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RESEARCH ARTICLE

Fine-Scale Spatial Genetic Structure in Emmer Wheat and the Role of Population Range Position

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Abstract The extent of spatial genetic structure (SGS) within plant populations depends on seed and pollen dispersal distance, breeding type, level of self-fertilization and effective plant density. Self-fertilizing species with gravity-dispersed seeds are expected to have both small effective population sizes and low pollen movement leading to high genetic structure. Higher SGS can be expected in more patchy and peripheral populations because of lower plant density and population sizes, and lower intensity of gene flow. We tested these predictions analyzing SGS in two core and two peripheral populations of predominantly self-fertilizing emmer wheat. Analysis of SGS with 11 nuclear microsatellites revealed (1) a negative linear relationship between kinship coefficients, calculated for pairs of individuals, and the logarithm of geographical distance between members of the pairs, in all studied populations; and (2) a significant autocorrelation for a distance up to 5 m (core populations) or 20 m (peripheral populations). Pollen flow, estimated from comparison of nuclear and chloroplast variation, was spatially limited, as was seed dispersal. Our results support a hypothesized relationship between SGS intensity and breeding system, the mode of seed dispersal and the population range position (core vs. periphery).

Keywords Gene flow · Neighborhood size · Pollen flow · *Sp* statistics · Spatial autocorrelation

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Introduction

Analysis of gene dispersal is important for understanding the role of gene flow in species distribution over space. Gene dispersal at the fine scale (i.e. within populations) is particularly important for detecting a spatial scale at which major gene dispersal occurs and this scale is inferred from a change in genetic distance or relatedness among neighboring individuals, compared with more spatially distant individuals. An interplay between limited gene flow and local genetic drift leads to a balance which, according to the theory of isolation by distance, results in spatial genetic structure (SGS). The rate of decrease of genetic similarity with distance is a measure of strength of SGS (Loiselle et al. 1995; Rousset 2000; Hardy 2003). The extent of SGS within plant populations depends on seed and pollen dispersal distance, breeding type, level of self-fertilization and effective plant density (Vekemans and Hardy 2004).

The relative contribution of contemporary seed and pollen dispersal to gene flow, despite recognized importance for plant population demographic and genetic processes is still poorly understood (but see Doligez et al. 1998; Ravigne et al. 2006; Epperson 2007; Lopez et al. 2008). Especially lacking are studies investigating a relationship among seed/pollen dispersal, breeding type and population size/range position. Self-fertilizing species are expected to have both smaller effective populations sizes (Ingvarsson 2002) and lower pollen movement leading to higher genetic structure than out-crossing species (Hamrick and Godt 1996). Higher SGS can be expected in more patchy and peripheral populations because of lower plant density and population sizes, and lower intensity of gene flow (Doligez et al. 1998; Vekemans and Hardy 2004). Although still limited, there are increasing reports on SGS in fragmented versus continuous (Williams et al. 2007;

Born et al. 2008; De-Lucas et al. 2009) and core versus peripheral populations (Gapare and Aitken 2005; Pandey and Rajora 2012).

Here we investigate gene dispersal patterns in an annual grass *Triticum turgidum* L. ssp. *dicoccoides* (hereafter *T. diccocoides*). This species is an ideal model species to study SGS in core versus peripheral populations of a predominantly self-fertilizing plant species. Distribution of this species is well known (Özkan et al. 2011) with peripheral populations being smaller, more isolated and patchily distributed than core populations (Feldman and Kislev 2007; Özkan et al. 2011). *T. diccocoides* was intensively studied for extent and structure of genetic variation at large (Fahima et al. 2002; Ozbek et al. 2007, 2011) and fine geographical scale (Anikster et al. 1991; Felsenburg et al. 1991; Nevo et al. 1991; Li et al. 2000a, b, c; Volis et al. 2004). However, a majority of the studies conducted at the fine geographical scale examined an association between environmental parameters (e.g. topography, soil type) and allele frequencies using different molecular markers, and only one analyzed the spatial structure and assessed an intensity of gene flow in this species (Golenberg 1987). In this study, a sharp decrease in gene flow was observed beyond the first distance class of 5–7 m. However, this study was done in a single population with limited sample size and distance coverage and using low polymorphism markers (allozymes).

