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Relationship between the genetic diversity of *Artemisia halodendron* and climatic factors

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ABSTRACT

Artemisia halodendron (Asteraceae) is a dominant sand-fixing semi-shrub species native to the Horqin Sandy Land of northeastern China. In this study, we evaluated levels of genetic variation within and among sampled *A. halodendron* populations from two different hydrothermal regions of the Horqin Sandy Land using inter-simple sequence repeat (ISSR) markers. We also investigated possible relationships between genetic diversity of this species and climatic factors. Our analysis revealed that *A. halodendron* is highly genetically diverse, with populations from a low hydrothermal level region having higher genetic diversity index values than those from a high hydrothermal level region. An analysis of molecular variation (AMOVA) revealed relatively high levels (>89.83%) of within-population genetic variation. Based on cluster analysis, the 13 studied *A. halodendron* populations can be clustered into two clades. Genetic diversities of all populations have been influenced by many climatic factors, and Nei's genetic diversity (*h*) is strongly correlated with annual temperature range (ART). These results have important implications for restoration and management of degraded ecosystems in arid and semi-arid areas.

1. Introduction

Artemisia halodendron (Asteraceae) is a climax and dominant sand-fixing semi-shrub species native to the Horqin Sandy Land of northeastern China. It is an important component of vegetation rehabilitation efforts in the Horqin Sandy Land because of several highly valuable ecological traits, which include its high drought tolerance, anti-wind erosion utility, sand burial-resistance (Dong et al., 2000; Li et al., 2002; H.L. Zhao et al., 2006), and status as a key species for plant community establishment and landscape formation (Li, 1991). *A. halodendron* is distributed in mobile, semi-mobile and fixed dunes, and lowlands. A special combination of conditions with respect to water fertility and heat in Inner Mongolia, and the Horqin Sandy Land characterizes the main part of the distribution range (Fu, 1993). The life history traits of *A. halodendron* include long-lived, perennial, wind-pollinated, seed reproduction, vegetative propagation and broad ecological amplitude (Fu, 1993). Previous studies on *A. halodendron* have focused on its population distribution patterns (Chao et al., 1999; Cao et al., 2008), biomass allocation (Li et al., 2005), breeding distribution (Li et al., 2005), morphological characteristics and physiological adaptations (Zhou et al., 1999), root longevity (Huang et al., 2009), and establishment (Li et al., 2002) in the Horqin Sandy Land. The relationship between *A. halodendron* genetic diversity and climatic factors has not yet been reported, however.

The Horqin Sandy Land is located in an agro-pastoral transition zone between the Inner Mongolian Plateau and the Northeast Plains (42°41′–45°45′N, 118°35′–123°30′E). It covers an area of approximately 139,300 km^2, of which about 71,884 km^2 is desertified (Wang, 2003; Zhao et al., 2003). The landscape in this area is characterized by sand dunes alternating with gently undulating lowland areas (Li et al., 2005). The region, which is located in the continental temperate zone, experiences a semi-arid monsoon climate with a mean annual temperature of 3–7 °C and mean annual rainfall of 350–500 mm (Zhao et al., 2003). Over recent decades, this region has undergone severe desertification (Li et al., 2000, 2004), a northward-moving phenomenon affecting

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interlocked agro-pastoral areas of northern China during the past few centuries (Zhao et al., 2000, 2002).

In this study, we assessed *A. halodendron* population genetic variation along temperature and humidity gradients in Horqin Sandy Land using inter-simple sequence repeat (ISSR) markers. We addressed the following questions: (1) what is the level of genetic diversity within and among *A. halodendron* populations in different hydrothermal regions? (2) what are the relationships between uncovered genetic diversity and climatic factors, and how are they correlated? These results were interpreted with the aim of providing baseline genetic information for restoration and management of degraded ecosystems in arid and semi-arid regions.

