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Genetic diversity of two annual *Salsola* species (Chenopodiaceae) among habitat types in desert plant communities

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Abstract: Desert annual plant species are valuable pasture forage and sources for restoration of degraded pastures. Examining the impact of environmental stresses on genetic diversity and population structure can identify species populations suitable for habitat restoration. We examined allozyme diversity and population structure of two annual species, halo-xerophyte *Salsola incanescens* and xero-halophyte *S. paulsenii*, in association with the type of desert plant community, which reflects the water-salt conditions in the soil. We sampled 19 wild populations in 15 xerophytic, xero-halophytic and halophytic plant communities in Kyzylkum desert (Uzbekistan, Central Asia). The species studied had low genetic variability (P_{95} = 0.05–0.10, H_O = 0.008–0.025) and high population structure (F_{ST} = 0.585–0.739). Wright's fixation index indicated deviations from Hardy–Weinberg equilibrium with a deficit of heterozygotes (F_{IS} = 0.79) in *S. incanescens* populations. No evidence of isolation by distance was found for the species studied (Mantel's r = 0.02–0.04). Estimates of genetic variability in populations from xerophytic and halophytic plant communities differed significantly (2- to 10-fold, $P < 0.05$) for both species. Moreover, allele–habitat associations at the GOT and Me loci were found in both species. Decreased levels of within-population variability were found in both species in halophytic communities. In sub-optimal habitats, the decline in genetic differentiation and increase in gene flow were found for both*S. incanescens* (xerophytic plant communities) and *S. paulsenii* (halophytic communities). Our results indicate that genetic diversity rather than population differentiation is affected by the habitat type.

Key words: genetic diversity; halophytes; isozymes; natural selection; salinity; water deficit; Central Asia.

Introduction

Climate variability, drought and temperature extremes are predicted to have a greater impact and proceed more rapidly under climate change in arid and semi-arid environments (Stern 2006; Christmann et al. 2009; IPCC 2014). Frequent changes in temperature regimes and precipitation patterns in recent decades in the Central Asian arid region pose serious threats such as habitat fragmentation (landscape disturbance effects) and loss of plant resources. Habitat fragmentation and changes in vegetation composition may prevent gene flow between isolated populations, reducing their genetic diversity (Odat et al. 2004; Leonardi et al. 2012). Vegetation of Kyzylkum desert of the Irano-Turanian geographical province (Central Asia) represents a series of xerophytic, xero-halophytic, halophytic and hyper-halophytic plant communities reflecting the water-salt balance in soil, which depends on salinisation and desalinisation processes and changes in groundwater table depth (Akzhigitova et al. 2003). There are two stress factors that may simultaneously affect the plants: deficit of available water and salinity. In small quantities, salt is necessary for the optimal growth of wild desert halophytes, but it can lead to stress in large quantities. Moreover, the level of salinity for optimal growth varies among different halophytic species (Flowers & Colmer 2008; Aslam et al. 2011).

Desert species with different degrees of drought and salt tolerance often occupy the same plant communities and are characterised by overlapping ecological niches. Xero-halophytic species are characterised by heterogeneous and mosaic habitats. The influence of environmental variability and habitat type on the genetic diversity and genetic differentiation between local populations has been demonstrated (Nevo et al. 1981; Prentice & Cramer 1990; Prentice et al. 1995; Linhart & Grant 1996; Odat et al. 2004). The effect of local environmental stresses (such as water deficit and soil salinity) on diversity and population structure of the

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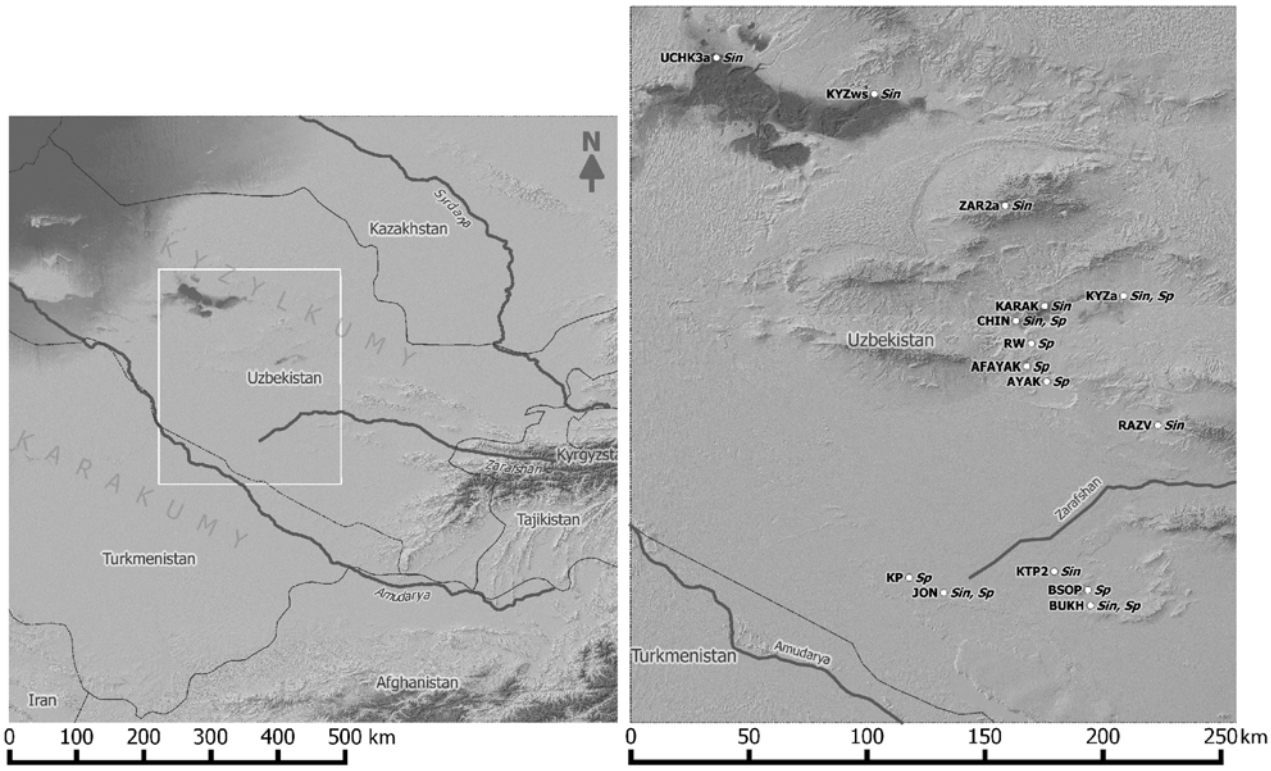

Fig. 1. Distribution map of plant communities where populations of *Salsola incanescens* and *S. paulsenii* were sampled for this study in Kyzylkum desert in Uzbekistan (Central Asia). Capital letters indicate the plant communities. *Sin* – *S. incanescens* populations, *Sp* – *S. paulsenii* populations.