The aim of the present study was to analyze SGS and assess impact of seed and pollen dispersal on the pattern of genetic diversity in core versus peripheral populations of *T. diccocoides* using SSR markers. First, we investigated the scale of within-population gene flow by multivariate spatial autocorrelation analysis and a test for isolation by distance in two core and two peripheral populations. Second, we estimated the relative contributions of pollen-mediated and seed-mediated gene flow by comparing the patterns revealed by nuclear versus chromosomal SSR markers. Our hypotheses were: (1) since *T. diccocoides* is predominantly (>95 %) self-pollinated with gravity dispersed seeds, this species is expected to have fine-scale within-population SGS with low pollen to seed migration ratio; (2) the peripheral populations of the species are expected to have higher levels of SGS than the core populations due to their lower population size and density; and potentially restricted gene dispersal.

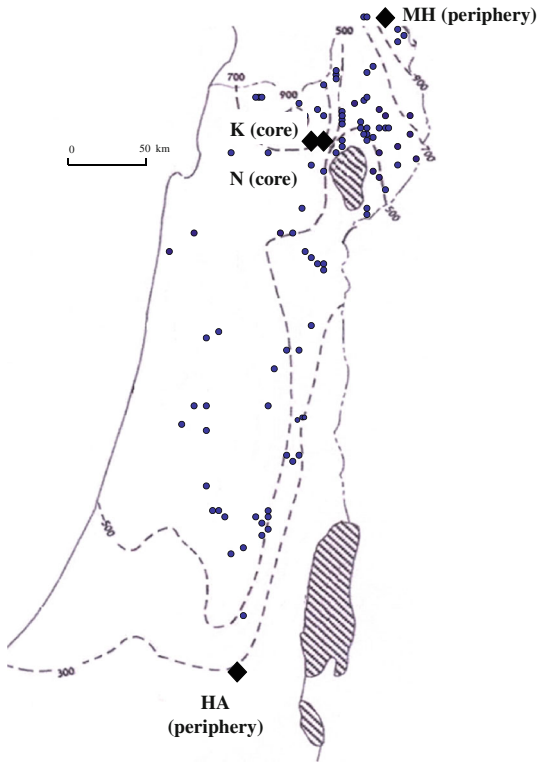

Fig. 1 Map of Israel showing isohyets of multiyear averages of annual rainfall amount (mm), distribution of *T. diccocoides* and study populations. Dots mark known populations of *T. diccocoides* based on data of the Institute for Cereal Improvement (Israel)

Materials and Methods

Study Species and Sampling

Wild emmer wheat, *T. turgidum* L. ssp. *dicoccoides* is a predominantly self-pollinating (>95 % for the whole *Triticum-Aegilops* group, Mendlinger and Zohary 1995) annual grass in which spikes at maturity disarticulate into arrow-shaped dispersal units (spikelets) having a sharp-edged rachis and containing 2 or rarely 3 grains. The spikelets are shattered close to the mother plant and the awns balance and propel them into the ground as they fall (Elbaum et al. 2007).

The species is found in habitats with annual precipitation ranging 200 to over 1,300 mm, at altitudes between −100 and 1,400 m, and in several soil types, although most populations are found on terra-rossa and basalt soils (Feldman and Sears 1981; Feldman and Kislev 2007). We have chosen two populations representing the species distributional core in the Upper Jordan Valley catchment area (Ammiad Karst and Ammiad North), and two populations from the two opposite edges of species distributional range (cold edge Mount Hermon and hot edge Har Amasa; Fig. 1).

Sampling was done in 2007. From each sampled plant we took a separately bagged spike and precise GIS coordinates of its location. The number of sampled plants was 70, 68, 57 and 94 (K, N, MH and HA locations, respectively).

