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Bioclimatic regions influence genetic structure of four Jordanian *Stipa* species

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ABSTRACT

Strong environmental gradients can affect the genetic structure of plant populations, but little is known as to whether closely related species respond similarly or idiosyncratically to ecogeographic variation. We analysed the extent to which gradients in temperature and rainfall shape the genetic structure of four *Stipa* species in four bioclimatic regions in Jordan. Genetic diversity, differentiation and structure of *Stipa* species were investigated using amplified fragment length polymorphism (AFLP) molecular markers. For each of the four study species, we sampled 120 individuals from ten populations situated in distinct bioclimatic regions and assessed the degree of genetic diversity and genetic differentiation within and among populations. The widespread ruderals *Stipa capensis* and *S. parviflora* had higher genetic diversity than the geographically restricted semi-desert species *S. arabica* and *S. lagascae*. In three of the four species, genetic diversity strongly decreased with precipitation, while genetic diversity increased with temperature in *S. capensis*. Most genetic diversity resided among populations in the semi-desert species (Φ_{ST} = 0.572/0.595 in *S. arabica*/*lagascae*) but within populations in the ruderal species (Φ_{ST} = 0.355/0.387 *S. capensis*/*parviflora*). Principal coordinate analysis (PCoA) and STRUCTURE analysis showed that *Stipa* populations of all species clustered ecogeographically. A genome scan revealed that divergent selection at particular AFLP loci contributed to genetic differentiation. Irrespective of their different life histories, *Stipa* species responded similarly to the bioclimatic gradient in Jordan. We conclude that, in addition to predominant random processes, steep climatic gradients might shape the genetic structure of plant populations.

INTRODUCTION

The genetic structure of plant populations is shaped by both random processes, such as genetic drift and gene flow, and by selective evolutionary forces (Barrett & Kohn 1991). Spatial environmental variation, and thus ecological diversification between habitats, is crucial for the maintenance of genomic diversity at the species level, due to evolutionary forces such as divergent selection and local adaptation (Linhart & Grant 1996; Gram & Sork 2001). To estimate the importance of such evolutionary forces, studies in species occurring in sharply contrasting environments may reveal the influence of ecological conditions on plant genetic structure (Nevo 2001). Environmental differences also strongly affect demographic processes like germination and seedling recruitment, which in turn affect genetic differentiation among plant populations (Montesinos *et al.* 2009), often due to random processes such as founder effects or genetic drift (Lawton-Rauh 2008). Hence, the genetic structure of any plant species reflects its interaction with the environment (Andrew *et al.* 2010).

Within populations, genetic diversity is considered to be important for adaptations to environmental change, and consequently for the long-term survival of plant populations (Bauert *et al.* 1998). Genetic diversity is strongly influenced by reproductive mode and mating system (Loveless & Hamrick 1984; Hamrick & Godt 1989). In addition, genetic diversity is assumed to increase with abiotic and biotic heterogeneity and in stressful environments (Nevo 2001; Kis-Papo *et al.* 2003). Thus, environmental conditions such as temperature and precipitation also affect genetic diversity (Wang *et al.* 2006; Zhao *et al.* 2006; Liu *et al.* 2009). However, as genetic variation is mostly studied with anonymous molecular markers, the relative contribution of selective and neutral processes to the observed patterns is difficult to assess. Genome scans or outlier analyses allow for the identification of individual marker loci, which are more strongly differentiated than is otherwise expected from neutral models of evolution (Beaumont & Nichols 1996; Pérez-Figueroa *et al.* 2010). Thus, in many species, a small proportion of molecular markers has been identified to be potentially adaptive and to contribute to correlations

between environmental variables and genetic diversity (*e.g.* Jump *et al.* 2006; Michalski *et al.* 2010; Shi *et al.* 2011). Several studies have revealed effects of environmental gradients on the genetic diversity of individual grass species under arid conditions (Nevo *et al.* 1998; Nevo 2001; Turpeinen *et al.* 2001, 2003; Baek *et al.* 2003; Sharma *et al.* 2004; Huebner *et al.* 2009). In addition, some studies have tried to control for evolutionary history by comparing levels of diversity between widespread and rare congeners (Karron 1987; Gitzendanner & Soltis 2000). Here, we expand on these ideas by presenting a study of the effects of a steep bioclimatic gradient on the genetic diversity, differentiation and structure of four *Stipa* species from Jordan with different life histories.

Jordan is situated in the transition zone between arid and semiarid bioclimates, and is thus characterised by strong spatial variation in aridity over short geographic distances. Although the distance between the northern and the southern border is only about 350 km, annual rainfall can be as high as 600 mm in the north-western mountains and as low as 50 mm in the southern and eastern desert regions (Dahamsheh & Aksoy 2007). Thus, due to the strong variation in bioclimate, there are conspicuous changes in the vegetation and in the composition of the flora over relatively short distances (Zohary 1973; Freiwan & Kadioglu 2008).

The genus *Stipa* is widely represented throughout zonal grasslands and semi-deserts of the northern hemisphere (Lavrenko & Karamysheva 1993). Our study covered four of the six *Stipa* species found in Jordan, which were selected because they occurred in at least three of the four distinct bioclimatic regions. We hypothesised that the bioclimatic gradient in Jordan has a strong impact on the genetic diversity, differentiation and structure of *Stipa* species. As there is no detailed genomic information on *Stipa* species, we used amplified fragment length polymorphism (AFLP), a reliable technique for assessing genetic variation among and within populations (Vos *et al.* 1995; Turpeinen *et al.* 2003). We addressed the following questions across four *Stipa* species: (i) is genetic variation within populations related to bioclimatic variables and population size; (ii) does genetic variation within populations differ among *Stipa* species with different life histories; (iii) how is genetic variation distributed among and within populations; (iv) is the genetic structure related to bioclimatic regions; and (v) does divergent selection along environmental gradients acting on particular AFLP loci contribute to genetic differentiation?