Table 1. Description of sampled species and sampling sites in Kyzylkum desert.

Species	Code of population (plant communities)**	Type of plant communities	Geographic coordinates	Altitude (m)	Sample size
Salsola incanescens Iljin* Section *Caroxylon* (I) (halo-xerophyte)	KTP2	Xerophytic	N 39°44.323′ E 64°32.221′	242	26
	RAZV		N 40°27.267′ E 65°02.635′	300	30
	ZAR2a	Xero-halophytic	N 41°31.562′ E 64°17.894′	521	30
	KYZws		N 42°04.306′ E 63°39.420′	110	30
	CHIN		N 40°57.720′ E 64°21.032′	153	30
	BUKH		N 39°34.744′ E 64°42.854′	219	31
	KYZa		N 41°05.026′ E 64° 52.552′	121	25
	UCHK3a	Halophytic	N 42°14.944′ E 62°53.142′	35	30
	KARAK		N 41°02.033′ E 64°29.430′	103	31
	JON		N 39°38.087′ E 63°59.847′	205	30
Salsola paulsenii Litw. Section *Salsola* (VI) (xero-halophyte)	KP	Xerophytic	N 39°41.348′ E 63°49.629′	194	33
	RW		N 40°51.097′ E 64°25.533′	193	26
	AYAK	Xero-halophytic	N 40°39.638′ E 64°29.795′	167	31
	KYZa,		N 41° 05.026′ E 64° 52.552′	121	31
	CHIN,		N 40°57.720′ E 64°21.032′	153	30
	BUKH		N 39°34.744′ E 64°42.854′	219	31
	AFAYAK	Halophytic	N 40°42.084′ E 64°23.722′	172	29
	BSOP		N 39°37.295′ E 64°40.024′	216	25
	JON		N 39°38.087′ E 63°59.847′	205	29

*The nomenclature of species has been adopted according to the Flora of Uzbekistan (1953).
**Population code corresponds to the code of the plant community. Code of plant communities is the abbreviated name of the nearest village. The same codes for populations of two species (bold type) mean that these populations were found in the same plant community.

desert xero-halophytes is still not well understood. For annual species, these factors are especially important because of small population size and isolation of populations. Small population size may cause a decrease in genetic variability through genetic drift, and isolation may lead to increased differentiation among populations with genetic drift having a greater impact than gene flow on population structure. The loss of diversity may reduce the evolutionary potential for adaptation to changing environments, cause the loss of fitness and increase extinction risk (Ouborg et al. 2006; Dostalek et al. 2014).

Annual *Salsola* species (Chenopodiaceae) are typical representatives of xero-halophytic flora of the Irano-Turanian deserts and valuable pasture forage species (Gintzburger et al. 2003; Akhani et al. 2007; Toderich et al. 2009). Genetic polymorphism has been studied only for some annual *Salsola* species. The genetic variability estimates in annual *Salsola* are highly variable from monomorphic in *S. aperta* (Toderich et al. 2012) to a moderate level of polymorphism ($P = 0.22$–0.31, $H_E = 0.129$–0.159) in *S. pestifer* and *S. tragus* (Ryan & Ayres 2000, Wojnicka-Poltorak et al. 2002, Toderich et al. 2012), corresponding to average values for annuals that have predominantly outcrossing sexual reproduction and widespread geographic range ($P = 0.4$, $H_E = 0.132$) (Hamrick & Godt 1989). These differences may be related to the habitat type and/or ecological niche of these species. *S. aperta* is a desert psammophytic species with a narrow ecological niche, whereas *S. pestifer* and *S. tragus* are semi-desert and ruderal species with widespread geographic ranges. Desert *Salsola* species differ considerably in ecological plasticity and width of ecological niche. Among these species, there are obligate psammophytes (*S. aperta* and *S. praecox*) and species (e.g. *S. incanescens, S. lanata, S. paulsenii* and *S. scleranthа*) that can grow in various habitats with different levels of soil salinity and drought. In Kyzylkum desert, the type of plant communities reflects the relative dominance of these stresses. Xerophytic plant communities grow in habitats with a high water deficit and low or non-saline soils, xero-halophytic plant communities prefer habitats with moderate water deficit and soil salinity and halophytic plant communities grow in habitats with high soil salinity. When different populations experience different environmental conditions, local selection regimes can constrain the underlying genomic architecture, thereby promoting local adaptation (Schluter 2000). The extent of local adaptation is largely shaped by the interaction between selection and gene flow along selective gradients (Lenormand 2002; Forester at al. 2016). When selection is strong, local adaptation may occur under migration–selection balance due to selection against maladapted genes (Yeaman & Whitlock 2011). Identifying and analysing the alleles that underlie functional trait variation and adaptation are necessary for understanding the process of local adaptation (Marden 2013; Gompert 2016). The study of population genetics of functional genes, when the role of selection can be distinguished from the effects of drift, allows improved insights into the effects of loss of genetic diversity on evolutionary potential (Ouborg et al. 2006).

In the current study, we used plant communities as indicators of local stresses (deficit of available water and salinity) for investigation of the effect of habitat type on the genetic diversity and population structure of two typical desert annual *Salsola* species. We chose species with different salt and drought tolerances although they can be found in the same habitats. Isozymes were used as genetic markers since they can reflect changes in environmental conditions (Spooner et al. 2005). We expected to find differences in the level of genetic diversity between populations from different habitats, as well as a decreased level of within-population variability, an increased gene flow in sub-optimal habitats and an increased level of genetic differentiation among habitats.