2. Materials and methods

2.1. Population sampling

A total of 290 individuals were sampled from 13 natural *A. halodendron* populations. Populations 1–8 were located in a low hydrothermal synthesis index region (average 25.29), while populations 9–13 were in a high hydrothermal synthesis index region (average 32.8). Hydrothermal synthesis index values were calculated from the formula

$$S = \sum_{t=1}^{12} 0.18 r_t / 1.045^{T_t}$$

where t = month, r_t = monthly rainfall, and T_t = monthly mean temperature (Bailey, 1979). We sampled 19 to 30 individuals from each population in July 2011 (Table 1, Fig. 1). Climatic data were obtained from CMA (China Meteorological Administration) and given in Table 2. The time period used to derive the climatic means was 1971–2000. Annual temperature range was calculated from the formula

$$\mathrm{ART} = (\mathrm{MTWM} - \mathrm{MTCM})$$

where MTWM = warmest monthly mean temperature, MTCM = coldest monthly mean temperature. Warm index values were calculated from the formula

$$WI = \sum_{i=1}^{12} (t_i - 5)$$

where t = above 5 °C monthly mean temperature. Cold index values were calculated from the formula

$$CI = \sum_{i=1}^{12} (5 - t_i)$$

where t = below 5 °C monthly mean temperature. Humidity index values were calculated from the formula

$$HI = AP/WI$$

where AP = mean annual rainfall (Xu, 1983). Young healthy leaves were randomly sampled from plants spaced at least 30 m apart, and immediately stored with silica gel in zip-lock plastic bags for later DNA extraction.

Table 1
Origin of materials and number of samples for 13 populations of *Artemisia halodendron* from the Horqin Sandy Land.

Population	No. of plants	Latitude (°N)	Longitude (°E)	Mean altitude (m)	Habitats
Pop1	30	42°45′46″	120°35′07″	385	Semi-mobile dune
Pop2	19	42°58′11″	120°40′45″	357	Semi-mobile dune
Pop3	21	42°55′45″	119°11′37″	367	Fixed dune
Pop4	22	43°10′10″	119°55′49″	434	Mobile dune
Pop5	20	42°47′12″	120°36′02″	452	Mobile dune
Pop6	19	42°31′06″	120°23′51″	330	Lowland between mobile dunes
Pop7	23	42°39′34″	120°10′23″	495	Mobile dune
Pop8	30	42°54′15″	119°46′47″	479	Mobile dune
Pop9	20	44°30′46″	121°13′44″	346	Semi-fixed dune
Pop10	21	43°41′33″	122°33′19″	253	Fixed dune
Pop11	20	43°15′54″	122°11′22″	177	Fixed dune
Pop12	20	42°51′26″	122°30′32″	175	Fixed dune
Pop13	25	43°15′42″	121°25′11″	251	Fixed dune

2.2. DNA extraction and ISSR-PCR amplification

Total DNA was extracted using an AxyPrep genomic DNA mini kit (Axygen, Beijing, China). DNA was quantified spectrophotometrically; samples yielding high quantities of good quality DNA were used in consecutive experiments. After screening 100 ISSR primers from the University of British Columbia (UBC primer set no. 9) for well-amplified and polymorphic bands among plant populations, we selected 14 primers for use with all individuals. To ensure data quality, we have done some planning when run on gels. Then we will state the specific methods. There were 25 bands in each gel, and included one marker and twenty-four bands came from eight populations (randomized samples three individuals from each population when run on gels) in the same primer, and according to this order analogized.

ISSR amplifications were performed in 25-μL reaction volumes containing 40 ng genomic DNA, 1.0 U Taq polymerase, 3 mM $MgCl_2$, 500 μM of each dNTP, 20 mM Tris–HCl (pH 8.3), 100 mM KCl, and 0.3 μM primer. Amplification conditions consisted of an initial step of 3 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at the appropriate annealing temperature (see Appendix S1 for details), and 2 min at 72 °C, and a final 7 min extension step at 72 °C. ISSR reactions were performed at least twice for all individuals and for all the primers to determine the reproducibility of banding patterns. Amplification products along with 100-bp DNA ladder were electrophoretically resolved on 1.8% agarose gels containing ethidium bromide (0.5 μg/mL final concentration) at 100 V for 2 h, and photographed under ultraviolet light.

2.3. Data analysis

During analysis of the resulting gels, only clear and reproducible bands were considered. Amplified fragments were scored for presence (1) or absence (0) of bands, and the data transformed into a 0/1 binary character matrix. The resulting binary data matrix was analyzed using POPGENE Version 1.32 (Yeh and Yang, 1999). Genetic diversity of each population was estimated according to percentage of polymorphic loci (P), observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (h), and Shannon's diversity index (I). Analysis of molecular variance (AMOVA) was performed to analyze among-population sources of variation using ARLEQUIN with 1000 bootstrap replicates (Schneider et al., 2000). A correlation analysis between genetic diversity indices and climatic factors was conducted using SPSS 17.0.