MATERIAL AND METHODS

Study region and plant material

Jordan is of great interest to vegetation ecology because it is the meeting place of four major phytogeographic regions: the Mediterranean (subhumid and semiarid Mediterranean), the Irano-Turanian (arid Mediterranean), the Saharo-Arabian and the Sudanian regions (Zohary 1973; Al-Eisawi 1985, 1996; Freiwan & Kadioglu 2008). In the most recent bioclimatic analyses by Al-Eisawi (1985, 1996), nine subdivisions are considered that fall under four main bioclimatic regions, representing a gradient of decreasing precipitation and increasing temperature: (i) subhumid Mediterranean bioclimate; (ii) semiarid Mediterranean bioclimate; (iii) arid Mediterranean bioclimate; and (iv) Saharan Mediterranean bioclimate (hereafter referred to as subhumid, semiarid, arid and Saharan bioclimate, respectively).

Four *Stipa* species differing in life form and geographical distribution were studied. The first two species – tufted annual *Stipa capensis* Thump. (2n = 34, 36; = *Stipellula capensis* (Thunb.) Röser & Hamasha, comb. nov., Hamasha *et al.* 2012; Roeser 2012) and the perennial tussock grass *Stipa parviflora* Desf. (2n = 28, 44; = *Stipellula parviflora* (Desf.) Röser & Hamasha, comb. nov., Hamasha *et al.* 2012; Roeser 2012) – are widespread ruderals in the Irano-Turanian, Saharo-Arabian and Mediterranean regions that have very similar climatological requirements and distribution patterns. The other two restricted species – *Stipa arabica* Trin. & Rupr. (2n = 44) and *Stipa lagascae* R. & Sch. (2n = 44), (both Section *Barbatae* Junge emend. Freitag; Freitag 1985) – are perennial tussock grasses. *S. arabica* is a tufted perennial steppe grass of the Irano-Turanian region, which extends to the Mediterranean and Saharo-Arabian region, whereas *S. lagascae* is a perennial bunchgrass that occurs throughout the Mediterranean region and extends to the West Irano-Turanian region (Zohary 1962; Scholz 1991).

Stipa species are pollinated by wind and are facultatively cleistogamous, producing both chasmogamous and cleistogamous flowers (Ponomarev 1961), but ratios of selfing *versus* outcrossing flowers are not known. Flowering and seed dispersal of all the study species usually takes place between March and June (Zohary 1962). Caryopses are characterised by their long hygroscopic awns, which facilitate dispersal by wind and animals. In the lab, seed germination of all four *Stipa* species occurred under a wide range of temperature regimes, while in the field, germination seems to be mainly controlled by ambient climatic factors (Hamasha & Hensen 2009).

We sampled ten populations of each species along an eco-geographical transect from north-eastern to southern Jordan (Fig. 1) at elevations ranging from −415 m a.s.l. to 1655 m a.s.l. and from a total of 27 sites. Plant samples covered most of the natural distribution of *Stipa* species in Jordan and represented three of the four bioclimatic regions for each species (Al-Eisawi 1985, 1996). A population was defined as a group of plants separated from their closest conspecifics by more than 1 km. The minimum distance between two populations of the same species was 10 km, with the maximum distance being 236 km. For the AFLP analysis, we sampled leaves of 12 individuals per population between April and July 2007. Distances between individual tussocks were always >1 m, and samples were collected from areas no >400 m². Most populations were of moderate (100–500 individuals) or large size (>500 individuals); only three of the 40 populations were small (<100 individuals; Table 1).

For each site, we characterised environmental and climate conditions by elevation, mean annual rainfall, and mean annual January and August temperature, based on Al-Eisawi (1985, 1996), Sharma *et al.* (2004) and Hijmans *et al.* (2005); Fig. 1, Table 1). To account for co-linearity among these environmental variables, we carried out a principal components analysis (PCA) on environmental variables, with rainfall being log-transformed to improve normality. For further analyses of site conditions, we extracted the scores of the PC axes. The first two axes explained more than 97% of the environmental variation in all four species. Climate-related variables had high

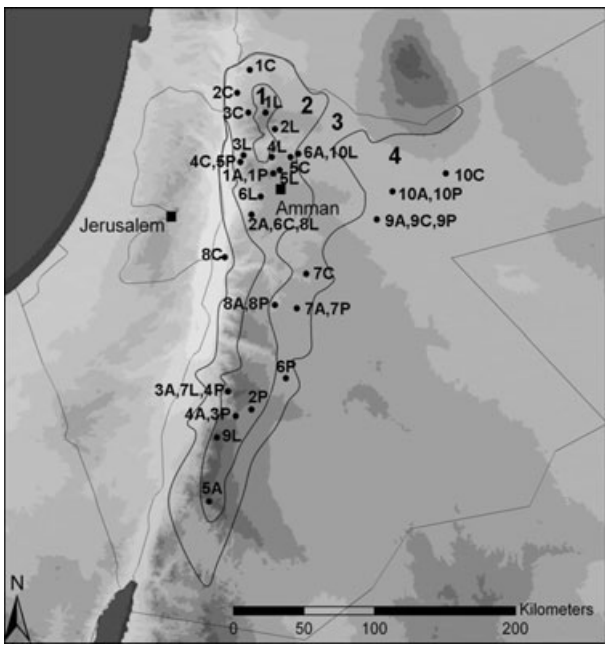

Fig. 1. Geographic distribution of the sampled populations of (A) *Stipa arabica*, (C) *S. capensis*, (L) *S. lagascae* and (P) *S. parviflora* in Jordan. For bioclimatic and population codes, refer to Table 1.

loadings on the first PCA axis (PC 1), along which elevation increased and temperature decreased (data not shown). Rainfall strongly increased along PC 2. Environmental distance between population pairs was calculated as Euclidean distance of the factor scores.

