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Full Length Research Paper

Effects of landscape fragmentation on genetic diversity of *Stipa krylovii roshev* (*Stipa* L.) in agro-pastoral ecotone in Northern China

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***Stipa krylovii Roshev* (*Stipa* L.) is one of the most important grass species for rangeland ecology and animal husbandry. But some populations of this species are under threat due to landscape fragmentation and habitat isolation resulting from the reclamation and cultivation in ecotone. To determine if and how these disturbances have impacted on genetic structure of *S. krylovii* populations, an inter-simple sequence repeat (ISSR) markers was used to characterize them for the first time in China. *S. krylovii* populations from 10 isolated patches were compared with population from unbroken natural rangeland. Eight primers screened were employed to assess genetic diversity within and among populations. The results revealed the total genetic diversity (Ht) and the within population genetic diversity (Hs) were 0.2054 and 0.1388 respectively, while the coefficient of genetic differentiation (Gst) were 0.3241 suggesting more variability within the populations than among them. The cluster pattern of *S. krylovii* indicated that the populations with larger habitat size were grouped together with CK population in one cluster; likewise, populations with moderate habitat size and smaller area were also grouped together respectively, while the smallest population formed itself into a solo cluster due to genetically different from others. These findings may be early signs of fragmentation effects that in the future will result in species loss even if the present populations are maintained.**

Key words: *Stipa krylovii roshev*, genetic diversity, landscape fragmentation, patch, habitat isolation, ISSR marker, rangeland, ecotone.

INTRODUCTION

Habitat loss and habitat isolation caused by landscape fragmentation not only affect the ecological processes of biotic and abiotic factors in ecosystem, but also exert an influence on genetic structure and genetic variation of species, which will make a difference to their adaptability (Saunders et al., 1991; Liener et al., 2002). Genetic effects followed habitat fragmentation are mainly manifested in bottleneck effect which caused in the process of fragmentation, genetic drift effect and inbreeding effect of remnant populations which produced by shrinking habitat, and limited gene flow as a result of habitat isolation. Theoretical predictions indicate that habitat fragmentation will be accompanied by an erosion of genetic variation and the increase of genetic differentiation among populations (Young et al., 1996).

Stipa krylovii roshev, belongs to *Stipa* L., is a perennial, thicket, xerophytic bunch grass. It is the main constructive species of typical rangeland in central Asia steppe and has the extensive distribution due to its high drought resistance. The main distribution area of *S. krylovii* is the typical steppe of Mongolian Plateau, including the Liaohe Plain of Northeast China, the Loess Plateau, the Tibetan Plateau and Xinjiang Province is also distributed (Lu and Wu, 1996). *S. krylovii* steppe is not only the zonal vegetation formation of typical steppe zone and mountain steppe zone of desert region, but also the variant of grazing succession of partial *Stipa grandis* steppe. It plays an important role both in maintaining the rangeland ecology and developing animal husbandry.

A series of research work on *Stipa* L has been carried out in recent years (Ma et al., 1995; Zhao et al., 2003; Han et al., 2004; Wang et al., 2006). However, little atten-

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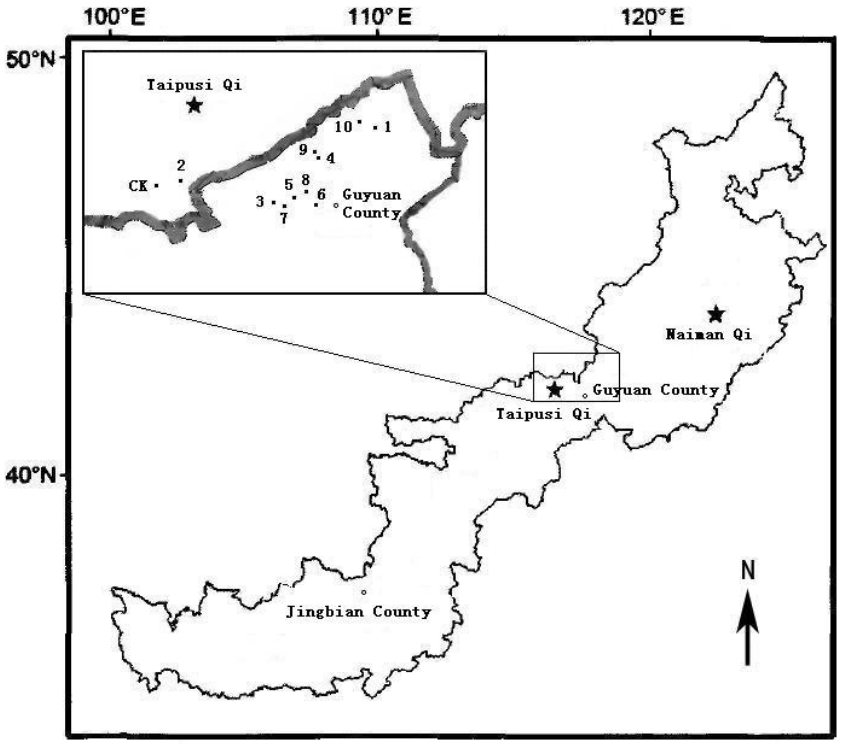