3. Results

3.1. Genetic diversity

ISSR band profiles revealed high levels of polymorphism in the surveyed *A. halodendron* populations. The 14 selected ISSR primers

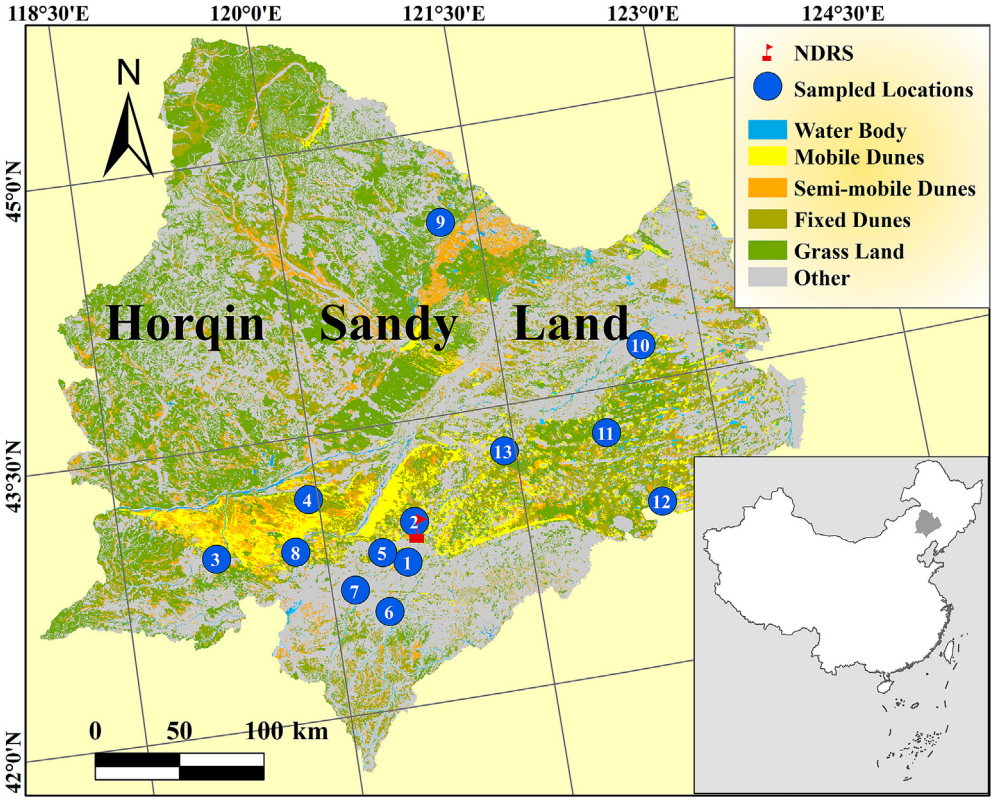

Fig. 1. Geographic distribution of *Artemisia halodendron* in Horqin Sandy Land (gray area in the map of China) and the sampling sites of 13 populations.

generated a total of 157 clear and distinguishable fragment bands, of which 103 (65.61%) were polymorphic. The size of the amplified fragments ranged from 200 to 2000 bp, with seven fragments generated on average per primer. The highest number of bands was generated from the primers UBC827 and UBC873. The highest percentage of polymorphic bands was generated from the primer UBC810 (Appendix S1).

Calculated genetic diversities within and among the 13 *A. halodendron* populations are given in Table 3. The number of polymorphic loci (n) ranged from 16 to 59, and percentages of polymorphism (P) ranged from 10.19% to 37.58%. Observed number of alleles (Na) varied from 1.1019 to 1.3758; effective number of alleles (Ne) ranged from 1.0602 to 1.1729. Nei's genetic diversity (h) and Shannon's diversity index (I) values were 0.0904–0.1687 and 0.0921–0.1955, respectively. At the species level, Nei's genetic diversity (h) was calculated to be 0.2204, and Shannon's diversity index (I) was 0.3236 (Table 3). With respect to hydrothermal regions, n was 88 for the low hydrothermal level region populations, and 80 for high hydrothermal level populations; in the low hydrothermal level region, P was 56.05%, whereas in the high

Table 2
Climatic factors value (average ± SD) for the 13 population sites from the Horqin Sandy Land, obtained from China Meteorological Administration.

