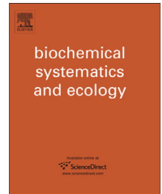
Biochemical Systematics and Ecology 66 (2016) 1–7

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco



Genetic diversity and population differentiation of *Capparis spinosa* (Capparaceae) in Northwestern China

Qian Wang [a, b], Ming-Li Zhang [a, c, *], Lin-Ke Yin [a, **]

[a] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 26 February 2016
Accepted 27 February 2016
Available online xxx

Keywords:
Capparis spinosa
Genetic diversity
Population differentiation
Geographical isolation
Gene flow
Tianshan region

ABSTRACT

To explore the influence of vicariance on differentiation patterns of taxa in arid regions, we systematically assessed the genetic diversity and variability of *Capparis spinosa*, a typical xerophyte that is widely distributed in the Tianshan Mountains and adjacent areas. In total, 300 individuals from 25 populations were sampled, and 14 haplotypes were identified using two cpDNA sequences (*rpS*12-*rpL*20 and *ndh*F). A high level of total genetic diversity ($H_T = 0.859$) was detected, and this was attributed to the extensive distribution range, which included numerous large populations. The SAMOVA results suggested that the 25 populations were clustered into 4 major geographical groups; a similar divergence trend was found by constructing a BEAST phylogenetic tree and a network diagram. The AMOVA results revealed that significant genetic differentiation occurred among groups. Our results indicated a considerable correlation between genetic divergence and geographical distribution. Isolation due to complex mountain and desert geography might limit gene exchange between disjunct populations, resulting in high differentiation between geographical groups.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Genetic diversity and variability within and among populations is essential for the long-term survival and adaptive evolution of plant species (Ge et al., 2011). Recent studies have concentrated on species biodiversity hotspots in South America, South Africa and East Asia (Gao et al., 2014); the diversification patterns and drivers of eremophytes in arid regions have also attracted increasing attention (Meng et al., 2015). Containing the world's largest mountains in arid regions, the Tianshan region is located in the Eurasian hinterland and was formed by the intense collision between India and Asia during the Cenozoic (Sun et al., 2004). In response to the geographical separation caused by various topographic conditions, drought-tolerant flora associated with the temperate continental arid climatic zone exhibits intriguing patterns of diversity. To

* Corresponding author. Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.
** Corresponding author. Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.
E-mail addresses: zhangml@ibcas.ac.cn (M.-L. Zhang), yinlk@ms.xjb.ac.cn (L.-K. Yin).

http://dx.doi.org/10.1016/j.bse.2016.02.034
0305-1978/© 2016 Elsevier Ltd. All rights reserved.

appreciate the mechanisms underlying the diversification and differentiation of representative taxa in this region, it is necessary to understand the profound influence of geographical complexity on xerophytes.

Capparis spinosa L., a perennial creeping subshrub that is widely distributed in the Mediterranean and in arid West and Central Asia (Fici, 2001), is a xerophytic Tertiary relic (Wu et al., 2010). In particular, wild *C. spinosa* is mainly distributed on the southern and northern flanks of the Tianshan Mountains and in adjacent desert areas in arid Northwestern China. This plant material has been utilized as a source of traditional medicine and is considered to be an excellent material for wind screens and sandy soil stabilization (Panico et al., 2005; Zhang and Hai, 2002). Extensive research concerning this species has focused on its ecological reproductive, physiological stress and pharmacological aspects (Panico et al., 2005; Rhizopoulou et al., 2006; Zhang and Tan, 2009). Previous reports involving the intraspecific variation of *C. spinosa* have been based on morphological parameters (Fici, 2001) and several molecular markers, i.e., RAPD and ISSR markers (Bhoyar et al., 2012; Özbek and Kara, 2013), which provide little information on the genetic structure of *C. spinosa*.

Maternally inherited chloroplast DNA is effective for detecting the intraspecific variation of plant species and is used to estimate the contribution of seed dispersal to total gene flow (McCauley, 1995). This method has been widely used for the reconstruction of important phylogenetic patterns of many taxa (Ge et al., 2011; Shi and Zhang, 2015; Su et al., 2011). In the present study, we report the first use of a cpDNA technique to quantify the intraspecific variability of *C. spinosa*. On the basis of sequence variation yielded from *rpS*12-*rpL*20 and *ndh*F, we aimed to 1) evaluate the genetic diversity and phylogeny within this species and 2) explore the drivers of genetic differentiation among geographical populations in the Tianshan region.

