Plant Ecology and Evolution 147 (2): 187–201, 2014
http://dx.doi.org/10.5091/plecevo.2014.838

REGULAR PAPER

Genetic variation in Sinai's range-restricted plant taxa *Hypericum sinaicum* and *Origanum syriacum* subsp. *sinaicum* and its conservational implications

Mohamed S. Zaghloul[1,*], Peter Poschlod[2] & Christoph Reisch[2]

[1]Botany Department, Suez Canal University, Ismailia, Egypt
[2]Institut für Botanik, Universität Regensburg, DE-93053 Regensburg, Germany
*Author for correspondence: Zaghloul_mohamed@yahoo.com

Background and aims – It is a key conservation aim to maintain genetic diversity within populations of rare and threatened species. The flora of the Sinai Peninsula is unique and, therefore, of strong interest. However, in only few studies genetic structure and variation within and among populations of Sinai plants have been analysed. In the study presented here, we analysed the genetic structure of *Hypericum sinaicum* and *Origanum syriacum* subsp. *sinaicum*, which are two rare respectively near-endemic and endemic medicinal perennial plants with overlapping ranges restricted to the mountainous region of southern Sinai in Egypt.
Methods and key results – We used AFLP markers and calculated standard genetic diversity measures. Both species exhibited much higher genetic diversity and lower genetic differentiation than generally reported for endemic plants. Although the taxa differed in distribution range and density of populations in the study region (local scale), molecular variation within populations was not significantly different between both taxa. *H. sinaicum*, the taxon with the narrower range and fewer populations, exhibited a stronger population differentiation than *O. syriacum* subsp. *sinaicum*, the taxon with the wider range and more populations at the scale of the study. Populations of both species followed the isolation-by-distance model. Bayesian clustering of individuals was successful in identifying several populations with distinct gene pools in both taxa.
Conclusions – We discussed recommendations for conservation of each taxon and concluded that the conservation of genetic diversity occurring naturally in these taxa should still be possible by a combination of *in situ* and *ex situ* conservation efforts.

Key words – Conservation genetics, medicinal plants, Hypericaceae, Lamiaceae, Sinai, AFLPs.

INTRODUCTION

In arid environments, floras are often composed of a "skeleton" of very few common species with wide ecological amplitude and many rare and/or endemic species with a limited distribution in time and/or space. This is mainly a consequence of rain scarcity and irregularity in space and time (e.g. Danin 1983). In mountainous arid environments the large outcrops of smooth-faced rocks function as a refuge for more mesophilic and rare species where runoff flows into the few available fissures which comprise very favourable habitats for plants (Danin 1983). Many taxa occurring in arid regions exhibit a historically fragmented or disjunct distribution (Hopper et al. 1996). Such a range fragmentation would be expected to lead to strong genetic structure within species (Moran & Hopper 1983) as already reported for several species (e.g. Coates 2000, Zaghloul et al. 2012).

The populations of rare and endangered species are often very small, which strongly affects genetic variation and, therefore, the long-term survival of these species. Populations consisting of only few individuals are strongly subjected to the effects of genetic drift (leading to reduction in genetic variation) and suffer more likely from inbreeding, inbreeding depression, or genetic swamping by more common congeners (Barrett & Kohn 1991). Stochastic events such as drought, fire, floods, or rapid environmental change can reduce population size further, producing genetic bottlenecks. The loss of genetic variation through stochastic factors and the deleterious effects of inbreeding in small populations are potential threats that may compromise the long-term viability of populations of these endemic species and elevate the

genetic vulnerability of populations to rapid environmental change. This loss is either through the direct loss of potentially adaptive alleles or via the more general increase in extinction vulnerability owing to increased selective load interacting with decreased genetic effective population size. Maintenance of genetic diversity within populations is thus a key conservation aim, as it will enhance their ability to adapt to future environmental changes (e.g. Frankel et al. 1995). On the contrary, reduction of genetic diversity within populations may significantly reduce the ability of the population to resist and recover from perturbations such as pest and disease outbreaks (Altizer et al. 2003, Burdon & Thrall 2001) or extreme weather events (McLaughlin et al. 2002, Reusch et al. 2005) and may increase their risk of extinction *per se* (Newman & Pilson 1997).

