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Genetic Differentiation of Black Saxaul, *Haloxylon aphyllum* (Chenopodiaceae), along a Soil Salinity Gradient in the Kyzylkum Desert

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Abstract—Populations of *Haloxylon àphyllum* in the Kyzylkum Desert have been found to be markedly deficient in heterozygotes at a medium level of genetic diversity ($P_{95} = 0.56$, $A = 1.67$, $H_o = 0.14$, $H_e = 0.28$). Spatial genetic differentiation of these populations have been revealed along a soil salinity gradient (from 0 to 0.5 mmol Na^+/g), with their genetic diversity reaching a maximum ($H_o = 0.21–0.25$, $H_e = 0.25–0.27$) in areas with a moderate salinity level (0.05–0.1 mmol Na^+/g). Locus Got-2 can serve as a marker of this differentiation ($Fst_{Got-2} = 0.4$).

Keywords: genetic differentiation, *Haloxylon aphyllum*, soil salinity, the Kyzylkum Desert

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The Kyzylkum Desert lies in the Turan Lowland, Central Asia. Its landscape is a plain crossed by sets of aligned low hills and closed depressions. The vegetation has a complex spatial structure, which is conditioned by micro- and mesotopographic heterogeneity of the environment and associated processes, such as salinization and desalinization, changes in groundwater table depth, etc. (Akzhigitova et al., 2003). Species with the C_4 type of photosynthesis are an essential component of halophyte–xerophyte plant communities indicative of salinization–desalinization processes in Kyzylkum soils (Akzhigitova et al., 2003; Toderich et al., 2009).

Among these plants, a special place belongs to the black saxaul, *Haloxylon aphyllum* (Minkw.) Iljin (Chenopodiaceae), a major edificator species that also plays a very important role as a soil-stabilizing and forage plant and a source of firewood for local residents (Nikitin, 1966; Gintzburger et al., 2003). This treelike shrub (1–9 m tall) with succulent photosynthesizing shoots can grow in topographic depressions on sandy, solonchak, or gypsum soils, forming large populations extending over tens of kilometers (Nikitin, 1966; Gintzburger et al., 2003). Natural populations have a certain optimum of genetic diversity established in the course of evolution, and disturbances in the genetic balance of a population system lead to its degradation, which has consequences for the state of the species and ecosystem as a whole (Altukhov, 2003). To gain an insight into such microevolutionary processes, it is necessary to identify ecological factors responsible for local genetic differentiation of populations, which takes place in response to heterogeneity of the environment (e.g., differences in moisture or nutrient supply) manifested even within distances of no more than several hundreds of meters (Nevo, Krugman, and Beiles, 1994; Mitton, Grant, and Yoshino, 1998; Prentice et al., 2000).

The effect of soil salinity on local genetic differentiation of populations has not been studied sufficiently. Therefore, the purpose of this study was to analyze the role of this factor in the genetic diversity and differentiation of *H. aphyllum* populations.

MATERIAL AND METHODS

The plant material was collected in two *H. aphyllum* populations named Kyzylkesek (41°10′ N, 64°90′ E; annual average air temperature 15.7°C, annual average precipitation 175 mm) and Bukhara (39°34′ N, 64°42′ E; annual average air temperature 15.3°C, annual average precipitation 157 mm) (Gintzburger et al., 2003). The contents of sodium ions (Na^+) in photosynthetic organs of *H. aphyllum* and in three soil horizons (0–20, 20–40, and 40–60 cm) were determined using a Hitachi 207 atomic absorption spec-

trometer (Japan). Measurements were made in water extracts from 100-mg samples. In each population, we delimited three areas with similar sandy soils and pit- and mound topography but with different levels of soil salinity (Fig. 1). In each area (subpopulation), seeds from 10–25 individual plants were collected for analysis. The distances between the plants and subpopulations varied from 5 to 50 m and from 1 to 5 km, respectively. The total sample consisted of 50–147 seeds per subpopulation (165–275 seeds per population).