Genetic Markers

DNA was extracted from fresh leaves of 6 week old plants using the modified CTAB protocol of Rogers and Benedich 1985). Eleven polymorphic nuclear (Röder et al. 1995,

1998) and four chloroplast (Ishii et al. 2001) SSR loci (Table 1) were amplified with polymerase chain reaction (PCR) (Röder et al. 1995; Schuelke 2000). The PCR products were detected and sized by the ABI PRISM 3700 DNA Analyzer at the Hebrew University, Jerusalem, Israel. The data were analyzed using Peak Scanner™ Software v1.0 (Applied Biosystems).

Statistical Analysis

We employed two approaches for detecting and describing SGS in the studied populations. Analysis of spatial autocorrelation utilized the multivariate procedure of Smouse and Peakall (1999) and Peakall et al. (2003). The SGS patterns were compared between populations in a pair-wise fashion as described by Smouse et al. (2008) using the test of heterogeneity of spatial autocorrelation. The statistical significance of autocorrelation coefficient (r) for each of seven distance classes and correlogram heterogeneity test criteria (ω) was tested by 10,000 random permutations and obtaining 95 % confidence intervals after bootstrapping (10,000 repeats). The analyses were conducted by GENALEX 6.5 software (Peakall and Smouse 2006).

Further assessment of SGS followed the procedure described by Vekemans and Hardy (2004), based on pair-wise kinship coefficients between individuals and implemented in the SPAGEDI software (Hardy and Vekemans 2002). The multi-locus kinship coefficient values calculated as described in Loiselle et al. (1995) were regressed on the natural logarithm of geographic distance separating the two individuals to estimate the regression slopes (b). To visualize SGS, kinship coefficient values were averaged over a set of seven distance classes and plotted against geographical distance. A jackknife procedure (over loci) was used to estimate standard errors and 10,000 permutations to assess significance of the each distance class multi-locus kinship coefficient.

The extent of gene dispersal was estimated from the regression slope as $Nb = -(1 - F_0)/b$ where Nb is the neighborhood size in terms of number of individuals, in a continuous two-dimensional population (Wright, 1943), and F_0 is the average kinship coefficient between adjacent individuals. In our study, we estimated F_0 for the first distance class (1 m distance) as the closest approximation to "adjacent" plants (Vekemans and Hardy 2004; Oddou-Muratorio and Klein 2008) and designated it $F_{(1)}$. The lower and upper bounds for the 95 % confidence intervals (CI) of Nb were computed as $(F_{(1)} - 1)/(b \pm 2\mathrm{SE}_b)$, SE_b being the standard error of the regression slope b (Hardy et al. 2006). We also calculated the 'Sp' statistic, which is the inverse of the neighborhood size Nb under isolation by distance in two-dimensional space (Vekemans and Hardy 2004), i.e. the ratio $-b/(1 - F_{(1)})$. This statistic is very useful in comparison of SGS and gene dispersal across populations and species.

Gene dispersal under migration-drift equilibrium σ was estimated using an iterative approach implemented in the SPAGEDI software. Estimation of σ requires knowledge of effective population density D_e. The effective density was estimated from the adult population density, D, using approximation $D \cdots N_e/N$ (ratio of the effective to the census population size) (Hardy et al. 2006). Under self-fertilization N_e is predicted to equal $N(2\text{-}s)/2$ (Pollak 1987), thus in a predominantly self-fertilizing species $N_e \approx N/2$. As density of emmer varies not only spatially but also from year to year (Noy-Meir et al. 1991), we used a range of D (2, 4 and 10 plants/m^2) for calculation of $D_e = D/2$. These values are representative of the plant density observed in the field (Noy-Meir et al. 1991).

The effective population size (*Ne*) for each of the four populations was estimated from nuclear SSR data using the maximum likelihood approach of MIGRATE program (Beerli and Felsenstein 2001). The *Ne* was calculated from the number of mutants per generation θ (i.e. $4N_e\mu$) values using an average mutation rate for microsatellites in wheat of 10^{-4} per generation (Thuillet et al. 2002), as $\theta/(4 \times 10^{-4})$, assuming an infinite allele mutational model. For the estimation of θ, each run was conducted with ten short chains of 500 and three long chains of 5,000, and the burn-in at the beginning of each chain of 10,000. The migration matrix assumed one-directional gene flow from core (Ammiad population) to periphery (MH and HA populations), and bidirectional symmetric gene flow between K and N habitats. Ten simulations were performed and their results combined.

