Environmental gradients shape the genetic structure of two medicinal Salvia species in Jordan

Article *in* Plant Biology · October 2016

DOI: 10.1111/plb.12512

6 authors, including:

Mohammad Al-Gharaibeh
Martin Luther University Halle-Wittenberg
13 PUBLICATIONS **279** CITATIONS
SEE PROFILE

Christoph Rosche
Martin Luther University Halle-Wittenberg
8 PUBLICATIONS **32** CITATIONS
SEE PROFILE

Susanne Lachmuth
Martin Luther University Halle-Wittenberg
20 PUBLICATIONS **133** CITATIONS
SEE PROFILE

Karsten Wesche
Senckenberg Research Institute
161 PUBLICATIONS **1,653** CITATIONS
SEE PROFILE

plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Environmental gradients shape the genetic structure of two medicinal *Salvia* species in Jordan

M. M. Al-Gharaibeh[1,2], H. R. Hamasha[3], C. Rosche[1,4], S. Lachmuth[1,5], K. Wesche[5,6] & I. Hensen[1,5]

1 Institute of Biology, Geobotany and Botanical Garden, Martin Luther University Halle Wittenberg, Halle/Saale, Germany
2 Department of Natural Resources and Environment, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan
3 Biology Department, Jerash University, Jerash, Jordan
4 Department of Botany, Faculty of Science, Charles University in Prague, Prague, Czech Republic
5 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
6 Senckenberg Museum of Natural History Görlitz, Görlitz, Germany

Keywords
AFLP; drought; flowering phenology; genetic diversity; phytogeographic regions; *Salvia spinosa*; *Salvia syriaca*.

Correspondence
M. M. Al-Gharaibeh, Martin Luther University Halle Wittenberg, Institute of Biology, Geobotany and Botanical Garden, Am Kirchtor 1, 06108 Halle/Saale, Germany.
E-mail: mohammad.al-gharaibeh@botanik.uni-halle.de

Editor
X. Wang

Received: 19 August 2016; Accepted: 1 October 2016

doi:10.1111/plb.12512

ABSTRACT

- Environmental gradients, and particularly climatic variables, exert a strong influence on plant distribution and, potentially, population genetic diversity and differentiation. Differences in water availability can cause among-population variation in ecological processes and can thus interrupt populations' connectivity and isolate them environmentally. The present study examines the effect of environmental heterogeneity on plant populations due to environmental isolation unrelated to geographic distance.
- Using AFLP markers, we analyzed genetic diversity and differentiation among 12 *Salvia spinosa* populations and 13 *Salvia syriaca* populations from three phytogeographical regions (Mediterranean, Irano-Turanian and Saharo-Arabian) representing the extent of the species' geographic range in Jordan. Differences in geographic location and climate were considered in the analyses.
- For both species, flowering phenology varied among populations and regions. Irano-Turanian and Saharo-Arabian populations had higher genetic diversity than Mediterranean populations, and genetic diversity increased significantly with increasing temperature. Genetic diversity in *Salvia syriaca* was affected by population size, while genetic diversity responded to drought in *S. spinosa*. For both species, high levels of genetic differentiation were found as well as two well-supported phytogeographical groups of populations, with Mediterranean populations clustering in one group and the Irano-Turanian and Saharo-Arabian populations in another. Genetic distance was significantly correlated to environmental distance, but not to geographic distance.
- Our data indicate that populations from moist vs. arid environments are environmentally isolated, where environmental gradients affect their flowering phenology, limit gene flow and shape their genetic structure. We conclude that environmental heterogeneity may act as driver for the observed variation in genetic diversity.

INTRODUCTION

The maintenance of genetic diversity is a fundamental prerequisite for species evolution and an important goal of conservation efforts (Frankham *et al.* 2010). It determines the ability of natural populations to cope with multiple biotic and abiotic stress factors (Khan *et al.* 2015), and is considered vitally important to their long-term survival (Bauert *et al.* 1998). Declining within-population genetic diversity is often associated with reduced population fitness (Leimu *et al.* 2006), and lower genetic diversity in populations can significantly reduce their adaptability to environmental change (Izawa *et al.* 2007) and increase the risk of extinction (Gilpin & Soule 1986).

Populations' genetic diversity and differentiation are controlled by interactions between gene flow, genetic drift and natural selection (Eckert *et al.* 2008), which are strongly influenced by demography and the level of environmental heterogeneity within the distribution range (Manel *et al.* 2003; Sommer *et al.* 2013). Widespread species are typically subject to climatic and geographic variation within their distribution range (*e.g.* Holman *et al.* 2003; Still *et al.* 2005). Abiotic (*e.g.* high temperature, drought and salinity) and biotic (*e.g.* competition) stresses can adversely affect the growth and productivity of plants and trigger a variety of morphological, phenological and molecular changes (Ahmad & Prasad 2012; Little *et al.* 2015; Wheeler *et al.* 2015). Hence, temperature and moisture count among the main environmental factors that can affect genetic differentiation and diversity among populations (Still *et al.* 2005). Landscape barriers present within a population's range (Duarte *et al.* 2015) can result in the isolation of populations by environment (Wang & Bradburd 2014). Such isolation can limit gene flow (Duarte *et al.* 2015) and lead either to low genetic diversity or to stronger genetic differentiation (Till-Bottraud & Gaudeul 2002).

Gene flow through seed and pollen ensures connectivity and largely shapes the genetic structure within and among plant

populations (Scheepens *et al.* 2012). However, populations of many plant species are spatially isolated by geographic distance, and seed dispersal among these populations is often restricted (Manel *et al.* 2003). Therefore, gene flow within and among populations is primarily dependent on and regulated by pollen dispersal (Scheepens *et al.* 2012). Long-distance dispersal in this context is, however, rare (Hardy *et al.* 2004). As such, pollen dispersal even over short distances may be dependent on and governed by the environmental differences between habitats (Wang & Bradburd 2014). Environmental gradients within species' ranges can cause gradual shifts in flowering time (Primack 1980; Cortés *et al.* 2013) leading to reduced pollen exchange and potentially to reproductive isolation (Franks & Weis 2009).

In any case, geographic differences in climate exert a strong influence on plant distribution (Djamali *et al.* 2012) and can define and determine a plant's phytogeographic range (Danin 1992). Overlapping ranges are combined in phytogeographic regions, which are clearly different in terms of bioclimatic envelope, as shown for Middle and Southwest Asia (Djamali *et al.* 2012). Altitudinal and latitudinal differences cause further environmental differentiation (Al-Eisawi 1996). Similarly, there are four distinct phytogeographic regions in Jordan (Zohary 1973; Al-Eisawi 1996), which create a sharp environmental transition across the country. Although spatially structured, these transitions are not simply associated with geographic distance and allow us to disentangle isolation by environment from isolation by distance.

Salvia spinosa L. and *Salvia syriaca* L. (Lamiaceae) are two medicinal plant species (Oran & Al-Eisawi 1998; Ulubelen 2003) occurring within different habitat types, representing a gradient of decreasing precipitation and increasing temperature (Al-Gharaibeh *et al.* 2016). In Jordan, the range of their natural distribution spans three phytogeographic regions (Mediterranean, Irano-Turanian and Saharo-Arabian). The climate conditions, altitudes of their habitats, soil types and population sizes for both species are highly variable among these regions (Zohary 1973; Danin 1992; Al-Gharaibeh *et al.* 2016). Studies over such environmentally heterogeneous geographic ranges are promising to reveal the influence of ecological conditions on plant genetic structure (Nevo 2001; Hamasha *et al.* 2013). For the present study, we hypothesised that the effect of climatic and altitudinal heterogeneity among these regions would have a role in shaping patterns of genetic diversity and differentiation in *Salvia* species. Using AFLP (amplified fragment length polymorphism), we attempted to answer the following questions: (i) how is genetic diversity distributed among and within populations of the two *Salvia* species; (ii) is genetic diversity related to population size or to differences in water availability and temperature among bioclimatic regions; and (iii) do differences in climate conditions and altitudinal ranges among the three phytogeographic regions have an influence on their genetic differentiation?