2. Materials and methods

2.1. Plant material

In total, 300 individuals from 25 natural populations of *C. spinosa* were investigated, covering almost the entire range of the distribution of the species in Northwestern China (Table 1, based on records from the Chinese Virtual Herbarium, http://www.cvh.org.cn/). In each population, leaf samples were randomly collected from 12 individuals that were separated from each other by at least 100 m. For each plant, approximately 10 g of fresh leaf material was gathered and immediately dried in the field using silica gel. We also selected *Capparis bodinieri* H. Lév. as an outgroup for subsequent phylogenetic analysis. All voucher specimens were deposited in the Herbarium of the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences (XJBI).

Table 1
Geographical locations, sample sizes, cpDNA haplotypes and diversity indices for 25 populations of *C. spinosa*.

Population code	Locality	Latitude/longitude	Altitude (m)	Sample size	Haplotype	H_d (±SD)	π (±SD)
Overall				300		0.8381 ± 0.0081	0.0044 ± 0.0023
Group I				60		0.2164 ± 0.0684	0.0005 ± 0.0004
1 YGS	Yengisar	38° 34′/76°06′	2010	12	A	0	0
2 AKT	Akto	38°47′/75°17′	2441	12	A	0	0
3 WQ	Wuqia	39°44′/75°40′	1945	12	A, N	0.4091 ± 0.1333	0.0009 ± 0.0007
4 KS	Kashgar	39°42′/76°06′	1363	12	A, B	0.4848 ± 0.1059	0.0011 ± 0.0008
5 JS	Jiashi	39°56′/78°01′	1162	12	A	0	0
Group II				120		0.7714 ± 0.0179	0.0023 ± 0.0013
6 KP	Kalpin	40°35′/79°36′	1131	12	D, E	0.3030 ± 0.1475	0.0011 ± 0.0008
7 AKS	Aksu	40°52′/80°01′	1076	12	C, D, M	0.7273 ± 0.0580	0.0026 ± 0.0016
8 BC	Baicheng	41°59′/83°03′	1369	12	C, D	0.5455 ± 0.0615	0.0004 ± 0.0004
9 LT	Luntai	41°14′/84°12′	921	12	C, I	0.3030 ± 0.1475	0.0016 ± 0.0011
10 KRL	Korla	42°01′/84°38′	1072	12	C, D, H	0.5303 ± 0.1359	0.0006 ± 0.0005
11 HJ	Hejing	42°31′/86°16′	1403	12	C, E	0.4091 ± 0.1333	0.0018 ± 0.0012
12 TKS	Toksun	42°49′/88°38′	95	12	D, E	0.5455 ± 0.0615	0.0020 ± 0.0013
13 TRP	Turpan	42°52′/89°11′	−84	12	D, E, L,	0.6212 ± 0.1176	0.0021 ± 0.0013
14 SS	Shanshan	43°01′/90°39′	483	12	E, J, K	0.5909 ± 0.1079	0.0024 ± 0.0015
15 HM	Hami	42°31′/94°09′	773	12	D, E, F	0.7273 ± 0.0580	0.0022 ± 0.0014
Group III				72		0.5458 ± 0.0504	0.0023 ± 0.0013
16 JMS	Jimsar	43°55′/89°07′	945	12	E	0	0
17 URMQ	Urumqi	43°44′/86°57′	1060	12	D, E	0.4091 ± 0.1333	0.0015 ± 0.0010
18 MNS	Manas	44°11′/86°08′	642	12	E	0	0
19 SHZ	Shihezi	44°16′/85°57′	356	12	D	0	0
20 SW	Shawan	44°19′/85°35′	584	12	E	0	0
21 KRMY	Karamay	44°56′/84°46′	296	12	G	0	0
Group IV				48		0.1560 ± 0.0666	0.0007 ± 0.0005
22 WS	Wusu	44°19′/84°19′	685	12	B, G	0.4848 ± 0.1059	0.0022 ± 0.0014
23 BL	Bole	44°42′/82°05′	470	12	B	0	0
24 YN	Yining	43°37′/82°08′	750	12	B	0	0
25 GL	Gongliu	43°37′/81°49′	710	12	B	0	0