To calculate the ratio of pollen flow to seed flow, we used the formula from El Mousadik and Petit 1996): Pollen/seed migration ratio $= [2(1/\Phi_{STc} - 1)\text{–}(1/\Phi_{STn} - 1)]/(1 - 1/\Phi_{STc})$, where Φ_{STn} and Φ_{STc} are levels of among-population differentiation calculated from nuclear and chloroplast markers, respectively. Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to get the Φ_{STn} and Φ_{STc} values. Because of spatially limited seed dispersal in emmer (several meters from the mother plant) and predominant self-fertilizing, for estimation of contemporary and not historical gene flow we could not use the populations separated by tens and hundreds of kilometers. Therefore estimation of pollen to seed flow was done comparing population differentiation between N and K populations separated by about 1 km.

Results

Population Genetic Diversity

Genetic diversity statistics for the four populations are given in Table 1. The average number of alleles per locus A and expected heterozygosity He were similar in all

Table 1 Repeat motif and statistics of genetic diversity of SSR loci in the four populations

Locus	Repeat motif	Populations															
		HA (n = 88)				K (n = 68)				N (n = 63)				MH (n = 56)			
		A	Ho	He	F_I	A	Ho	He	F_I	A	Ho	He	F_I	A	Ho	He	F_I
Nuclear[a]																	
GWM018	(CA)nGA(TA)k	2	0	0.291	1	5	0	0.686	1	3	0	0.343	1	5	0	0.725	1
GWM095	(AC)n	4	0	0.628	1	5	0.074	0.618	0.881	3	0.016	0.344	0.954	2	0	0.427	1
GWM124	(CT)n(GT)k	2	0	0.167	1	4	0	0.648	1	4	0	0.653	1	4	0	0.659	1
GWM136	(CT)n	2	0	0.023	1	2	0	0.481	1	2	0	0.094	1	2	0	0.487	1
GWM162	(CA)nAA(CA)k	2	0	0.378	1	2	0	0.496	1	2	0	0.402	1	2	0	0.036	1
GWM186	(GA)n	6	0	0.739	1	5	0	0.588	1	6	0	0.731	1	6	0	0.719	1
GWM218	(CT)n	3	0.012	0.369	0.968	3	0	0.474	1	3	0.016	0.439	0.964	3	0.018	0.614	0.970
GWM251	(CA)n	2	0	0.317	1	3	0	0.384	1	3	0	0.148	1	3	0	0.510	1
GWM340	(GA)n	2	0	0.285	1	2	0	0.344	1	2	0	0.500	1	2	0	0.361	1
GWM540	(CT)n(CC)(CT)k	2	0	0.317	1	3	0	0.442	1	3	0.082	0.646	0.873	3	0	0.487	1
GWM537	(CA)n(TA)k	4	0	0.661	1	3	0	0.344	1	4	0	0.719	1	4	0	0.436	1
Locus mean (SE)		2.818 (0.400)	0.001 (0.001)	0.380 (0.065)	0.997 (0.003)	3.364 (0.364)	0.007 (0.007)	0.500 (0.036)	0.989 (0.011)	3.182 (0.352)	0.010 (0.007)	0.456 (0.066)	0.981 (0.012)	3.273 (0.407)	0.002 (0.002)	0.496 (0.059)	0.997 (0.003)
Chloroplast[b]																	
WCt2	(T)n	4		0.489		2		0.029		4		0.717		4		0.634	
WCt6	(C)n	3		0.292		3		0.382		2		0.200		3		0.484	
WCt13	(A)n	2		0.492		2		0.327		2		0.350		2		0.249	
WCt22	(T)n	3		0.282		3		0.485		3		0.580		3		0.385	
Locus mean (SE)		3.0 (0.408)		0.389 (0.059)		2.5 (0.289)		0.306 (0.098)		2.177 (0.514)		0.462 (0.116)		3.0 (0.408)		0.438 (0.081)	