MATERIAL AND METHODS

Study area

Jordan hosts both arid and semi-arid Mediterranean ecosystems within a relatively small geographic area (Alhamad 2006), which has given rise to some remarkable and diverse habitats (Ababsa 2013). The country's topography is governed by a desert plateau, with highlands in the western areas comprising arable land and Mediterranean evergreen forests (Danin 1992; Al-Eisawi 1996). Altitudes range from 415 m a.s.l. at the Dead Sea to 1854 m a.s.l. at Um al-Dami Mountain (Wadi Rum). The overall climate is eastern mediterranean and is characterised by mild and moderately rainy winters with hot rainless summers (Al-Eisawi 1996). Bioclimatic analyses by Al-Eisawi (1996) indicated the presence of nine bioclimatic subdivisions in Jordan that fall under four main phytogeographic regions, namely the Mediterranean, the Irano-Turanian, the Saharo-Arabian and the Sudanian. The environmental heterogeneity of the phytogeographic regions is remarkable, with vegetation cover and density, soil texture, altitude, temperature and annual rainfall being the principal characterising factors (Zohary 1973; Al-Eisawi 1996). According to Al-Eisawi (1996, data from 31 metrological stations), altitude in three of the four phytogeographic regions ranges from 700–1700 m in the Mediterranean, 400–700 m in the Irano-Turanian and 600–700 m in the Saharo-Arabian region, while rainfall varies between 300–600 mm, 150–250 mm and 50–100 mm, and mean summer temperatures are 20, 25 and >30 °C, respectively (Table 1). We also calculated a drought Index (DI; Cortés *et al.* (2013), which clearly indicates increasing aridity from the Mediterranean region to the Saharo-Arabian region (Table 1).

Study species

Salvia spinosa (2n = 2x = 20; Al-Turki *et al.* 2000) and *Salvia syriaca* (2n = 2x = 22; Afzal-rafii 1980) are short-lived perennial herbs that can grow from 30 to 60 cm and 30 to 80 cm, respectively (Zohary & Feinbrun 1966). Their flowering season extends from April to June for *S. spinosa* and until July for *S. syriaca*. Flowers of both species receive visits from honeybees and bumblebees in the field (Al-Gharaibeh *et al.* 2016), which is supported by Claßen-Bockhoff *et al.* (2004), who cite bee pollination for the majority of *Salvia* species. Both *Salvia* species produce mucilaginous seeds (Al-Gharaibeh *et al.* 2016). Flowering stems typically break off at a point below the panicle in *S. spinosa* and seeds are subsequently dispersed by wind-blown tumbling, while gravity is the only dispersal mechanism for *S. syriaca* seeds (personal observation). In addition, field observations indicate that early summer rainfall can hamper seed dispersal in *S. spinosa* due to production of mucilage, which firmly attaches the seeds to the dry calyx. Populations of *S. spinosa* inhabit the Irano-Turanian region and extend to the Mediterranean and Saharo-Arabian regions. Despite having the same chorotype as *S. spinosa*, *S. syriaca* is more common in the Mediterranean region but much rarer in the Saharo-Arabian region (Zohary & Feinbrun 1966; Danin 1992). In the Mediterranean and Irano-Turanian regions, seed-producing populations of both species were highly fragmented, with population sizes (number of individuals) being relatively small due to agricultural practices that treat both species as weeds because of their allelopathic properties (Qasem 2001). In addition, seed production is very low in *S. syriaca* (Al-Gharaibeh *et al.* 2016).

Plant sampling and DNA extraction

In the periods April–June 2012 and May–June 2013, we took leaf samples from 12 individuals each from 12 and 13 natural

Table 1. Environmental conditions, population size and genetic diversity parameters (H_e, PPB, BR) for the study populations of *Salvia spinosa* and *S. syriaca*.

		Geographic			Climate						Genetic diversity		
Population	Pop. code	Lt	Ln	Al	Rn	DI	Tm	Ta	Tj	Pop. size	H_e	PPB	BR
Salvia spinosa													
Mediterranean region													
Fjaej	Med1	30.57	35.63	1265	307	32.88	15.5	19.4	4.4	19	0.15	42.4	1.339
Abo Bana	Med2	30.87	35.67	1150	220	56.91	16.0	20.7	6.5	17	0.15	42.4	1.323
Kings Road	Med3	30.08	35.43	1615	216	37.93	15.5	21.6	5.8	67	0.14	41.9	1.285
Gafgafa	Med4	32.37	35.92	780	369	27.98	17.5	24.4	9.1	18	0.17	61.0	1.471
Mean											**0.15**	**46.93**	**1.35**
Irano-Turanian region													
JUST	Ira1	32.48	35.98	585	272	52.52	17.4	27.0	4.0	173	0.18	62.8	1.35
Sarrot	Ira2	32.17	35.95	535	262	59.61	17.9	27.0	4.0	23	0.21	72.1	1.406
Humret Sahen	Ira3	32.1	35.66	650	297	55.07	19.0	26.5	11.7	19	0.21	71.5	1.413
Al Jezzah	Ira4	31.68	35.96	715	168	67.39	18.0	23.6	8.4	53	0.22	69.2	1.431
Mean											**0.21**	**68.9**	**1.4**
Saharo-Arabian region													
Al-Azaraq	Sah1	31.87	36.74	560	99	88.43	18.5	25.1	8.7	15	0.27	72.1	1.567
BorquCastle	Sah2	32.6	38	545	96	88.55	18.5	26.0	7.0	13	0.23	69.8	1.453
Wadi Rum	Sah3	29.6	35.38	960	48	89.63	23.0	39.2	9.3	12	0.14	41.9	1.295
Safawi	Sah4	31.99	36.85	615	86	88.46	18.5	27.0	9.2	31	0.26	75.0	1.535
Mean											**0.23**	**64.7**	**1.46**
Overall Mean											**0.19**	**60.18**	**1.41**
Salvia syriaca													
Mediterranean region													
Madaba	Med1	31.75	35.76	780	306	39.98	17	22.9	8.5	>2000	0.27	74.6	1.609
Ras Yousif	Med2	32.72	35.87	820	459	37.59	16.5	22.4	7.1	80	0.20	64.4	1.358
Airport Highway	Med3	31.87	35.88	920	262	43.55	16	23.6	8.4	35	0.12	23.7	1.267
Abo Bana	Med4	30.87	35.67	1150	220	56.91	16	20.7	6.5	19	0.11	20.3	1.214
Burgush	Med5	32.4	35.7	875	480	19.73	16	22.0	4.0	44	0.13	50.8	1.249
Beer Dabaghat	Med6	30.39	35.5	1610	264	32.91	14.5	19.4	4.4	300	0.10	16.9	1.189
Mean											**0.16**	**41.78**	**1.31**
Irano-Turanian region													
Sarrot	Ira1	32.17	35.95	535	262	59.61	17.9	27.0	4.0	63	0.19	50.8	1.448
Shafa Badran	Ira2	32.04	35.91	760	250	49.21	17.5	24.0	8.4	75	0.17	45.8	1.374
Rehab	Ira3	32.35	36.02	795	234	54.43	17.5	24.0	4.0	277	0.17	47.5	1.339
Karak	Ira4	31.2	35.73	745	168	57.95	18	24.1	9.5	250	0.16	49.2	1.33
Dieban	Ira5	31.59	35.79	635	256	61.77	18.5	24.9	10.5	>300	0.22	62.7	1.515
Mean											**0.18**	**51.2**	**1.4**
Saharo-Arabian region													
Mafraq 1	Sah1	32.33	36.19	720	168	69.12	18.5	24	4.0	42	0.19	47.5	1.392
Mafraq 2	Sah2	32.37	36.21	525	166	75.66	17.5	24.8	7.4	51	0.15	42.4	1.3
Mean											**0.17**	**44.95**	**1.35**
Overall Mean											**0.17**	**45.89**	**1.35**

Pop = population; Med = Mediterranean, Ira = Irano-Turanian, Sah = Saharo-Arabian phytogeographic region; Geographic: Lt = latitude (decimal degrees), Ln = longitude (decimal degrees), Al = Altitude (m); Climate, Rn = mean annual rainfall (mm), DI = Drought index; Tm = mean annual temperature (°C), Ta = temperature of mean hottest month (August) (°C), Tj = mean coldest month (January) (°C); Genetic diversity, H_e = mean gene diversity, PPB = percentage polymorphic bands, BR = band richness.

Mean values for genetic diversity parameters per region and per species are presented in bold.

populations of *S. spinosa* and *S. syriaca*, respectively, for AFLP analysis. The sampling approach covered the entire range of the species' natural distribution in Jordan and represented a broad altitudinal range of 526 m a.s.l. to 1616 m a.s.l. (Table 1, Fig. 1). Four populations per phytogeographic region were sampled for *S. spinosa*. For *S. syriaca*, we sampled six populations from the Mediterranean region and five from the Irano-Turanian region, while only two populations were found in the Saharo-Arabian region, where the species is rare (Table 1). Onset and termination of the flowering period were monitored and documented for all populations in the growing seasons 2012 and 2013 (Fig. 2). Number of individuals per population was directly counted for the smaller populations or estimated for large populations by counting a 25 × 25 m quadrat and extrapolating from that. The populations sampled differed in size; *S. spinosa* ranged from 12 to 173 individuals while *S. syriaca* ranged from 19 to over 2000 individuals per population (Table 1). The minimum distance between two conspecific populations was 10 km, with the maximum distance being over 300 km.