Data on genetic variation within and among populations of rare and endangered species allow the comparison of these species with other species having similar life history characteristics and similar geographic ranges. Comparisons with congeneric species sharing a common evolutionary past and having similar mating systems and seed-dispersal mechanisms are perhaps the most informative (Karron 1987) but are not always feasible. Although the flora of the Sinai Peninsula is unique and of strong conservation interest, only few studies on the biology and genetics of the native taxa especially the rare ones, have been conducted to date (Zaghloul et al. 2006, Zaghloul et al. 2007, Zaghloul et al. 2012). The study presented here is the first where AFLPs have been applied to analyse the genetic variation of rare and endangered Sinai plant taxa. We describe the local-scale genetic variation and structure of *Hypericum sinaicum* and *Origanum syriacum* subsp. *sinaicum,* which are two respectively near-endemic and endemic rare plant taxa that co-occur on high-montane rock outcrops in the south of the Sinai Peninsula. These taxa were chosen because they are both perennial herbaceous medicinal plants restricted to mountainous habitats growing in small populations but with different local-scale distribution ranges. Populations of *H. sinaicum* are smaller, more isolated and restricted in distribution than those of *O. syriacum* subsp. *sinaicum*. For both species, there are no congeneric species native to the study area.

MATERIALS AND METHODS

Study species

***Hypericum sinaicum* Hochst. & Steud. ex Boiss.** – *Hypericum sinaicum* is an endangered near-endemic species growing in small dripping springs on cliffs and sheltered moist crevices in the mountainous area of southern Sinai, Egypt (Boulos 1999, Zaghloul 1997). Its distribution is associated closely with moisture content, sand and silt fractions, carbonate, exposure degree, and organic matter content (Zaghloul 1997). Outside southern Sinai, it has been known so far only from Jabal Lawz in the extreme NW of Saudi Arabia (Robson 1996) and Dana Nature Reserve in SW Jordan (Danin 1997). Up to our knowledge, no study on the reproductive biology (including information on pollen and seed dispersal vectors, and putative clonal growth) of the species has been published yet. Nevertheless, an analysis of 55 species of the genus *Hypericum* demonstrated a high plasticity of reproduction pathways (Matzk et al. 2003). Moreover, male sterility and apomixis were reported for the genus (Hoar 1931, de Moraes et al. 2009) including facultative apomixis in *H. perforatum* (Noack 1939, Barcaccia et al. 2006).

H. sinaicum is a potential medicinal plant for the alleviation of nervous disorders, depression, and infectious diseases. Hypericin, protohypericin, pseudohypericin, protopseudohypericin, and hyperforin have been isolated from *H. sinaicum* (Alali et al. 2009). Hypericin has been used as an antidepressant, antiviral and anti-inflammatory agent (Bombardelli & Morazzoni 1995) and has shown promise in a preliminary brain tumor trial (Couldwell et al. 2011). Due to prevailing drought conditions and habitat destruction due to human activities, *H. sinaicum* has become one of the most endangered species in Egypt.

***Origanum syriacum* L. subsp. *sinaicum* (Boiss.) Greuter & Burdet** – The subspecies is gynodioecious with protandrous flowers (Rodríguez-Riaño & Dafni 2007). It is endemic to anticlines of the northern Sinai and the lower and upper Sinai massif (Boulos 2002, Danin 1983). Its distribution is correlated with exposure aspect and it reaches its optimal performance at NW aspects (Zaghloul et al. 2010). Leaves have an aromatic odour with slightly bitter and very hot taste and are used for various flavouring purposes. It is also used as a strewing herb for the pungent fragrance of its bruised leaves (Bailey & Danin 1981). In folk medicine, dry leaves are used as spice, digestive, condiment and pain relief, and in treatments for stomach disorders, and diabetes. It has been integrated in a Bedouins' mixture of forty different herbs which are collected during spring and used as a general recipe for improvement of the body health (Omran & Moustafa 2006). Hot tea is used for chest diseases (Batanouny 1999).

Study area

The study was carried out in St. Katherine Protectorate which is a part of the southern Sinai massif that is characterized by its altitudinal gradient starting from 1500 m a.s.l. till the highest peak (Mount St. Katherine) that is 2641 m a.s.l. The St. Katherine Protectorate is located in the centre of the mountainous region of the southern Sinai between 33°30' and 34°30'E, and between 28°50' and 29°50'N. The Protectorate area is described as predominantly smooth-faced granite outcrops forming mountains such as Mount Serbal, Mount Ras Safsafa and Mount El-Rabah. Black mountains consisting of old volcanic rocks are rather common. Mountains support mainly Irano-Turanian steppe vegetation dominated by *Seriphidium herba-album* accompanied by *Gymnocarpos decandrus*. Lower elevations are dominated by *Seriphidium herba-album* and accompanied by *Zilla spinosa* and *Fagonia mollis* on stony alluvium of ridges, *Agathophora alopecuroides* and *Atraphaxis spinosa* on soils derived from dark volcanic rocks (Mount St. Katherine), and *Stachys aegyptiaca* and *Tanacetum sinaicum* on terraces. Characteristic trees and shrubs include: *Crataegus* × *sinaica*, *Ficus palmata*, *Rhamnus dispermus*, *Cotoneaster orbicularis* and *Rhus tripartita* (Moustafa 1990).