Material and methods

Study species

Salsola incanescens and *S. paulsenii* (Chenopodiaceae) are annual plants widely distributed throughout Central Asian deserts (Flora of Uzbekistan 1953; Gintzburger et al. 2003). They occur in various plant communities, xerophytic, xero-halophytic and halophytic, but differ in tolerance to salt and drought. Both species are drought tolerant (xerophytes) and salt tolerant (halophytes), but *S. incanescens* is more salt tolerant and prefers places with moderate-to-strong soil salinisation (salt marshes) in Kyzylkum desert. *S. paulsenii* is less salt tolerant, but more drought resistant and prefers slightly saline soils and sandy dunes. Therefore, we use the terms 'halo-xerophyte' for *S. incanescens* and 'xero-halophyte' for *S. paulsenii*. Flowering time of these species is between June and August and seeds are set in September-October (Flora of Uzbekistan 1953; Gintzburger et al. 2003). They are characterized by a predominantly outcrossing mode of reproduction and anemophily.

Study area and sampling

The study area was located in south-west and central parts of Kyzylkum desert in Uzbekistan, which extends between the coordinates of 42°15′51.047″ N and 62°48′44.242″ E, and 39°30′38.16″ N and 65°9′33.175″ E (Fig. 1). The region is characterized by an extreme continental climate with very cold winters and hot summers. Precipitation in the form of snowfall is seen usually in winter and rains are common in spring and autumn. Precipitation is higher in the south-western part (200–250 mm) as compared with the central part (100–120 mm) of Kyzylkum desert (Gintzburger et al. 2003). Soils are sandy, grey-brown and solonchak types, which are typical for Central Asian arid regions.

We sampled 10 populations of *S. incanescens* and 9 populations of *S. paulsenii* in three types of plant communities: xerophytic, xero-halophytic and halophytic (Table 1, Fig. 1). Xerophytic plant communities occupy fixed and semi-fixed sandy soils with no or slight salinisation. Xero-halophytic plant communities grow on soils with moderate-to-strong soil salinisation. Sites are located in a wide range of elevations from depressions to highlands. Halophytic plant communities grow on solonchak soils with high levels of salinisation, usually distributed on lowlands and depressions. More than 100 seeds from 10 to 15 mother plants from each population were collected and combined to generate a

seed pool. From this seed pool, 50 seeds were germinated, and all good germinated seeds were analysed. The number of good germinated seeds varied among populations. Final sample sizes for each population are specified in Table 1.

Electrophoresis procedures

On the basis of starch gel electrophoresis of isozymes from randomly chosen embryos variability of the following enzymatic systems was studied: glutamate oxaloacetate transaminase (GOT (AAT), E.C. 2.6.1.1), diaphorase (DIA, E.C. 1.6.99), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), superoxide dismutase (SOD, E.C. 1.15.1.1), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), malate dehydrogenase (MDH, E.C. 1.1.1.37), and malic enzyme (Me, E.C. 1.1.1.40). The seeds were cleaned of their wings and soaked in water for 12 h, and homogenized in 80 μL of Tris-HCl buffer with KCl, $MgCl_2$, EDTA, Triton X-100, and PVP. Enzymes were separated in 10% starch gel using two buffer systems (Muona and Szmidt 1985; Wojnicka-Poltorack et al. 2002). Staining of particular enzymes as well as genetic interpretation of the results followed standard techniques (Muona & Szmidt 1985; Soltis & Soltis 1990).

Data analysis

Allele frequencies and standard genetic diversity parameters were estimated at the species and population levels following Nei & Roychoundhury (1974) and Wright (1984) using POPGENE 1.32 (Yeh et al. 1999). The following parameters were calculated: 99th and 95th percentiles of polymorphic loci (P_{99}, P_{95}), mean numbers of allele loci (A) and expected and observed heterozygosity (H_E, H_O); estimation of these parameters was conducted for each locus and then averaged across loci for each population. Population level estimates of these parameters were averaged over all populations to obtain means and standard error (SE). Species level estimates of these parameters were calculated based on total data for each species (sample size is 293 embryos for *S. incanescens* and 265 embryos for *S. paulsenii*).

F-statistics (F_{IS}, F_{IT},and F_{ST})were used to analyze genetic structure in populations (Nei 1987). These measures represent relative excess of homozygotes or heterozygotes compared with panmictic expectations relative to all populations (F_{IT}), within populations (F_{IS}), and among populations (F_{ST}). One estimate of Nm (the number of migrants per generation) was estimated using the extent of genetic differentiation among populations: $N_m = 0.25(1-F_{ST})/F_{ST}$ (Nei 1987). Nei's (1987) genetic distances (D) were estimated between all pairs of populations to generate average clusterings using the UPGMA methods (modified from NEIGHBOR procedure of PHYLIP Version 3.5) (Yeh et al. 1999). The existence of a significant correlation of genetic distances between pairs of populations with the corresponding geographical distances was investigated using Mantel test (GenAlEx 6.4 (Peakall & Smouse 2006)).

Analysis of variance (ANOVA) with a post hoc Tukey's test for pairwise comparison (SigmaPlot [11.0], Systat Software (USA)) was performed to compare the response variables for genetic variation (P_{95}, H_E and H_O) from different habitat types for both species (explanatory variables). Differences between populations from different habitat types

Table 2. Summary of genetic diversity among 19 populations and species-level estimates for two desert annual *Salsola* species based on 13 allozyme loci.

Populations*	P_{99}**	P_{95}	H_E	H_O	A
		Salsola incanescens			
BUKH	0.23	0.23	0.102	0.007	1.23
KARAK	0.31	0.08	0.019	0.005	1.31
KYZa	0.15	0.00	0.009	0.003	1.15
CHIN	0.08	0.00	0.007	0.003	1.08
JON	0.15	0.15	0.061	0.000	1.15
KTP2	0.23	0.15	0.065	0.027	1.31
RAZV	0.15	0.15	0.050	0.018	1.15
ZAR2a	0.08	0.08	0.035	0.010	1.08
UCHK3	0.08	0.08	0.010	0.005	1.08
KYZws	0.08	0.08	0.010	0.000	1.08
Population mean (± SE)	**0.15** (± 0.06)	**0.10** (± 0.04)	**0.037** (± 0.015)	**0.008** (± 0.004)	**1.16** (± 0.05)
Species level***	0.62	0.23	0.139	0.008	1.21
		S. paulsenii			
KYZa	0.07	0.07	0.028	0.039	1.07
CHIN	0.07	0.07	0.034	0.067	1.07
RW	0.13	0.13	0.069	0.068	1.20
AYAK	0.00	0.00	0.000	0.000	1.00
AFAYAK	0.07	0.07	0.014	0.016	1.07
BUKH	0.07	0.07	0.009	0.010	1.07
BSOP	0.00	0.00	0.000	0.000	1.00
KP	0.07	0.07	0.021	0.026	1.07
JON	0.00	0.00	0.000	0.000	1.00
Population mean (± SE)	**0.05** (± 0.02)	**0.05** (± 0.02)	**0.019** (± 0.016)	**0.025** (± 0.013)	**1.06** (± 0.03)
Species level	0.27	0.13	0.045	0.025	1.33