For electrophoretic allozyme analysis, the seeds were cleaned of their wings, 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. Pieces of Whatman M3 paper (11 × 12 mm) were soaked in the homogenate and placed into sample wells. Enzymes were separated in 10% starch gel using two buffer systems (Muona and Szmidt, 1985). In system 1, the electrode buffer was 160 mM Tris–50 mM citric acid, pH 8.0; the gel buffer was prepared by diluting 10 ml of the electrode buffer with 90 ml H2O. In system 2, the electrode buffer was 300 mM boric acid–60 mM NaOH, pH 8.2; the gel buffer was 80 mM Tris–9 mM citric acid, pH 8.7. Electrophoresis was performed at 90 V, 40–50 mA in buffer system 1 or at 210 V, 70–80 mA in buffer system 2 for 4–6 hours at 5°C.

Seven enzyme systems were analyzed: glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), diaphorase (DIA, EC 1.6.99), glutamate dehydrogenase (GDH, EC 1.4.1.2), superoxide dismutase (SOD, 1.15.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), and phosphoglucomutase (PGM, EC 5.4.2.2). Histochemical staining and genetic interpretation of the results were performed as described (Goncharenko, Padutov, and Potenko, 1993; Wojnicka-Poltorak et al., 2002), with certain modifications. The zones of enzyme activity and corresponding gene loci were designated by enzyme acronyms and numbered in order of decreasing electrophoretic mobility, from anode to cathode. Alleles were designated according to the mobility of encoded allozymes relative to that of the most widespread variant, which was taken as 100 arbitrary units. The level of genetic variation was estimated by calculating the following parameters: the proportion of polymorphic loci (P_{95}), the average number of alleles per locus (A), the effective number of alleles (n_e), and the average observed (H_o) and expected (H_e) heterozygosities. To analyze the structure and differentiation (subdivision) of populations, Wright's (1984) indices Fis, Fit, and Fst were used. The significance of differences in allele and genotype frequencies between the samples was evaluated by χ^2 test for heterogeneity.

RESULTS

Allozyme variation. Analysis of seven enzyme systems in the two *H. aphyllum* revealed nine loci; four of them (*Got-1, Sod, G6pd-1*, and *G6pd-2*) were monomorphic in both populations, and one (*Pgm*), only in the Kyzylkesek (K) population. Other loci were represented in populations and subpopulations by two to three alleles occurring with different frequencies (Table 1). On the whole, we revealed 16 alleles, including 10 alleles common to all subpopulations. Alleles Dia^{90}, $6pgd^{95}$, and Pgm^{95} were absent in the K population, and allele Dia^{110}, in the Bukhara (B) population. Allele Got-2^{90} was found in subpopulations 2K, 3K, and 2B. A total of 18 genotypes were detected in both populations. Two of them were heterozygous (Got-$2^{100/90}$ and $Gdh^{100/90}$) and occurred most frequently in subpopulations 2K and 2B (Table 1). The results of χ^2 tests confirmed the diagnostic role of *Dia, 6pgd*, and *Pgm* loci at the population level. Subpopulations within each of the two populations (K and B) proved to differ significantly at loci *Got-2, Dia*, and, to a lesser extent, *6pgd* and *Pgm*.

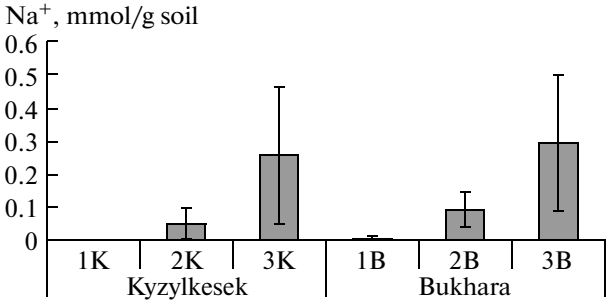

Fig. 1. Sodium ion contents in soils under *Haloxylon aphyllum* populations Kyzylkesek (the central Kyzylkum, Navoi region, Uzbekistan; 41°10′ N, 64°90′ E) and Bukhara (the southwestern Kyzylkum, Bukhara region, Uzbekistan; 39°34′ N, 64°42′ E) and their subpopulations (1K–3K and 1B–3B) growing in areas with different levels of soil salinity.