Figure 1. Distribution of 10 sampling sites (patches) and CK in agro-pastoral ecotone of northern China

tion has been given to reveal the genetic structure of *S. krylovii* which collected from patches of landscape fragmentation so far. In the present study, the constructive species (*S. krylovii*) from 10 populations were employed to be detected their genetic diversity and genetic structure response to the influence of habitat fragmentation. The objective of this study was to provide referenced data for the restoration and management of populations as well as useful elements for landscape ecological study in the future.

A variety of DNA markers during the last decade have been used successfully to understand genetic structure and differentiation among various plant species (Welsh and McClelland, 1990; Heath et al., 1993; Gupta et al., 1994; Wolfe and Liston, 1998; Esselman et al., 1999; Pasakinskiene et al., 2000; Aga et al., 2005). Among these molecular techniques, the inter-simple sequence repeat (ISSR) method has been adopted widely due to its special advantages. ISSR PCR is technically simple and requires no sequence information to design polymerase chain reaction (PCR) primers but performed using single primers based on a simple repeat with the addition of a degenerate nucleotide or a selective anchor (Zietkiewicz et al., 1994). These primers amplify the region between microsatellites. In addition, the ISSR technique yields highly reproducible results and reveals abundant polymorphisms combined with the separation of amplification products on a polyacrylamide gel (Wolff et al., 1995).

The method has been shown to generate similar or higher levels of polymorphism compared with RFLP or RAPD (Godwin et al., 1997; Nagaoka and Ogihara, 1997; Hollingsworth et al., 1998). Therefore, ISSR markers have been commonly used in studies, such as variety identification, genetic mapping, gene location and genetic diversity (Camacho and Liston, 2001; Wolfe and Randle, 2001; Wang, 2002; He, 2008).

MATERIALS AND METHODS

Study site

The study site is located at the southeastern margin of Inner Mongolia Plateau, which lies in the typical agro-pastoral ecotone in Hebei Province of northern China and is considered as one of the ecological fragile zone. It is characterized by a continental, semi-arid, monsoon climate in the temperate zone, and with Cambids soil dominated. The zonal vegetation has the physical appearance of typical Inner Mongolian steppes with *S. krylovii*. As the constructive species, *Leymus chinensis* as the dominant species and all kinds of forbs.

In this area, 10 “island” patches (populations) with different size were randomly selected (Figure 1), which were remained in the process of fragmentation due to human reclamation in the rangeland and each patch area was measured by GPS (etrex Venture, Uni-Strong Company, China) for three times. In order to ensure the experimental data are scientific and objective, the following conditions must be met to eliminate the differences among patches during they were selected: (i) to exist under the similar natural conditions (climate and terrain), (ii) to be isolated by surrounding farmland at

Table 1. Distribution information of patches (populations) and CK in agro-pastoral ecotone of northern China.

Patch code	Location	Longitude	Latitude	Altitude (m)	Area (hm^2)
pop1	Yushugou	115°49′29”	41°50′52”	1365	1.2532
pop2	Wuxing Village	115°24′7”	41°48′38”	1419	2.4272
pop3	Banjietan Village	115°36′38”	41°40′26”	1408	3.1126
pop4	Beigang Village	115°40′35”	41°45′38”	1386	4.5598
pop5	Huoxingyong Village	115°38′13”	41°40′47”	1397	4.7857
pop6	Wentielu	115°39′55”	41°38′56”	1407	6.8661
pop7	Banjietan	115°37′25”	41°40′24”	1411	7.6368
pop8	Yuanbaoshan	115°39′43”	41°40′28”	1401	10.6000
pop9	Key Grassland Experiment Station	115°40′25”	41°45′57”	1388	35.4000
pop10	Luotuoshan	115°47′29”	41°51′47”	1381	79.8882
CK	Bugeding	115°11′23”	41°41′52”	1416	——

*1-10 represents the patch arranged according to the area; CK represents the population from unbroken steppe.

least for 30 years in order to study the “isolation effect” on genetic structure of species, (iii) and the vegetation within each patches was primitive community instead of artificially cultivated or fallow vegetation. The control area (that is, CK) was located in the steppe with unbroken landscape in Bugeding region (belonging to Taipusi Qi, Inner Mongolia) (Table 1).