*Population code corresponds to the code of the plant community. The same codes for populations of two species mean that these populations were found in the same plant community. **P_{99}, P_{95} = 99^{th} and 95^{th} proportion of polymorphic loci, H_E = expected heterozygosity, H_O = observed heterozygosity, A = mean number of alleles per locus.
***Species level estimates of these parameters were calculated based on total data for each species (sample size is 293 embryos for *S. incanescens* and 265 embryos for *S. paulsenii*).

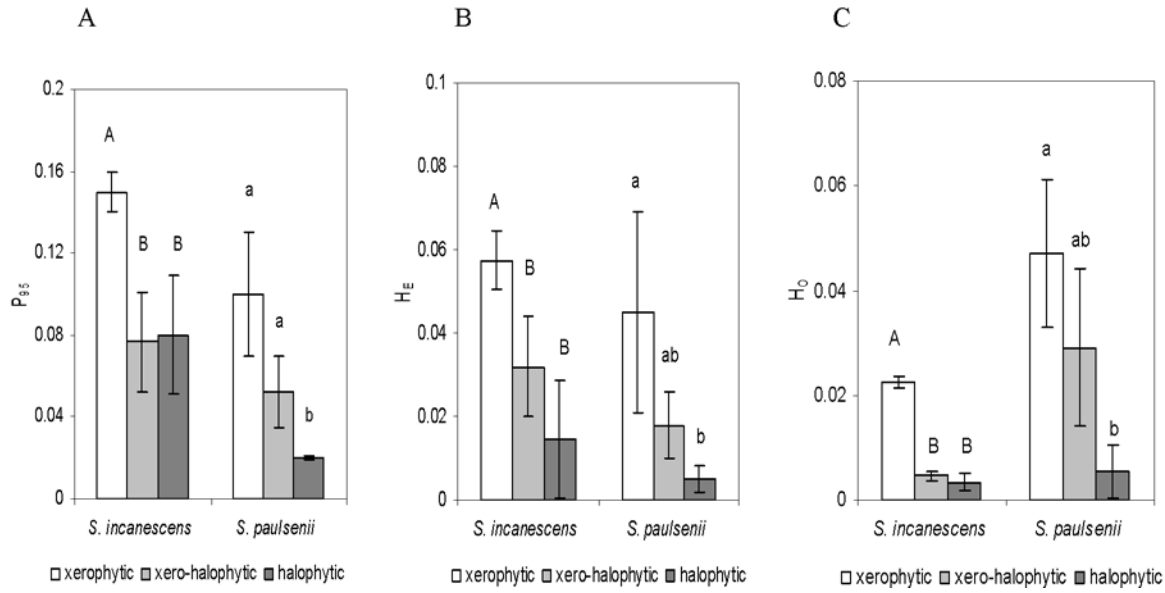

Fig. 2. Level of genetic polymorphism of *Salsola incanescens* and *S. paulsenii* from different habitats: xerophytic, xero-halophytic and halophytic plant communities. (A) P_{95}, 95th percentiles of polymorphic loci; (B) H_E, mean expected heterozygosity and (C) H_O, mean observed heterozygosity. The values presented are means (± SE) of two to five replicates. Different capital letters above the bars represent significant differences ($P < 0.05$, Tukey's pairwise comparison) in genetic parameters between *S. incanescens* populations from different habitats. Different lowercase letters above the bars represent significant differences ($P < 0.05$, Tukey's pairwise comparison) in genetic parameters between *S. paulsenii* populations from different habitats.

were considered significant at $P < 0.05$ and marked on the diagrams by different letters above the bar (capital letters in case of *S. incanescens*, and lowercase letters in case of *S. paulsenii*). Habitat type was a fixed factor acting on the genetic parameters regardless of plant species. Regression analysis was carried out using SigmaPlot [11.0]

Results

Genetic diversity

Twenty-three alleles at 13 loci were resolved across 10 populations of *S. incanescens*. At the species level, 62% of the loci were polymorphic ($P_{99} = 0.62$), and expected heterozygosity was 0.139 (Table 2). Within-population genetic diversity estimates obtained across all populations were significantly lower: P_{99} = 0.08–0.31, A = 1.08–1.31, H_E = 0.007–0.102 (Table 2). Nineteen alleles at 15 loci were resolved across 9 populations of *S. paulsenii*. The genetic diversity estimates were lower in *S. paulsenii* than those in *S. incanescens*. At the species level, 27% of the loci were polymorphic, and expected heterozygosity was 0.045 (Table 2). Population level genetic diversity estimates were also lower: P_{99} = 0–0.13, A = 1.00–1.2 and H_E = 0–0.069 (Table 2). Three populations of *S. paulsenii* (AYAK, BSOP and JON) were monomorphic across all loci. A total of 37 alleles were detected across the 19 *Salsola* populations studied, but only five alleles were common for both species. Observed heterozygosities (H_O = 0–0.027) across loci were lower than expected for all *S. incanescens* populations (Table 2). In polymorphic *S. paulsenii* populations, the observed heterozygosities (H_O = 0.010–0.068) were equal or higher than the level of expected heterozygosity (H_E = 0.009–0.069; Table 2).

Genetic structure

The mean Wright's fixation indices (F_{IS}, F_{IT} and F_{ST}) across loci in *S. incanescens* were high and significantly different from zero (0.786, 0.944 and 0.739, respectively). In *S. paulsenii*, Wright's fixation indices not only were lower ($F_{IS} = -0.293$, $F_{IT} = 0.464$ and $F_{ST} = 0.585$) but also were significantly different from zero. High values of F_{ST} indicate high genetic differentiation of populations in both species. Moreover, low gene flow ($N_m = 0.09$ in *S. incanescens* and $N_m = 0.18$ in *S. paulsenii*) was observed. The genetic distance (D) between *S. paulsenii* populations (0.0001–0.1352) was less than between *S. incanescens* populations (0.005–0.3905). No correlation was found between geographic and genetic distance for all pairwise populations in the *Salsola* species studied (Mantel test $r = 0.02$, $P = 0.96$ in *S. incanescens* and $r = 0.04$, $P = 0.83$ in *S. paulsenii*).