H_d, haplotype (gene) diversity; π, nucleotide diversity.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from powdered tissues following a modified CTAB (cetyltrimethyl ammonium bromide) protocol (Doyle and Doyle, 1987). After screening using 17 pairs of primers (Table S1), two cpDNA regions (*rpS*12-*rpL*20 and *ndh*F (329F, 927R)) were selected as having greater sequence variation between individuals in the populations. Polymerase chain reactions were performed in a total volume of 30 μL containing 1.5 μL of 10 × reaction buffer, 2 μL of $MgCl_2$ (25 mmol/L), 3 μL of dNTP mixture (2.5 mmol/L), 2.2 μL of each of forward and reverse primers (50 ng/μL), 0.3 μL of *Taq* DNA polymerase (5 U/μL) and 1.0 μL of template DNA (5 ng/μL). PCR amplification was performed using a Gene-Amp PCR system 9700 DNA Thermal Cycler (Applied Biosystems, Foster City, CA, USA) and the following conditions: 4 min of initial denaturation at 94 °C; 30 cycles of 30 s denaturation at 94 °C, 30 s of annealing at 52 °C, and 90 s of elongation at 72 °C; and a final extension for 10 min at 72 °C. All products were purified using a PCR product purification kit and then sequenced using an ABI Prism 3730xl automated sequencer at Sangon Biotech Co., Ltd., Shanghai, China.

2.3. Data analysis

DNA sequences were edited using SeqMan software (Lasergene, DNASTAR Inc., Madison, Wisconsin, USA). Multiple sequences were aligned using the program Clustal X 1.81 (Thompson et al., 1997) and manually adjusted. Unique cpDNA haplotypes from all individuals were identified using DnaSP 5.0 (Librado and Rozas, 2009). All resulting different sequences were submitted to the GenBank database under the accession numbers as follows: KU866560–KU866562 for *rpS*12-*rpL*20 and KU866548–KU866558 for *ndh*F; and the accession numbers of *C. bodinieri* were: KU866563 for *rpS*12-*rpL*20 and KU866559 for *ndh*F.

To determine the maximum differences and thereby divide the sampled populations into defined groups (*K*) based on geographical homogeneity and haplotype composition, spatial analysis of molecular variance was implemented in SAMOVA 1.0 (Dupanloup et al., 2002). We set the range of *K* as 2–5 by repeated analysis until a maximal F_{CT} value was obtained; configurations with a single population in one of the groups were excluded to maintain the group structure (Heuertz et al., 2004; Iwasaki et al., 2012).

Genetic indices of haplotype diversity (H_d) and nucleotide diversity (π) were calculated in Arlequin 3.1 (Excoffier et al., 2005) at the species, group and population levels. The indices of gene diversity and degree of differentiation, including the average within-population gene diversity (H_S), total gene diversity (H_T), genetic differentiation over populations (G_{ST}), and number of substitution types (N_{ST}), were computed using Permut 1.0 (Pons and Petit, 1996). The significance of the *P* values was validated using the *U* Test as performed in the Haplonst procedure (available at www.pierroton.inra.fr/genetics/labo/Software/index.html). The number of female migrants per generation was deduced using the expression $Nm = 0.5 (1-G_{ST})/ G_{ST}$ (Hamilton and Miller, 2002). To assess the genetic variation among and within populations, AMOVA was performed as implemented in the program Arlequin 3.1 (Excoffier et al., 2005).

Relationships among haplotypes were determined using a median-joining network (MJN) method and projected based on the topology using Network 4.6.1.0 (Bandelt et al., 1999). Furthermore, to reconstruct the dendrogram of cpDNA haplotypes, the BEAST 1.6.1 program (Drummond and Rambaut, 2007) was implemented for phylogenetic analysis. A Markov Chain Monte Carlo (MCMC) method was performed using an annectent tree priority rule and an HKY substitution model. The MCMC chains were run for 20,000,000 generations, and trees were sampled every 1000 generations. A maximum clade credibility tree was generated using TreeAnnotator version 1.6.1 with a burn-in of 1000 trees. Finally, the trees were edited in FigTree 1.3.1 (http://tree.bio.ed.as.uk/software/figtree/). The statistical support of the clades was determined based on Bayesian posterior probability.