Population	AMT[a] (±SD) (°C)	ART[b] (±SD) (°C)	*WI*[c] (±SD) (°C)	*CI*[d] (±SD) (°C)	*AP*[e] (±SD) (mm)	*HI*[f] (±SD) (mm/°C)	*S*[g] (±SD)
Pop1	7.5 ± 0.4	36.1 ± 1.8	56.3 ± 6.7	86.2 ± 0.3	269.9 ± 40.2	4.9 ± 1.2	21.8 ± 1.9
Pop2	7.5 ± 0.4	36.1 ± 1.8	56.3 ± 6.7	86.2 ± 0.3	269.9 ± 40.2	4.9 ± 1.2	21.8 ± 1.9
Pop3	7.5 ± 0.3	34.3 ± 4.7	52.2 ± 3.5	82.1 ± 5.7	371.1 ± 77.4	7.1 ± 5.1	30.7 ± 10.5
Pop4	6.4 ± 0.5	34.7 ± 4.5	58.7 ± 3.7	75.7 ± 6.6	369.9 ± 102.3	6.3 ± 0.9	31.3 ± 9.1
Pop5	7.5 ± 0.4	36.1 ± 1.8	56.3 ± 6.7	86.2 ± 0.3	269.9 ± 40.2	4.9 ± 1.2	21.8 ± 1.9
Pop6	7.5 ± 0.4	36.1 ± 1.8	56.3 ± 6.7	86.2 ± 0.3	269.9 ± 40.2	4.9 ± 1.2	21.8 ± 1.9
Pop7	7.5 ± 0.4	36.1 ± 1.8	56.3 ± 6.7	86.2 ± 0.3	269.9 ± 40.2	4.9 ± 1.2	21.8 ± 1.9
Pop8	6.4 ± 0.5	34.7 ± 4.5	58.7 ± 3.7	75.7 ± 6.6	369.9 ± 102.3	6.3 ± 0.9	31.3 ± 9.1
Pop9	6.6 ± 0.5	36.5 ± 2.3	61.2 ± 4.2	80.2 ± 8.5	382.5 ± 145.9	6.3 ± 1.9	29.8 ± 9.7
Pop10	6.6 ± 0.5	34.9 ± 3.9	62.4 ± 4.3	81.9 ± 7.7	373.4 ± 114.5	6.0 ± 1.5	31.0 ± 11.2
Pop11	6.6 ± 0.5	34.9 ± 3.9	62.4 ± 4.3	81.9 ± 7.7	373.4 ± 114.5	6.0 ± 1.5	31.0 ± 11.2
Pop12	6.9 ± 0.6	38.5 ± 2.3	60.4 ± 7.2	80.4 ± 2.8	448.1 ± 39.7	7.4 ± 1.6	36.1 ± 0.5
Pop13	6.9 ± 0.6	38.5 ± 2.3	60.4 ± 7.2	80.4 ± 2.8	448.1 ± 39.7	7.4 ± 1.6	36.1 ± 0.5

[a] AMT = mean annual temperature.
[b] ART = annual temperature range.
[c] *WI* = warm index.
[d] *CI* = cold index.
[e] *AP* = mean annual rainfall.
[f] *HI* = humidity index.
[g] *S* = hydrothermal synthesis index.

Table 3
Genetic diversity indices of *Artemisia halodendron* populations.

