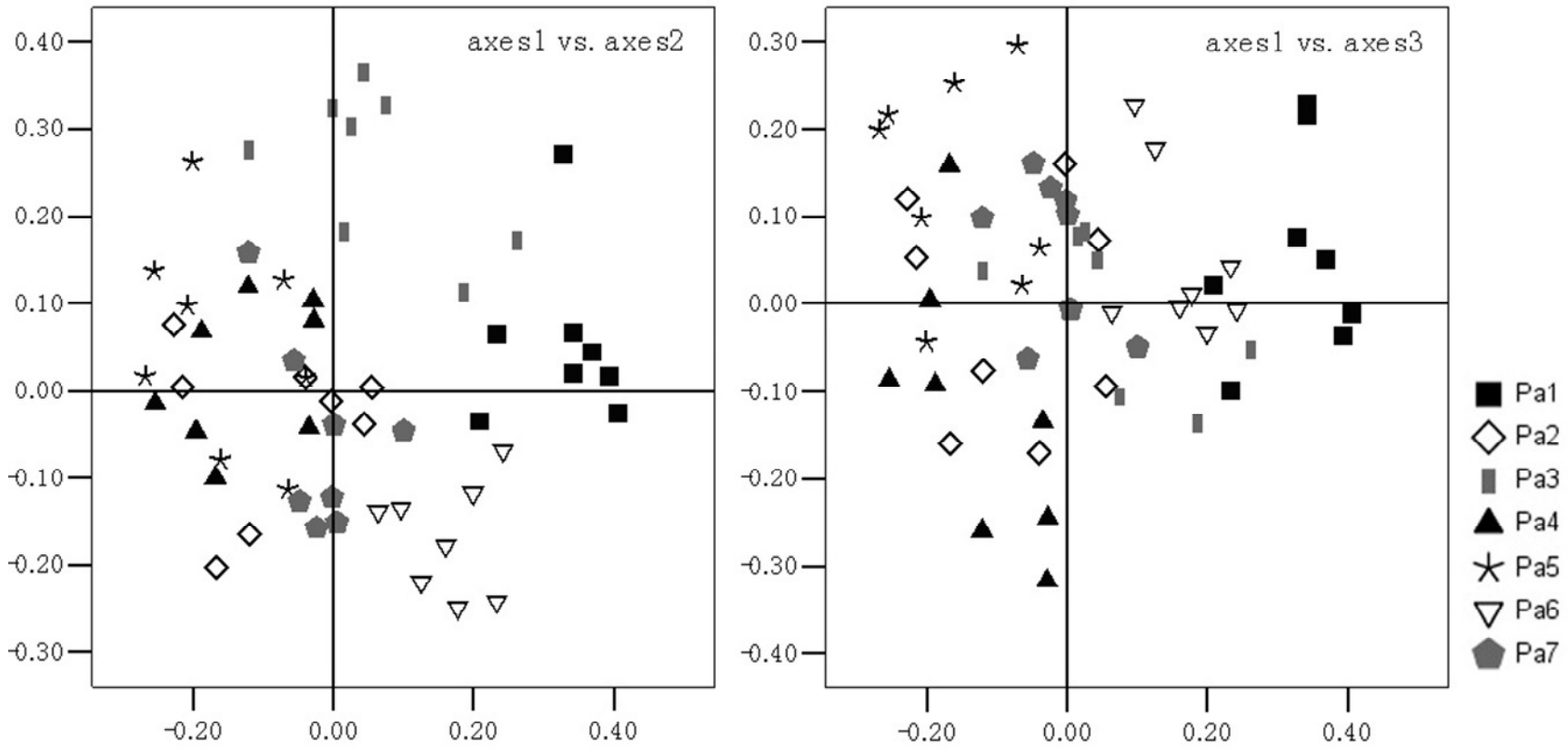

Figure 2. Principal coordinate analysis (PCoA) of *P. acaulis* random amplified polymorphic DNA (RAPD) data based on square-root transformed genetic similarity (% variance explained: axis1 22.9%; axis 2 19.2%; axis 3 16.9%; axis 4 8.1%)