3. Results

3.1. Genetic variation and genetic diversity analysis

The total length of the two cpDNA regions was 1331 bp (728 bp for *rpS*12-*rpL*20 and 603 bp for *ndh*F). Based on the combined sequences, 23 polymorphic sites were detected, including 22 nucleotide substitutions and 1 indels (Table S2). Altogether, 14 haplotypes (A-N) were identified from the 25 populations. The distribution of the predominant haplotypes exhibited a high degree of geographical structure. Haplotype A was only found on the south side of the western Tianshan Mountains (in populations 1–5); haplotypes C and D were distributed over the south side of the middle and eastern Tianshan Mountains (shared by populations 6–13 and 15); haplotype E was most frequently distributed on the north side of the Tianshan Mountains (in populations 16–18 and 20); and haplotype B was present in the Ili Valley and its surroundings (in populations 22–25) (Fig. 1, Table 1).

SAMOVA clustered the 25 sampled populations into 4 major geographical groups: (I) that on the south side of the western Tianshan Mountains (populations 1–5), (II) that on the south side of the middle and eastern Tianshan Mountains (populations 6–15), (III) that on the north side of the Tianshan Mountains (populations 16–21), and (IV) that in the Ili Valley and its surroundings (populations 22–25) (Fig. 1, Table 1). The highest F_{CT} value (0.5023) was obtained when *K* was set at 4.

Nei's indices of genetic diversity showed that the haplotype diversities (H_d) varied across the 25 populations, ranging from 0 to 0.7273, and the nucleotide diversities (π) ranged from 0 to 0.0026. At the species level, high values of haplotype diversity

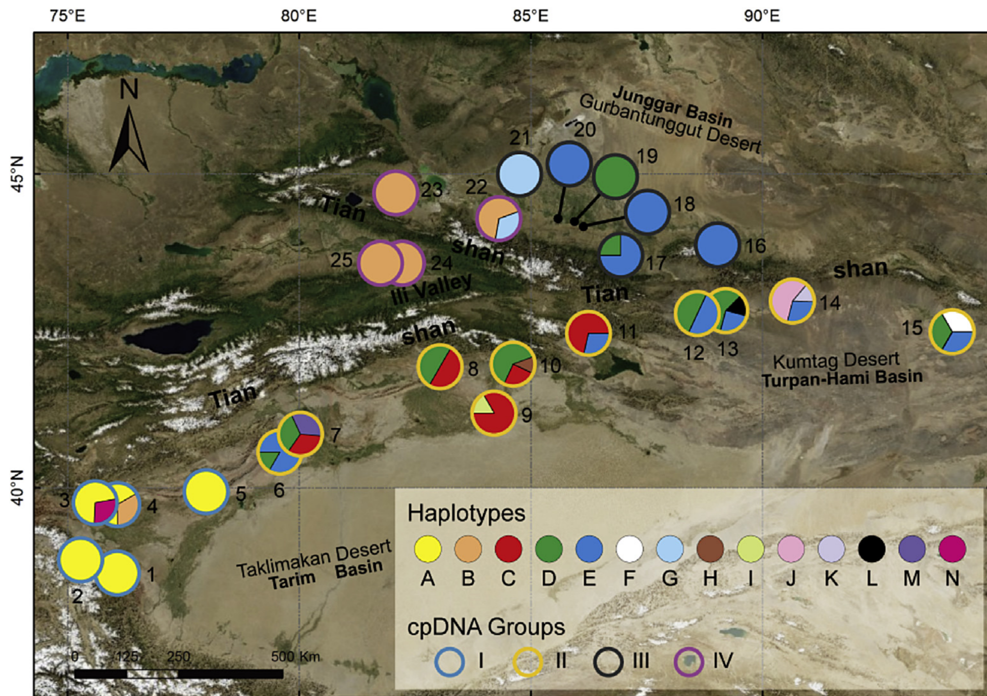


Fig. 1. The geographical distributions of 14 cpDNA haplotypes in 25 populations of *C. spinosa*. The population codes correspond to those shown in Table 1. The colors used in the pie charts show the proportions of haplotypes in each population. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