The St. Katherine Protectorate area has a diversity of landforms (slopes, terraces, gorges, plains, ridges, and wadis), geologic structures, geomorphologic formations, and altitudinal

gradients that result in several microhabitats, each of which has its peculiar environmental conditions and plant cover and results in a fairly rich and unique vegetation and flora. The vegetation is characterized by the dominance of four families; Asteraceae, Lamiaceae, Fabaceae, and Brassicaceae (Moustafa 1990). The flora is rich, especially in medicinal, rare, and endemic species. It comprises 520 species (41.2% of the total flora of Sinai) which have been subjected to threats causing declines in population number and size. So, only 323 species were recorded in the recent surveys (Moustafa et al. 1998, 1999, 2001, Abd El-Wahab et al. 2004). The area harbours 26 endemic species (42.6% of the total species endemic to Egypt, Moustafa et al. 2001). Among the 323 species recorded in St. Katherine Protectorate, 115 species (35.6%) are considered as medicinal species (Abd El-Wahab et al. 2004).

While Sinai is broadly characterized by an arid to extremely arid climate with long hot rainless summers and mild winters (Danin 1983, Issar & Gilad 1982), the St. Katherine area is characterized by a unique climate. It is the coolest area in the Sinai and Egypt due to its high elevation. The lowest mean minimum temperature is recorded in January and February (1.4°C); while the highest mean maximum temperature in June and July is 30.8 and 31.8°C, respectively (Abd El-Wahab 1995). Its climate is influenced by the Mediterranean Sea and by the orographic impact of high elevation.

Some of the threats affecting rare and endemic plants in the St. Katherine area and Sinai deserts in general are specific to populations of medicinal plants, but the majority affects the functional communities and ecosystems in which these populations ultimately exist and interact with other species and the abiotic environment (Abd El-Wahab et al. 2004). These threats can be classified in two categories. The first includes the natural threats; drought, floods, and natural enemies (rodents and insects). A cycle of drought and flood years has been observed in the area. While the drought itself has effects on sparse vegetation in arid to extremely arid

Table 1 – Summary description of populations sampled for *H. sinaicum* and *O. syriacum* from the Sinai.

Species	Population	Abbrev.	Longitude	Latitude	No. sampled individuals
Origanum syriacum	W. El-arbaien	OArb	33°57’0.09”E	28°33’4.66”N	14
	W. Shaq Mousa (S1)	OSha1	33°57’49.99”E	28°31’48.77”N	15
	W. Shaq Mousa (S2)	OSha2	33°57’49.89”E	28°31’45.15”N	15
	W. Shaq Mousa (S3)	OSha3	33°57’49.73”E	28°31’41.03”N	13
	W. Shaq Mousa (S4)	OSha4	33°57’49.81”E	28°31’37.03”N	9
	W. Garagnia (S1)	OGar1	33°58’11.28”E	28°31’19.03”N	15
	W. Garagnia (S2)	OGar2	33°58’14.12”E	28°31’46.94”N	15
	Gebel Mousa (Farsh Elia)	OMou	33°58’30.01”E	28°32’41.21”N	15
	W. El-Talaa	OTal	33°55’55.87”E	28°34’0.23”N	15
	W. Telah (S1)	OTel1	33°56’0.83”E	28°34’7.23”N	15
	W. Telah (S2)	OTel2	33°55’51.54”E	28°34’19.11”N	15
	W. Topooq (Slebaat)	OTop	33°56’1.14”E	28°32’42.86”N	15
	W. Zwateen (S1)	OZwa1	33°55’45.69”E	28°32’15.19”N	14
	W. Zwateen (S2)	OZwa2	33°55’10.42”E	28°32’45.33”N	15
	Farsh El-Romana	ORom	33°53’15.98”E	28°31’50.24”N	15
	W. Edghemeyiat	OEdg	33°54’30.86”E	28°32’15.89”N	15
	W. Obwale’e	OObw	33°54’35.37”E	28°32’16.30”N	15
Total	17				245
Hypericum sinaicum	W. Garagnia (S1)	HGar	33°58’11.28”E	28°31’19.03”N	15
	W. Shaq Mousa (S4)	HSha	33°57’49.81”E	28°31’37.03”N	15
	Kahf El-Ghola	HKah	33°56’57.67”E	28°32’44.99”N	11
	Ain Shekayia	HAin	33°55’59.04”E	28°32’35.62”N	15
	W. Edghemeyiat	HEdg	33°54’30.86”E	28°32’15.89”N	13
	W. Obwale’e	HObw	33°54’35.37”E	28°32’16.30”N	15
	Farsh El-Romana	HRom	33°53’15.98”E	28°31’50.24”N	15
	W. Abu Hepaiq	HAbu	33°52’30.02”E	28°33’38.03”N	15
	W. Ze’ater	HZea	33°52’59.28”E	28°33’45.77”N	15
	Hagar El-Nemr	HHag	33°53’11.12”E	28°33’51.90”N	15
	Um Selah	HSel	33°53’22.79”E	28°34’28.03”N	15
	Sekekrayia	HSek	33°54’25.25”E	28°33’37.25”N	15
Total	12				174