Populations		Sample size	n[a]	P[b] (%)	Na[c] (±SD)	Ne[d] (±SD)	h[e] (±SD)	I[f] (±SD)
Low hydrothermal level region	Pop1	30	50	31.85	1.3185 ± 0.4674	1.1645 ± 0.2949	0.1001 ± 0.1657	0.1535 ± 0.2435
	Pop2	19	38	24.20	1.2420 ± 0.4297	1.1569 ± 0.3096	0.0904 ± 0.1711	0.1337 ± 0.2477
	Pop3	21	24	15.29	1.1529 ± 0.3610	1.0977 ± 0.2560	0.1565 ± 0.1415	0.1838 ± 0.2056
	Pop4	22	29	18.47	1.1847 ± 0.3893	1.1202 ± 0.2818	0.1687 ± 0.1553	0.1012 ± 0.2245
	Pop5	20	43	27.39	1.2739 ± 0.4474	1.1763 ± 0.3232	0.1019 ± 0.1774	0.1510 ± 0.2570
	Pop6	19	28	17.83	1.1783 ± 0.3840	1.1186 ± 0.2881	0.1668 ± 0.1544	0.0985 ± 0.2219
	Pop7	23	21	13.38	1.1338 ± 0.3415	1.0854 ± 0.2470	0.1486 ± 0.1339	0.1720 ± 0.1933
	Pop8	30	28	17.83	1.1783 ± 0.3840	1.1019 ± 0.2501	0.1609 ± 0.1412	0.0921 ± 0.2076
	Overall	184	88	56.05	1.5605 ± 0.4979	1.3258 ± 0.3658	0.1925 ± 0.1967	0.2899 ± 0.2823
High hydrothermal level region	Pop9	20	59	37.58	1.3758 ± 0.4859	1.2252 ± 0.3483	0.1308 ± 0.1894	0.1955 ± 0.2732
	Pop10	21	16	10.19	1.1019 ± 0.3035	1.0602 ± 0.2029	0.1355 ± 0.1130	0.1534 ± 0.1657
	Pop11	20	24	15.29	1.1529 ± 0.3610	1.1056 ± 0.2758	0.1588 ± 0.1485	0.1860 ± 0.2127
	Pop12	20	40	25.48	1.2548 ± 0.4371	1.1729 ± 0.3361	0.0967 ± 0.1793	0.1420 ± 0.2565
	Pop13	25	39	24.84	1.2484 ± 0.4335	1.1620 ± 0.3147	0.0933 ± 0.1726	0.1382 ± 0.2499
	Overall	106	80	50.96	1.5096 ± 0.5015	1.3086 ± 0.3898	0.1758 ± 0.2051	0.2613 ± 0.2914
All populations	Average	22	34	21.51	1.2151 ± 0.4019	1.1344 ± 0.2649	0.1315 ± 0.1572	0.1474 ± 0.2117
	Species level	290	103	65.61	1.5924 ± 0.4930	1.3905 ± 0.4031	0.2204 ± 0.2122	0.3236 ± 0.3005

[a] n = The number of polymorphic loci.
[b] P = The percentage of polymorphic loci.
[c] Na = Observed number of alleles.
[d] Ne = Effective number of alleles.
[e] h = Nei's gene diversity.
[f] I = Shannon's information index.

hydrothermal level region it was 50.96%. Populations of *A. halodendron* from the low hydrothermal level region had higher genetic diversity indices than those in the high hydrothermal level region.

AMOVA showed that most of the variation (>89.83%) was within populations (Table 4). Significant genetic differences were detected between the two groups defined as low and high hydrothermal level regions, with the variance among groups being 8.42% ($p = 0.004$). To further reveal the relationships among populations, cluster analysis (UPGMA) was used to generate a dendrogram based on Nei's genetic distance (Fig. 2). The 13 populations of *A. halodendron* were clustered into two clades. Clade I comprised seven populations (1–5, 7, 8) from the low hydrothermal region and population 12 from the high hydrothermal region, while clade II consisted of four populations (9, 10, 11, 13) from the high hydrothermal region and population 6 from the low hydrothermal region.

3.2. Climatic factors and genetic diversity

Genetic diversities of the 13 *A. halodendron* populations from the Horqin Sandy Land were influenced by the following climatic factors: mean annual temperature (AMT), annual temperature range (ART), warm index (*WI*), cold index (*CI*), mean annual rainfall (*AP*), humidity index (*HI*), and hydrothermal synthesis index (*S*) (Table 5). The results showed that *I* was significantly affected by changes in AMT, with its values ranging from 0.0967 to 0.1630. Increased in ART were accompanied by significant increase in *Ne* and significant decrease in *h*, which changed from 1.0971 to 1.1675 and from 0.0950 to 0.1561. With decreasing *WI* and *CI*, *I* were significantly changed, the lowest mean for *I* were 0.1289 (*WI* was 55–60) and 0.0967 (*CI* < 80). *AP* in the range of 300–400 mm was associated with the highest value of *h* (0.1519), which was significantly higher than that observed in populations over 400 mm *AP*. *S* in the range of 30–35 was associated with the lowest value of *Ne* (1.0971) and the highest value of *h* (0.1561), which was significantly lower and higher than that observed in *S* value > 35. *HI* had no significant effect on *A. halodendron* genetic diversity.

Correlation analyses evidenced that there were negative correlations between genetic diversity indices and *S* and between *h* and all climatic indices, and positive correlation between other genetic diversity indices and other climatic indices except between *WI* and *I* (Appendix S2). Of these correlations between *Ne* and ART and between *h* and ART were significant, the correlation coefficients (r-values) were 0.633 ($p < 0.05$) and −0.731 ($p < 0.01$) (Appendix S2).

Table 4
Analysis of molecular variance (AMOVA) for *Artemisia halodendron* populations.