DISCUSSION

RAPD polymorphisms and genetic diversity within populations

RAPD analysis was found to provide a solution for detecting the genomic diversities and structures in populations of *P. acaulis*. The 104 different phenotypes among 110 plants on 61 available fragments were investigated by using 11 RAPD primers (Table 1). The result that 6 clonal individuals represented in 110 samples suggested that, *P. acaulis* was capable of growing and reproducing clonally.

To understand the reproduction strategies of *P. acaulis*, it is essential to determine the relative success in establishment from vegetative propagation and seeds. In plants, the prevalence of vegetative reproduction often resulted in local genetic uniformity, although some clonal species sometimes exhibited a great intra-population genetic differentiation sometimes (Ellstrand and Roose, 1987; Widén et al., 1994). The results of our study showed that, a high proportion of diversity was detected within populations (83.39%) and the remainder part of the genetic diversity was observed among populations (Table 3), which was similar with other reports that estimated for desertification grassland or desert in these areas tended to be lower from northern

Table 3. Summary of the analysis of molecular variance (AMOVA) for *P. acaulis* populations.

Level of variation	d.f.	Sum of squares	Variance components	Variation (%)	Φ Statistics	P- value
Among regions	2	46.911	0.349	4.64	Φ_{CT} = 0.046	0.0002
Among populations within regions	4	81.462	0.901	11.97	Φ_{SC} = 0.126	0.0001
Within populations	97	608.751	6.276	83.39	Φ_{ST} = 0.166	0.0001
Total	103	737.125	7.526			

Significances based on 9999 permutations. d.f., Degree of freedom.

Table 4. Pairwise population matrix of geographical distance (km, above diagonal) and pairwise Φ_{ST} values (below diagonal).

Population	Pa1	Pa2	Pa3	Pa4	Pa5	Pa6	Pa7
Pa1		5.3	5.5	12.6	17.7	24.0	15.2
Pa2	0.144**		6.5	12.5	14.2	20.4	13.6
Pa3	0.154**	0.074**		7.2	13.3	19.6	15.7
Pa4	0.233**	0.058**	0.113**		9.9	15.2	15.8
Pa5	0.239**	0.125**	0.169**	0.141**		6.4	16.6
Pa6	0.207**	0.158**	0.240**	0.249**	0.275**		18.2
Pa7	0.190**	0.082**	0.136**	0.117**	0.178**	0.137*	

**P= 0.0001; *P= 0.0034 based 9999 permutations.

China and Mongolia mountain endemics (Wesche et al., 2006). The partition levels and partitions of genetic diversity within and among populations in our study were comparable to those obtained in several other studies based on RAPD of long-lived and outcrossing clonal species (Kreher et al., 2000; Nybom and Bartish, 2000; Albert et al., 2004).

Propagule recruitment from genets of perennial clonal plants could decrease genetic depletion within populations and there was a kind of specific reproductive strategy in these plants (Price and Marshall, 1999). In our study, the genetic diversity of population revealed a high level. This was probably related to vegetation reproduction of this species, which appeared to prolong generation periods and lowered the renewal ratio of population. As another result, the genotypes of species did not easily lost, which would be helpful for the maintenance of alleles (Auge et al., 2001). However, compared with the non-clonal plants of similar generation length, the clonal plants had less influence to fragmentation (Kudoh et al., 2001).