DNA extraction, AFLP analysis and genotyping

We applied the same extraction protocol and AFLP procedure as described in Hensen *et al.* (2011), except that we extracted DNA from 100 mg frozen leaf material; restriction and ligation were performed for a total of 4 h, and 0.45 pmol of *Eco*RI-adapter and 4.55 pmol *Mse*I adapter were used for ligation. The four primer pair combinations for fingerprinting were AGC/CAG, AAG/CAG, AGC/CTA and AAG/CTA for *S. capensis* and *S. parviflora* and AGC/CAG, AAG/CAG, AGC/CAC and AAG/CAC for *S. arabica* and *S. lagascae*. Polymorphic DNA bands were scored as present (1) or absent (0) for each DNA sample, excluding the smeared and weak ones, by visual inspection. The reproducibility of AFLP markers for the four primer pair combinations was investigated by three repeated analyses of all steps from DNA isolation to data scoring for eight individuals per species. The mean genotyping error rate per individual (observed number of differences between replicates/total number of comparisons; Bonin *et al.* 2004) was 4%, which is similar to AFLP error rates previously reported for other plant species (Bonin *et al.* 2004; Minder & Widmer 2008). The four primer pairs used in the AFLP analysis of *S. capensis*, *S. parviflora*, *S. arabica* and *S. lagascae* resulted in 335, 330, 396 and 423 reliable bands, respectively. Of these bands, 232 (69.3%), 242 (73.3%), 291 (73.5%) and 328 (77.5%) were polymorphic and were consequently used in the analysis. The number of polymorphic bands per primer pair ranged between 45 and 68, 46 and 72, 48 and 103, and 67 and 91, respectively.

Genetic variation and role of environmental conditions

Genetic variation within populations was first assessed as percentage of polymorphic bands (PPB). Second we applied a Bayesian approach (Holsinger & Wallace 2004) to estimate the *Hs* – analogous to Nei's *He* – using the program HICKORY, version 1.1 (Holsinger & Wallace 2004). This approach does not assume Hardy-Weinberg equilibrium (HWE) or a fixed value of *f*. Values for burn-in, sampling and thinning were 5,000, 100,000 and 20, respectively. Based on the deviance information criterion (DIC), we compared model fits of alternative models. We choose the full model (Holsinger & Lewis 2003) for *S. capensis*, *S. parviflora* and *S. arabica* because *f* values (0.11–0.15) indicated deviance from HWE, and the $f = 0$ model for *S. lagascae* as the *f* value (0.076) indicated HWE.

Complementary effects of environmental conditions and of population size on genetic diversity within populations were tested by multiple regressions. In the multiple regressions we used the first and second PCA component and population size classes as predictors of genetic diversity *Hs*. Non-significant terms were step-wise removed from the model (backward elimination, $P > 0.05$). PCA and multiple regression analyses were carried out in R 2.9.1 (R Development Core Team 2009).

Genetic population structure and role of environmental conditions

In order to visualise patterns of genetic distances among individuals, populations and regions, a principal coordinate analysis (PCoA) was performed on the AFLP data set. To further infer ancestry groups without prior population information, we performed Bayesian cluster analyses using STRUCTURE 2.3.3 (Pritchard *et al.* 2000). We chose the admixture model with correlated allele frequencies with 10,000 burn-in iterations and 50,000 MCMC iterations. We performed ten replicate runs for each *K* (the putative number of ancestral gene pools) ranging from 1 to 10. Alpha was found to be ≥ 1 in preliminary runs, and therefore was set to 1 for the main analyses. The most likely number of clusters was determined by jointly assessing the absolute value (ΔK<20 were not considered) and peak position of ΔK (Evanno *et al.* 2005). As STRUCTURE identifies the upper hierarchical levels, we submitted the clusters identified in the first round to a second STRUCTURE analysis.

Genetic differentiation among populations was quantified first with HICKORY, using θ^B which is the best estimate of Wright's F_{ST} (Holsinger & Lewis 2003). We then applied an analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) as implemented in GenAlEx 6.1 (Peakall & Smouse 2006). To assess the level of differentiation based on environmental conditions, we calculated AMOVAS grouping populations by bioclimatic region (Table 1). The relationship between genetic differentiation among populations (pair-wise Φ_{ST} values) and both geographic and environmental distances was examined with Mantel tests. Because geographic and environmental distance were not independent ($r = 0.376$–0.795, Mantel $P < 0.01$), we employed partial Mantel tests to examine the relationships between genetic and environmental distances while controlling for geographic distance. Mantel tests were carried out with the function MANTEL from the VEGAN package (Oksanen *et al.* 2010) in R.

Table 1. Environmental conditions and genetic diversity (PPB, *Hs*) in Jordanian study populations of *Stipa capensis*, *S. parviflora*, *S. arabica* and *S. lagascae*.