Plant material and sampling

Sample collection was conducted in mid-July 2007, thirty to forty-five random plant material of *S. krylovii* were collected from the population of each patch in an intervals at least be maintained 10 m. An additional sample was obtained from CK region was also included for comparison. The young leaf tissue were sealed along with the self-indicating silica gel (for drying) respectively (1:10) in the labeled polythene bag. Then, all samples were brought back to laboratory and stored at -20℃ until further use.

DNA extraction

Total genomic DNA was extracted from the dried leaf tissue using modified CTAB (cetyltrimethyl ammonium bromide) method described by Murray and Thompson (1980). Approximately 300 mg of the youngest leaf tissue was ground in liquid nitrogen using mortar and pestle. The fine leaf powder was transferred to a 1.5 ml centrifuge tube and incubated at 65□for 30 min after adding 2×CTAB isolation buffer (100mM Tris-Hcl, pH8.0, 1.4M NaCl, 20mM EDTA, 2% CTAB, 0.2% 2-mercaptoethanol). After incubation, equal volume of chloroform-isoamylalcohol (24:1) was added to the incubated mixture, and after mixed thoroughly by inverting the tube gently for a number of times, it was centrifuged at 12000 rpm for 10 min. The suspension was collected and transferred to a sterilized tube, and then it was extracted again with chloroform–isoamylalcohol and was centrifuged as above. Nucleic acids were precipitated by the addition of two volumes of cooled ethanol and one-tenth volume of 0.2 M sodium acetate. After being centrifuged for 5 min, the precipitated DNA was rinsed with 75% ethanol and air dried. The DNA was later dissolved in TE Buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH 8.0) and quantified by electrophoresis (0.8% agarose gel) and ultraviolet spectrophotometer (Ultrospec 3100, Biochrom Ltd. Britain); a final concentration of 30 ng/μl was determined and stored at -20℃.

PCR amplification with ISSR primers

A total number of 51 ISSR primers, obtained from the University of British Columbia (UBC) primer sets and from Sangon Biological Engineering Technology (Shanghai, China), were screened initially using a few DNA samples to select the appropriate primers suitable for *S. krylovii.* Eventually, 8 ISSR primers that produced good resolution and reproducible bands were selected (Table 2). This was done after the conditions of PCR amplification were optimized by an orthogonal design combined with single factor experiments.

Twenty-five DNA samples from each of eleven populations were subsequently used for the ISSR-PCR with different primers. The PCR amplification was performed in a total reaction volume of 25 μl, containing 50 ng templates DNA, 1 U Taq DNA polymerase, 1.5 mM Mg^{2+}, 0.2 mM dNTP, 0.8 μmol/L primer, 1×buffer and sterile, distilled H_2O. All the PCR amplifications were carried out in a MJ Research Thermal-Cycler, PTC 200 (MJ Research Inc. Watertown, Massachusetts, USA) following different cycles as follows: an initial denaturation of 5 min at 94℃, 35 cycles for 45 s at 94℃, 45 s annealing at 50.5 to 54.5℃ (according different primers), and 90 s extension at 72℃ were performed, this was followed by 7-min extension at 72℃, then a final incubation at 4℃.

Electrophoresis

The amplified products were firstly checked on 2% agarose gels visualized with ethidium bromide staining under UV light before conducted the vertical slab gel electrophoresis. Then the PCR amplifications were separated on a 10% denaturing polyacrylamide gel (acrylamide : Bis 29 : 1) in 1×TBE buffer for 3 – 4 h at 60 W and gels were stained using silver nitrate, meanwhile, a 100-base-pair ladder was loaded on the gel for use as a standard for the estimation of the sizes of bands. Banding patterns were photographed into a computer imaging file.