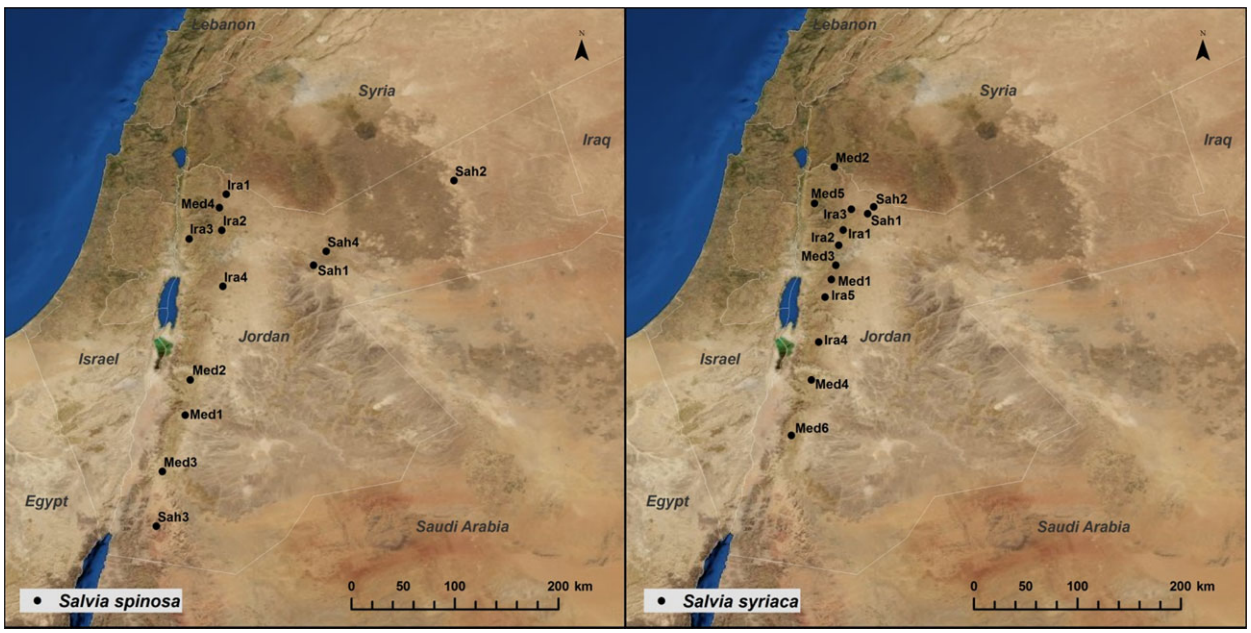

Fig. 1. Geographic distribution of the sampled populations of *Salvia spinosa* (left map) and *Salvia syriaca* (right map). Med = Mediterranean, Ira = Irano-Turanian, Sah = Saharo-Arabian phytogeographic region.

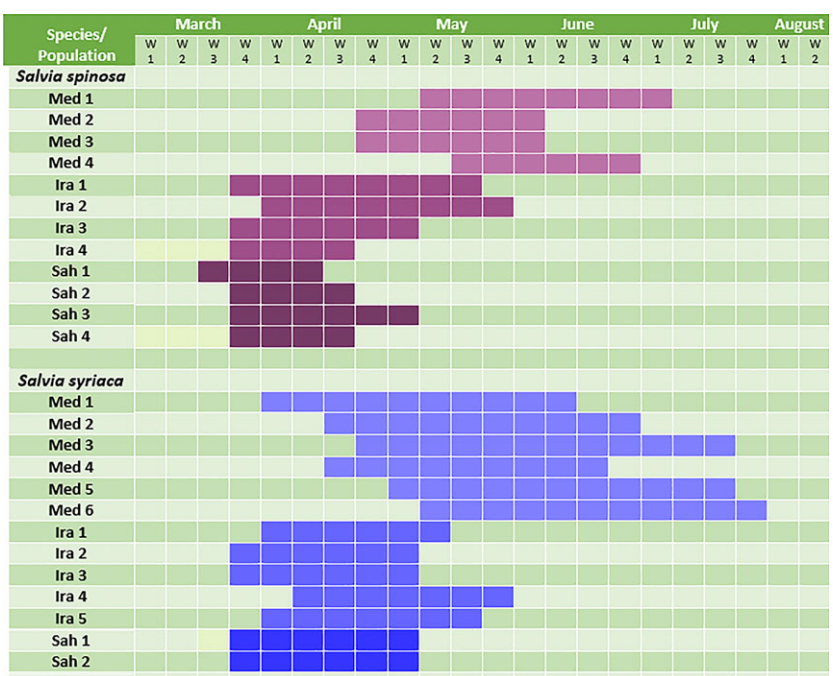

Fig. 2. Onset and length of the flowering period. Two years of field observations of the flowering time for 12 and 13 populations of *Salvia spinosa* and *Salvia syriaca*, respectively. Med = Mediterranean, Ira = Irano-Turanian, Sah = Saharo-Arabian phytogeographic region.

Total genomic DNA was extracted as described in Stein *et al.* (2014); roughly 20 mg of 300 desiccated leaf samples were ground to a fine powder and used for DNA isolation. Extracted genomic DNA was double-digested using the restriction enzymes *Mse*I and *Eco*RI, with the ends of the resulting fragments being ligated to form a double-stranded adapter. For fragment amplification, 16 primer combinations were screened, resulting in four primer combinations for fingerprinting AAG/CAT, AGC/CAT, AAG/CCA and ACT/CCA. AFLP main amplification products in plates (96-well plates) were purified by centrifugation on a column of Sephadex G-50 Superfine powder, and the purified amplification products were analysed using a MegaBACE 1000 sequencer (GE Healthcare, Chicago, IL, USA).

Genotyping AFLP

Peaks generated from the MegaBACE sequencer were converted into 0/1 matrices with a MegaBACE Fragment Profiler (version 1.2; Amersham Bioscience, Chicago, IL, USA). Using these binary data matrices, polymorphic DNA bands were given scores of 0 (absence) or 1 (presence). To avoid errors and

any bias resulting from the manual scoring process, an automated scoring system was generated following the approach of Ley & Hardy (2013) in SPAGeDi 1.4 software (Hardy & Vekemans 2002), with at least 25% of the individuals being replicated. The broad-sense heritability of each marker was then estimated and expressed as an F_{ST} value following 1000 random permutations. The four primer pairs used in the AFLP analyses of *S. spinosa* and *S. syriaca* resulted in 172 and 59 polymorphic bands, respectively.

Environmental variables

For each site, we characterised environmental and climate conditions by altitude, mean annual precipitation (rainfall) and mean annual January and August temperature, based on Al-Eisawi (1996) and FetchClimate (Grechka *et al.* 2016; Table 1). Moreover, potential evapotranspiration values were retrieved through FetchClimate (Grechka *et al.* 2016). The normalised annual mean drought index (DI) for each site was calculated according to Cortés *et al.* (2013, Table 1). With the aim of determining which variables were highly correlated, we carried out a principal components analysis (correlation matrix PCA) on environmental variables in R (version 3.0.2, package *vegan*; R Core Team 2013; Oksanen *et al.* 2013). The first two axes explained 80% of the environmental variation in both species. Along the first PCA axis (PC1) mainly elevation increased and temperature decreased (data not shown), while the DI strongly increased with decreasing rainfall along PC2. We thus retained temperature and DI as key variables for further analyses. Environmental distance between population pairs was calculated as Euclidean distance of the factor scores.

Spatial distribution of genetic diversity

A number of different approaches exist for analysing AFLP data (Bonin *et al.* 2007). In our study we determined key parameters following (i) allele frequency approaches for estimating Nei's gene diversity (H_e), and (ii) band-based approaches, by estimating the percentage of polymorphic bands (PPB), band richness (BR) among populations according to Lynch & Milligan (1994) with 10,000 permutations using AFLP-SURV 1.0 (Vekemans *et al.* 2002). Initially, a one-way ANOVA was used to determine whether there were any significant differences between the means of the three phytogeographic regions for each parameter (H_e, PPB and BR). For further statistical tests regarding the spatial distribution of genetic diversity in response to temperature (PCA 1), drought (PCA 2) and number of individuals (explanatory variables), we used the Pearson method and assessed pair-wise correlation and linear regression to check the effect of each variable on genetic diversity. Pearson pair-wise correlation and linear regression analyses were carried out in R-3.0.2 (R Core Team 2013). The correlations of genetic diversity parameters as well as explanatory variables were checked with and without log transformation, and the best fitting model was chosen. The same approach was also used to test the significance of each explanatory variable on the distribution pattern of genetic diversity. Akaike's information criterion (AIC; Akaike 1974) was used to compare alternative models (with and without log transformation) for the same explanatory variable and to identify the model that best explained variation in this response.