			Geographical			Climate					Genetic diversity	
Population	Pop. code	Bio-climate	Lt	Ln	Elv	Rn	Tm	Ta	Tj	Sz	PPB	*Hs*
Stipa capensis												
Um Qais	1C	2	32.65	35.72	368	431	19.2	26.2	11.5	3	47.8	0.232
Dair Abu Said	2C	2	32.51	35.64	-21	320	21.9	29.2	13.8	2	37.9	0.210
Baun	3C	2	32.39	35.71	431	417	19.0	26.0	11.2	3	51.7	0.237
Humrit Sahin	4C	2	32.09	35.66	352	280	19.6	26.5	11.7	2	37.5	0.204
Beren	5C	2	32.12	35.98	715	307	17.2	24.5	9.0	2	37.9	0.208
Mount Nebo	6C	2	31.77	35.73	511	301	18.3	24.9	10.5	2	32.8	0.193
Swaqah	7C	3	31.41	36.08	800	170	16.3	23.1	8.1	2	37.5	0.206
Dead Sea	8C	4	31.51	35.56	-415	91	24.6	31.5	16.4	2	44.4	0.230
Qasr Amra	9C	4	31.74	36.53	648	91	17.7	25.1	8.7	3	51.3	0.250
Safawi	10C	4	32.02	36.97	631	75	18.7	27.0	9.2	3	50.9	0.254
Mean											43.0	0.222
S. parviflora												
Jubaiha	1P	2	32.02	35.87	1044	434	15.6	22.8	7.4	2	33.9	0.205
Rashadiah	2P	2	30.59	35.73	1161	229	15.2	22.2	7.3	1	30.2	0.191
Tafila	3P	2	30.55	35.63	1237	263	14.4	21.2	6.7	3	44.6	0.223
Dana	4P	2	30.70	35.58	1292	220	14.0	20.7	6.5	2	45.9	0.221
Humrit Sahin	5P	2	32.09	35.66	352	280	19.6	26.5	11.7	3	48.8	0.236
Hasa	6P	3	30.78	35.95	856	173	17.0	24.0	8.7	1	32.6	0.194
Qatranah	7P	3	31.20	36.02	806	157	16.7	23.6	8.4	3	48.4	0.232
Adar-Karak	8P	3	31.22	35.88	675	179	17.4	24.1	9.5	2	44.2	0.220
Qasr Amra	9P	4	31.74	36.53	648	91	17.7	25.1	8.7	3	47.9	0.231
Azraq Shamali	10P	4	31.91	36.63	636	90	17.9	25.5	8.7	3	57.4	0.258
Mean											43.4	0.221
S. arabica												
Jubaiha	1A	2	32.02	35.87	1044	434	15.6	22.8	7.4	2	28.5	0.153
Mount Nebo	2A	2	31.77	35.73	511	301	18.3	24.9	10.5	2	31.6	0.162
Dana	3A	2	30.70	35.58	1292	220	14.0	20.7	6.5	2	40.9	0.189
Tafila	4A	2	30.55	35.63	1237	263	14.4	21.2	6.7	3	40.9	0.188
Ras Naqab	5A	2	30.03	35.46	1585	185	14.1	21.6	5.8	3	44.3	0.199
Zarqa N	6A	3	32.14	36.03	524	199	18.0	25.2	9.7	2	30.6	0.162
Qatranah	7A	3	31.20	36.02	806	157	16.7	23.6	8.4	2	33.0	0.165
Adar-Karak	8A	3	31.22	35.88	675	179	17.4	24.1	9.5	2	30.6	0.167
Qasr Amra	9A	4	31.74	36.53	648	91	17.7	25.1	8.7	2	37.1	0.185
Azraq Shamali	10A	4	31.91	36.63	636	90	17.9	25.5	8.7	2	42.3	0.191
Mean											36.0	0.176
S. lagascae												
Samta	1L	1	32.39	35.82	1086	473	15.2	22.4	7.1	2	32.9	0.171
Souf	2L	2	32.29	35.88	672	427	17.4	24.5	9.3	2	28.7	0.155
Subayhi	3L	2	32.13	35.68	492	379	18.7	25.6	10.8	2	22.0	0.145
Salhoub	4L	2	32.12	35.86	738	412	17.2	24.4	9.1	2	25.3	0.160
Shafa Badran	5L	2	32.04	35.91	856	358	16.7	24.0	8.4	2	27.1	0.167
Naur	6L	2	31.88	35.79	449	275	18.7	25.5	10.7	2	30.8	0.167
Dana	7L	2	30.70	35.58	1292	220	14.0	20.7	6.5	3	40.6	0.201
Mount Nebo	8L	2	31.77	35.73	511	301	18.3	24.9	10.5	1	21.7	0.155
Shoubak	9L	2	30.42	35.51	1655	300	12.3	19.4	4.4	3	31.7	0.181
Zarqa N	10L	3	32.14	36.03	524	199	18.0	25.2	9.7	2	47.6	0.226
Mean											30.8	0.173

Abbreviations: bioclimatic regions: 1 = Subhumid 2 = Semiarid 3 = Arid 4 = Saharan; geographic: Lt = latitude (decimal degrees), Ln = longitude (decimal degrees), Elv = Elevation (m); climate: Rn = mean annual rainfall (mm); Tm = mean annual temperature (°C), Ta = temperature of mean hottest month (August) (°C), Tj = mean coldest month (January) (°C); Sz = estimate of population size: 1 = small (<100 individuals) 2 = intermediate (100–500 individuals) 3 = large (>500 individuals); Genetic diversity: PPB = percentage of polymorphic bands; *Hs* = mean gene diversity.

Divergent selection at individual AFLP loci

To test whether divergent selection had an effect on patterns of genetic differentiation, we performed genome scans to identify outlier loci with particularly strong differentiation, and then related their population band frequencies to environmental variables. Genome scan analyses typically suffer from the detection of false positives and may be inefficient when population differentiation is high (Pérez-Figueroa *et al.* 2010). We therefore used a staged approach. First, we used BAYESCAN (Foll &

Gaggiotti 2008), which implements a Bayesian method that directly estimates the posterior probability of selection for each locus. For each species, BAYESCAN was run with default settings. Loci were classified as either behaving neutrally (Bayes factor <3, log10(BF)<0.5), showing some evidence of selection (0.5 < log10(BF)<2), or showing decisive evidence of selection (log10(BF) >2). Next, for each locus, we performed Pearson correlation tests of population band frequency (arcsin-transformed) with the scores of the two environmental factors (PC1, PC2) and determined the number and proportion of significant correlations ($P < 0.05$) to either of the factors for the three classes of evidence for selection. Next, to control for the large number of analyses we developed a null model to determine the expected number of correlations found to be significant when population band frequencies were not actually related to the environment. For each species, we therefore randomly permuted the population band frequencies and performed the correlation analysis as above 100 times and determined the mean (±95% confidence interval) number of correlations found to be significant.

RESULTS

Genetic variation and role of environmental conditions

Genetic diversity values were higher in the widespread ruderal than in the semi-desert *Stipa* species. The mean values of percentage polymorphic bands (PPB) and gene diversity (*Hs*) for the ruderal species *S. capensis* and *S. parviflora* were 42.9 ± 8.9 (95% CI) and 43.3 ± 5.3 and 0.222 ± 0.013 and 0.221 ± 0.013, respectively (Table 1). Values for the semi-desert species *S. arabica* and *S. lagascae* were lower (PPB = 35.9 ± 3.5 and 30.8 ± 5.0; *Hs* = 0.176 ± 0.010 and 0.173 ± 0.015).