Data analysis

Only intense and reproducible bands were scored visually for presence (1) or absence (0) at positions, and scores were assembled in a rectangular data matrix. Initially, data were processed in MS Excel for calculation of the polymorphic band of individual primers, average polymorphic band per primer and percentage of polymor-

Table 2. List and obtained results of the 8 ISSR primers used in this study.

Primer Code sequence (5′-3′)		Annealing temperature (℃)	Band size (bp)	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands%
UBC814	(CT)8A	52.5	280 - 1500	13	11	84.62
UBC822	(TC)8A	50.5	620 - 1500	8	8	100.00
UBC825	(AC)8T	53.5	300 - 1500	15	15	100.00
UBC827	(AC)8G	54.5	200 - 1250	13	12	92.31
UBC841	(GA)8YC	54.5	220 - 1200	17	16	94.12
UBC842	(GA)8YG	54.5	150 - 1600	26	21	80.77
UBC844	(CT)8RC	52.5	230 - 1600	20	19	95.00
UBC846	(CA)8RT	51.0	400 - 1500	16	16	100.00
Total			150 - 1600	128	118	92.19

*Y=(C, G), R = (A, T)

Table 3. Genetic parameters of 11 *S. Krylovii* populations revealed by ISSR.

Population	Plant samples	Na	Ne	No. of loci	PPB (%)	He	H’
pop1	25	1.5703	1.3302	73	57.03	0.1893	0.2829
pop2	25	1.4297	1.2058	55	42.97	0.1247	0.1922
pop3	25	1.4531	1.2277	58	45.31	0.1327	0.2015
pop4	25	1.4062	1.2112	52	40.62	0.1247	0.1900
pop5	25	1.4062	1.1966	52	40.62	0.1202	0.1852
pop6	25	1.4375	1.2050	56	43.75	0.1242	0.1922
pop7	25	1.4141	1.1811	53	41.41	0.1088	0.1686
pop8	25	1.4766	1.2604	61	47.66	0.1550	0.2344
pop9	25	1.5469	1.2714	70	54.69	0.1640	0.2527
pop10	25	1.4453	1.2152	57	44.53	0.1306	0.2014
CK	25	1.4922	1.2609	63	49.22	0.1529	0.2316
Total	275	1.9219	1.3275	118	92.19	0.2054	0.3278

*Na; observed number of alleles, Ne; effective number of alleles, No. of loci, number of polymorphic loci, PPB; percentage of polymorphic loci, He; Nei’s genetic diversity index, H′; Shannon’s diversity index.

phism. The resulting data matrix of the ISSR was analyzed using POPGENE 32 (Yeh et al., 2000), which including the Nei’s (1973) genetic diversity parameters; the mean expected heterozygosity (He); Shannon’s diversity index (H'); the total genetic diversity (Ht); the within population genetic diversity (Hs); the genetic diversity among populations (Dst) and the coefficient of genetic differentiation (Gst). The amount of gene flow among populations was calculated using $Nm= (1-G_{ST})/4G_{ST}$ after Slatkin and Barton (1989). Based on the matrix of genetic distance (Nei, 1978), cluster analyses were performed using unweighted pair/group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973) of the NTSYS-pc statistical package (Rohlf, 1998).

RESULTS

Inter-simple sequence repeat analysis

Eight of the fifty-one primers which produced polymerphic, reproducible and clear bands were screened for ISSR-PCR amplification. The eight primers yielded a total of 128 reliable loci (bands) from the 275 samples (11 populations) of *S. krylovii* DNA amplified, of which 118 (92.19%) were polymorphic with a range from 150 to 1600 bp in length and a mean of 16 bands (fragments) per primer (Table 2). Among the eight ISSR primers, UBC842 gave the maximum number of 26 bands and the minimum number of 8 bands by UBC822.