($H_d = 0.8381$) and nucleotide diversity ($\pi = 0.0044$) were observed. Within groups, the diversity was highest in Group II ($H_d = 0.7714$; $\pi = 0.0023$) and lowest in Group IV ($H_d = 0.1560$; $\pi = 0.0007$). At the population level, populations 7 (AKS, $H_d = 0.7273$) and 15 (HM, $H_d = 0.7273$) in Group II showed particularly high levels of gene diversity (Table 1). In addition, the total genetic diversity ($H_T = 0.859$, s.e. $= 0.0209$) was high; whereas, the average gene diversity within populations ($H_S = 0.284$, s.e. $= 0.0550$) was low (Table 2).

3.2. Phylogenetic analysis

According to the results of the main divergence, validated with a BEAST tree (Fig. 3) and a network diagram (Fig. 2) based on sequence variation, the 14 haplotypes were clustered into 5 major clades: haplotype B alone constituted the first clade; haplotypes A and N formed the second clade; haplotypes C, D, H and J clustered together in the third clade; haplotypes E, F, I, K and M formed the fourth clade; and haplotypes G and L constituted the fifth clade.

3.3. Differentiation among groups, among populations and within populations

The AMOVA results revealed that more of the variation was inter-populational (83.39%) rather than intra-populational (16.61%). Differentiation between the geographical groups was high; the results showed that 66.33% of the total variation occurred among the four groups and that 17.06% of the total variation existed among populations within groups. The fixation indices F_{ST} (0.83390, $P < 0.001$) and F_{CT} (0.66326, $P < 0.001$) also revealed significant differentiation among populations and

Table 2
Estimates of genetic diversity and differentiation for the total distribution and within four geographical groups of *C. spinosa*.

Regions	H_S	H_T	G_{ST}	N_{ST}	Nm
Group I (1–5)	0.179	0.225	0.205	0.195	1.9390
Group II (6–15)	0.530	0.796	0.334	0.290	0.9970
Group III (16–21)	0.068	0.633	0.892	0.905	0.0605
Group IV (22–25)	0.121	0.167	0.273	0.273	1.3315
Total distribution	0.284	0.859	0.670	0.800	0.2463

H_S, average genetic diversity within populations; H_T, total genetic diversity; G_{ST}, inter-population differentiation; N_{ST}, number of substitution types; Nm, number of migrants per generation.

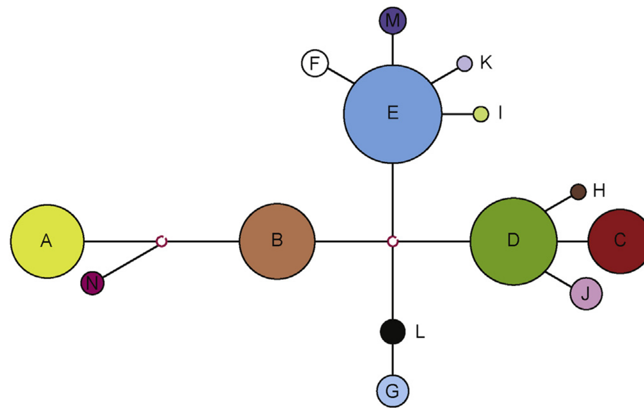


Fig. 2. Phylogenetic relationships of 14 haplotypes recovered using a median-joining network (MJN). Circle size reflects haplotype frequency in all samples.