Genetic diversity among habitat types

The level of genetic diversity in populations of both species differed among the types of plant communities. Estimates of genetic variability (P_{95} and H_E) in the populations from xerophytic plant communities were significantly higher (approximately 2- to 5-fold) than those from halophytic communities for both species (ANOVA, $P < 0.05$, Fig. 2A and B). Mean observed heterozygosity in *S. paulsenii* and *S. incanescens* was also significantly higher (approximately 7- to 10-fold) in xerophytic communities than that in halophytic plant communities (Fig. 2C). For both species, less than 16%

Table 3. Comparison of genetic diversity estimates of *Salsola* species (Chenopodiaceae) and some annual chenopods.

Species	Ecotype	P*	Ho	Source
	Annual *Salolsa* species			
Salsola aperta	psammophyte, xero-halophyte	0.00	—	Toderich et al. 2012
S. incanescens	halo-xerophyte	0.15	0.008	This study
S. komarovii	psammophyte	0.30	0.113	Kim & Chung 1995
S. lanata	halophyte	0.21	0.031	Wojnicka-Poltorak et al. 2002
S. paulsenii	xero-halophyte	0.05	—	Toderich et al. 2012
		0.05	0.025	This study
S. pestifer	xero-halophyte,	0.06	0.129	Wojnicka-Poltorak et al. 2002
	ruderal	0.22	—	Toderich et al. 2012
S. praecox	psammophyte, xero-halophyte	0.13	0.159	Wojnicka-Poltorak et al. 2002
S. tragus	xero-halophyte, ruderal	0.31	0.038	Ryan & Ayres 2000
	Perennial *Salsola* species			
S. arbuscula	xero-halophyte	0.36	—	Toderich et al. 2012
S. gemmascens	xero-halophyte	0.18	—	Toderich et al. 2012
S. orientalis	xero-halophyte	0.29	—	Toderich et al. 2012
S. richteri	xero-halophyte	0.29	—	Toderich et al. 2012
	Other annual chenopods			
Atriplex tatarica	mesophyte, ruderal	0.47	0.151	Mandak et al. 2005
Bassia sedoides	xero-halophyte, ruderal	0.14	0.005	Shuyskaya et al. 2015
Salicornia europaea	halophyte	0.23	0.000	Wolff & Jefferies 1987
S. ramosissima	halophyte	0.20**	—	Kruger et al. 2002
Suaeda salsa	halophyte	0.68	0.082	Song & Zang 2007

*P = proportion of polymorphic loci, H_O = observed heterozygosity **by RAPD markers

Table 4. Allele frequencies at Got-1(2) and Me-2 loci in populations of *Salsola incanescens* and *S. paulsenii* associated with the habitat type.

Plant communities	*S. incanescens*					*S. paulsenii*				
	Got-1			Me-2		Got-2		Me-2		
	A	B	C	A	B	A	B	A	B	C
Xerophytic	0.98	0.02	0.00	1.00	0.00	0.82	0.18	0.22	0.47	0.25
Xero-halophytic	0.78	0.01	0.21	0.79	0.21	0.98	0.02	0.21	0.79	0.00
Halophytic	0.67	0.00	0.33	0.67	0.33	1.00	0.00	0.04	0.00	0.96

of diversity was from among populations from different habitats (F_{ST} = 0.08 in *S. incanescens* and 0.16 in *S. paulsenii*). In both species, significant differences in alleles frequencies (χ^2 test, $P < 0.05$) for GOT and Me loci were found in populations from different plant communities (Table 4).

Discussion

Genetic diversity and structure

We examined the level of genetic diversity and population structure of two desert annual species *S. incanescens* and *S. paulsenii*. We used isozymes as genetic markers. Isozymes are often less polymorphic than DNA markers; however, they can reflect changes in environmental conditions (Spooner et al. 2005; Marden 2013), and the use of isozymes provides more opportunities to reveal the effect of the environment (such as the habitat type) on genetic diversity.

Mean estimates of genetic variability in studied populations of *Salsola* species were lower (P = 0.05–0.15, H_E = 0.019–0.037 and H_O = 0.008–0.025) than in other species of the genus *Salsola*: perennials (P = 0.18–0.36 in *S. arbuscula, S. gemmascens, S. orientalis* and *S. richteri* (Toderich et al. 2012)) and in some annuals (P = 0.06–0.31 and H_O = 0.038–0.159) in *S. praecox* and *S. pestifer* (Wojnicka-Poltorak et al. 2002, Toderich et al. 2012), *S. tragus* (Ryan & Ayres 2000) and *S. komarovii* (Kim & Chung 1995) (Table 3). On average, the genetic diversity parameters in the populations of the annual species studied were lower than those in others annuals with predominantly outcrossing reproduction and widespread geographic range (P of 0.4 and H_E of 0.132) (Hamrick & Godt 1989). This result may be partially due to the fact that seeds (embryos) were used in our analysis instead of adult plants. Genetic polymorphism estimates of seed do not always correspond to the genetic estimates of adult plants. Indeed, for the annual *Atriplex tatarica* (Chenopodiaceae), an increase in the number of heterozygotes and the partitioning of genetic diversity among populations with increasing population age (seeds, seedlings, mature plants and fruiting plants) has been shown. However, the differences in the number of alleles per polymorphic locus and gene diversity

were not significant (Mandak et al. 2006).