Genetic diversity in the populations of *S. Krylovii*

The genetic diversity of 11 populations (patches) of *S. Krylovii* was analyzed using POPGENE 32. The results in Table 3 showed that the genetic diversity has a larger differentiation among the populations when it was expressed by PPB, where the highest value was 57.03% (pop1) and the lowest value was 40.62% (pop4, pop5), each of them was lower than the total percentage of polymorphic bands (92.19%). The number of polymorphic loci was between 52 (pop4, pop5) and 73 (pop1) among these popu-

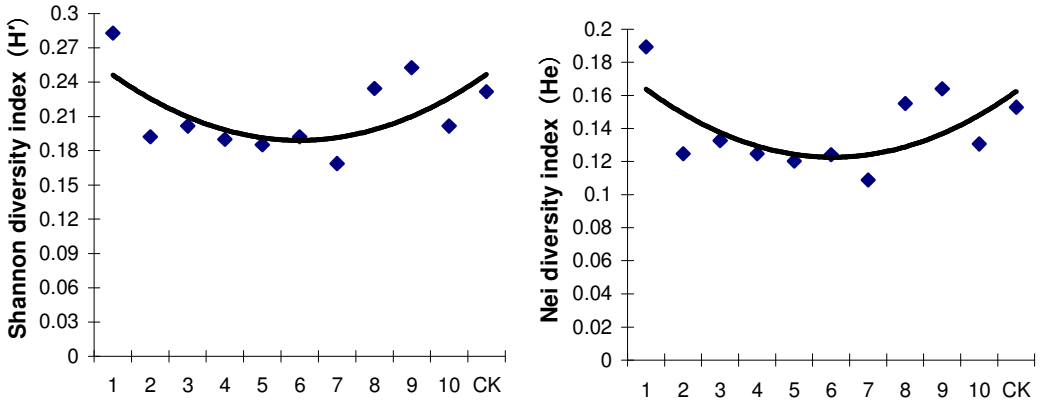

Figure 2. Trend analysis of genetic diversity in 11 *S. Krylovii* populations expressed by Shannon diversity index (H') and Nei's diversity index (He). (1-10 represents the 10 *S. Krylovii* populations arranged in the size; CK represents the population from unbroken steppe)

lations. The Shannon diversity index (H ') was 0.3278, ranging from 0.1686 (pop7) to 0.2829 (pop1) in each population. Nei's diversity index (expected heterozygosity, He) which represents the degree of genetic diversity is an indicator commonly used to assess the genetic differentiation among populations. It denotes the proportion occupied by genetic variation between populations in the total genetic variation. The results of this study showed that: pop1 has the highest Nei's genetic diversity while pop7 got the lowest value; this is similar to results revealed by Shannon diversity index.

These results indicated that there was some difference in the genetic diversity of *S. Krylovii* among 11 populations. The genetic diversity revealed by He, H′ and He was almost consistent, but slightly has the difference with PPB and Na measured. Although the parameter PPB can visually display a certain degree of genetic diversity with simple calculation, it is only an estimated value in measure of genetic diversity due to its uncertainty in calculating the degree of evenness in bands frequency and its vulnerability to the effect of sample size. Whereas the Shannon diversity index which based on phenotype frequency of bands and the Nei's diversity index which based on Hardy-Weinberg assumption are more reliable in weighting genetic diversity among populations (Qian and Ge, 2001). The genetic variation of each population revealed by He and H′ were analyzed and shown in Figure 2. As habitat area shrinking, the genetic diversity of *S. Krylovii* gradually decreased at first and then began to rise; the genetic diversity in larger patches (populations) can maintain a considerable high level with population in CK.

Genetic differentiation and gene flow among populations of *S. Krylovii*

Table 4 showed the assessment of the genetic structure for the 11 populations using POPGENE 32. The total genetic diversity (Ht) was found to be 0.2054, and the within-population genetic diversity (Hs) was 0.1388, while the coefficient of gene differentiation (Gst) for all loci was 0.3241 indicating that the variations between populations and those within populations contributed 32.41 and 67.59% respectively. The number of migrants per generation (Nm) is an estimated value from Gst to measure the gene flow, the higher its value, the less genetic differentiation among populations. According to the previous study and estimation of gene flow (Wright, 1931), if Nm> 1, it can prevent the differentiation among populations caused by genetic drift. The amount of gene flow (Nm) among *S. Krylovii* populations was found to be 1.0425 (>1), which indicated that there was a certain frequency of gene flow among the separated patches and the exchange of genetic material would reduce the effect of genetic drift as well as the serious genetic differentiation among isolated populations.

Table 4. Analysis of genetic differentiation among the *S. Krylovii* populations based on ISSR.

Population	Ht	Hs	Gst	Nm
Mean St. Dev	0.2054 (0.0290)	0.1388 (0.0168)	0.321	1.0425

*Ht; total gene diversity, Hs; gene diversity within populations, Gst; coefficient of population differentiation, Nm, estimate of gene flow from Gst.