ecosystems, it also aggravates any other threat, especially human-induced ones. The second includes the disturbances due to human impact which are recorded all over southern Sinai; over-grazing, over-collecting, uprooting, feral donkeys, over-cutting for fuel wood, urbanization (construction of new settlements, infrastructure, and digging new wells), quarries, tourism, solid wastes (due to urbanization and tourism activities), and other land use types. These disturbances lead to the destruction of natural habitats and the disappearance of plant communities in which endemic plants live and interact (Abd El-Wahab et al. 2004).

Sampling design

To study the genetic diversity within and the molecular differentiation among populations, twelve *H. sinaicum* and seventeen *O. syriacum* subsp. *sinaicum* populations were sampled from eighteen sites (table 1, fig. 1) within the St. Katherine Protectorate. Fifteen individuals were sampled from each population (table 1) except for few cases (HKah and HEdg in *H. sinaicum* and OArab, OSha3, OSha4 and OZwa1 in *O. syriacum* subsp. *sinaicum*) where the population sizes were too small. Within each population, sampled individuals were chosen randomly. Sampling of vegetatively propagated ramets had been minimized by sampling the distinct and apparently unique genets only. Leaves were collected from each individual and stored in plastic bags with silica gel until running AFLP analysis at Regensburg University. Although the local-scale distribution range (spatial distribution within the Protectorate) of *O. syriacum* subsp. *sinaicum* is wider than that of *H. sinaicum*, populations of the two taxa were sampled over the same geographic range for comparative purposes. The sampling area is approximately 60 km^2 and represents the core of the Protectorate.