Comparison of genetic estimates in annual chenopods species with different salt and drought tolerances revealed that halophytes have a higher percentage of polymorphic loci but lower observed heterozygosity: $P = 0.21$–0.68 and $H_O = 0$–0.082 in *S. lanata* (Wojnicka-Poltorak et al. 2002), *Salicornia europaea* (Wolff & Jefferies 1987), *S. ramosissima* (Kruger et al. 2002) and *Suaeda salsa* (Song & Zang 2007) (Table 3). In general, annual xero-halophytic chenopods, which prefer non-, low or moderate saline habitats, are characterised by a 2.3-fold higher level of observed heterozygosity ($H_O = 0.07 \pm 0.02$) than halophytic chenopods ($H_O = 0.03 \pm 0.02$; Table 3). In our study, *S. incanescens*, which prefers more saline soils, is characterised by lower mean heterozygosity ($H_O = 0.008 \pm 0.002$) compared with *S. paulsenii* (Table 2). Furthermore, a 79% deficit of heterozygotes was found in *S. incanescens* populations. There was no relationship ($R^2 = 0.13$, regression analysis) between observed heterozygosity and the sample size in *S. incanescens.* The deficit of heterozygotes in *S. incanescens* populations may be partially a consequence of inbreeding (this species is mainly cross-pollinated) and/or natural selection. One of the possible reasons for the loss of genetic polymorphisms in halophytes may be their restricted ecological distribution (open habitats in coastal and inland salt marshes) (Wolff & Jefferies 1987).

At the species level, genetic polymorphism was significantly higher (2.3- to 5.4-fold) than that at the population level in both species, indicating that different populations have a different set of alleles, which may be adapted to local conditions. A diverse mosaic of habitats may contribute to the promotion of genetic heterogeneity, provided that different alleles and genotypes have different relative fitnesses in different microhabitats (Bazzaz & Sultan 1987; Linhart & Grant 1996; Prentice et al. 2000). The differences in edaphic conditions and water-salt balance in Kyzylkum desert (Akzhigitova et al. 2003), as well as small size and isolation of populations of the studied annual species, likely led to an increase in diversity between distant populations rather than within populations. The fact that 58%–74% of the total variation was due to differences among populations indicates a significant isolation of populations in the species studied, especially in *S. incanescens* ($F_{ST} = 0.739$). Thus, in these species, the level of gene flow was very low ($N_m = 0.09$–0.18). The lack of correlation between genetic and geographic distance in both *Salsola* species studied suggests that gene flow does not play a significant role in shaping structure among populations. Further, the low gene flow in these species ($N_m < 1.0$) is not of sufficient magnitude to counterbalance genetic drift (Wright 1984). According to genetic drift theory, loss of rare alleles, increased differentiation and weakening of the isolation-by-distance component of genetic structure among populations can be expected (Barrett & Chalesworth 1991; Ellstrand & Elam 1993). Small populations can also be more susceptible to genetic drift (Ouborg et al. 2006; Masel 2011; Leonardi et al. 2012; Nazareno & Reis 2014). Our results revealed that the desert annual *Salsola* species studied fit some of these expectations. There is no evidence for an isolation-by-distance pattern for both species but they have high population differentiation ($F_{ST} = 0.585$–0.739). Thus, genetic drift may play some role in shaping the genetic structure of the populations of the studied species.

Genetic diversity in relation to the habitat type

We studied the level of genetic diversity and population structure of two desert annual *Salsola* species in relation to the habitat type (desert plant communities), which reflect the balance of local stresses (deficit of available water and salinity). Several studies have provided evidence that environmental variability such as soil type, pH, nutrient, moisture and soil depth (Nevo et al. 1981; 1994; Prentice et al. 1995, 2000) and habitat (Odat et al. 2004; Leonardi et al. 2012; Lega et al. 2014) have the potential to influence the genetic diversity and genetic differentiation between local populations. Habitat selection and environmental heterogeneity may contribute to the promotion of genetic heterogeneity (Bazzaz & Sultan 1987; Linhart & Grant 1996; Prentice et al. 2000).

We found that estimates of genetic variability in populations from xerophytic and halophytic plant communities differ significantly ($P < 0.05$) for both species (Fig. 2). Populations in xerophytic plant communities were characterised by 2- to 10-fold higher genetic variability (Fig. 2) than others. This result was expected for *S. paulsenii*, which prefers slightly saline soils and sandy dunes. However, *S. incanescens* is more typically found in xero-halophytic and halophytic plant communities rather than in the xerophytic communities because of the need for salt. The reason for such an increase in genetic diversity may be the presence of perennial C_4 species (such as *Haloxylon aphyllum, H. persicum*, *S. richteri* and *S. paletskiana*) in xerophytic plant communities on low or non-saline soils, whose leaf fall is an additional source of salt and humus. These tree-like xero-halophytes can change soil properties (level of pH, salinity, alkalinity, moisture and humus) owing to their leaf fall (Akzhigitova et al. 2003; Yue et al. 2004; Rasooli & Jafari 2009). The soil properties in under-crown areas and between crowns can vary greatly, increasing the environmental heterogeneity for the populations of annual plants, in particular for *S. incanescens.* The higher genetic heterogeneity of *S. incanescens* in xerophytic communities may be maintained by spatial variability in selection pressure (Van Valen 1965). Those xerophytic communities more favourable for *S. paulsenii* are confirmed by the high observed heterozygosity ($P < 0.05$, Fig. 3C). Parameters measuring within-population variability were more variable within plant communities than among different plant communities. Low genetic differentiation among populations from different plant communities (8–16%) suggests that the effect of the habitat type is more apparent for genetic diversity rather than for population differentiation.

The different impact of habitat type on populations can be explained by a different balance between genetic drift and gene flow or by the effect of natural selection. The dominant effect of genetic drift compared with gene flow is evident in *S. incanescens* populations within xero-halophytic and halophytic plant communities (N_m = 0.08 and 0.06, respectively), as well as in *S. paulsenii* populations within xero-halophytic plant communities (N_m = 0.13). Gene flow is strong in *S. incanescens* populations within xerophytic plant communities and in *S. paulsenii* populations within halophytic plant communities (N_m = 1.5 and 2.73, respectively) where it seems to be sufficient to counterbalance random genetic drift. These data show that random genetic drift does not play a key role in different impacts on habitat type.