Mean values are provided with SE obtained by jackknifing over loci (in parentheses)

A number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, F_I inbreeding coefficient

[a] Source reference Röder et al. (1998)

[b] Source reference Ishii et al. (2001)

Table 2 Population estimates of the number of mutants per generation (θ) and effective population size (*Ne*), and an estimate of between-population (K vs. N) differentiation calculated from nuclear and chloroplast markers (Φ_{SN} and Φ_{SC}, respectively)

Population	θ (CI 95 %)	*Ne* (CI 95 %)	
HA	0.44 (0.41–0.49)	1,100 (1,025–1,225)	
K	0.82 (0.76–0.89)	2,050 (1,900–2,225)	
N	0.81 (0.70–0.93)	2,025 (1,750–2,325)	
MH	0.78 (0.71–0.89)	1,950 (1,775–2,225)	
K versus N	Φ_{SN}	Φ_{SC}	m_p/m_s
	0.183	0.162	0.788

Ne estimates assumed SSR mutation rate of 10^{-4} per generation

populations, with range 2.8–3.4 and 0.380–0.500, respectively. Heterozygotes were rare in all four populations (observed heterozygosity Ho and inbreeding coefficient F_I ranged 0.001–0.007 and 0.981–0.997, respectively).

The estimates of θ (with 95 % CI) for two core populations were 0.82 (0.76–0.89) and 0.81 (0.70–0.93) (K and N, respectively), and for two peripheral populations were 0.78 (0.71–0.89) and 0.44 (0.41–0.49) (MH and HA, respectively). The resulting *Ne* estimates assuming SSR mutation rate of 10^{-4} per generation were 2,050 (1,900–2,225), 2,025 (1,750–2,325), 1,950 (1,775–2,225) and 1,100 (1,025–1,225) individuals (K, N, MH and HA, respectively) (Table 2).

Spatial Genetic Structure

We found significant autocorrelation up to 20 m in two peripheral populations (HA and MH). In the two core populations, autocorrelation was detected at shorter distances, in the distance classes of 1 and 2 m in the K population, and 1, 2 and 5 m in the N population (Fig. 2). The two peripheral populations MH and HA did not differ in their SGS (heterogeneity test, $\omega = 12.2$, $p > 0.5$), while all other pairs of populations did differ (heterogeneity test, $\omega = 90.6$, 66.3, 65.0, 63.9 and 72.9, for all $p < 0.001$, HA vs. N, HA vs. K, N vs. K, MH vs. N and MH vs. K, respectively).

A highly significant linear decrease of estimated kinship coefficients between pairs of individuals with the logarithm of increasing geographical distance was detected in all four populations (Table 3; Fig. 2) with variance explained by the regression slopes (R^2) between 0.07 and 0.11.

The neighborhood size *Nb* estimates were the lowest in HA (7.6) and the highest in N population (37.3), and did