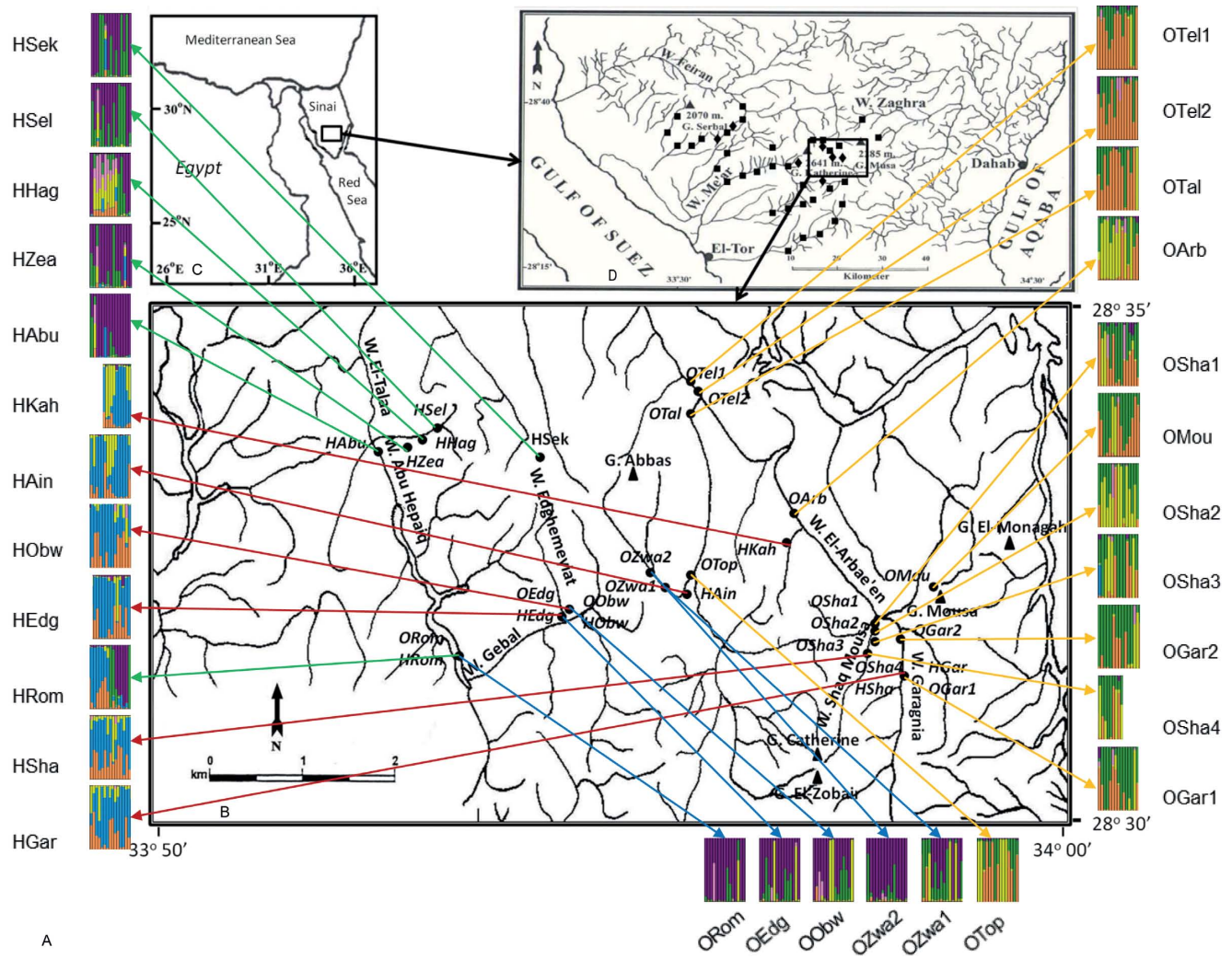

Figure 1 – A, posterior estimates of individual admixture coefficients for K=6 genetic clusters in *H. sinaicum* (left-hand side) and K=4 genetic clusters in *O. syriacum* subsp. *sinaicum* (right-hand side) plotted on (B) location map of the studied populations in southern Sinai. "H" denotes *H. sinaicum* and "O" denotes *O. syriacum* subsp. *sinaicum* populations. The same arrow colour (green and red for *H. Sinaicum* and orange and blue for *O. syriacum* subsp. *sinaicum*) represents populations which form a group at the first level of the UPGMA analysis; C, location map of the study area; D, map representing the known distribution range for *H. sinaicum* (♦) and *O. syriacum* subsp. *sinaicum* (■).

DNA isolation and AFLP and data analyses

DNA was isolated from the dried plant material of individual plants using the cetyltriammonium bromide (CTAB) method (Rogers & Bendich 1994). The AFLP procedure was performed according to the protocol from Beckman Coulter and following Reisch (2007). After a screening of twelve (in *H. sinaicum*) and eleven (in *O. syriacum* subsp. *sinaicum*) primer combinations performed on eight individuals, we selected three different fluorescently labelled combinations; M-CTA/E-ACC, M-CAT/E-ACG, and M-CTG/E-ACA for *H. sinaicum* and M-CTC/E-AAC, M-CTG/E-AGG, and M-CTC/E-ACA for *O. syriacum* subsp. *sinaicum* for further analyses.

The fluorescently labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Synthetic gels with AFLP fragments for each primer combination were analysed in BIONUMERICS 3.0 (Applied Maths, Kortrijk, Belgium). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent. When individuals did not give clear or not reproducible and easily-scored signals, all bands of this fragment size were excluded from the analysis. Genotyping error rate was calculated following Bonin et al. (2004) as the number of fragment (presence-absence) differences in duplicated samples, replicating 22 samples in *O. syriacum* subsp. *sinaicum* (i.e. 9% of sample size) and 15 in *H. sinaicum* (i.e. 8.6% of sample size).

The analysis of the AFLP markers was done under the assumption that each amplified band, regardless of its relative intensity, corresponds to a dominantly inherited allele at a single locus. Polymorphic loci were scored as "1" for the presence and "0" for the absence of the marker band. The basic data structure finally consisted of a binary (0/1) matrix, representing the scored AFLP markers. This matrix was used to analyse genetic structure and diversity within and among populations.

Genetic diversity and structure

Using GenAlEX 6.2 (Peakall & Smouse 2006), standard measures of genetic diversity for each population were calculated including the number of different alleles (*Na*), the number of effective alleles (*Ne*) = 1 / (p^2 + q^2), Shannon's information index (*SI*) = -1(p Ln (p) + q Ln(q)), the expected heterozygosity (*He*) = 2pq, the unbiased expected heterozygosity (*UHe*) = (2N/(2N-1)) *He*, and percentage of polymorphic loci (*PB*). All the measurements were assessed and presented as mean and standard error (SE) over loci within populations and over loci and populations within the species (Nei 1973). One-tailed T-tests with unequal variances were used to test for differences in summary statistics between populations of the two studied species. The genetic differences (PhiPT) among populations were estimated using the Analysis of Molecular Variance (AMOVA) procedure (Excoffier et al. 1992) to investigate the hierarchical partitioning of genetic variation among populations. PhiPT was calculated as the proportion of the variance among populations, relative to the total variance. PhiPT represents the correlation between individuals within a population, relative to the total and it is analogous to the fixation index (F_{ST}) for measuring population differentiation when the data are haploid or binary (Maguire et al. 2002). To compare the results and for making sure that differences are not due to unbalanced sampling design (twelve *H. sinaicum* populations *vs*. seventeen *O. syriacum* subsp. *sinaicum*; and 158 *vs*. 201 alleles), we repeated the analyses with a randomized selected subset of *O. syriacum* subsp. *sinaicum* populations.