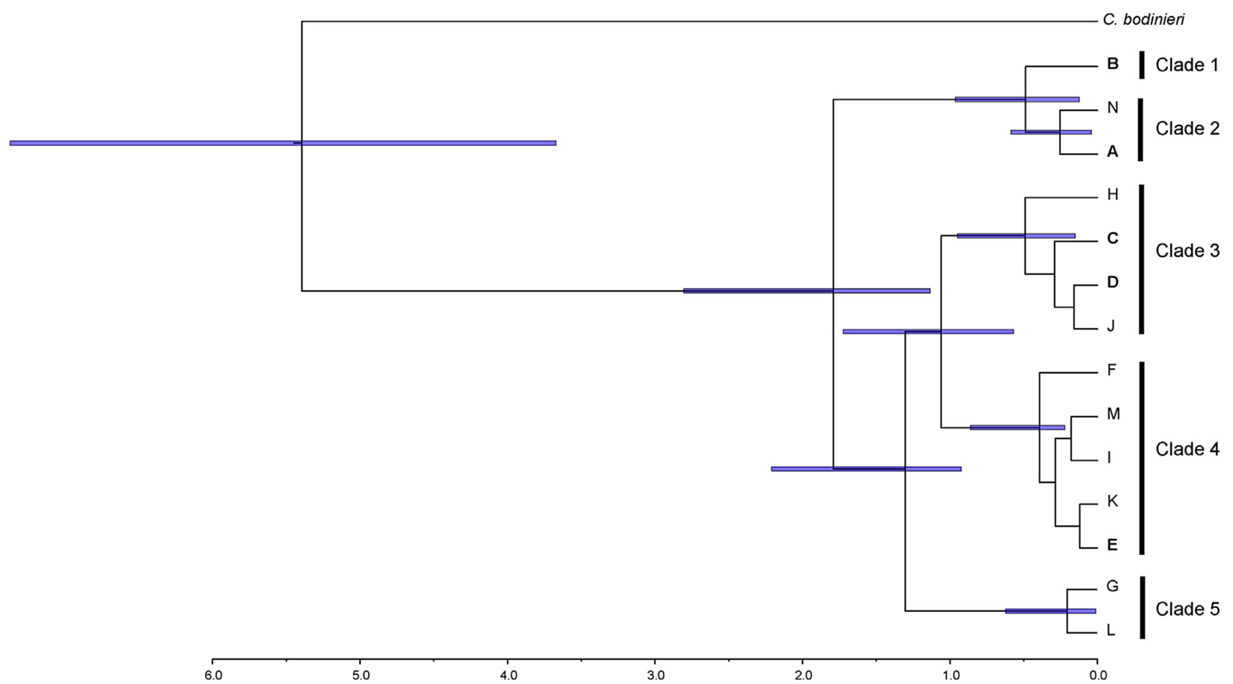


Fig. 3. The genetic divergence of haplotypes according to a BEAST dendrogram that was based on the cpDNA sequence of *rpS*12-*rpL*20 + *ndh*F. Blue bars indicate 95% posterior credibility intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

groups. Additionally, the level of inter-population differentiation (G_{ST} = 0.670, s.e. = 0.063) and the amount of gene flow (*N*m = 0.2463) calculated using Permut software (Table 2) indicated the presence of a considerable molecular difference and a low level of gene exchange across the studied populations.

4. Discussion

The total genetic diversity appeared to be somewhat higher than those of most other xeromorphic species, such as *Helianthemum songaricum* (H_T = 0.651, Su et al., 2011), *Zygophyllum xanthoxylon* (H_T = 0.518, Shi and Zhang, 2015), and *Atraphaxis manshurica* (H_T = 0.437, Xu and Zhang, 2015). High levels of genetic diversity have been strongly correlated with the accumulation of variation, which may have been largely determined by the extensive distribution range and effective populations (Frankham, 1996). As a long-lived perennial subshrub, *C. spinosa* has had a wide distribution range in arid regions since the Tertiary (Wu et al., 2010); therefore, we suggest that the current surviving individuals may have retained abundant genetic material from their ancestors during their long-term evolution. According to our field investigation, the Tianshan region harbors a number of effective populations with large sizes (3, 4, 6–15, 17, 22), especially populations 7 (over 200 individuals)