Genetic differentiation, clustering analyses and isolation by distance

To assess the level of differentiation and grouping of populations by phytogeographic region, we estimated the Molecular Variance (AMOVA based on Φ_{ST} distance; Excoffier *et al.* 1992) using Arlequin (version 3.5; Excoffier & Lischer 2010). The Sørensen coefficient (Sørensen 1948) was determined with the R package *vegan* (Oksanen *et al.* 2013), which is similar to Jaccard dissimilarity but places more emphasis on shared bands. The dissimilarity matrix was square root-transformed and served as a basis for principal coordinates analysis (PCoA) to investigate the pattern of genetic differentiation among individuals, populations and phytogeographic regions for the two *Salvia* species. In parallel, individuals of both species were also assigned to genetically homogeneous clusters *via* the model-based clustering algorithm provided in the STRUCTURE 2.3.3 program (Pritchard *et al.* 2000). The Bayesian analysis parameters were set by choosing an admixture model with correlated allele frequencies among populations with 100,000 burn-in length periods and 500,000 MCMC. Each run was iterated 20 times, with K ranging from 1 (assuming one panmictic population) to 12 (assuming every population formed an own cluster). We set K at 1 to 12 for *S. spinosa* and to 13 for *S. syriaca*. Subsequently, a Pr (X|K) index with respect to each K value was used to calculate ΔK using the formula described in Evanno *et al.* (2005) for the best number of clusters.

The relationships between genetic distance among populations and both geographic and environmental distances were examined with Mantel tests (Mantel 1967). As geographic and environmental distance were not independent (Mantel $P < 0.01$), a simple and a partial Mantel test were performed to examine whether genetic differentiation depended on geographic or environmental isolation using the *vegan* package (Oksanen *et al.* 2013) in R. We then created a pair-wise F_{ST} distance matrix and a geographic distance matrix; significance of correlations was determined based on 1000 permutations.

RESULTS

Flowering period

Based on 2 years of observation, the flowering period in terms of onset, length and offset varied among and within the regions' populations (Fig. 2). In both species, populations from Irano-Turanian and Saharo-Arabian regions started to flower earlier than in the Mediterranean parts. Moreover, the flowering time of all populations from Irano-Turanian and Saharo-Arabian regions overlapped in April. In the period between May and mid-June flowering time overlapped for all Mediterranean populations. Within the Mediterranean regions, populations found in forest habitats with northwest-facing slopes (Med 1and Med 4 in *Salvia spinosa*, Med 6 in *Salvia syriaca*) showed a delay in the onset of their flowering and a longer duration of the flowering period.

Spatial distribution of genetic diversity

The overall mean gene diversity (H_e), percentage polymorphic bands (PPB) and band richness (BR) were 0.19, 60.18 and 1.41 in *S. spinosa*, and 0.17, 45.89 and 1.35 in *S. syriaca*, respectively

(Table 1). In both species, mean values for each of the genetic diversity parameters (H_e, PPB and BR) indicated that Mediterranean populations showed the lowest genetic diversity compared to those of Irano-Turanian and Saharo-Arabian regions (Table 1). In *S. spinosa*, the one-way ANOVA detected a significant difference between the Mediterranean and Saharo-Arabian regions for H_e ($F_{2,\ 9} = 4.25$, $P = 0.05$) and PPB ($F_{2,\ 9} = 4.78$, $P < 0.05$), while in *S. syriaca* the differences were not significant between the three regions for all parameters. The only exception to this general pattern was the Wadi Rum population (Saharo-Arabian), where the estimated values for all diversity parameters of this population were more similar to the Mediterranean populations (Table 1).

Pearson pair-wise correlation indicated that the log-transformed genetic diversity parameters (H_e, PPB and BR) for both species were positively correlated at $P < 0.001$ in all cases (H_e – PPB, r = 0.938; H_e –BR, r = 0.902; PPB–BR, r = 0.833 for *S. spinosa*; and H_e –PPB, r = 0.929; H_e –BR, r = 0.959; PPB–BR, r = 0.845 for *S. syriaca*). Linear regression of the genetic diversity (H_e, PPB and BR) parameters with the explanatory variables (DI, temperature and number of individuals) revealed a significant effect of temperature (positive, $P < 0.01$) on all parameters in *S. syriaca* (Table 2). In addition, only band richness (BR) in the former species was significantly related to the number of individuals (positive, $P < 0.05$), while drought had no significant effect at all. In *S. spinosa*, temperature, drought and number of individuals had no significant effect on the three parameters (H_e, PPB and BR) when all sampled populations were included in the analyses. When excluding one outlier population (Wadi Rum) from the data set, the effects of drought (positive, $P < 0.05$) and temperature (positive, $P < 0.01$) were also significant (Table 2).

Genetic differentiation, clustering analyses and isolation by distance

Genetic differentiation (ϕ_{ST}) in the populations of *S. spinosa* and *S. syriaca* was 0.71 and 0.64, respectively, which was highly significant ($P < 0.001$; Table 3). After grouping the populations according to their bioclimatic region affinity (Mediterranean, Irano-Turanian and Saharo-Arabian), *S. spinosa* and *S. syriaca* showed strong differentiation among the groups (55.18% and 35.65% of the explained variance in AMOVA, respectively; Table 3). However, the percentage of differentiation among populations within the groups was somewhat lower at 20.33% for *S. spinosa* and 32.66% for *S. syriaca*.

For both species, the PCoA revealed the existence of two well-separated groups among the three phytogeographic regions; the Mediterranean populations clustered into one group while the Irano-Turanian and Saharo-Arabian populations formed the other group (Fig. 3). The first two PCoA axes accounted for 71% of the genetic variation for *S. spinosa* and 37% for *S. syriaca*. Most strikingly, for *S. spinosa*, the Wadi Rum population from the Saharo-Arabian region grouped among the Mediterranean populations. Results of the PCoA were also confirmed using the Bayesian STRUCTURE analysis, and the optimum ΔK value, was K = 2, where all individuals belonging to the two *Salvia* species were differentiated into two dissimilar clusters (Fig. 4), The Irano-Turanian and Saharo-Arabian populations formed one group, while the other group included only Mediterranean populations. This pattern of differentiation was very obvious in *S. syriaca*, while for *S. spinosa* the Wadi Rum population with Saharo-Arabian affinity again formed part of the Mediterranean group.

Simple and partial Mantel tests revealed that in both study species, genetic differentiation between populations was not significantly related to geographic distance (Table 4). Genetic differentiation was correlated to environmental distance when controlling for effects of geographic distance (Table 4).

DISCUSSION

Our AFLP data revealed a strong phytogeographic pattern among the populations of *Salvia spinosa* and *Salvia syriaca*. There was a clear genetic split between populations belonging to more moist environments (Mediterranean) and those of arid environments (Saharo-Arabian and Irano-Turanian), which may indicate limited gene flow between Mediterranean

Table 2. Linear regression analyses, predicted effect of each parameter (longitude, latitude, altitude, rainfall, temperature and number of individuals) on genetic diversity (Band Richness, Gene diversity and Percentage polymorphic bands).

	Salvia spinosa								*Salvia syriaca*			
	Gene diversity (H_e)+				Gene diversity (H_e)−				Gene diversity (H_e)			
	AIC	df	F-statistic	*P*-value	AIC	df	F-statistic	*P*-value	AIC	df	F-statistic	*P*-value
Temperature	3.63	10	0.63	0.446	−6.65	9	13.68	<0.01	−2.25	11	15.76	<0.01
Drought Index	−0.44	10	4.93	0.05067	−11.34	9	25.76	<0.001	8.32	11	0.8707	0.3708
Number of individuals	4.23	10	0.12	0.739	2.94	9	0.49	0.501	4.71	11	4.67	0.054
	% polymorphic bands (PPB)+				% polymorphic bands (PPB)−				% polymorphic bands (PPB)			
Temperature	4.89	10	0.7	0.422	−8	9	19.11	<0.01	7.52	11	21.05	<0.001
Drought Index	3.69	10	1.828	0.2062	−1.37	9	6.3	<0.05	21.30	11	0.1047	0.7524
Number of individuals	5.66	10	0.04	0.847	4.45	9	0.06	0.813	19.18	11	2.067	0.178
	Band richness (BR)+				Band richness (BR)−				Band richness (BR)			
Temperature	−27.59	10	0.63	0.637	−34.64	9	12.61	<0.01	−31.85	11	13.48	<0.01
Drought Index	−29.08	10	2.04	0.1836	−31.31	9	6.978	<0.05	−22.93	11	0.9684	0.3462
Number of individuals	−27.7	10	0.73	0.414	−26.97	9	1.76	0.217	−28.33	11	7.13	<0.05

AIC = Akaike information criterion; df = Degrees of freedom; **+** = for all *S. spinosa* populations; − = without Wadi Rum population.