In a multiple regression, both environmental gradients (PC1 = elevation and temperature variables, PC2 = rainfall) negatively affected genetic diversity of *S. capensis* ($R^2 = 0.959$; PC 1: $b = -0.003$, $t = -3.203$, $P = 0.018$; PC 2: $b = -0.008$, $t = -4.638$, $P = 0.003$), and genetic diversity increased in large populations ($b = 0.03$, $t = 9.78$, $P < 0.001$). Genetic diversity of *S. parviflora* was not affected by environmental gradients but increased in large populations ($R^2 = 0.768$; $b = 0.04$, $t = 4.69$, $P = 0.002$). The rainfall gradient negatively, and large population size positively, affected the genetic diversity of *S. arabica* ($R^2 = 0.808$; PC 2: $b = -0.011$, $t = -4.164$, $P = 0.004$; large size: $b = 0.018$, $t = 2.83$, $P = 0.025$). In *S. lagascae*, the observed genetic diversity also decreased along the rainfall gradient ($R^2 = 0.558$; PC 2: $b = -0.017$, $t = -3.18$, $P = 0.013$); population size had no effect.

Genetic population structure and role of environmental conditions

Populations of all of our study species were strongly differentiated (Table 2). The semi-desert species *S. arabica* ($\Phi_{ST} = 0.572$; $\theta^B = 0.458$) and *S. lagascae* ($\Phi_{ST} = 0.595$; $\theta^B = 0.457$) showed higher differentiation than the ruderal species *S. capensis* ($\Phi_{ST} = 0.355$; $\theta^B = 0.272$) and *S. parviflora* ($\Phi_{ST} = 0.387$; $\theta^B = 0.303$). Hierachical AMOVAs revealed that differentiation between different bioclimatic regions was significant for all four *Stipa* species, accounting for 4% (*S. capensis*) to 30% (*S. arabica*) of genetic variation (Table 2). Mantel and partial Mantel tests revealed that in all the study species, genetic differentiation between populations was not significantly related to geographic distance (Table 3). Genetic differentiation was correlated to environmental distance when controlling for effects of geographic distance in all species except *S. lagascae* (Table 3).

For all species, the first two PCoA axes accounted for 55–60% of the genetic variation (Fig. 2). The populations of *S. capensis* fell into two major groups, with the first group representing semiarid and arid populations and the second group including Saharan populations. In the Bayesian STRUCTURE analysis, the optimum number of clusters was found at $K = 5$ (Fig. 3a). Here, semiarid and arid individuals differentiated into two different clusters, while the Saharan populations split into three different clusters. The ten populations of *S. parviflora* formed three major groups in the PCoA (Fig. 2b). The first two groups encompassed both semiarid and arid populations, whereas the third group included only Saharan populations. The STRUCTURE analysis (Fig. 3b) strongly resembled the PCoA ($K = 3$). The populations of *S. arabica* formed three major groups in the PCoA (Fig. 2c), consisting of the semiarid, arid and Saharan populations. In the STRUCTURE analysis (Fig. 3c), hierarchical clusters were identified resulting in a total of five clusters, largely resembling the results of the PCoA. The populations of *S. lagascae* formed five groups in the PCoA (Fig. 2d), four of which were single subhumid, semiarid and arid populations. In the STRUCTURE analysis, again, hierarchical clusters were found, with a total of three clusters (Fig. 3d).

Divergent selection at individual AFLP loci

Across the four species, between 19 and 95 of the polymorphic AFLP loci (8–29%) showed some evidence (0.5 < log10(BF) <2) of diversifying selection (Fig. 4). Between 34 and 58 loci (15–20%) showed decisive evidence (log10 (BF) >2). For the loci showing no evidence of selection, the proportion of significant correlations did not exceed the value expected by chance for all species except *S. arabica* (17%). For the loci showing some evidence of selection, three species showed more correlations than expected by chance (*S. arabica, S. lagascae*, each 16%, and *S. parviflora*, 14%). For the loci with decisive evidence of selection, correlations to the environmental gradients were evident in all species for 17% (*S. capensis*), 30% (*S. parviflora*), 49% (*S. arabica*) and 14% (*S. lagascae*) of these loci, suggesting diversifying selection. Overall, thus the percentage of polymorphic AFLP markers that are putatively under diversifying climate selection was 2.6%, 5.4%, 9.6% and 2.4% in *S. capensis*, *S. parviflora*, *S. arabica* and *S. lagascae,* respectively. The two climatic gradients, PC1 and PC2, were correlated to 21 and 39 loci, respectively, across the four species.

DISCUSSION

Our data on genetic variation as estimated by AFLP in four Jordanian *Stipa* species occupying different ecogeographic regions revealed the effects of environmental variables on levels and structuring of genetic diversity. We found these effects of environmental conditions on the genetic structure in all four species, irrespective of their differences in life-history strategies. Our study thus adds to a growing body of literature reporting that genetic diversity within grass species can be shown to be

Table 2. Analysis of molecular variance (AMOVA) of 120 individuals in 10 populations of (a) *Stipa capensis*, (b) *S. parviflora*, (c) *S. arabica* and (d) *S. lagascae*. For each species, an AMOVA without prior grouping and with groups based on bioclimatic regions is given. *P*-values are based on 1,000 permutations.