Unbiased genetic identity and distance (Nei 1972) were calculated between pairs of populations using GenAlEX 6.2 (Peakall & Smouse 2006). Pairwise genetic differences between individuals were used in a principal coordinate analysis (PCoA) in GenAlEX to validate and further define naturally occurring genetic clusters. To construct a UPGMA dendrogram, estimates of pairwise Nei genetic distance were calculated using the Lynch & Milligan (1994) method for recovering unbiased statistics from dominant markers, as implemented in the program AFLP-SURV version 1.0 (Vekemans 2002) and assuming Hardy–Weinberg equilibrium. Allele frequencies were estimated using the Bayesian method of Zhivotovsky (1999) assuming a non-uniform prior distribution and confidence intervals of genetic distances were estimated using 999 bootstrap replicates. Resampled distance matrices (999) computed by bootstrapping over AFLP loci were used as input for the NEIGHBOR program from the PHYLIP software package (Felsenstein 1993) to infer bootstrap confidence on UPGMA tree branches. Then the majority-rule consensus tree method as implemented in the CONSENSE procedure was used to get the final bootstrapped tree. The out tree was visualized using MEGA5.2 (Tamura et al. 2011).

Isolation by distance analysis

To investigate whether the differentiation between sampling sites follows the isolation-by-distance model (Slatkin 1993, Wright 1943), Mantel test (10000 permutations) was conducted in XLSTAT version 2013.6.03 (Addinsoft 2014). The test aimed to evaluate the correlation between the matrix of pairwise $F_{ST}/(1-F_{ST})$ against the matrix of the logarithm of geographic distances among populations represented as drainage lines through wadis (Mantel 1967). As the study area is a very rough mountainous area with plant and wild life mainly confined within wadi systems, dispersal (whether by insect or wind for pollen grains or water or grazing herds for seeds) is mainly within these wadi systems. So, the geographic distances between populations were assessed through tracing the wadi drainage lines following Zaghloul et al. (2006). The distance between sampled populations ranged from 0.2 km to 14.64 km between *H. sinaicum* populations with an overall mean of 7.6 km and 0.12 km to 12.63 km between *O. syriacum* subsp. *sinaicum* populations with an overall mean of 5.22 km. AFLP-SURV version 1.0 (Vekemans 2002) was used to estimate pairwise F_{ST} after Reynolds et al. (1983). Isolation by distance was tested across the whole study area among sampled populations.

Bayesian clustering of individuals

Multi-locus genotypes were used to infer clusters of individuals representing different gene pools. Monte Carlo Markov Chain (MCMC) estimation was applied as implemented in TESS version 2.3 (Chen et al. 2007, Durand et al. 2009). We used the CAR (conditional auto regression) admixture model which assumes that individual multilocus genotypes arise from the admixture of at most K_{max} (potentially) unobserved parental populations (Durand et al. 2009). The admixture model was run 100 independent times for each K_{max} value starting from the clustering pattern obtained by a neighbour-joining algorithm. Each run consisted of 50,000 sweeps with a burn-in period of 30,000. For each value of K_{max}, the Deviance Information Criterion (DIC) was computed. The estimated admixture coefficients over the 10% runs with the lowest values of the DIC were averaged using algorithms found in CLUMPP version 1.1.2 (CLUster Matching and Permutation Program; Jakobsson & Rosenberg 2007). This program takes an input file containing the estimated membership coefficients for multiple independent runs and averages them after correcting for label switching. The outputs of CLUMPP were represented in bar graphs using DISTRUCT version 1.1 (Rosenberg 2004). The optimum number of clusters (K_{opt}) was determined through plotting the DIC values against K_{max}. The DIC decreases sharply and then exhibits a plateau at K_{opt}.

RESULTS

AFLP data

We analyzed 174 individuals from twelve populations of *H. sinaicum*, which resulted in a total of 158 amplified AFLP fragments across primer combinations. Primer combinations ACC-CTA, ACG-CAT, and ACA-CTG produced 62, 62, and 34 fragments, respectively. In *O. syriacum* subsp. *sinaicum* (245 individuals from 17 populations), 201 reproducible fragments were amplified. The primer combinations AAC-CTC, AGG-CTG, and ACA-CTC produced 91, 51, and 59 fragments, respectively. Every individual from each species showed its own AFLP phenotype. The genotyping error rates in the studied samples were within the acceptable limits; 3.5% at the fragment level in *H. sinaicum* and 4.9% in *O. syriacum* subsp. *sinaicum*.