Table 3. AMOVA of (a) 144 individuals belonging to 12 populations of *Salvia spinosa*, and of (b) 156 individuals belonging to 13 populations of *Salvia syriaca*. For each species, an AMOVA without prior grouping and with groups based on phytogeographic region is given. *P*-values are based on 1000 permutations.

Species	df	Sum of squares	Variance	% variation	Φ Statistics	*P*-value
(a) *Salvia spinosa*						
Without groups						
Among populations	11	3868.293	29.1352	71.17	$\Phi_{ST} = 0.712$	<0.001
Within populations	128	1510.750	11.80273	28.83		
Phytogeographic region						
Among groups	2	2734.055	26.58935	55.18	$\Phi_{CT} = 0.551$	<0.001
Among populations	9	1134.238	9.79561	20.33	$\Phi_{SC} = 0.453$	<0.001
Within groups						
Within populations	128	1510.750	11.80273	24.49	$\Phi_{ST} = 0.755$	<0.001
(b) *Salvia syriaca*						
Without groups						
Among populations	12	1036.12	7.04693	63.98	$\Phi_{ST} = 0.640$	<0.001
Within populations	139	551.406	3.96695	36.02		
Phytogeographic region						
Among groups	2	518.125	4.46197	35.65	$\Phi_{CT} = 0.356$	<0.001
Among populations	10	517.995	4.08748	32.66	$\Phi_{SC} = 0.510$	<0.001
Within groups						
Within populations	139	551.406	3.96695	31.69	$\Phi_{ST} = 0.683$	<0.001

df = Degrees of freedom.

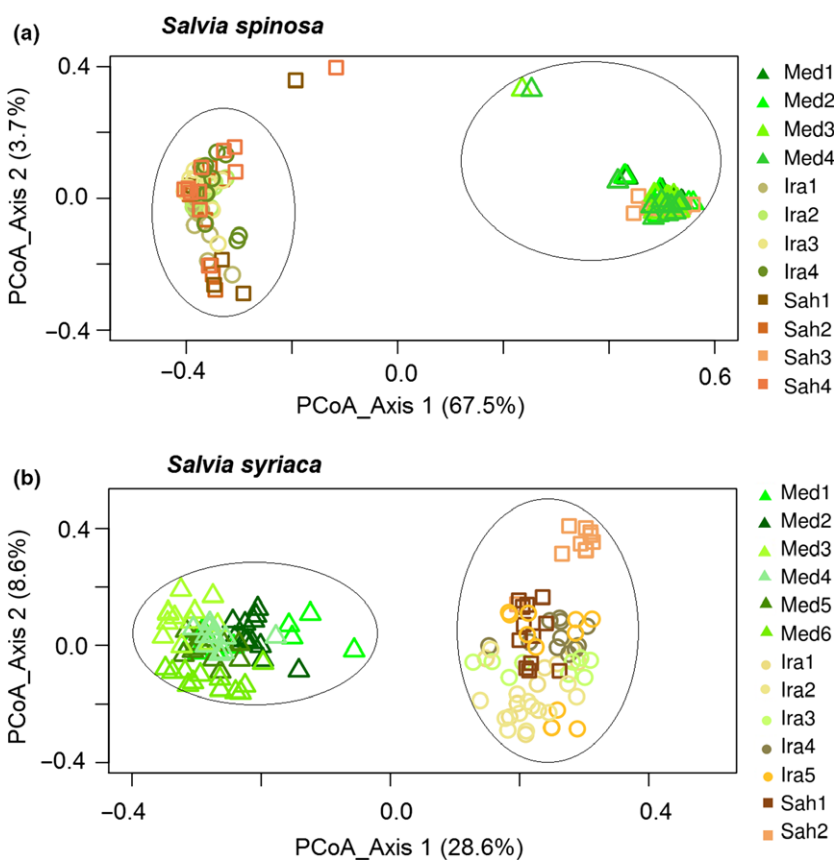

Fig. 3. Principal coordinates analysis for 12 and 13 populations of *Salvia spinosa* and *Salvia syriaca*, respectively Different symbols indicate bioclimatic regions. Med = Mediterranean, Ira = Irano-Turanian, Sah = Saharo-Arabian phytogeographic region.

populations and Saharo-Arabian/Irano-Turanian populations. Such a limitation in gene flow would give rise to fragmentation among habitats (Qian *et al.* 2013), which can lead to the evolution of distinct genetic lineages with substantial accumulation of differences in the genome (Mráz *et al.* 2007). Gene flow either by seed, pollen or both largely shapes the genetic structure within and among plant populations (Scheepens *et al.* 2012). Because most seeds only move short distances (Cain *et al.* 2000), seed dispersal is often found to be strongly spatially restricted (Scheepens *et al.* 2012), which makes the gene

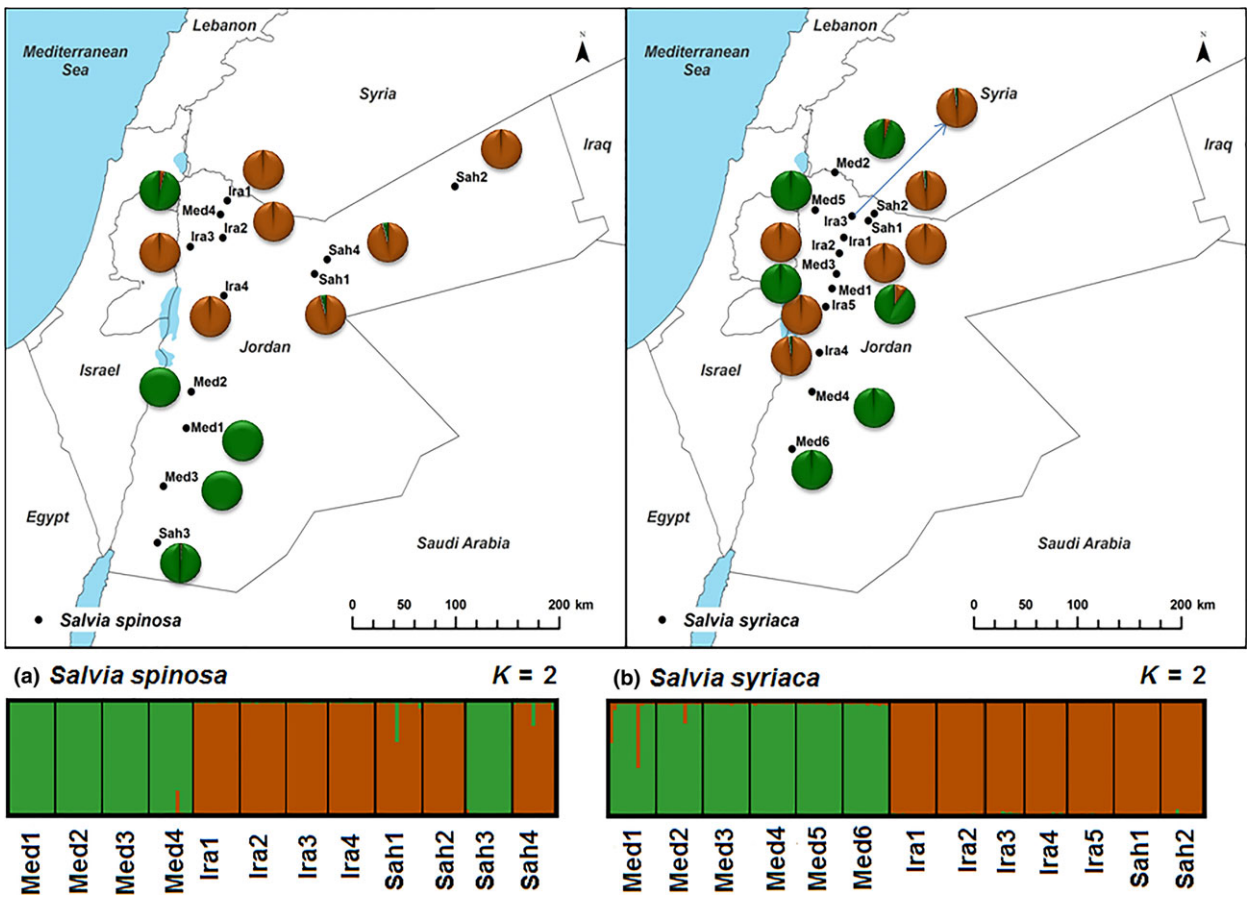

Fig. 4. Genetic clustering of 12 and 13 study populations of *Salvia spinosa* and *Salvia syriaca* according to STRUCTURE analysis (K = 2). Each bar denotes an individual and colours indicate the degree of affinity to each cluster. Pie charts indicate genetic membership at the population level. Med = Mediterranean, Ira = Irano-Turanian, Sah = Saharo-Arabian phytogeographic region.