Genetic differentiation among populations

A relatively high proportion (12%) of genetic differentiation was observed among populations of *P. acaulis* (Table 3). However, in other studies, the proportion was usually less than 5% for most plant species (Jelinski and Cheliak, 1992; Chen and Song, 1998). Genetic differentiation was known to attribute to the inter-population gene flow, genetic drift and inbreeding (Loveless and Hamrick, 1984). Genetic differentiation identified by RAPD analysis reflected the complex events involved in forms of dispersal (Bohonak, 1999). Isolation of population weakened gene flow, which limited communication of genetic information between populations and the effect of genetic drift or inbreeding would act on random fixation of alleles. Since the range of seed dispersal observed in our *P. acaulis* population was limited, the gene transfer might be deduced by pollen dispersal (Fore and Guttman, 1999).

Effects of isolation

We found that the genetic structure was partly influenced by geographical distances. Populations were distinct but not fully separated (Figure 2). It must be pointed out that, Pa7 had overlapped with other populations even though Pa7 was separated by croplands. This result confirmed that, geographical isolation did not always affect genetic

diversity (Hogbin et al., 1998).

According to Wright (1942), a positive correlation was detected between genetic distance and geographical distance when gene flow and genetic drift of populations reached equilibrium (Wright, 1942; Hutchison and Templeton, 1999). Our results of Mantel statistics showed that, genetic distance among populations was not correlated with geographical distance distinctly (R= 0.415, P= 0.0598), which might have resulted from the gene flow was not a dominant factor during genetic differentiation of species, especially in the moment when the species are faced with a strong biological selection against environmental change of habitat (Wright, 1951). The same conclusion was drawn from the research of *Agrostis tenuis* (Liu and Godt, 1983). Nevertheless, there were also some different reports about relativity between genetic distance and geographical distance in *Eucalyptus crucis* and *Gleditsia triacanthos* (Sampson et al., 1988; Schnabel and Hamrick, 1990). Φ_{ST} values based on RAPD were deemed to be related to life form, species breeding system and seed dispersal. An empirical value for long-lived perennials was 0.25 and mean value evaluated from species with a mixed breeding system showed 0.27, whereas the value for dispersal of seeds by wind was 0.25 (Nybom and Bartish, 2000). The phenomenon of low level of genetic differentiation (Φ_{ST}= 0.17) was caused by one or more factors of life history traits and it was a possible reason why genetic differentiation degree of *P. acaulis* population was less than other plant species in same area (Wang et al., 2005; Sui et al., 2009).

Conservation implication

Genetic exchange is extremely important to the maintenance of genetic diversity and often relates to the reproductive traits, such as allocation of reproductive biomass, dispersal of pollen and seeds, mature period of propagule (Reed and Frankham, 2003).

Increased use of agricultural land and invasion by exotic species had caused the fragmentation and destruction of population habitats, as a result, some populations became smaller and more isolated from each other than in the past (Chen, 2000). The fact that populations of *P. acaulis* had survived well till today suggests that they were not sensitive to the activities of humans and their live stocks, though the distribution extent of the populations has been partly affected by croplands. In that case, some modes of grassland management might be needed, which increased distribution continuity of population and reserved at least some small population as a kind of biology corridor.

The fragmented species with isolated generations ordinarily showed the adaptive traits which effectively maintained the genetic diversity and exchange, such as perennial life forms, amelioration of dispersal capacity or multiform reproductive modes (Nybom and Bartish, 2000). Therefore, further research should necessarily be made on reproduction adaptability and fitness of population, which would be useful in developing a conservation strategy.

ACKNOWLEDGEMENTS

This work was supported by grants from China Ministry of Science and Technology “973” project no. 2007CB106805. We would like to thank Mr. Shounan Shen, who kindly provided the test site and equipment support. We wish to thank Jin Wang and Li Lin for aid in process of samples collection, Dr. Jing Shi from the University of Tennessee for the advices of previous version of this paper.

REFERENCES

Albert T, Raspé O, Jacquemart AL (2004). Clonal diversity and genetic structure in *Vaccinium myrtillus* populations from different habitats. Belg. J. Bot., 137: 155-162.

Auge H, Neuffer B, Erlinghagen F (2001). Demographic and random amplified polymorphic DNA analyses reveal high levels of genetic diversity in a clonal violet. Mol. Ecol. 10: 1811-1819.