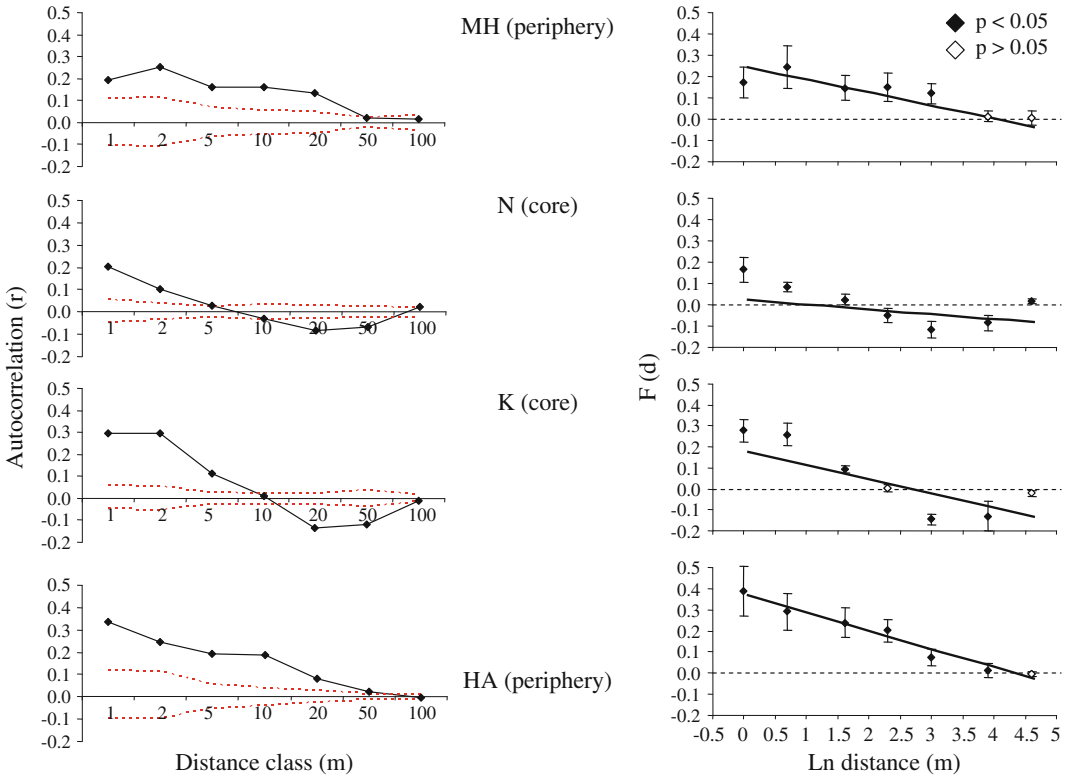

Fig. 2 *Left*: The autocorrelogram with 95 % confidence interval (*dotted lines*) for autocorrelation coefficient *r* (*solid line*) for the four populations studied. *Right*: Average kinship coefficients ± SE plotted against logarithmic distance between individuals and the estimated regression lines

Table 3 Estimates of SGS parameters for each population: *b*, slopes of the regression of kinship coefficient values on the logarithm of the spatial distance between individuals with associated determination coefficient R^2; average values of kinship coefficient between individuals separated by less than 1 m ($F_{(1)}$); neighborhood size (*Nb*); intensity of SGS (*Sp*); and gene dispersal distance σ assuming effective population density of 5, 2 and 1 plant/m^2

Population	b ± SE	R^2	p	$F_{(1)}$	*Nb* (95 % CI)	Sp	σ (5)	σ (2)	σ (1)
HA	−0.081 ± 0.018	0.086	<0.001	0.386	7.6 (6.2–9.7)	0.132	0.329	0.547	0.688
K	−0.051 ± 0.009	0.070	<0.001	0.278	14.1 (12.0–17.0)	0.071	0.309	0.434	0.629
N	−0.022 ± 0.005	0.017	<0.001	0.165	37.3 (30.5–49.6)	0.027	0.419	0.641	0.895
MH	−0.061 ± 0.019	0.110	<0.001	0.173	13.6 (10.3–19.7)	0.073	0.541	0.959	1.092

not differ between populations K and MH (14.1 and 13.6, respectively) (Table 3).

The *Sp* values ranged from 0.027 in N population to 0.132 in HA population with the average value across populations being 0.075. The average kinship coefficient between adjacent (1 m apart) individuals $F_{(1)}$ ranged 0.165–0.386.

The inferred gene dispersal (σ) was very low (range 0.309–1.092) for the effective plant density used (1, 2 and 5 plants/m^2).

Pollen to Seed Flow

The chloroplast genetic diversity was similar in the three populations (Table 1).

The Φ_{SN} and Φ_{SC} in an analysis of K and N population genetic differentiation were 0.183 and 0.162, respectively, providing the ratio of pollen flow to seed flow m_p/m_s 0.788 (Table 2).