Cluster analysis

Nei's genetic distance (D) and genetic identity (I) between populations were estimated as the important parameters. The value ranged from 0.0234 to 0.2190, and 0.8033 to 0.9769, respectively (Table 5). The UPGMA dendrogram based on the matrix of Nei's genetic distance identified the 11 pops into three groups and one outlier (Figure 3). The first cluster included three *S. Kry-*

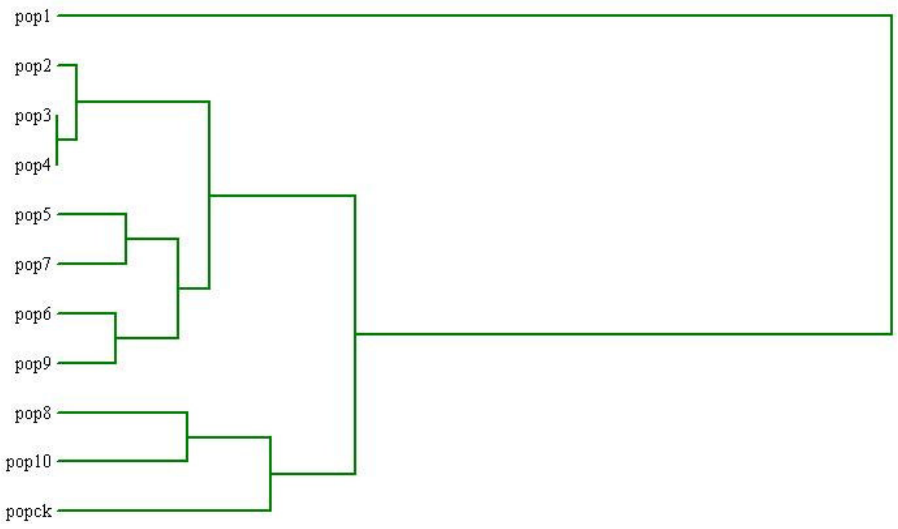

Figure 3. UPGMA dendrogran for 11 populations of *S. Krylovii* based on Nei's genetic distance (D).

Table 5. Nei's genetic distance (below diagonal) and genetic identity (above diagonal) among 11 populations of *S. Krylovii.*

Population	pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	pop9	pop10	CK
pop1	***	0.8346	0.8470	0.8360	0.8488	0.8223	0.8145	0.8033	0.8431	0.8131	0.8054
pop2	0.1807	***	0.9733	0.9731	0.9634	0.9541	0.9470	0.9325	0.9390	0.9279	0.9401
pop3	0.1661	0.0270	***	0.9769	0.9540	0.9496	0.9499	0.9330	0.9436	0.9446	0.9301
pop4	0.1791	0.0272	0.0234	***	0.9565	0.9394	0.9468	0.9284	0.9278	0.9438	0.9410
pop5	0.1640	0.0373	0.0471	0.0444	***	0.9653	0.9634	0.8988	0.9482	0.9073	0.9142
pop6	0.1956	0.0470	0.0517	0.0626	0.0354	***	0.9540	0.8952	0.9656	0.9024	0.9299
pop7	0.2052	0.0545	0.0514	0.0547	0.0372	0.0471	***	0.8987	0.9464	0.9073	0.9057
pop8	0.2190	0.0699	0.0693	0.0743	0.1067	0.1107	0.1068	***	0.8962	0.9517	0.9265
pop9	0.1707	0.0629	0.0580	0.0750	0.0532	0.0350	0.0550	0.1096	***	0.9152	0.9401
pop0	0.2069	0.0748	0.0570	0.0579	0.0972	0.1027	0.0973	0.0495	0.0886	***	0.9459
CK	0.2165	0.0618	0.0724	0.0608	0.0897	0.0727	0.0991	0.0764	0.0618	0.0556	***

lovii pops, that is, pop2, pop3 and pop4. In the second cluster pops, with more area, such as pop5, pop6, pop7 and pop9 were grouped together. In the third cluster, the population from pop8 first clustered together with pop10, then with pop CK. While the position of pop1 forms a separate cluster of its own indicated that it is genetically different from others. The clustering pattern revealed that there has generated a certain degree of genetic differentiation among *S. Krylovii* populations due to disturbance of fragmentation and habitat isolation.