Significant differences in genetic variability between different plant communities for both studied species indicate the possible role of natural selection in shaping the genetic structure of populations. The allele–habitat associations found at GOT and Me loci for both species (Table 4) confirm this interpretation. It has been shown that some enzymes could be under direct selection (Mitton & Grant 1984). For example, significant association between allozymes at the Pgi-2 locus and pH, moisture, soil depth and nutrient/water treatments was found for *Festuca oviana* (Prentice et al. 1995, 2000). Moreover, an allele–habitat association at the Got-2 locus was found in the xero-halophyte *Haloxylon aphyllum* (Shuyskaya et al. 2012). Both GOT (AAT) and Me (malic enzyme, NADP-ME) are important metabolic enzymes that are involved in the energy metabolism of plants. GOT plays a key role in primary nitrogen (N) assimilation and a crucial role in the metabolic regulation of carbon and nitrogen metabolism (Torre et al. 2007). Drought and salt stress are known to reduce plant growth and affect the whole metabolism. Plant responses to salt stress may lead to differences in nitrogen metabolism, namely ion uptake, N assimilation and amino acid and protein synthesis (Kusano et al. 2011). As a key component of regulation of C and N metabolism, GOT is involved in the restructuring of the metabolic homeostasis in plant responses to this stress (Gao et al. 2013). Malic enzyme (NADP-ME) is also associated with the plant metabolic response to stress. NADP-ME catalyses the oxidative decarboxylation of malate to produce pyruvate and NADPH, which is one of the most important factors in cell growth, proliferation and detoxification. It is used in the biosynthesis of osmotically active compounds, especially proline and mannitol (to maintain water balance). Severe stress that affects water balance leads to a loss of turgor and membrane damage related to the necessity for membrane lipid regeneration. Malic enzyme participates in this repair process by supplying pyruvate and NADPH for the biosynthesis of fatty acids and other primary and secondary metabolites (Doubnerova & Ryslava 2011). Indeed, metabolic enzyme loci are frequent targets of selection, and understanding how metabolic enzyme polymorphisms relate to phenotypes and fitness is necessary to examine ecological and evolutionary processes (Marden 2013).

In general, similar eco-genetic patterns are observed within both species: in sub-optimal habitats, two loci (at GOT and Me) become monomorphic and the highest gene flow and lowest F_{ST} are found. The highest level of genetic diversity was in xerophytic communities in both species. For *S. incanescens*, this may be a result of soil heterogeneity due to leaf fall of perennials. Thus, our results indicate that local conditions may influence the genetic diversity and population structure of studied species.

In conclusion, the results of this study demonstrate that two desert annual *Salsola* species have very low genetic variability and high population structure. No evidence for isolation by distance was found for the two species studied. The level of genetic diversity in populations from xerophytic and halophytic plant communities differs significantly for both species. Decline in genetic differentiation and increased gene flow were found in sub-optimal habitats for both species (xerophytic plant communities for *S. incanescens* and halophytic communities for *S. paulsenii*). This indicates that natural selection may play a significant role in shaping the genetic structure of the populations of the species studied. Further, our data highlight that genetic diversity of desert annual *Salsola* species may be affected by environmental stresses.

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References

Akhani H., Edwards G. & Roalson E.H. 2007. Diversification of the old world Salsoleae s.l. (Chenopodiaceae): molecular phylogenetic analysis of nuclear and chloroplast data sets and a revised classification. Int. J. Plant Sci. **168:** 931–956.

Akzhygitova N.I., Breckle S.W., Winkler G., Volkova E.A., Wucherer W., Kurochkina L.Ja., Makulbekova G.B., Ogar N.P., Rachkovskaya E.I., Safronova I.N. & Khramtsov V.N. 2003. Botanical geography of Kazakhstan and Middle Asia (desert region), Boston-Spectr, St. Peterburg, 424 pp.

Aslam R., Bostan N., Nabgha-e-Amen Maleeha Matia & Safdar W. 2011. A critical review on halophytes: Salt tolerant plants. J. Medic. Plants Res. **5:** 7108–7118.

Barrett S.C.H. & Charlesworth D. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature **352:** 522–524.

Bazzaz F.A. & Sultan S.E. 1987. Ecological variation and the maintenance of plant diversity, pp. 69–93. In Urbanska K.M. (ed.) Differentiation Patterns in Higher Plants, Academic Press, London, UK.

Christmann S., Martius C., Bedoshvili D., Bobojonov I., Carli C., Devkota K., Ibragimov Z., Khalikulov Z., Kienzler K., Manthrithilake H., Mavlyanova R., Mirzabaev A., Nishanov N., Sharma R.C., Tashpulatova B., Toderich K. & Turdieva M. 2009. Food Security and Climate Change in Central Asia and the Caucasus, CGIAR-PFU, ICARDA-CAC, Tashkent, 75 pp.

De la Torre F., Suarez M.F., de Santis L. & Canovas F.M. 2007. The aspartate minotransferase family in conifers: biochemical

analysis of a prokaryotic-type enzyme from maritime pine. Tree Physiol. **27:** 1283–1291.

Dostalek T., Munzbergova Z. & Plactova I. 2014. High genetic diversity in isolated populations of *Thesium ebracteatum* at the edge of its distribution range. Conservation Genet. **15:** 75–86.

Doubnerova V. & Ryslava H. 2011. What can enzymes of C4 photosynthesis do for C3 plants under stress? Plant Sci. **180:** 575–583.

Ellstrand N.C. & Elam D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Ann. Rev. Ecol. System. **24:** 217–242.

Flora of Uzbekistan (1953) Nauka, Tashkent, 548 pp.

Flowers T.J. & Colmer T.D. 2008. Salinity tolerance in halophytes. New Phytol. **179:** 945–963.

Gintzburger G., Toderich K.N., Mardonov B.K. & Makhmudov M.M. 2003. Rangelands of the arid and semi-arid zones in Uzbekistan, Centre de Cooperation Internationale en Resherche Agronomique pour le Development (CIRAD), Monpellier, 498 pp.

Gao S., Liu K.-T., Chung T.-W. & Chen F. 2013. The effects of NaCl stress on *Jatropha* cotyledon growth and nitrogen metabolism. J. Soil Sci. Plant Nutr. **13:** 99–113.

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

IPCC, 2014: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Field C.B. et al. (eds), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp.

Kim S.T. & Chung M.G. 1995. Genetic variation and population structure in Korean populations of sand dune species *Salsola komarovi* (Chenopodiaceae). J. Plant Res. **108:** 195–203.

Kusano M., Fukushima A., Redestig H. & Saito K. 2011. Metabolomic approaches toward understanding nitrogen metabolism in plants. J. Exp. Bot. **62:** 1439–1453.

Lega M., Fior S., Li M., Leonardi S. & Varotto C. 2014. Genetic drift linked to heterogeneous landscape and ecological specialization drives diversification in the Alpine endemic columbine *Aquilegia thalictrifolia*. Heredity **105:** 542–554.