Discussion

SGS and Pollen-to-Seed Flow in a Predominant Selfer

The SGS pattern detected in all four populations by both spatial autocorrelation analysis and a relationship of kinship coefficients with distance was consistent with isolation by distance. In no studied population was the autocorrelation coefficient significant for a distance between plants exceeding 20 m. These results evidence limited gene flow at short distances and fine-scale spatial organization of genetic variation in emmer wheat.

Gene flow has two components, and the relative importance of seed and pollen dispersal in creation of SGS can be inferred from joint usage of nuclear and chloroplast markers (Petit et al. 1993; Ennos 1994). The vast majority of SGS studies in general and those that estimated pollen-to-seed gene flow ratio in particular were done on out-breeding species (reviewed in El Mousadik and Petit 1996; Ouborg et al. 1999; Raspe et al. 2000). Ratio of pollen-to-seed flow analyzed in several out-crossing herbs was much higher than unity (Tarayre et al. 1997; McCauley 1997, 1998). In contrast to out-breeding species, pollen-to-seed migration ratio in predominantly self-fertilizing emmer wheat (this study) and wild barley (Volis et al. 2010) was close to unity. In both these species primary seed dispersal is by gravity, self-pollination is above 95 % and pollen life is short (less than 1 h in wheat and up to 26 h in barley (Fritz and Lukaszewski 1989; Parzies et al. 2005).

The averaged over populations SGS ($Sp = 0.075$) is remarkably similar to one detected in wild soybean ($Sp = 0.073$) (Zhao and Lu 2009) and wild barley ($Sp = 0.071$) (Volis et al. 2010). It is important to note, that in the latter studies the *Sp* values were estimated in three to four populations and averaged to provide the species *Sp*. These three species, emmer wheat, wild soybean and wild barley, share such characteristics as annual life cycle, predominant selfing and low dispersal distance of pollen (by wind) and seeds (by gravity). Higher SGS is theoretically expected and indeed was observed in a comparative species survey by Vekemans and Hardy (2004) in self-pollinated as compared with out-crossing, and in herbaceous as compared with tree species. In the survey of Vekemans and Hardy (2004) mode of seed dispersal also exhibited a trend (although this was not significant due to limited sample size) of increase in *Sp* from animal- and wind- to gravity-dispersed seeds. The conclusions of Vekemans and Hardy (2004) with respect to effects of breeding system, life form and seed dispersal on intensity of SGS are reinforced by high *Sp* values estimated for three self-pollinated, with gravity-dispersed seeds annual plants.

SGS in Core Versus Peripheral Populations

Although significant SGS was detected in all studied population, the extent of SGS differed in core and peripheral populations. While the SGS in the core populations was limited to 5 m distance, in both peripheral populations it was much larger (20 m).

Genetic diversity in core and peripheral populations was comparable, thus a higher level of genetic structuring in peripheral as compared with core populations appears to be due to lower intensity of within-population gene flow. The

latter is expected in the species periphery due to lower population density, lower number and higher isolation of patches of suitable environment. Consistent with this expectation, the arid edge peripheral population, which is extremely isolated (more than 10 km from the nearest population), distributed over a very limited area of less than 1 ha and comprises several hundred individuals, showed extreme values of neighborhood size (*Nb*) and intensity of SGS (*Sp*).

Studies comparing the fine-scale genetic structure of more than one population of a patchily distributed species are rare (Williams 1994; Williams and Guries 1994; Giles et al. 1998; Gapare and Aitken 2005; Zhao and Lu 2009). Especially rare are studies comparing fine-scale SGS across a species range. Our results agree with two studies of SGS in peripheral versus core populations. Gapare and Aitken (2005) found random distribution of genotypes in the core populations and positive SGS up to 500 m in the peripheral populations of Sitka spruce. Pandey and Rajora (2012) detected higher SGS in peripheral populations (up to 90 m), compared with core populations (up to 15 m) in the eastern white cedar. It appears to be a general rule that intensity of SGS increases from the species core to periphery if peripherality is associated with fragmentation of species habitat.

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