	df	Sum of squares	Variance	% Total	Φ Statistics	*P*-value
(a) *Stipa capensis*						
Analysis 1: without groups						
Among populations	9	1034.1	8.3	35.5	Φ_{ST} = 0.355	<0.001
Within populations	110	1663.3	15.1	64.5		
Analysis 2: bioclimatic regions						
Among regions	2	278.4	1.0	4.0	Φ_{CT} = 0.040	<0.001
Among populations	7	755.7	7.7	32.5	Φ_{SC} = 0.338	<0.001
Within populations	110	1663.3	15.1	63.5	Φ_{ST} = 0.365	<0.001
(b) *S. parviflora*						
Analysis 1: without groups						
Among populations	9	1273.2	10.4	38.7	Φ_{ST} = 0.387	<0.001
Within populations	110	1813.8	16.5	61.3		
Analysis 2: bioclimatic regions						
Among regions	2	418.8	2.4	8.5	Φ_{CT} = 0.085	<0.001
Among populations	7	854.4	8.8	31.8	Φ_{SC} = 0.348	<0.001
Within populations	110	1813.8	16.5	59.7	Φ_{ST} = 0.403	<0.001
(c) *S. arabica*						
Analysis 1: without groups						
Among populations	9	2379.5	20.7	57.2	Φ_{ST} = 0.572	<0.001
Within populations	110	1708.3	15.5	42.8		
Analysis 2: bioclimatic regions						
Among regions	2	1231.5	12.1	30.3	Φ_{CT} = 0.303	<0.001
Among populations	7	1148.0	12.4	30.9	Φ_{SC} = 0.443	<0.001
Within populations	110	1708.3	15.5	38.8	Φ_{ST} = 0.612	<0.001
(d) *S. lagascae*						
Analysis 1: without groups						
Among populations	9	2622.6	23.0	59.5	Φ_{ST} = 0.595	<0.001
Within populations	110	1724.3	15.7	40.6		
Analysis 2: bioclimatic regions						
Among regions	2	710.6	4.0	9.8	Φ_{CT} = 0.098	<0.001
Among populations	7	1911.9	21.5	52.1	Φ_{SC} = 0.578	<0.001
Within populations	110	1724.3	15.7	38.1	Φ_{ST} = 0.619	<0.001

Table 3. Tests for isolation by distance. Correlation coefficients and *P*-values of Mantel tests and partial Mantel tests for the correlation between genetic differentiation (gen) and both geographic distance (geo) environmental distance (env).

	gen–geo		gen–env		gen–env(given geo)		gen–geo(given env)	
	r	Mantel *P*	*r*	Mantel *P*	*r*	Mantel *P*	*r*	Mantel *P*
Stipa capensis	0.208	0.150	0.409	0.014	0.365	0.031	0.064	0.398
S. parviflora	0.152	0.164	0.313	0.036	0.277	0.049	−0.015	0.544
S. arabica	0.012	0.464	0.259	0.068	0.322	0.026	−0.198	0.866
S. lagascae	0.106	0.242	0.022	0.385	−0.103	0.721	0.146	0.197

associated with environmental variables (Nevo *et al.* 1998; Baek *et al.* 2003; Zhao *et al.* 2006; Huebner *et al.* 2009; Michalski *et al.* 2010).

Genetic variation and role of environmental conditions

Our results reveal that Jordanian *Stipa* species differ in their degree of genetic diversity. Populations of the closely related ruderals *S. capensis* and *S. parviflora* show higher diversity than the semi-desert species *S. arabica* and *S. lagascae*. At the population level, the percentage of polymorphic bands of our study *Stipa* species ranges between 31% and 43% and is higher than those obtained for the Eurasian *S. capillata* (AFLP; PPB = 21%; Wagner *et al.* 2011), *S. pennata* (AFLP; PPB = 21%; Wagner *et al.* 2012) and *S. pulcherrima* (AFLP; PPB = 9%; Durka *et al.* in press). Values of HICKORY's gene diversity index (*Hs*; 0.17–0.22) are higher than *He* values of several other *Stipa* species, *e.g. S. purpurea* (ISSR, *He* = 0.14; Liu *et al.* 2009), *S. pulcherrima* (AFLP, *He* = 0.03; Durka *et al.* in press) and *S. capillata* (AFLP, *He* = 0.08; Wagner *et al.* 2011; all studies mentioned used only polymorphic bands for data analysis).

The higher genetic diversity in the ruderal species compared to the semi-desert species could probably be explained by

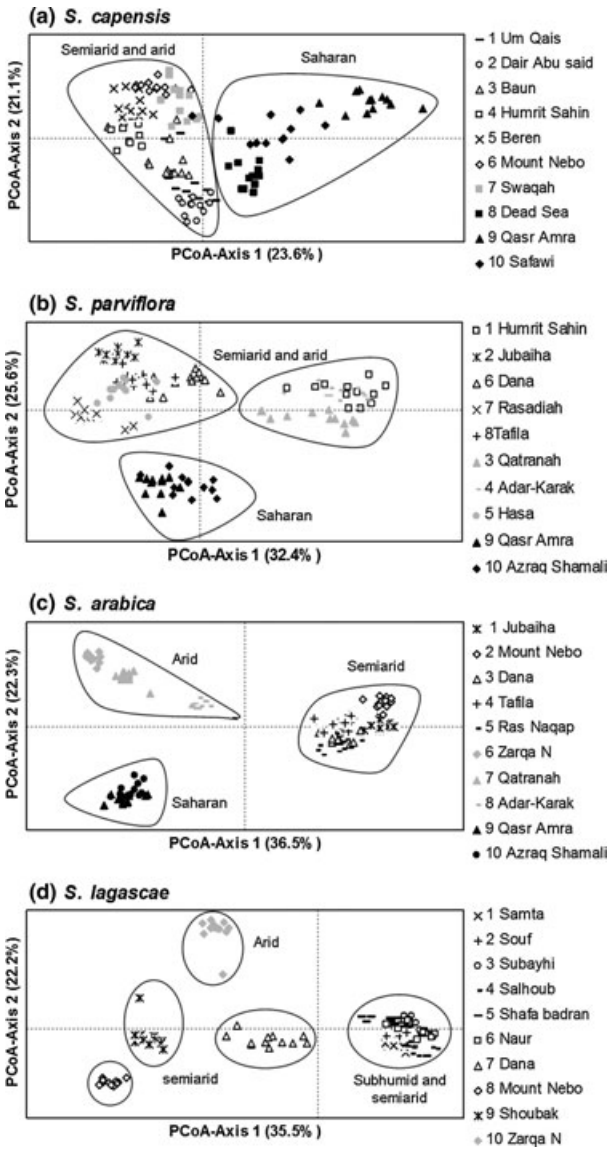

Fig. 2. Principal coordinates analysis for ten populations of a: *Stipa capensis* b: *S. parviflora* c: *S. arabica* and **d:** *S. lagascae*. Different symbols indicate various bioclimatic regions.