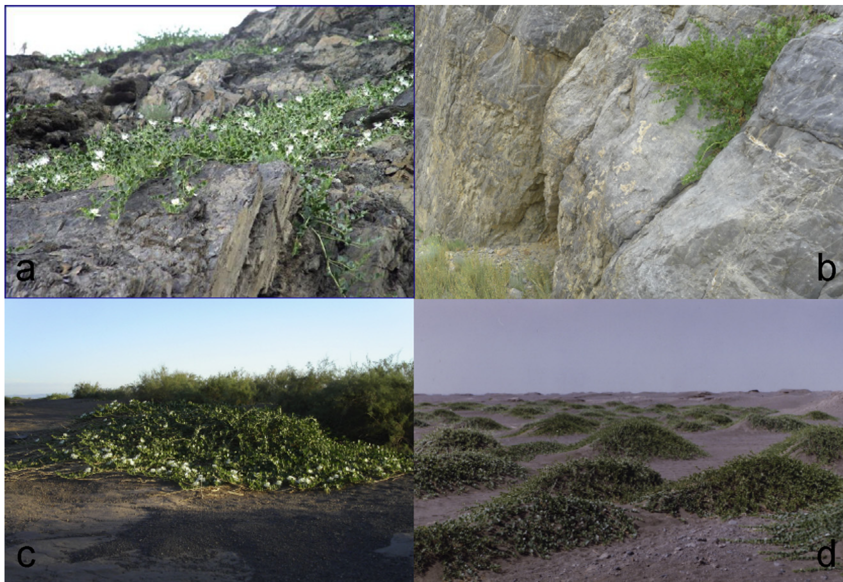


Fig. 4. Different topographic conditions over the *C. spinosa* habitat. (a, b) Denuded rocky slopes and (c, d) gravel desert in piedmont proluvial fans.

and 15 (over 200 individuals) of Group II. This region may be the distribution center of *C. spinosa* in China. On the other hand, the complex topographic conditions (including arid denuded rocky slopes and gravel deserts in piedmont proluvial fans, Fig. 4) and various altitude gradients (ranging from −84 to 2441 m, Table 1) of different locations may have indirectly caused the high level of genetic diversity in *C. spinosa*.

The genetic divergence of this species exhibited a structure similar to that of the geographical distribution of the high-frequency predominant haplotypes. The coefficient of inter-population differentiation was much higher than the mean value for dicotyledons ($G_{ST} = 0.273$) according to the statistical results reported by Hamrick and Godt (1989). Population differentiation is largely attributed to the amount of gene flow ($N_m = 0.2463$), which is mediated by seed dispersal for maternally inherited cpDNA in angiosperms (McCauley, 1995). Wright (1931) concluded that if $N_m < 1$, then the number of migrants per generation cannot counterbalance the continuous genetic differentiation caused by random genetic drift. In the survey region studied here, physical barriers separated populations into different partitions. Gene flow was restricted by spatial isolation between groups due to the inability of seeds to disperse across impassable mountains and sprawling sandy deserts by zoochory or anemochory; this situation promotes the inbreeding of individuals and generates genetic affinity between the offspring of relatively independent evolutionary units. Although limited gene flow, genetic drift and inbreeding may not give rise to rapid effects in each generation, the long-term vicariance, local adaptation, and gradual accumulation of these effects may manifest over time, consequently increasing genetic differentiation between divergent geographical groups.

C. spinosa has received increasing attention in recent years. Bhoyar et al. (2012) investigated the genetic variability of 9 *C. spinosa* sites using RAPD + ISSR markers, and their results showed that in the Ladakh region of India, more variation occurred within than between populations. Özbek and Kara (2013) found a low level of total genetic diversity ($H_T = 0.16$) of 15 *Capparis* populations in Turkey and reported that the gene flow ($Nm = 1.79$) between the populations was high based on a RAPD analysis. The different results obtained here may be due to the different marker systems selected; the greater distribution range, including complex geographically isolated settings; and the larger number of effectively investigated populations in the Tianshan region. However, our findings were consistent with the results of previous studies showing that natural isolation profoundly influences gene exchange and population differentiation of plant species (Cun and Wang, 2010).

In conclusion, our results suggest that genetic divergence and geographical distribution are closely correlated. High levels of genetic diversity may be significantly relevant to a wide distribution range including numerous large effective populations. In the Tianshan region, considerable degrees of population differentiation are likely attributable to the limited gene flow among divergent groups due to hindrance by vicariance.

Acknowledgments

This research was financially supported by the China National Key Basic Research Program (2014CB954201), and the Xinjiang Institute of Ecology and Geography, CAS.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.02.034.

References

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Bhoyar, M.S., Mishra, G.P., Naik, P.K., Murkute, A.A., Srivastava, R.B., 2012. Genetic variability studies among natural populations of *Capparis spinosa* from cold arid desert of trans-Himalayas using DNA markers. Natl. Acad. Sci. Lett. 35, 505–515.