Genetic diversity and population structure. The proportion of polymorphic loci (P_{95}) in both populations averaged 0.56, with the number of alleles per locus (A) and the effective number of alleles averaging 1.67 and 1.39, respectively (Table 2). The average observed heterozygosity proved to be lower than the expected heterozygosity: H_o= 0.11–0.17 vs. H_e = 0.20–0.27, which was indicative of deficiency in heterozygotes relative to the Hardy–Weinberg equilibrium. In both populations, all parameters of genetic variation were higher in subpopulations growing on moderately salinized soils (2K and 2B) (Table 2). The most significant differences were revealed in the level of heterozygosity: $H_o = 0.21–0.25$, $H_e = 0.25–0.27$ for 2K and 2B, and $H_o = 0.03–0.11$, $H_e = 0.08–0.14$ for 1K, 3K, 1B, and 3B. The main contribution to these differences was made by heterozygosity at locus *Got-2*, while the level of heterozygosity at *Gdh* significantly differed only between K subpopulations. Unlike the situation in whole populations, differences between the values of observed and expected heterozygosity in

Table 1. Allele frequencies at polymorphic loci and results of χ^2 test for their heterogeneity in populations and subpopulations of *Haloxylon aphyllum*

Locus	Allele/genotype*	Population							
		Kyzylkesek				Bukhara			
		1K	2K	3K	total	1B	2B	3B	total
Got-2	90	–	0.50	0.08	0.23	–	0.50	–	0.16
	100	1.00^{h}	0.50^{a}	$0.92^{a,b}$	0.77	$1.00^{b,c,j}$	$0.50^{c,d,j}$	$1.00^{b,f,j}$	0.84
	100/90*	–	1.00**	0.16	0.46	–	1.00**	–	0.33
Gdh	90	0.44	0.67	0.69	0.61	0.60	0.53	0.56	0.57
	100	0.56	0.33^{a}	0.31^{a}	0.39	0.40^{j}	0.47	0.44	0.43
	100/90*	0.21	0.44**	0.24	0.31	0.79	0.94	0.87	0.87
Dia	90	–	–	–	–	$0.07^{c,j}$	–	$0.71^{c,d,f}$	0.25
	100	1.00^{h}	0.45^{a}	1.00^{b}	0.81^{j}	$0.93^{a,b}$	$1.00^{b,d,j}$	$0.29^{a,b}$	0.75
	110	–	0.55	–	0.19	–	–	–	–
6pgd	95	–	–	–	–	–	$0.76^{c,d,j}$	–	0.24
	100	1.00	1.00	$0.71^{a,b}$	0.89^{j}	$0.13^{a,b}$	$0.03^{a,b}$	$0.06^{a,b}$	0.08
	110	–	–	0.29	0.11	$0.87^{c,j}$	0.21	$0.94^{c,f}$	0.68
Pgm	95	–	–	–	–	–	$0.42^{a,b,c}$	–	0.14
	100	1.00	1.00	1.00^{j}	1.00	1.00	$0.58^{d,f,j}$	1.00	0.86

Note: * Heterozygous genotype frequency; **differences from 1K and 3K are significant at $P < 0.05$; superscript letters indicate significant differences from (a) 1K, (b) 2K, (c) 3K, (d) 1B, (f) 2B, (j) B, and (h) K ($P < 0.05$–0.001).

Table 2. Parameters of genetic variation in *Haloxylon aphyllum* populations

Population	Number of individuals	Proportion of polymorphic loci(P_{95})	Average number of alleles per locus (A)	Effective number of alleles (n_e)	Average observed heterozygosity (H_o)	Average expected heterozygosity (H_e)
Kyzylkesek	275	0.44	1.33	1.24	0.11*	0.20
1K	60	0.11	1.00	1.09	0.03	0.08
2K	147	0.33	1.22	1.22	0.21	0.25
3K	68	0.33	1.11	1.16	0.06	0.14
Bukhara	165	0.56	1.56	1.36	0.17*	0.27
1B	52	0.33	1.22	1.14	0.11	0.12
2B	63	0.44	1.44	1.37	0.28	0.27
3B	50	0.33	1.22	1.17	0.12	0.14
Total for two populations	440	0.56	1.67	1.39	0.14	0.28

* $P < 0.05$.

subpopulations 2K, 1B, 2B, and 3B were insignificant, indicating that they are in a balanced state according to the Hardy–Weinberg law (Table 2).