Bohonak AJ (1999). Dispersal, gene flow, and population structure. Q. Rev. Biol., 74: 21-45.

Chen S, Xia T, Chen S, Zhou Y (2005). RAPD Profiling in Detecting Genetic Variation in *Endemic Coelonema* (Brassicaceae) of Qinghai-Tibet Plateau of China. Biochem. Genet., 43: 189-201.

Chen X (2000). Effects of habitat fragmentation on genetic structure of plant population s and implications for the biodiversity conservation. Acta Ecol. Sin., 20: 884-892.

Chen X, Song Y (1998). Microgeographic differentiation in a Cyclobalanopsis glauca population in western Huangshan , Anhui Province. J. Plant Resour. Environ., 7: 10-14.

De-Riek J, Calsyn E, Everaert I, Bockstaele EV, De-Loose M (2001). AFLP based alternatives for the assessment of Distinctness, Uniformity and Stability of sugar beet varieties. Theor. Appl. Genet., 103: 1245-1265.

Du M, Kawashima S, Yonemura S, Zhang X, Chen S (2004). Mutual influence between human activities and climate change in the Tibetan Plateau during recent years. Global Planet. Change 41: 241-249.

Ellstrand NC, Roose ML (1987). Patterns of genotypic diversity in clonal plant species. Am. J. Bot., 74: 123-131.

Excoffier L, Laval G, Schneider S (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. In: Evol. Bioinform. Online, 1: 47-50.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. Genetics 131: 479-491.

Fore SA, Guttman SI (1999). Genetic structure of *Helianthus occidentalis* (Asteraceae) in a preserve with fragmented habitat. Am. J. Bot., 86: 988-995.

Ge XJ, Yu Y, Yuan YM, Huang HW, Yan C (2005). Genetic diversity and geographic differentiation in endangered *Ammopiptanthus* (Leguminosae) populations in desert regions of northwest China as revealed by ISSR analysis. Ann. Bot,. 95: 843-851.

Hogbin PM, Ayre DJ, Whelan RJ (1998). Genetic variation and reproductive success of road verge populations of the rare shrub *Grevillea barklyana* (Proteaceae). Heredity, 80: 180-186.

Huang D, Wang K, Wu WL (2007). Dynamics of soil physical and chemical properties and vegetation succession characteristics during grassland desertification under sheep grazing in an agro-pastoral transition zone in Northern China. J. Arid Environ., 70: 120-136.