Table 4. Tests for isolation. Correlation coefficients and *P*-values of simple 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(geo)		gen-geo(env)	
	r	Mantel *P*	r	Mantel *P*	r	Mantel *P*	r	Mantel *P*
Salvia spinosa[a]	0.114	0.152	0.418	0.009	0.356	0.020	0.010	0.467
Salvia syriaca	−0.087	0.654	0.224	0.065	0.268	0.046	−0.174	0.844

[a]Without the outlier population (Wadi Rum).

flow within and into plant populations primarily dependent on pollen dispersal (Bacles & Ennos 2008).

Variation in flowering time and gene flow

In both study species, populations from drier and warmer habitats with sparse vegetation cover (Irano-Turanian and Saharo-Arabian regions) started their flowering period earlier than populations from moist and cooler habitats with dense vegetation cover (Mediterranean region). Also, we found variation among populations of the same region. However, strong flowering time differentiation within short distances is not uncommon (Cortés *et al.* 2013). Primack (1980) found apparent variation in flowering time among adjacent populations, depending on their altitude and climate conditions. Arid ecotypes usually start flowering earlier than ecotypes growing under more humid conditions (Volis 2007). However, photoperiod and temperature are two pivotal factors that trigger and regulate flowering time (Song *et al.* 2012). For example, under high temperatures and full sun exposure, onset of flowering in *Salvia* species starts earlier and is shorter (Pramuk & Runkle 2005). Moreover, soil salinity in Saharo-Arabian and Irano-Turanian regions (Al-Gharaibeh *et al.* 2016) can reduce the number of inflorescences (Ventura *et al.* 2014) and may consequently shorten the flowering period. Soil moisture can also affect the onset and duration of flowering time (Franks

et al. 2007). Hence, environmental heterogeneity among the geographic units of the species' range can lead to variation in flowering time (Primack 1980).

The higher variation in the flowering phenology among the Mediterranean populations of both species might also result from higher variation in topography and vegetation cover. Måren *et al.* (2015) revealed that slope aspect affects microclimate in terms of light intensity, soil and air temperature, humidity, soil moisture, evaporation and duration of the growing period. Indeed, these differences between north/south slope aspects were significantly higher in the Mediterranean region than in semiarid, arid and extreme-arid regions (Kutiel & Lavee 1999).

Variations in flowering time can limit the degree of gene flow within and among populations (Franks & Weis 2009). Hence, early-flowering plants are likely to mate with other early-flowering plants, while late-flowering plants are likely to mate with other late bloomers (Fox 2003). Thus, variation in flowering phenology can potentially increase reproductive isolation and effect genetic differentiation within and among populations (Franks & Weis 2009). This may have contributed to apparent genetic structuring.

Spatial distribution of genetic diversity

The overall mean gene diversity (H_e) for both *Salvia* species lies within the worldwide gene diversity range found for 51 *Salvia* species (*i.e.* H_e = 0.024–0.809; Braglia *et al.* 2011). However, these values are lower than the average gene diversity for short-lived perennials (Nybom 2004), since smaller, more fragmented populations are often found to have relatively low genetic diversity, as *e.g. Stipa* species (Nosrati *et al.* 2012; Hamasha *et al.* 2013). For the two study species, genetic diversity strongly increased with temperature, while in *S. spinosa* genetic diversity also increased as drought increased. These effects could have resulted from decreasing rainfall and increasing temperature towards the eastern and southern arid zones in Jordan, which creates steep climate and ecological clines over relatively short distances (Hamasha *et al.* 2013). The effect of environmental stress in enhancing genetic diversity in *S. spinosa* was more pronounced when the outlier population, Wadi Rum, was excluded. According to Danin (1999b) and Migliore *et al.* (2013), mountains located in Saharan areas, such as Wadi Rum, can offer microhabitats with relatively cool temperatures and sufficiently good levels of humidity where the underlying sandstone aquifer capacity is high (Danin 1999a) or there is sufficient run-off from crevices, creating microhabitats (Danin 2008).

The pattern of increasing genetic diversity with environmental stress in our study was found to be more evident and significant in *S. spinosa* than in *S. syriaca*. This can be explained by the differing distribution pattern of both species' populations. *Salvia spinosa* populations have a wider distribution range and extend further into eastern areas than *S. syriaca* populations, which have a more linear distribution, running from north to south (Fig. 1). In the southern Mediterranean Basin, plant genetic diversity is characterised by a clear increasing trend from the moister west to the drier east (Conord *et al.* 2012). In addition, Hamasha *et al.* (2013) found that genetic diversity in Jordanian *Stipa* populations increases from north to south and from west to east in response to increasingly stressful environments, namely decreasing rainfall towards the eastern and southern part of the country. Our study thus adds to a growing body of literature highlighting the role of environmental variables in shaping genetic diversity within plant species (Still *et al.* 2005; Conord *et al.* 2012; Hamasha *et al.* 2013; Jugran *et al.* 2013).

A PCA analysis revealed that one of the two essential principal directions of our study climate space is a gradient in temperature that is negatively correlated with altitude. Due to the significant positive effects of temperature on all estimates of genetic diversity, in both species, we thus assume that these variables are negatively affected by altitude, which is in accordance with a number of other studies (*e.g. Arabidopsis thaliana,* Gomaa *et al.* 2011; *Stipa capensis,* Hamasha *et al.* 2013; *Valeriana jatamansi,* Jugran *et al.* 2013). Declining genetic diversity toward higher altitudes may be explained by upward migration during Holocene warm periods (Hensen *et al.* 2011), or may be the result of lower environmental stress (temperature and drought) at higher altitudes in the Near East (Danin 1999a). Elsewhere, several studies have reported that genetic diversity increases under more stressful (*i.e.* dry and hot) environmental conditions (Nevo 2001; Badri *et al.* 2008; Peleg *et al.* 2008; Fitzgerald *et al.* 2011; Hamasha *et al.* 2013). Moreover, variation in habitat with respect to vegetation cover at differing altitudes can moderate environmental conditions (Wheeler *et al.* 2015). For instance, dense vegetation cover facilitates plant protection from temperature extremes (Wheeler *et al.* 2015), reduces soil moisture evaporation (Kutiel & Lavee 1999), affects the pollination process and, consequently, genetic diversity (Jugran *et al.* 2013). In the latter study, genetic diversity in *Valeriana jatamansi* populations from forest habitats (Mediterranean habitat in our study) was lower than that in grassland habitats (Irano-Turanian and Saharo-Arabian regions in our study). As such, we assume that the sharp transition in environmental conditions among the Jordanian phytogeographic regions has had a strong influence on the genetic diversity of the two study species.

Population sizes in *S. spinosa* had no effect on genetic diversity, while a significant positive correlation between population size and genetic diversity was found for *S. syriaca*. In accordance, the highest levels of genetic diversity were recorded for the two largest populations (Madaba >2000 individuals, H_e = 0.27, PPB = 74.6, BR = 1.609; and Dieban >300 individuals, H_e = 0.22, PPB = 62.7, BR = 1.515). While population size is known to strongly affect gene diversity (Leimu *et al.* 2006), Van Treuren *et al.* (1991) found that genetic diversity in *Salvia pratensis* populations was correlated more closely to plant density than overall number. This effect might be related to the rate of outcrossing pollination, since larger, denser populations can be more attractive to pollinators than smaller, more fragmented populations (Jennersten 1988; Wilcock & Neiland 2002).

Genetic differentiation, clustering analyses and isolation by distance

The Φ_{ST} values recorded in our study for both *S. spinosa* (0.712) and *S. syriaca* (0.640) are much higher than mean Φ_{ST} values observed in short-lived perennials elsewhere (0.41; Nybom 2004). Geographic distance can restrict a population's connectivity and can lead to high levels of differentiation, as found for *Salvia officinalis* by Liber *et al.* (2014). Moreover, the heterogeneity of habitat and environment among populations

may influence gene flow by way of disrupting or discriminating dispersal characteristics and selecting only more local strains, resulting in a pattern of isolation by environment (Wang & Bradburd 2014).

According to Mantel tests, genetic differentiation among populations of our study species was not related to geographic distance but significantly related to environmental distance. As such, the absence of isolation-by-distance strongly indicates that gene flow between populations *via* seed or pollen dispersal is infrequent (Hamasha *et al.* 2013) and populations are environmentally isolated. Based on the phytogeographic regions, AMOVA analysis for *S. spinosa* showed very high differentiation among groups (55.18%), while differentiation among populations was lower (20.33%), suggesting a higher level of gene flow within the same phytogeographic region than between regions. According to Duarte *et al.* (2015), variations in humidity and temperature among habitats can cause variation in flowering phenology and consequently limit gene flow, which leads to a high probability of reproductive isolation (Franks & Weis 2009). Therefore, we assume that the differences in drought and temperature between the Mediterranean and other phytogeographic regions isolated the *Salvia* populations environmentally and shaped their genetic structure. The high genetic differentiation among the populations of *S. syriaca* (32.66%), even after grouping them according to their regional affinity, suggests that gene flow between populations by seed or pollen dispersal within the same phytogeographic region is very rare.