Analysis of *H. aphyllum* intrapopulation differentiation with respect to two heterozygous loci revealed a considerable excess of heterozygotes at *Got-2* (Fis = –0.8…–1) in both populations (Fig. 2). Heterozygotes at *Gdh* were in excess in B subpopulations (Fis = –0.77) but proved to be in deficit in K subpopulations (Fis = 0.34). The inbreeding coefficient of an individual relative to population (Fit) indicated a slight excess in heterozygotes at *Got-2* (Fit = –0.1...–0.2). According to the coefficient of local population subdivision Fst, approximately 40% of variation at *Got-2* in both

populations was accounted for by variation among subpopulations (Fig. 2).

DISCUSSION

Genetic polymorphism. In general, basic parameters of *H. aphyllum* genetic diversity (except observed heterozygosity H_o) are at the average level recorded for species cross-pollinated by wind ($P_{95} = 0.49–0.66$, $A = 1.79–2.40$) (Hamrick, Godt, and Sherman-Broyles, 1992) but exceed those in arid Chenopodiaceae species such as *Salsola praecox, S. pestifer*, and *S. lanata* ($P_{95} = 0.1–0.2$, $H_o = 0.03–0.16$; Wojnicka-Poltorak et al., 2002) and in *Chenopodium* species ($P_{95} = 0.1–0.3$; Crawford and Wilson, 1979). The deficiency in heterozygotes revealed in *H. aphyllum* is not characteristic either of wind cross-pollinated species (on average, $H_o = 0.26–0.29$, $H_e = 0.15–0.16$) (Hamrick, Godt, and Sherman-Broyles, 1992) or of *S. praecox, S. pestifer*, and *S. lanata*. However, a similar shift of population genetic structure from the Hardy–Weinberg equilibrium toward inbreeding (*S. salsa* ($H_o = 0.08$, $H_e = 0.18$) has been observed in *S. salsa* (Zong and Zang, 2007). This is evidence for exposure to strong stress and consequent selection in favor of homozygotes, which are better adapted to the impact of this factor (Altukhov, 2003).

Intrapopulation variation and differentiation. Differences in the level of genetic diversity and, especially, heterozygosity among *H. aphyllum* subpopulations are indicative of local adaptation and genetic differentiation within populations. A relationship has been revealed between genetic variation and heterogeneity of the environment, primarily with respect to the amounts of accessible moisture (Nevo, Krugman, and Beiles, 1994; Mitton, Grant, and Yoshino, 1998) and soil nutrients (Prentice et al., 2000; Devi, Sinclair, and Valdez, 2010; Fisher et al., 2010). Therefore, microgeographic differentiation observed within *H. aphyllum* populations may be explained by heterogeneity of environmental factors that (1) account for differences in stress load between habitats, which provide for genetic heterogeneity, and (2) create landscape barriers to gene flow, thereby contributing to genetic differentiation among semi-isolated populations (Linhart and Grant, 1996; Altukhov, 2003). In view of small distances between subpopulations in both *H. aphyllum* populations and the absence of landscape barriers to cross pollination and winged seed dispersal by wind, genetic differentiation due to geographic isolation is hardly probable. However, the fact that the genetic structure of individual subpopulations is in a Hardy–Weinberg equilibrium provides evidence for subdivision of *H. aphyllum* populations into panmictic groups, i.e., for their local genetic differentiation, Moreover, both these populations show the Wahlund effect, whose essence is that subdivision of a population into individual randomly mating groups is formally equivalent to inbreeding in the population as a whole (Wahlund, 1928; cited from Altukhov, 2003, p. 38).