Hutchison D, Templeton A (1999). Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution. 53: 1898-1914.
Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223-270
Jelinski DE, Cheliak WM (1992). Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. Am. J. Bot. 79: 728-736.
Kreher SA, Foré SA, Collins BS (2000). Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. Mol. Ecol. 9: 1247-1252.
Kudoh H, Shibaike H, Takasu H, Whigham DF, Kawano S (2001). Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. J. Ecol. 87: 244-257.
League K, Veblen T (2006). Climatic variability and episodic *Pinus ponderosa* establishment along the forest-grassland ecotones of Colorado. For. Ecol. Manag. 228: 98-107.
Legendre P, Legendre L (1998). Numerical Ecology. (2ed), Elsevier Science BV, Amsterdam, The Netherlands pp 52-80.
Lewontin RC (1972). The apportionment of human diversity. Evol. Biol. 6: 381-389.
Li Y, Wang W, Liu Z, Jiang S (2007). Grazing Gradient versus Restoration Succession of *Leymus chinensis* (Trin.) Tzvel. Grassland in Inner Mongolia. Restor. Ecol. 16: 572-583.
Lioubimtseva E, Henebry GM (2009). Climate and environmental change in arid Central Asia: Impacts, vulnerability, and adaptations. J. Arid Environ., 73: 963-977.
Liu EH, Godt MJW (1983). The differentiation of population over short distances. In: Schonew-Cox CM, Chambers SM, Macbryde B (eds) Genetics and Conversation. Benjamin/Cummings, Menlo Park, California, USA, pp. 78-95.
Liu ZG, Li ZQ, Dong M, Nijs I, Bogaert J, Magdy IE (2007). Small-scale spatial associations between *Artemisia frigida* and *Potentilla acaulis* at different intensities of sheep grazing. Appl. Veg. Sci.,10: 139-148.
Loveless MD, Hamrick JL (1984). Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15: 65-95.
Mantel N (1967). The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.
Nybom H, Bartish IV (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant Ecol. Evol. Syst. 3: 93-114.
Peakall R, Smouse PE (2005). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, 6: 288-295.
Pluess AR, Stöcklin J (2004). Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. Am. J. Bot., 91: 2013-2021.
Price EAC, Marshall C (1999). Clonal plants and environmental heterogeneity-An introduction to the proceedings. Plant Ecol., 141: 3-7.
Reed DH, Frankham R (2003). Correlation between fitness and genetic diversity. Conserv. Biol., 17: 230-237.
Roldán-Ruiz I, Dendauw J, Bockstaele EV, Depicker A, Loose MD (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol. Breed., 6: 125-134.
Sampson JF, Hopper SD, James SH (1988). Genetic diversity and the conservation of *Eucalyptus crucis* Maiden. Aust. J. Bot. 36: 447-460.
Schnabel A, Hamrick JL (1990). Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). Am. J. Bot., 77: 1060-1069.
Sheng Y, Zheng W, Pei K, Ma K (2005). Genetic variation within and among populations of a dominant desert tree *Haloxylon ammodendron* (Amaranthaceae) in China. Ann. Bot. 96: 245-252.
SPSS (2005). SPSS 13.0 for Windows. In: SPSS Inc., Chicago, IL, USA.
Sui XQ, Wang K, Zheng SH, Li L, An SZ (2009). Effects of landscape fragmentation on genetic diversity of *Stipa krylovii* Roshev (*Stipa* L.) in agro-pastoral ecotone in Northern China. Afr. J. Biotechnol., 8: 3431-3439.
Wang Y, Zhao L, Wang H, Wang J, Huang D, Hong R, Teng X, Miki N (2005). Molecular Genetic Variation in a Clonal Plant Population of *Leymus chinensis* (Trin.) Tzvel. J. Integr. Plant Biol., 47: 1055-1064.
Washitani I, Ishihama F, Matsumura C, Nagai M, Nishihiro J, Nishihiro MA (2005). Conservation ecology of *Primula sieboldii*: synthesis of information toward the prediction of the genetic/demographic fate of a population. Plant Spec. Biol., 20: 3-15.
Wesche K, Hensen I, Undrakh R (2006). Range-wide genetic analysis provides evidence of natural isolation among populations of the Mongolian endemic *Potentilla ikonnikovii* Juz. (Rosaceae). Plant Spec. Biol., 21: 155-163.
Widén B, Cronberg N, Widén M (1994). Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. Folia Geobot. 29: 245-263.
Wright S (1942). Isolation by distance. Genetics, 28: 114-138.
Wright S (1951). The genetical structure of populations. Ann. Egu., 15: 323-354.
Xia T, Chen S, Chen S, Ge X (2005). Genetic variation within and among populations of *Rhodiola alsia* (Crassulaceae) native to the Tibetan Plateau as detected by ISSR markers. Biochem. Genet.. 43: 87-101.
Xu L, Wang Y, Wang X, Zhang L, Yue M, Gu F (2003). Genetic structure of *Reaumuria soongorica* population in Fukang desert, Xinjang and its relationship with ecological factors. Acta Bot. Sin.. 45: 787-794.
Zhao CZ, Zhang QP, Dong XG, Wang Q, Wang YY (2010). Soil seed bank of *Potentilla acaulis* community in arid grassland of the upstream of Shiyang River. Arid Land Geogr., 1:15-20.