	Source of variation	*df*	Percentage of variation	Fixation indices	*P*
[a]	Among populations	12	10.17	$F_{ST} = 0.1017$	$P < 0.001$
	Within population	277	89.83		
	Total	289			
[b]	Among groups	1	8.42	$F_{CT} = 0.0842$	$P = 0.004$
	Among populations with groups	11	1.81	$F_{SC} = 0.0925$	$P < 0.001$
	Total	277	89.80	$F_{ST} = 0.1088$	$P < 0.001$

[a] AMOVA from thirteen populations as one group.
[b] AMOVA from two groups as represented by hydrothermal synthesis index.

4. Discussion

Genetic variation is non-randomly distributed among populations and species (Nevo, 1998), with distribution of alleles and genotypes over space and time often affected by numerous factors such as breeding system, seed dispersal mechanism, geographic range, life form, and natural selection (Hamrick and Godt, 1989; Hamrick et al., 1991; Maki, 2003; Meloni et al., 2006).

Genetic diversity within populations is essential for adaptation to environmental change and, consequently, long-term species survival. Assessing genetic variation is thus an important component of plant conservation and ecological restoration. In our study, *A. halodendron* generally exhibited high levels of genetic diversity. At the species level, the percentage of polymorphism (*P*) in *A. halodendron* was considerably large when compared to *A. halodendron* in a different habitat gradient ($P = 57.14\%$) based on ISSR analysis (Huang et al., 2011). With respect to the percentage of polymorphic loci (*P*), Nei's diversity index (*h*) and Shannon's diversity index (*I*) can better respond to bands' richness and uniformity, and richness and uniformity are two important indicators to

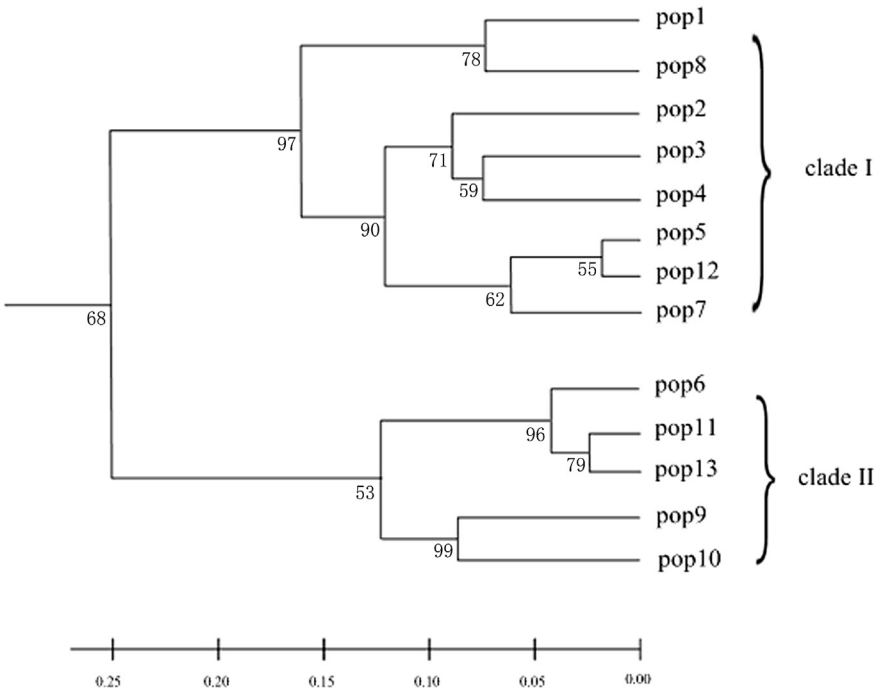

Fig. 2. UPGMA dendrogram of *Artemisia halodendron* populations based on Nei's (1972) genetic distances from ISSR data.

measure genetic diversity. In addition, Nei's diversity index (*h*) can also be associated with species biological characteristics. The value of *h* estimated in our study for *A. halodendron*, 0.2204, is similar to that reported from RAPD markers for *Stipa grandis* from Inner Mongolia (0.2305) (Zhao et al., 2008). Because it is relatively insensitive to bias produced by failures to detect heterozygous individuals (Dawson et al., 1995), another index—Shannon's diversity index (*I*)—is well suited for use with ISSR data. In *A. halodendron*,

Table 5
The comparison of genetic diversities from *Artemisia halodendron* populations within different climatic factors conditions.