Genetic diversity

Although the local-scale distribution range substantially differs between *H. sinaicum* (narrowly distributed) and *O. syriacum* subsp. *sinaicum* (widely distributed), the molecular variation within populations was not significantly different between both taxa (T-test: P_{SI} = 0.301, P_{He} = 0.356, P_{UHe} = 0.360, P_{PB} = 0.118). Populations HSek and OZwa2 had the lowest and HHag and OSha2 and OSha3 had the highest diversity in *H. sinaicum* and *O. syriacum* subsp. *sinaicum*, respectively (table 2). Shannon's Information Index (*SI*) ranged from 0.280 ± 0.023 (HSek) to 0.370 ± 0.023 (HHag) with a mean of 0.328 ± 0.007 in *H. sinaicum* and from 0.271 ± 0.021 (OZwa2) to 0.371 ± 0.021 (OSha2) with a mean of 0.322 ± 0.005 in *O. syriacum* subsp. *sinaicum* populations. The unbiased expected heterozygosity (UH_e) ranged from 0.194 ± 0.001 (HSek) to 0.259 ± 0.017 (HHag) with a mean of 0.231 ± 0.005 in *H. sinaicum* and from 0.190 ± 0.015 (OZwa2) to 0.264 ± 0.015 (OSha2) with a mean of 0.228 ± 0.004 in *O. syriacum* subsp. *sinaicum* populations. The percentage of polymorphic bands (*PB*) within populations was a little higher in *H. sinaicum* than in *O. syriacum* subsp. *sinaicum* populations. It ranged from 53.16% (HSek) to 66.46% (HHag) with a mean of 59.28% (± 1.36) in *H. sinaicum* and from 45.77% (OSha4) to 63.18% (OSha2 and OSha3) with a mean of 57.10% (± 1.17) in *O. syriacum* subsp. *sinaicum* populations.

Genetic structure

The AMOVA results revealed that a moderate proportion of the total genetic variation in both species is found among populations and that *H. sinaicum* populations have more structured genetic diversity than *O. syriacum* subsp. *sinaicum.* While 17% of the detected genetic variation in *H. sinaicum* is among populations, it was only 10% in *O. syriacum* subsp. *sinaicum* (table 3). The results did not differ when the analyses were repeated with a randomized selected subset of *O. syriacum* subsp. *sinaicum* populations.

In both species, the Principal Coordinate Analysis (PCoA, fig. 2) separated sampled individuals into two distinct groups (gene pools). The first two axes of PCoA explained 64.29% of variation in *H. sinaicum* and 63.28% in *O. syriacum* subsp. *sinaicum*. In *H. sinaicum*, individuals belonging to HGar, HSha, HKah, HAin, HEdg, and HObw populations were clustered in only one group while individuals from all other populations were distributed among the two groups. In *O. syriacum* subsp. *sinaicum*, individuals belonging to OTel2 were clustered in one group (left-hand-side), while all other individuals belonging to other populations were distributed among the two groups (fig. 2A).

In the Mantel test (fig. 3) a significant positive association was found between $F_{ST}/(1-F_{ST})$ and the logarithm of geographic distances among populations of both *H. sinaicum* and *O. syriacum* subsp. *sinaicum* (P = 0.003 and < 0.0001, respectively). This significant positive association reflects that populations of both species follow the isolation-by-distance model.

The genetic identity of pairwise comparisons was lower in *H. sinaicum* than *O. syriacum* subsp. *sinaicum* populations. While it ranged from 0.865 (HEdg and HSek) to 0.983 (HGar and HSha) with an overall mean of 0.930 in *H. sinaicum* (table 4), it ranged from 0.921 (OArab and OTel2) to 0.987 (OEdg and OObw) with an overall mean of 0.966 in *O. syriacum* subsp. *sinaicum* populations (table 5). Average genetic identity values for HObw (0.923) and HAbu (0.922) were low relative to all other *H. sinaicum* populations. In *O. syriacum* subsp. *sinaicum*, OArb (0.947) and OZwa2 (0.948) populations had lower values than other populations.

UPGMA dendrograms were constructed to examine genetic relationships among studied populations of each species. In *H. sinaicum*, bootstrap support was above 50% for 90% of the nodes. At the first level of clustering, HHag, HAbu, HZea, HRom, HSel, and HSek populations of *H. sinaicum*, were clustered in one group which matches the

Table 2 – Genetic diversity parameters in the studied populations.
SE = Standard error, *Na* = number of different alleles, *Ne* = number of effective alleles, *SI* = Shannon's information index, *He* = expected heterozygosity, *UHe* = unbiased expected heterozygosity, and *PB* = percentage of polymorphism.