Leonardi S., Piovani P., Scalfi M., Piotti A., Giannini R. & Menozzi P. 2012. Effect of habitat fragmentation on the genetic diversity and structure of peripheral populations of beech in Central Italy. Heredity **103:** 408–417.

Linhart Y.B. & Grant M.C. 1996. Evolutionary significance of local genetic differentiation in plants. Ann. Rev. Ecol. System. **27:** 237–277.

Mandak B., Bimova K., Plackova I., Mahelka V. & Chrtek J. 2005. Loss of Genetic Variation in Geographically Marginal Populations of Atriplex tatarica (Chenopodiaceae). An. Bot. **96:** 901–912.

Mandak B., Bimova K., Mahelka V. & Plackova I. 2006. How much genetic variation is stored in the seed bank? A study of *Atriplex tatarica* (Chenopodiaceae). Mol. Ecol. **15:** 2653–2663.

Marden J.H. 2013. Nature's inordinate fondness for metabolic enzymes: why metabolic enzyme loci are so frequently targets of selection. Mol. Ecol. **22:** 5743–5764.

Masel J. 2011. Genetic drift. Curr. Biol. **21:** R837–R838.

Mitton J.B. & Grant M.C. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Ann. Rev. Ecol. System. **15:** 479–499.

Muona O. & Szmidt A. 1985. A multilocus study of natural populations of *Pinus sylvestris*. Population genetics in forestry. Lecture Notes in Biomathematics **60:** 226–240.

Nazareno A.G. & Reis M.S.D. 2014. At risk of population decline? An ecological and genetic approach to the threatened palm species *Butia eriospatha* (Arecaceae) of Southern Brazil. Heredity **105:** 120–129.

Nei M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, 512 pp.

Nei M. & Roychoudhury A.K. 1974. Sampling variances of heterozygosity and genetic distance. Genetics **76:** 379–390.

Nevo E., Brown A.H.D., Zohary D., Storch N. & Beiles A. 1981. Microgeographic edaphic differentiation an allozyme polymorphysms of wild barley (*Hordeum spontaneum*, Poaceae). Plant Syst. Evol. **138:** 287–292.

Nevo E., Krugman T. & Beiles A. 1994. Edaphic natural selection of allozyme polymorphisms in *Aegilops peregrina* at a Galilee microsite in Israel. Heredity **72:** 109–112.

Odat N., Jetschke G. & Hellwig F.H. 2004. Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities. Mol. Ecol. **13:** 1251–1257.

Ouborg N.J., Vergeer P. & Mix C. 2006. The rough edges of the conservation genetics paradigm for plants. J. Ecol. **94:** 1233–1248.

Peakall R. & Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes **6:** 288–295.

Prentice H.C. & Cramer W. 1990. The plant community as a niche bioassay: environmental correlates of local variation in *Gypsophila fastigiata*. J. Ecol. **78:** 313–325.

Prentice H.C., Lonn M., Lefkovitch L.P. & Runyeon H. 1995. Associations between allele frequencies in *Festuca ovina* and habitat variation in the alvar grass-lands on the Baltic island of Oland. J. Ecol. **83:** 391–401.

Prentice H.C., Lonn M., Lager H., Rosen E. & van der Maarel E. 2000. Changes in allozyme frequencies in *Festuca ovina* populations after a 9-year nutrient/water experiment. J. Ecol. **88:** 331–347.

Rasooli B. & Jafari M. 2009. Investigation of the variations of soil factors under *Haloxylon aphyllum* cultivation. Desert **14:** 113–117.

Ryan F.J. & Ayres D.R. 2000. Molecular markers indicate two cryptic, genetically divergent populations of Russian thistle (*Salsola tragus*) in California. Can. J. Bot. **78:** 59–67.

Soltis D.E. & Soltis P.S. 1990. Isozymes in plant biology. Springer, London, 268 pp.

Song B. & Zang Z. 2007. Measuring morphology and genetic biodiversity of *Suaeda salsa* population in the Huanghue River Delta. Rus. J. Ecol. **4:** 277–284.

Spooner D., van Treuren R. & de Vicente M.C. 2005. Molecular markers for genebank management. IPGRI Technical Bulletin No. 10. International Plant Genetic Resources Institute, Rome, Italy.

Stern N. 2006. Review on the economics of climate change. Cambridge, 576 pp.

Shuyskaya E.V., Gismatullina L.G., Toderich K.N., Voronin P.Yu. & Soldatova N.V. 2012. Genetic Differentiation of Black Saxaul, *Haloxylon aphyllum* (Chenopodiaceae), along a Soil Salinity Gradient in the Kyzylkum Desert. Rus. J. Ecol. **43:** 302–306.

Toderich K.N., Shuyskaya E.V., Ismail S., Gismatullina L.G., Radjabov T., Bekchanov B.B. & Aralova D.B. 2009. Phytogenic resources of halophytes of Central Asia and their role for rehabilitation of sandy desert degraded rangelands. J. Land Degrad. Develop. **20:** 386–396.

Toderich K.N., Shuyskaya E.V., Faisal Taha, Shoaib Ismail, Gismatullina L.G. & Li E.V. 2012. Adaptive fruit structural mechanisms of asiatic Salsola species and its germplasm conservation and utilization. J. Arid Land Studies. **22:** 73–76.

Van Valen L. 1965. Morphological variation and width of ecological niche. Am. Natural. **99:** 377–390.

Wojnicka-Poltorak A., Chudzinska E., Shuiskay E., Barchak H., Toderich K.N. & Prus-Glowacki W. 2002. Izoenzymatic and cytolodical studies of some Asiatic species of the genus *Salsola*. Acta Soc. Botanic. Polonae **71:** 115–120.

Wolff S.L. & Jefferies R.L. 1987. Morphological and isozyme variation in *Salicornia europaea* (s.l.) (Chenopodiaceae) in North America. Can. J. Bot. **65:** 1410–1419.

Wright S. 1984. Evolution and the genetics of populations. Univ. Chicago Press, Chicago, 511.

Yeh F.C., Yang R.C. & Boyle T. 1999. POPGEN, version 1.32. Microsoft Windows-based freeware for population genetic analysis. University of Alberta/CIFOR, Edmonton.

Yue D., Hui C. & Li Z. 2004. Niche construction for desert plants in individual and population scales: theoretical analysis and evidences from saksaul (*Haloxylon ammodendron*) forest. Isr. J. Plant Sci. **52:** 235–244.

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