Climatic factors		*Na*[a] (±SD)	*Ne*[b] (±SD)	*h*[c] (±SD)	*I*[d] (±SD)	*P*[e] (±SD)	Compared populations
AMT[f] (°C)	<6.5	1.1815 ± 0.0045a	1.1111 ± 0.0129a	0.1648 ± 0.0055a	0.0967 ± 0.0064a	18.1500 ± 0.4525a	4,8
	6.5–7	1.2166 ± 0.1055a	1.1333 ± 0.0637a	0.1274 ± 0.0227a	0.1488 ± 0.0261b	21.6567 ± 10.5490a	9–13
	>7	1.2268 ± 0.0731a	1.1452 ± 0.0378a	0.1230 ± 0.0335a	0.1630 ± 0.0302b	22.6760 ± 7.3065a	1–3,5–7
ART[g] (°C)	34.3–35	1.1541 ± 0.1042a	1.0971 ± 0.0622b	0.1561 ± 0.0165a	0.1433 ± 0.0470a	15.4140 ± 10.4217a	3,4,8,10,11
	36–38	1.2537 ± 0.0669a	1.1545 ± 0.0361ab	0.1231 ± 0.0320ab	0.1507 ± 0.0248a	25.3717 ± 6.6889a	1,2,5–7,9
	>38	1.2516 ± 0.0675a	1.1675 ± 0.0399a	0.0950 ± 0.0463b	0.1401 ± 0.0338a	25.1600 ± 6.7529a	12,13
WI[h] (°C)	<55	1.1529 ± 0.0000a	1.0977 ± 0.0000a	0.1565 ± 0.0000a	0.1838 ± 0.0000a	15.2900 ± 0.0000a	3
	55–60	1.2156 ± 0.0647a	1.1320 ± 0.0342a	0.1339 ± 0.0349a	0.1289 ± 0.0317b	21.5643 ± 6.4695a	1,2,4–8
	>60	1.2268 ± 0.1055a	1.1452 ± 0.0637a	0.1230 ± 0.0277a	0.1630 ± 0.0261ab	22.6760 ± 10.5490a	9–13
CI[i] (°C)	<80	1.1815 ± 0.0045a	1.1110 ± 0.0129a	0.1648 ± 0.0055a	0.0967 ± 0.0064b	18.1500 ± 0.4525a	4,8
	80–85	1.2145 ± 0.0991a	1.1373 ± 0.0602a	0.1286 ± 0.0283a	0.1665 ± 0.0249a	21.4450 ± 9.9054a	3,9–13
	>85	1.2293 ± 0.0739a	1.1403 ± 0.0376a	0.1216 ± 0.0339a	0.1417 ± 0.0277a	22.9300 ± 7.3872a	1,2,5–7
AP[j] (mm)	<300	1.2293 ± 0.0739a	1.1403 ± 0.0376a	0.1216 ± 0.0339ab	0.1417 ± 0.0277a	22.9300 ± 7.3872a	1,2,5–7
	300–400	1.1911 ± 0.0960a	1.1185 ± 0.0595a	0.1519 ± 0.0265a	0.1520 ± 0.0421a	19.1083 ± 9.5985a	3,4,8–10,11
	>400	1.2516 ± 0.0675a	1.1675 ± 0.0399a	0.0950 ± 0.0463b	0.1401 ± 0.0338a	25.1600 ± 6.7529a	12,13
HI[k] (mm/°C)	<5	1.2293 ± 0.0739a	1.1403 ± 0.0376a	0.1216 ± 0.0339a	0.1417 ± 0.0277a	22.9300 ± 7.3872a	1,2,5–7
	5–7	1.1987 ± 0.1030a	1.1226 ± 0.0642a	0.1509 ± 0.0284a	0.1456 ± 0.0419a	19.8720 ± 10.2972a	4,8–10,11
	>7	1.2187 ± 0.0551a	1.1442 ± 0.0351a	0.1155 ± 0.0372a	0.1547 ± 0.0270a	21.8700 ± 5.5137a	3,12,13
S[l]	<30	1.2537 ± 0.0669a	1.1545 ± 0.0361ab	0.1231 ± 0.0320ab	0.1507 ± 0.0248a	25.3717 ± 6.6889a	1,2,5–7,9
	30–35	1.1541 ± 0.1042a	1.0971 ± 0.0622b	0.1561 ± 0.0165a	0.1433 ± 0.0470a	15.4140 ± 10.4217a	3,4,8,10,11
	>35	1.2516 ± 0.0675a	1.1675 ± 0.0399a	0.0950 ± 0.0463b	0.1401 ± 0.0338a	25.1600 ± 6.7529a	12,13