differences in their distribution, population sizes and seed bank longevity. Our results are in line with those reporting that genetic diversity of widespread species is higher than that of geographically restricted species (Gitzendanner & Soltis 2000; Cole 2003). In addition, population sizes of the widespread ruderal species were larger than those of the semi-desert species (Table 1; personal observation, H. Hamasha). One of the ruderals, *S. capensis,* is known to build up a large seed bank (Boeken *et al.* 2004) and may produce very large populations in climatically favourable years. In contrast, the semi-desert perennials are protected against climatic uncertainty rather by their long life span than by a seed bank (Hegazy *et al.* 2009). In addition, limited gene flow among semi-desert perennial populations made them more susceptible to genetic drift. Our findings suggest that semi-desert species are impacted more by genetic drift than are ruderal species.

In *S. capensis*, genetic diversity was negatively correlated with elevation and with the different temperature variables, all of which explain the first principal component. Separating the roles of these strongly related environmental variables on plant adaptation would require manipulative experiments (Huebner *et al.* 2009). Rainfall is the major contributor to the second principal component and negatively affected the genetic diversity in *S. capensis*, *S. arabica* and *S. lagascae*. These effects could be explained by decreasing rainfall towards the eastern and southern arid zones in the country, creating steep climatic and ecological clines over relatively short distances. Our results suggest that genetic diversity of Jordanian *Stipa* populations increases in stressful environments, particularly in *S. capensis, S. arabica* and *S. lagascae*. Relationships between the degree of DNA polymorphism as revealed by RAPDs and ecogeographical variables have been reported for *Stipa krylovii* (Wang *et al.* 2006) and *S. grandis* (Zhao *et al.* 2006). Moreover, our results are in line with SSR studies of wild barley (*Hordeum spontaneum*) in Israel (Turpeinen *et al.* 2001; Huebner *et al.* 2009) and in Jordan (Baek *et al.* 2003). Although several studies found higher genetic diversity in *H. spontaneum* and *Triticum dicoccoides* in habitats with stressful (*i.e.* dry and hot) environmental conditions (Nevo *et al.* 1998; Turpeinen *et al.* 2001, 2003; Sharma *et al.* 2004), other studies along gradients of environmental stress did not find differences (Volis *et al.* 2002). Associations between environmental variables and molecular polymorphisms could be a result of neutral evolutionary processes, but such associations may also indicate selection and adaptive processes. Environmental unpredictability in stressful habitats might select for higher levels of genetic diversity, possibly because of their buffering effects in heterogeneous environments (Nevo 2001).

Genetic population structure and role of environmental conditions

The Φ_{ST} values, between 0.355 and 0.595, and θ^B values for our *Stipa* species, between 0.272 and 0.458, were higher than mean Φ_{ST} values of long-lived perennials (0.25; Nybom 2004). High genetic differentiation among populations was also found in several other *Stipa* species (Wang *et al.* 2006; Zhao *et al.* 2006; Liu *et al.* 2009; Hensen *et al.* 2010; Wagner *et al.* 2011, 2012), and we assume that high θ^B or Φ_{ST} values are typical for the facultatively cleistogamous genus *Stipa*. However, we found deviance from HWE only in three of our study species, and only with comparatively low *f* values (0.11–0.15). Although our study species are known to be capable of selfing, the data suggest that they maintain a high degree of outcrossing.

Genetic differentiation among populations was not related to geographic distance in any of the four species. The absence of isolation by distance strongly suggests that gene flow between populations by seed or pollen dispersal is very rare. The Jordanian landscape, comprising high mountain ridges, steep valleys and isolated habitat patches, may contribute to limited gene flow among populations, resulting in the strong differentiation among populations (Hensen *et al.* 2011). We expect processes of random genetic drift to be more important in the semi-desert species, due to smaller population sizes and probably smaller seed banks.

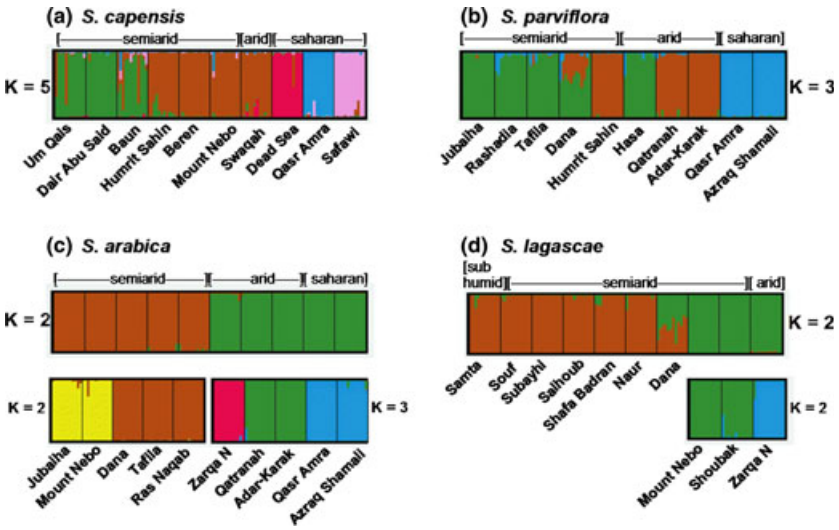

Fig. 3. Bar plots showing the results of the Bayesian cluster analysis with the program STRUCTURE for a: *S. capensis*, b: *S. parviflora*, c: *S. arabica* and d: *S. lagascae*. Colours indicate different clusters (*i.e.* admixture groups) and bar heights indicate the probability that each individual belongs to the respective admixture group.