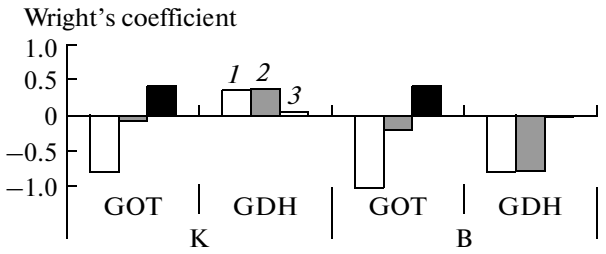

Fig 2. Differentiation of *Haloxylon aphyllum* populations according to Wright's coefficients (*1*) Fis, (*2*) Fit, and (*3*) Fst.

The fact that the level of heterozygosity is more than 50% higher in subpopulations growing on moderately saline soils, compared to other subpopulations, is evidence that such ecological conditions are optimal for *H. aphyllum*, whereas low or high soil salinities are pessimal and affect the genetic diversity of its populations, resulting in a significant reduction of heterozygosity. Such nonlinear correlations in the level of genetic diversity have been described for wild wheat in a gradient of atmospheric precipitation (Peleg et al., 2008). These authors interpret their results in the context of the intermediate-disturbance hypothesis (IDH) (Grime, 1977; Connell, 1978; Huston, 1979), according to which biological diversity reaches a peak in ecosystems exposed to moderate stress, in contrast to low- or high-stress ecosystems. The results of our studies can also be considered within the framework of this hypothesis, provided soil salinity is a factor determining genetic differentiation of *H. aphyllum* populations.

Role of soil salinity. Under conditions of the study region, two stress factors are likely to affect *H. aphyllum* plants: soil salinization itself and deficit of accessible soil moisture. Salinized soils contain a variety of salts (Na_2SO_4, $MgSO_4$, $CaSO_4$, KCl, Na_2CO_3, etc.), but NaCl prevails, and the toxic effect of soil salinity on plants is due primarily to the accumulation of Na^+ ions (Zhang, Flowers, and Wang, 2010). To check the effect of this factor on genetic subdivision of *H. aphyllum* populations, we analyzed Na^+ accumulation by green plant parts along the gradient of soil salinity. The results showed, however, that the contents of these ions in plants from all subpopulations were approximately equal (Fig. 3), being apparently determined by the limit of *H. aphyllum* capacity to absorb water from the soil. Plants growing on a physically dry soil (e.g., on microelevations) or in moist but salinized habitats (most solonchaks) experience water stress due to the same cause, namely, a low water potential of the soil. The relationship between soil salinity and soil water potential is nonlinear, since the water potential of dry soil can be equal to or even higher than that of solonchak. Therefore, the accessibility of moisture to plants is determined by such a combination of soil moisture and salinity at which the water potential of the soil is higher than that of photosynthetic plant organs. In our model, these conditions are satisfied in areas with

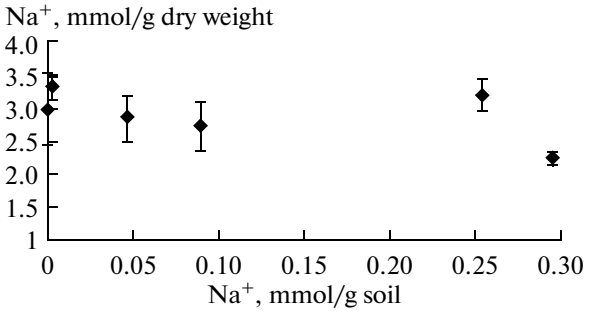

Fig. 3. Sodium ion contents in photosynthetic organs of *Haloxylon aphyllum* along soil salinity gradient.

moderate soil salinity, where the genetic diversity of *H. aphyllum* reaches the highest level.

Thus, soils with moderate salinity appear to be optimal for *H. aphyllum* because subpopulations growing on such soils are the main reserve of ecological plasticity for the population as a whole (21–28% heterozygosity). The general deficit of heterozygotes in *H. aphyllum* populations develops due to their subdivision into panmictic groups (the Wahlund effect). Locus *Got-2* can serve as a marker of this subdivision, since Wright's Fst for this locus (Fst = 0.4) is indicative of its selective significance or linkage with adaptively important genes..

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