Different letters within a variable indicate significant differences at $P < 0.05$; same letters means not significant.
[a] *Na* = Observed number of alleles.
[b] *Ne* = Effective number of alleles.
[c] h = Nei's gene diversity.
[d] I = Shannon's information index.
[e] *P* = The percentage of polymorphic loci.
[f] AMT = Mean annual temperature.
[g] ART = Annual temperature range.
[h] WI = Warm index.
[i] CI = Cold index.
[j] AP = Mean annual rainfall.
[k] HI = Humidity index.
[l] S = Hydrothermal synthesis index.

we calculated a value of 0.3236 for *I*. This is similar to the mean *I* value for outcrossing species given by Bussell (1999). Other authors (Hamrick and Godt, 1990; Nybom and Bartish, 2000) note that levels of genetic variation are strongly dependent on plant life form, geographic range, pollen dispersal mechanisms, and natural selection. *A. halodendron* possesses life history traits that include longevity, a perennial habit, wind pollination, seed-based reproduction, vegetative propagation, and a broad ecological amplitude. This combination of traits should reportedly enable the species to achieve a high level of genetic diversity (Babbel and Selander, 1974; Pearse et al., 2004; Ge et al., 1999). *A. halodendron* does exhibit high levels of genetic diversity; this characteristic made it a dominant species of the Horqin Sandy Land. At the regional level, *A. halodendron* populations from the low hydrothermal level region had greater genetic diversity than those from the high hydrothermal level region. These genetic differences between the two hydrothermal levels, which were significant, may be due to environmental differences (Bao et al., 2010; Li and Shi, 2009; Li and Wulantuya, 2011). The low hydrothermal level region is undergoing severe desertification, strong human disturbance, and high rates of habitat fragmentation (Wang et al., 2010; Zhao et al., 2000). Because of the changing environment, genetic differentiation would be expected to increase (Wang et al., 2009). In our study, however, the relationship between population genetic diversity was not well matched with hydrothermal conditions. These observations are similar to those of other studies examining relationships between populations in species with large distribution areas (Díaz et al., 1999; Qiu et al., 2004; Chen et al., 2009).

An organism's ecological characteristics are shaped by the interaction between genetic traits and external organic or inorganic factors. In other words, ecological characteristic are the comprehensive exhibition of the interaction between genomes and complex environments (Xu et al., 2003). With regard to the whole ecosystem, relationships between genomes and environmental factors should therefore be considered as an important component of ecological research (Li and Peng, 2001). In our study, several climatic factors were found to have influenced genetic diversities of the 13 *A. halodendron* populations in the Horqin Sandy Land. This is mainly because population combination has been changed when 13 *A. halodendron* populations were classified in accordance with the climatic factors. Some studies have found positive (Frankham, 1996; Karron, 1997) or negative correlations (Widén and Andersson, 1993; Bijlsma et al., 1997) between population size and genetic variation. Our correlation analysis uncovered positive association between most genetic diversity indices and most climatic factors. These results are consistent with the previously-reported relationship between RAPD diversity and ecological factors in *S. grandis* from Inner Mongolia (N.X. Zhao et al., 2006). Our study detected significant or highly significant relationships between some genetic diversity indices (*Ne* and *h*) of the 13 studied *A. halodendron* populations and annual temperature range (ART), suggesting that genetic variation in *A. halodendron* depends primarily on this climatic factor. The negative correlation found between genetic diversity indices and hydrothermal synthesis index (*S*) is similar to the relationship previously seen between RAPD diversity and some soil ecological factors in *Reaumuria soongorica* from the Fukang Desert (Xu et al., 2003; Qian et al., 2008). The *S* index applied here, is one of a high number of humidity or aridity indices which all reflect the balance between water input (precipitation) and loss from the system through evapotranspiration (increasing with increasing temperature). Thus, this class of index values gives simplified indications of site water balance. The collaborative impact of different environmental factors on plant population genetic diversity requires further research.

5. Conclusions

In conclusion, there are significant genetic differences between *A. halodendron* populations from different hydrothermal level regions. Genetic diversity of all populations was affected by various climatic factors. Genetic differentiation in *A. halodendron* is the result of the collaborative impacts of climatic factors and genetic traits. Additional research is needed to determine levels of genetic variation in *A. halodendron* throughout its entire distributional range.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2013.12.005.

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