DISCUSSION

Landscape fragmentation and genetic diversity

Landscape fragmentation induced by various factors could simplify the population structure of species through reducing the chances of intraspecific gene exchange and increasing genetic drift effect of isolated populations, meanwhile, the genetic isolation presented by fragmentation easily resulting in pollen sterility, self-incompatibility and other biological consequences among plant populations (Li et al., 1999; Warburton et al., 2000). These heredity consequences will make an impact on genetic diversity, fitness as well as population differentiation. It is considered that the intraspecific genetic differentiation is not only the prelude of species differentiation, but also the basic for dynamic regulation of population (Zhong, 1992). Species formation, development and extinction always accompanied by environment selection and adaptive process during the whole life history.

Genetic diversity is the sum of genetic information carried by organism, it plays a decisive role in species and community diversity, and meanwhile it is the basis of population survival, development and evolution (Barrett and

Kidwell, 1998; Yan, 2005). Genetic diversity includes the distribution pattern of genetic variation (i.e. the genetic structure of populations) as well as the level of genetic variation, the direct expression form (Hamrick and Loveless, 1989). The evaluation of genetic diversity is significant for us to understand the adaptability of species, the distribution of genetic resources and the origin of species. The level of genetic diversity, representing the abundance of gene in a specific environment of this species, is form-ed in the long process of evolution and adaptivity, it deter-mines the ability to adapt to environmental changes and potential development trends of the species.

In the present study, the eight primers yielded a total of 128 loci (bands) from 11 *S. krylovii* populations, the value of PPB was 92.19% indicated that there was a high level of genetic diversity in this species. However, this value was lower than reported (PPB = 99.22%) using RAPD analysis to the same species by HAN (2003), it is because that the latter *S. krylovii* Samples collected from the non-fragmented habitat and escaped from the effect of isolation, whereas those in fragmented patches possibly lacking some genetic loci due to the isolated environment, which lowered the level of genetic diversity.

Because the ISSR mark is the dominant mark, for the cross-pollination plant, an existing locus couldn't represent its homozygosis, however, the Shannon diversity index (H′) can overcome this defect and estimates genetic diversity by taking the occurrence frequency of amplified products as the phenotype frequency of locus, it is feasible for the wind pollinated plant. Another parameter to evaluate genetic diversity is the Nei's diversity index (He), which reflecting the richness and evenness of allele in a population, and the value obtained is proportional to the degree of loci variation (Wang, 1996). In our study, the value of H′ and He was 0.3278 and 0.2054 respectively, which is lower than the results (H′ = 0.4764 and He = 0.3120) from previous research (Han, 2003). This suggested that there was a tendency downward in the level of genetic diversity due to habitat isolation.

Trend analysis based on H′ and He was carried out to 11 populations of *S. Krylovii* (Figure 2). The level of genetic diversity among *S. Krylovii* populations didn't show the descending trend along with the shrinking habitat size as we imagined, but performed a trend decreased gradually at first and then began to rise. It can be observed from the "U" curve that the higher level of genetic diversity of *S. Krylovii* could be maintained on the larger patches as much as that of population in CK region. While, the *S. Krylovii* population in the small patch also obtained a similar high level of genetic diversity, which was disagree with the previous report (Hedrick, 2005a). The reason might be that the *S. Krylovii* in order to adapt to the pressure of environmental selection (landscape fragmentation), some individuals lacking fitness have been eliminated, while the survivors own more genetic variation and have a far genetic relationship displayed a higher level of genetic diversity. Just as found in our investigation, both the density and the quantity of *S. Krylovii* reduced with the decrease of habitat area. In addition, even though fragmentation and isolation could reduce part of genetic diversity, the species would generate some new loci and variation to respond to the environmental extremely se-lection, especially when this pressure reaches a certain threshold value. Although the genetic diversity of *S. Kry-lovii* from small patch increased in form, a marked change have been occurred probably in the intraspecific genetic material and genetic structure, and this increase trend was quite contrary to the population of CK region genetically, the following analysis based on genetic distance also proved this point.