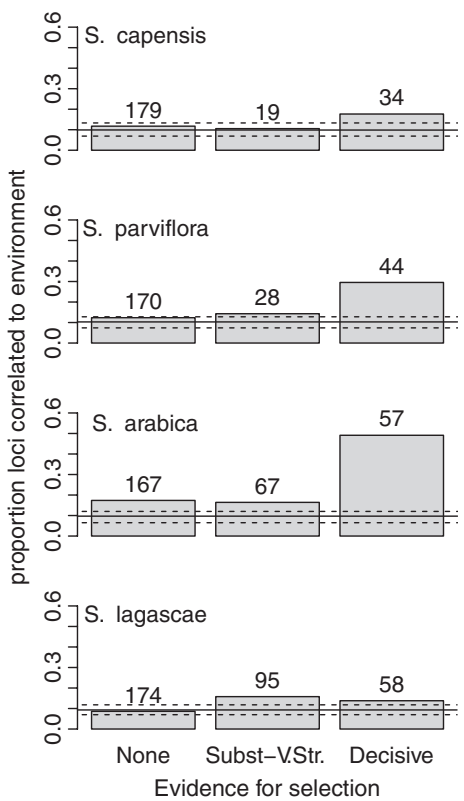

Fig. 4. Proportion of loci significantly correlated to either of the two environmental gradients (PC1, PC2). Loci were classified according to evidence for selection based on the BayesFactor (Log10BF) of BayeScan analyses: no evidence (Log10BF<0.5), substantial to very strong evidence (0.5 < Log10BF<2) and decisive evidence (Log10BayesFactor >2). Numbers above bars indicate the total number of loci per class. Horizontal lines indicate the proportion of significant correlations expected by chance. In this null model band frequencies were randomised among populations within species (mean ± 95% CV, n = 100 randomisations).

The genetic structure of Jordanian *Stipa* populations was found to be shaped by both demographic process and climate factors. PCoA and STRUCTURE analysis confirmed that the populations of Jordanian *Stipa* species clustered ecogeographically, generally in line with their bioclimatic regions, because those sharing the same climate environment grouped together in the same cluster, irrespective of their geographic distances. Genetic clustering within some of the bioclimatic regions suggests that gene flow is not effective enough to genetically connect populations, even within bioclimatic regions. The Mediterranean climate is characterised by strong inter-annual and seasonal weather fluctuations (Etterson 2004; Peleg *et al.* 2008). Consequently, variability in soil moisture imposes fluctuating selection pressures between different bioclimatic regions, influencing phenological patterns such as flowering time and timing of seed germination (Hamasha & Hensen 2009). More specifically, seed germination of all four *Stipa* species was negatively correlated with annual precipitation, and populations with the highest seed germination were always of arid and Saharan Mediterranean origin. Differences in such demographic processes may produce patterns of genetic variation that resemble patterns due to natural selection (Nielsen 2001; Przeworski 2002). Population genetic structure developed under restricted gene flow cannot be distinguished from population structures produced by spatially heterogeneous selection (Turner *et al.* 1982; Sokal & Wartenburg 1983).

The separation of semiarid and arid populations from Saharan populations was more pronounced in *S. arabica* and *S. lagascae* than in *S. capensis* and S. *parviflora*. Less clustering in *S. capensis* and S. *parviflora* could be attributed to their wider distributional range and the similarity of ecological conditions in their populations. Thus, particularly for *S. arabica* and *S. lagascae*, genetic differentiation between bioclimatic regions may have been caused by local adaptation rather than solely by demographic processes. In accordance with this explanation, Saharan populations always clustered together and formed a separate group from the semiarid and arid populations, a pattern highly unlikely to be due to random processes.

A close association between genetic structure and environmental factors has also been found in a number of other grass species. In a recent SSR study from Jordan, 16 barley populations formed distinct clusters in mesic and xeric regions, indicating that clustering represents climatic and edaphic divergence (Baek *et al.* 2003). Similarly, analysis of the SSR

data revealed that temperature and precipitation gradients have shaped the genetic make-up of wild barley in Israel (Huebner *et al.* 2009). Genetic differentiation among populations of *Arrhenatherum elatius* was correlated with temperature and precipitation gradients, suggesting adaptive process of populations in response to climate gradients (Michalski *et al.* 2010). The findings of our study are consistent with these previous studies and indicate that genetic structure, revealed by AFLP analyses, are a result of both environmental selective drivers and neutral differentiation (see also Abratowska *et al.* 2012). Hence, the genetic structure of plant populations is not randomly distributed but is associated with ecogeographic factors.

Divergent selection at individual AFLP loci

The ultimate mechanisms causing ecogeographic differentiation in *Stipa* species are difficult to reveal because AFLPs are assumed to be selectively neutral. Previous AFLP studies suggested that such patterns could be due to selection of loci closely linked to neutral markers or due to lack of gene flow among populations occurring in contrasting environments (Volis *et al.* 2005; Vasemägi 2006; Odat *et al.* 2010). Along a steep gradient of rainfall extending to arid conditions, variation at genes related to drought stress, such as dehydrin genes (Vornam *et al.* 2011), may be under particularly strong selection. Our study species differed in the proportion of putatively selected markers, and in both the annual and perennial species relatively low (2.4–2.6% for *S. lagascae*, and *S. capensis*) and high (5.4% and 9.6% for *S. parviflora* and *S. arabica*) proportions of selected markers were found. The AFLP markers that decisively departed from a model of neutral evolution were more likely to be affected by environmental gradients than markers that behaved neutrally, suggesting climatic diversifying selection as a causal factor of genetic differentiation (Michalski *et al.* 2010). Thus, the genetic patterns detected with AFLPs reflect both demographic and selective processes in *Stipa* populations in response to bioclimatic conditions.

CONCLUSIONS

We conclude that genetic variation of Jordanian *Stipa* populations, as estimated by AFLPs, is influenced not only by the predominant random processes, but also by bioclimatic conditions. In accordance with our expectations, genetic diversity was higher in dry habitats in three of the four species, suggesting that environmental stress might select for high levels of genetic diversity. Population differentiation in all four study species was high, probably due to both genetic drift and diversifying selection in different climates. Thus, demographic process and climate variation among bioclimatic regions are crucial determinants of genetic structure of *Stipa* populations.

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