As differentiation in genetic structure can be detected from AFLP markers (Herrmann *et al.* 2010), PCoA and Bayesian STRUCTURE analysis revealed that populations of both species clustered phytogeographically. The same pattern of clustering was also found in the genetic structure of Jordanian *Stipa* species (Hamasha *et al.* 2013). According to Al-Gharaibeh *et al.* (2016), similar clustering was found in our study for the germination behaviour of *S. spinosa* seeds, where at the low temperatures (8/4 °C), seeds from Irano-Turanian and Saharo-Arabian populations started to germinate 8 days earlier than those from the Mediterranean region.

A number of floristic analyses have proposed that the mountainous region of Wadi Rum represents a putative Mediterranean refugium in the Near Eastern Deserts (Danin 1999a,b, 2008; Löwenstern *et al.* 2000), but this assumption is not supported by our results on genetic diversity for the area. However, the genetic clustering of the Wadi Rum population (Saharo-Arabian) with other Mediterranean populations in our study may be a result of similarity in microclimate conditions of this population with climate conditions of other Mediterranean populations (Danin 1999a,b, 2008). In addition, Al-Quran (2012) mentioned that most Wadi Rum inhabitants rely on medicinal plants that grow in the region and in adjacent areas, which may have led to seeds being anthropogenically transported from adjacent populations, *e.g.* the King's Road population that had very similar genetic diversity values to that of the Wadi Rum population.

CONCLUSIONS

We conclude that the estimated genetic diversity of Jordanian *Salvia* populations is influenced by the environmental heterogeneity that prevails across the three phytogeographic regions and leads to high levels of genetic diversity in the regions characterised by higher temperature and drought. Population differentiation in both study species was high among the phytogeographic regions, indicating genetic divergence, since the populations from both Irano-Turanian and Saharo-Arabian regions clustered together. We therefore assume that the genetic differentiation is positively and strongly correlated with environmental dissimilarity. For effective management, we recommend that populations in each region should be maintained to ensure the preservation of genetic diversity in these medicinal plant species.

ACKNOWLEDGEMENTS

We gratefully acknowledge Birgit Müller and Steffen Heinicke for technical assistance with the AFLP analyses, and Mohammad Albashabsheh and Um Ayed Al-Gharaibeh for assistance with fieldwork. We also thank the German Academic Exchange Service (DAAD) for financial support to the first author.

REFERENCES

Ababsa M. (2013) *Atlas of Jordan history*. Territories and Society. Presses De L'IFPO, Beyrouth, Lebanon.

Afzal-rafii Z. (1980) In Chromosome number reports LXVII. *Taxon*, **29**, 365–366.

Ahmad P., Prasad M. (Eds) (2012) *Abiotic stress responses in plants: metabolism, productivity and sustainability*. Springer, New York, NY, USA.

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723.

Al-Eisawi D. (1996) *Vegetation of Jordan*. UNESCO—Regional Office for Science and Technology for the Arab States, Cairo, Egypt.

Al-Gharaibeh M.M., Hamasha H.R., Lachmuth S., Hensen I. (2016) Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan. *Plant Species Biology*. doi: 10.1111/1442-1984.12123

Alhamad M.N. (2006) Ecological and species diversity of arid Mediterranean grazing land vegetation. *Journal of Arid Environments*, **66**, 698–715.

Al-Quran S. (2012) Species level conservation of four rare endangered medicinal plants in Wadi Rum, southern Jordan. *Acta Botanica Hungarica*, **54**, 26–31.

Al-Turki T.A., Filfilan S.A., Mehmood S.F. (2000) A cytological study of flowering plants from Saudi Arabia. *Willdenowia - Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem*, **30**, 339–358.

Bacles C.F.E., Ennos R.A. (2008) Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape. *Heredity*, **101**, 368–380.

Badri M., Zitoun A., Ilahi H., Huguet T., Aouani M.E. (2008) Morphological and microsatellite diversity associated with ecological factors in natural populations of *Medicago laciniata* Mill. (Fabaceae). *Journal of Genetics*, **87**, 241–255.

Bauert M.R., Kälin M., Baltisberger M., Edwards P.J. (1998) No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. *Molecular Ecology*, **7**, 1519–1527.

Bonin A., Ehrich D., Manel S. (2007) Statistical analysis of amplified fragment length polymorphism data, a toolbox for molecular ecologists and evolutionists. *Molecular Ecology*, **16**, 3737–3758.

Braglia L., Casabianca V., De Benedetti L., Pecchioni N., Mercuri A., Cervelli C., Ruffoni B. (2011) Amplified fragment length polymorphism markers for DNA fingerprinting in the genus *Salvia*. *Plant Biosystems*, **145**, 274–277.

Cain M.L., Milligan B.G., Strand A.E. (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany*, **87**, 1217–1227.

Claßen-Bockhoff R., Speck T., Tweraser E., Wester P., Thimm S., Reith M. (2004) The staminal lever mechanism in *Salvia* L. (Lamiaceae), a key innovation for adaptive radiation? *Organisms Diversity & Evolution*, **4**, 189–205.

Conord C., Gurevitch J., Fady B. (2012) Large-scale longitudinal gradients of genetic diversity, a meta-