Genetic differentiation and gene flow

The genetic structure of population refers to the distribution pattern (in space or time) of genetic variation or gene and genotypes, which is subject to the combined effects of mutation, gene flow, natural selection and genetic drift (Liu and Zhao, 1999). The evolutional potential of the species or population, to a large extent, depends on its genetic structure (Hamrick and Godt, 1990). It is an important step to confirm the genetic structure of a population, and then we can understand its biology characteristics and explore the evolutional process and mechanisms. The genetic structure of population is mainly reflected by genetic differentiation within and between populations. The coefficient of genetic differentiation (Gst) as the most commonly used index expressed by the genetic variation between population accounts for the proportion in the total variation among populations. In our study, Ht was found to be 0.2054 and the within-population genetic diversity (Hs) was 0.1388 (contributed 67.59%) indicating that the genetic diversity between populations occupied a large proportion of the total. *S. Krylovii* is the cross-pollination plant tends to anemophily, this pollination mode enable it to have the higher rate of gene recombination to adapt to the environmental changes by producing a large number of recombinants. Furthermore, *S. Krylovii* is a perennial grass characterized by high seed setting rate, longevity and wide distribution, as the species of late succession stage, it possesses the life history characteristics and essential conditions for genetic variation at a high level.

In the present study, Gst for all loci was 0.3241 indicating that the variations within populations contributed 67.59%, but still 32.41% occurred between populations. Another study on the same grass showed that the Gst was 0.2566 in the absence of isolation effect (Han, 2003), which was lower contrasted to the value we observed. It will be seen from this that the genetic variation among populations would increase when under the long-term isolation.

Gene flow, the genetic counterpart of dispersal, is an

important content for study the genetic structure of species, which may lead to the 'genetic rescue' of genetically eroded populations (Richards, 2000; Ingvarsson, 2001). If we say that natural selection is the foremost evolutionary force leading to population differentiation, then gene flow is the most important factor to counteract the effect of selection (Grant, 1991). In term of plants, gene flow is transferred by the movement or migrant of pollen, seeds, spores, nutrition body and other carriers. In the present study, results of gene flow were estimated using the average number of migrants per generation (Nm) based on Wri-ghts (1931) formula. The obtained value was 1.0425 (>1), which was much higher than the value (0.065) from in-breeding plants. It is generally believed that if Nm > 1, gene flow could resist genetic drift and reduce inbreeding depression in order to maintain the diversity of genetic variation; whereas Nm 1, genetic drift will become the main factors that influencing genetic structure of species (Leigh et al., 1993; Liu and Zhao, 1999).

The reason that *S. Krylovii* populations maintained the relatively high gene flow under the long-term isolation may be relate to its own breeding system and life history characteristics, which were viewed as the important factors to influence genetic structure of populations. Generally, populations achieve gene flow through pollen spread and seed dispersal, and the former especially has an important impact on the distribution of genetic variation both within and between populations. *S. Krylovii* is the cross-pollination plant and especially pollinated by the wind, the farmland can not be an effective barrier to block gene flow among populations, even if self-crossing exist, it is low-level and couldn't reduce the genetic diversity rapidly within populations.

Genetic distance/identity and Cluster pattern

Nei's genetic distance (D) and genetic identity (I), reflecting the degree of hereditary difference, were estimated among 11 isolated *S. Krylovii* populations. The value presented a great varying range from 0.0234 to 0.2190 and 0.8033 to 0.9769 respectively. On the whole, pop1 showed the large distance genetically compared with others isolates. The clusters of the dendrogram are basically consistent with the above results. It is clear that the pops with larger area were grouped together with CK pop in one cluster (Figure 3); likewise, pops with moderate area and smaller area were also grouped together respectively; while pop1 formed itself into a solo cluster due to genetically different from others, which hinted us that it has occurred genetic variation remarkably or generated some new gene mutations under the extreme pressure from limited habitat. However, more molecular assay and detailed studies need to carry out to give evidence.

Conclusions

The ISSR-PCR reaction system in *S. Krylovii* was established for the first time in China through an orthogonal design combined with single-factor experiments. This optimized amplification system is stable and can meet the requirements of analysis for this species.

The eight ISSR primers employed to explore the eleven *S. Krylovi* populations revealed that there is a high level of genetic diversity in this species, which reflected *S. Krylovii* has the great potential to adapt to environmental changes, and meanwhile the high genetic diversity will be the "source" for it to survive and evolve in the long run. The present study has also clearly demonstrated that habitat isolation caused by landscape fragmentation could bring about negative impact on genetic structure of *S. Krylovii*. On the one hand, habitat isolation could reduce genetic diversity among *S. Krylovii* populations; on the other hand, it could increase genetic differentiation between them. The work presented here adds to a growing body of evidence concerning the relationship between "island" habitat and genetic structure of species inside it. At the same time, this research also underlines the need to protect rangeland ecology and local vegetation, especially in the fragile ecotone.

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