Cun, Y.Z., Wang, X.Q., 2010. Plant recolonization in the Himalaya from the southeastern Qinghai-Tibetan Plateau: geographical isolation contributed to high population differentiation. Mol. Phylogenet. Evol. 56, 972–982.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571–2581.

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

Fici, S., 2001. Intraspecific variation and evolutionary trends in *Capparis spinosa* L. (Capparaceae). Plant Syst. Evol. 228, 123–141.

Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conserv. Biol. 10, 1500–1508.

Gao, X.Y., Meng, H.H., Zhang, M.L., 2014. Diversification and vicariance of desert plants: evidence inferred from chloroplast DNA sequence variation of *Lagochilus ilicifolius* (Lamiaceae). Biochem. Syst. Ecol. 55, 93–100.

Ge, X.J., Hwang, C.C., Liu, Z.H., Huang, C.C., Huang, W.H., Hung, K.H., Wang, W.K., Chiang, T.Y., 2011. Conservation genetics and phylogeography of endangered and endemic shrub *Tetraena mongolica* (Zygophyllaceae) in Inner Mongolia, China. BMC Genet. 12, 1.

Hamilton, M.B., Miller, J.R., 2002. Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measures of population structure. Genetics 162, 1897–1909.

Hamrick, J.L., Godt, M.J.W., 1989. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates, Sunderland, pp. 43–63.

Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X., Vendramin, G.G., 2004. Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe. Mol. Ecol. 13, 3437–3452.

Iwasaki, T., Aoki, K., Seo, A., Murakami, N., 2012. Comparative phylogeography of four component species of deciduous broad-leaved forests in Japan based on chloroplast DNA variation. J. Plant Res. 125, 207–221.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

McCauley, D.E., 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol. Evol. 10, 198–202.

Meng, H.H., Gao, X.Y., Huang, J.F., Zhang, M.L., 2015. Plant phylogeography in arid Northwest China: retrospectives and perspectives. J. Syst. Evol. 53, 33–46.

Özbek, Ö., Kara, A., 2013. Genetic variation in natural populations of *Capparis* from Turkey, as revealed by RAPD analysis. Plant Syst. Evol. 299, 1911–1933.

Panico, A.M., Cardile, V., Garufi, F., Puglia, C., Bonina, F., Ronsisvalle, G., 2005. Protective effect of *Capparis spinosa* on chondrocytes. Life Sci. 77, 2479–2488.

Pons, O., Petit, R.J., 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144, 1237–1245.

Rhizopoulou, S., Ioannidi, E., Alexandredes, N., Argiropoulos, A., 2006. A study on functional and structural traits of the nocturnal flowers of *Capparis spinosa* L. J. Arid. Environ. 66, 635–647.

Shi, X.J., Zhang, M.L., 2015. Phylogeographical structure inferred from cpDNA sequence variation of *Zygophyllum xanthoxylon* across north-west China. J. Plant Res. 128, 269–282.

Su, Z.H., Zhang, M.L., Sanderson, S.C., 2011. Chloroplast phylogeography of *Helianthemum songaricum* (Cistaceae) from northwestern China: implications for preservation of genetic diversity. Conserv. Genet. 12, 1525–1537.

Sun, J.M., Zhu, R.X., Bowler, J., 2004. Timing of the Tianshan Mountains uplift constrained by magnetostratigraphic analysis of molasse deposits. Earth Planet. Sci. Lett. 219, 239–253.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Wright, S., 1931. Evolution in mendelian populations. Genetics 16, 97–159.

Wu, Z.Y., Sun, H., Zhou, Z.K., Li, D.Z., Peng, H., 2010. Floristics of Seed Plants from China. Science Press, Beijing.

Xu, Z., Zhang, M.L., 2015. The effect of past climatic oscillations on spatial genetic structure of *Atraphaxis manshurica* (Polygonoideae) in the Horqin sandlands, Northern China. Biochem. Syst. Ecol. 60, 88–94.

Zhang, L.Y., Hai, Y., 2002. Plant communities excluded in the book of “The vegetation and its utilization in Xinjiang”: I. The desert plant communities. Arid. Land Geogr. 25, 84–89.

Zhang, T., Tan, D.Y., 2009. An examination of the function of male flowers in an andromonoecious shrub *Capparis spinosa*. J. Integr. Plant Biol. 51, 316–324.