analysis across six phyla in the Mediterranean basin. *Ecology and Evolution*, **2**, 2600–2614.
Cortés A.J., Monserrate F.A., Ramírez-Villegas J., Madriñán S., Blair M.W. (2013) Drought tolerance in wild plant populations: the case of common beans (*Phaseolus vulgaris* L.). *PLoS One*, **8**, e62898.
Danin A. (1992) Flora and vegetation of Israel and adjacent areas. *Bocconea*, **3**, 18–42.
Danin A. (1999a) Desert rocks as plant refugia in the Near East. *Botanical Review*, **65**, 93–170.
Danin A. (1999b) Sandstone outcrops – a major refugium of Mediterranean flora in the xeric part of Jordan. *Israel Journal of Plant Sciences*, **47**, 179–187.
Danin A. (2008) Desert rocks – A habitat which supports many species that were new to science in the last 40 years. *Turkish Journal of Botany*, **32**, 459–464.
Djamali M., Brewer S., Breckle S.W., Jackson S.T. (2012) Climatic determinism in phytogeographic regionalization: a test from the Irano-Turanian region, SW and Central Asia. *Flora*, **207**, 237–249.
Duarte J.F., de Carvalho D., Vieira F.de.A. (2015) Genetic conservation of *Ficus bonijesulapensis* R.M. Castro in a dry forest on limestone outcrops. *Biochemical Systematics and Ecology*, **59**, 54–62.
Eckert C.G., Samis K.E., Lougheed S.C. (2008) Genetic variation across species' geographical ranges, the central-marginal hypothesis and beyond. *Molecular Ecology*, **17**, 1170–1188.
Evanno G., Regnaut S., Goudet J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE, a simulation study. *Molecular Ecology*, **14**, 2611–2620.
Excoffier L., Lischer H. (2010) Arlequin suite ver 3.5, a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.
Excoffier L., Smouse P.E., Quattro L.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes, applications to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
Fitzgerald T.L., Shapter F.M., McDonald S., Waters D.L.E., Chivers I.H., Drenth A., Nevo E., Henry R.J. (2011) Genome diversity in wild grasses under environmental stress. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 21140–21145.
Fox G.A. (2003) Assortative mating and plant phenology: evolutionary and practical consequences. *Evolutionary Ecology Research*, **5**, 1–18.
Frankham R., Ballou J.D., Briscoe D.A. (2010) *Introduction to conservation genetics*. Cambridge University Press, Cambridge, UK.
Franks S.J., Weis A.E. (2009) Climate change alters reproductive isolation and potential gene flow in an annual plant. *Evolutionary Applications*, **2**, 481–488.
Franks S.J., Sim S., Weis A.E. (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1278–1282.
Gilpin M.E., Soule M.E. (1986) Minimum viable populations, processes of species extinction. In: Soule M.E. (Ed.), *Conservation biology: the science of scarcity and diversity*. Sinauer Associates, Sunderland, MA, USA, pp 19–34.
Gomaa N.H., Montesinos-Navarro A., Alonso-Blanco C., Pico F.X. (2011) Temporal variation in genetic diversity and effective population size of Mediterranean and subalpine *Arabidopsis thaliana* populations. *Molecular Ecology*, **20**, 3540–3554.
Grechka D.A., Berezin S.B., Emmott S., Lyutsarev V., Smith M.J., Purves D.W. (2016) Universal, easy access to geotemporal information: FetchClimate. *Ecography*, **39**, 904–911.
Hamasha H.R., Schmidt-Lebuhn A.N., Durka W., Schleuning M., Hensen I. (2013) Bioclimatic regions influence genetic structure of four Jordanian *Stipa* species. *Plant Biology*, **15**, 882–891.
Hardy O.J., Vekemans X. (2002) spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.
Hardy O.J., González-Martínez S.C., Fréville H., Boquien G., Mignot A., Colas B., Olivieri I. (2004) Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal. *Journal of Evolutionary Biology*, **17**, 795–806.
Hensen I., Cierjacks A., Hirsch H., Kessler M., Romoleroux K., Renison D., Wesche K. (2011) Historic and recent fragmentation coupled with altitude affect the genetic population structure of one of the world's highest tropical tree line species. *Global Ecology and Biogeography*, **21**, 455–464.
Herrmann D., Poncet B.N., Manel S., Rioux D., Gielly L., Taberlet P., Gugerli F. (2010) Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. *Genome*, **53**, 302–310.
Holman J., Hughes J., Fensham R. (2003) A morphological cline in *Eucalyptus*, a genetic perspective. *Molecular Ecology*, **12**, 3013–3025.
Izawa T., Kawahara T., Takahashi H. (2007) Genetic diversity of an endangered plant, *Cypripedium macranthos* var. *rebunense* (Orchidaceae): background genetic research for future conservation. *Conservation Genetics*, **8**, 1369–1376.
Jennersten O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, **2**, 359–366.
Jugran A.K., Bhatt I.D., Rawal R.S., Nandi S.K., Pande V. (2013) Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. *Flora*, **208**, 13–21.
Khan M.K., Pandey A., Thomas G., Akkaya M.S., Kayis S.A., Ozsensoy Y., Hamurcu M., Gezgin S., Topal A., Hakki E.E. (2015) Genetic diversity and population structure of wheat in India and Turkey. *AoB Plants*, **7**, plv083. doi,10.1093/aobpla/plv083.
Kutiel P., Lavee H. (1999) Effect of slope aspect on soil and vegetation properties along an aridity transect. *Israel Journal of Plant Sciences*, **47**, 169–178.
Leimu R., Mutikainen P.I.A., Koricheva J., Fischer M. (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology*, **94**, 942–952.
Ley A.C., Hardy O.J. (2013) Improving AFLP analysis of large-scale patterns of genetic variation – a case study with the Central African lianas *Haumania* spp (Marantaceae) showing interspecific gene flow. *Molecular Ecology*, **22**, 1984–1997.
Liber Z., Židovec V., Bogdanović S., Radosavljević I., Pruša M., Filipović M., Han Dovedan I., Jug-Dujaković M., Šatović Z. (2014) Genetic diversity of Dalmatian sage (*Salvia officinalis* L.) as assessed by RAPD markers. *Agriculturae Conspectus Scientificus*, **79**, 77–84.
Little C.J., Wheeler J.A., Sedlacek J., Cortés A.J., Rixen C. (2015) Small-scale drivers: the importance of nutrient availability and snowmelt timing on performance of the alpine shrub *Salix herbacea*. *Oecologia*, **180**, 1015–1024.
Löwenstern A.B., Al-Eisawi D.M., Garbari F. (2000) Studies on the Flora of Jordan 14. The species of the Hisma Basin (Wadi Rum desert). *Webbia*, **55**, 195–277.
Lynch M., Milligan B.G. (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.
Manel S., Schwartz M.K., Luikart G., Taberlet P. (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.
Mantel N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
Måren I.E., Karki S., Prajapati C., Yadav R.K., Shrestha B.B. (2015) Facing north or south: does slope aspect impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley? *Journal of Arid Environments*, **121**, 112–123.
Migliore J., Baumel A., Juin M., Fady B., Roig A., Duong N., Médail F. (2013) Surviving in mountain climate refugia, new insights from the genetic diversity and structure of the relict shrub *Myrtus nivellei* (Myrtaceae) in the Sahara desert. *PLoS One*, **8**, e73795.
Mráz P., Gaudeul M., Rioux D., Gielly L., Choler P., Taberlet P., IntraBioDiv Consortium (2007) Genetic structure of *Hypochaeris uniflora* (Asteraceae) suggests vicariance in the Carpathians and rapid post-glacial colonization of the Alps from an eastern Alpine refugium. *Journal of Biogeography*, **34**, 2100–2114.
Nevo E. (2001) Evolution of genome–phenome diversity under environmental stress. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 6233–6240.
Nosrati H., Feizi M.A.H., Mazinani M., Haghighi A.R. (2012) Effect of population size on genetic variation levels in *Capparis spinosa* (Capparaceae) detected by RAPDs. *EurAsian Journal of BioSciences*, **6**, 70–75.
Nybom H. (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, **13**, 1143–1155.
Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Wagner H. (2013) *Vegan: community ecology package. R package version. 2.0-10*. R Foundation for Statistical Computing, Vienna, Austria.
Oran S., Al-Eisawi D. (1998) Check-list of medicinal plants in Jordan. *Dirasat Medical and Biological Sciences*, **25**, 84–112.
Peleg Z., Saranga Y., Krugman T., Abbo S., Nevo E., Fahima T. (2008) Allelic diversity associated with aridity gradient in wild emmer wheat populations. *Plant, Cell and Environment*, **31**, 39–49.
Pramuk L.A., Runkle E.S. (2005) Photosynthetic daily light integral during the seedling stage influences subsequent growth and flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. *HortScience*, **40**, 1336–1339.

Primack R.B. (1980) Variation in the phenology of natural populations of Montane shrubs in New Zealand. *Journal of Ecology*, **68**, 849–862.

Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Qasem J.R. (2001) Allelopathic potential of white top and Syrian sage on vegetable crops. *Agronomy Journal*, **93**, 64–71.

Qian X., Wang C., Tian M. (2013) Genetic diversity and population differentiation of *Calanthe tsoongiana*, a rare and endemic orchid in China. *International Journal of Molecular Sciences*, **14**, 20399–20413.

R Core Team (2013) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Scheepens J.F., Frei E.S., Armbruster G.F.J., Stöcklin J. (2012) Pollen dispersal and gene flow within and into a population of the alpine monocarpic plant *Campanula thyrsoides*. *Annals of Botany*, **110**, 1479–1488.

Sommer S., McDevitt A.D., Balkenhol N. (2013) Landscape genetic approaches in conservation biology and management. *Conservation Genetics*, **14**, 249–251.

Song Y., Gao Z., Luan W. (2012) Interaction between temperature and photoperiod in regulation of flowering time in rice. *Science China Life Sciences*, **55**, 241–249.

Sørensen T.A. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content, and its application to analyses of the vegetation on Danish commons. *Kongelige Danske Videnskabernes Selskab*, **5**, 1–34.

Stein K., Rosche C., Hirsch H., Kindermann A., Köhler J., Hensen I. (2014) The influence of forest fragmentation on clonal diversity and genetic structure in *Heliconia angusta*, an endemic understorey herb of the Brazilian Atlantic rain forest. *Journal of Tropical Ecology*, **30**, 199–208.

Still D.W., Aoyama N., Kim D.H. (2005) Genetic variation in *Echinacea angustifolia* along a climatic gradient. *Annals of Botany*, **96**, 467–477.

Till-Bottraud I., Gaudeul M. (2002) Intraspecific genetic diversity in alpine plants. In: Körner C., Spehn E.M. (Eds), *Mountain biodiversity, a global assessment*. Parthenon, London, UK, pp 23–34.

Ulubelen A. (2003) Cardioactive and antibacterial terpenoids from some *Salvia* species. *Photochemistry*, **64**, 395–399.

Van Treuren R., Bijlsma R., Van Delden W., Ouborg N.J. (1991) The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity*, **66**, 181–189.

Vekemans X., Beauwens T., Lemaire M., Roldan-Ruiz I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*, **11**, 139–151.

Ventura Y., Myrzabayeva M., Alikulov Z., Omarov R., Khozin-Goldberg I., Sagi M. (2014) Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. *AoB Plants*, **6**, plu053.

Volis S. (2007) Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology*, **21**, 381–393.

Wang I.J., Bradburd G.S. (2014) Isolation by environment. *Molecular Ecology*, **23**, 5649–5662.

Wheeler J.A., Schnider F., Sedlacek J., Cortés A.J., Wipf S., Hoch G., Rixen C. (2015) With a little help from my friends: community facilitation increases performance in the dwarf shrub *Salix herbacea*. *Basic and Applied Ecology*, **16**, 202–209.

Wilcock C., Neiland M.R.M. (2002) Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, **7**, 270–277.

Zohary D. (1973) *Geobotanical foundations of the middle east*. CRC Press, Stuttgart, Germany.

Zohary M., Feinbrun D. (1966) *Flora Palaestina, part three: text*. Israel Academy of Sciences and Humanities, Jerusalem, Israel.