Species	Pop. code	*Na*		*Ne*		*SI*		*He*		*UHe*		*PB*	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Hypericum sinaicum	HGar	1.544	0.044	1.379	0.032	0.316	0.024	0.215	0.017	0.222	0.017	56.96	
	HSha	1.551	0.045	1.373	0.030	0.319	0.023	0.215	0.016	0.223	0.017	58.86	
	HKah	1.532	0.045	1.371	0.030	0.316	0.023	0.214	0.016	0.224	0.017	56.33	
	HAin	1.500	0.046	1.385	0.032	0.315	0.024	0.216	0.017	0.223	0.018	54.43	
	HEdg	1.494	0.046	1.375	0.032	0.310	0.024	0.212	0.017	0.220	0.018	53.80	
	HObw	1.551	0.043	1.369	0.031	0.313	0.024	0.212	0.017	0.219	0.017	56.96	
	HRom	1.595	0.044	1.425	0.031	0.358	0.023	0.244	0.016	0.252	0.017	62.66	
	HAbu	1.627	0.042	1.436	0.032	0.360	0.024	0.246	0.017	0.254	0.017	64.56	
	HZea	1.601	0.047	1.438	0.032	0.364	0.023	0.248	0.017	0.256	0.017	65.19	
	HHag	1.608	0.047	1.441	0.031	0.370	0.023	0.251	0.016	0.259	0.017	66.46	
	HSel	1.570	0.047	1.364	0.030	0.318	0.023	0.212	0.016	0.220	0.017	62.03	
	HSek	1.456	0.050	1.322	0.030	0.280	0.023	0.187	0.016	0.194	0.017	53.16	
Total	12	1.552	0.013	1.390	0.009	0.328	0.007	0.223	0.005	0.231	0.005	59.28	1.36
Origanum syriacum	OArb	1.587	0.040	1.446	0.028	0.366	0.021	0.251	0.015	0.260	0.015	62.69	
	OSha1	1.567	0.040	1.411	0.028	0.342	0.021	0.233	0.015	0.241	0.015	60.20	
	OSha2	1.612	0.037	1.455	0.028	0.371	0.021	0.255	0.015	0.264	0.015	63.18	
	OSha3	1.607	0.038	1.448	0.028	0.369	0.021	0.253	0.015	0.263	0.015	63.18	
	OSha4	1.408	0.041	1.363	0.030	0.282	0.022	0.197	0.016	0.208	0.017	45.77	
	OGar1	1.562	0.039	1.416	0.028	0.343	0.021	0.235	0.015	0.243	0.016	59.20	
	OGar2	1.562	0.037	1.402	0.028	0.333	0.021	0.228	0.015	0.236	0.015	57.71	
	OMou	1.547	0.038	1.398	0.028	0.329	0.021	0.225	0.015	0.233	0.016	56.72	
	OTal	1.522	0.039	1.328	0.026	0.285	0.020	0.191	0.014	0.198	0.015	54.73	
	OTel1	1.527	0.039	1.345	0.027	0.298	0.021	0.200	0.014	0.207	0.015	55.72	
	OTel2	1.483	0.042	1.340	0.028	0.286	0.021	0.193	0.015	0.200	0.015	53.23	
	OTop	1.522	0.041	1.400	0.029	0.325	0.022	0.223	0.015	0.231	0.016	56.22	
	OZwa1	1.507	0.039	1.381	0.029	0.311	0.022	0.213	0.015	0.221	0.016	53.73	
	OZwa2	1.413	0.045	1.324	0.027	0.271	0.021	0.184	0.015	0.190	0.015	49.25	
	ORom	1.577	0.040	1.371	0.027	0.319	0.020	0.214	0.014	0.222	0.015	61.19	
	OEdg	1.532	0.041	1.370	0.028	0.312	0.021	0.211	0.015	0.219	0.015	57.21	
	OObw	1.562	0.041	1.401	0.028	0.337	0.021	0.229	0.015	0.236	0.015	60.70	
Total	17	1.535	0.010	1.388	0.007	0.322	0.005	0.220	0.004	0.228	0.004	57.10	1.17
T-Test (1-tailed) *P*						0.301		0.356		0.360		0.118	

Table 3 – Summary AMOVA table.
Note: r.s.s. = randomly selected subset.

Source	Species	df	SS	MS	Est. Var.	%	PhiPT	P
Among Pops	*H. sinaicum*	11	722.050	65.641	3.396	17%		
	O. syriacum subsp. *sinaicum*	16	815.695	50.981	2.144	10%		
	O. syriacum subsp. *sinaicum* (r.s.s)	11	553.270	50.297	2.083	10%		
Within Pops	*H. sinaicum*	162	2662.381	16.434	16.434	83%		
	O. syriacum subsp. *sinaicum*	228	4582.109	20.097	20.097	90%		
	O. syriacum subsp. *sinaicum* (r.s.s)	165	3230.696	19.580	19.580	90%		
Total	*H. sinaicum*	173	3384.431		19.830	100%	0.171	0.001
	O. syriacum subsp. *sinaicum*	244	5397.804		22.241	100%	0.096	0.001
	O. syriacum subsp. *sinaicum* (r.s.s)	176	3783.966		21.663	100%	0.096	0.001

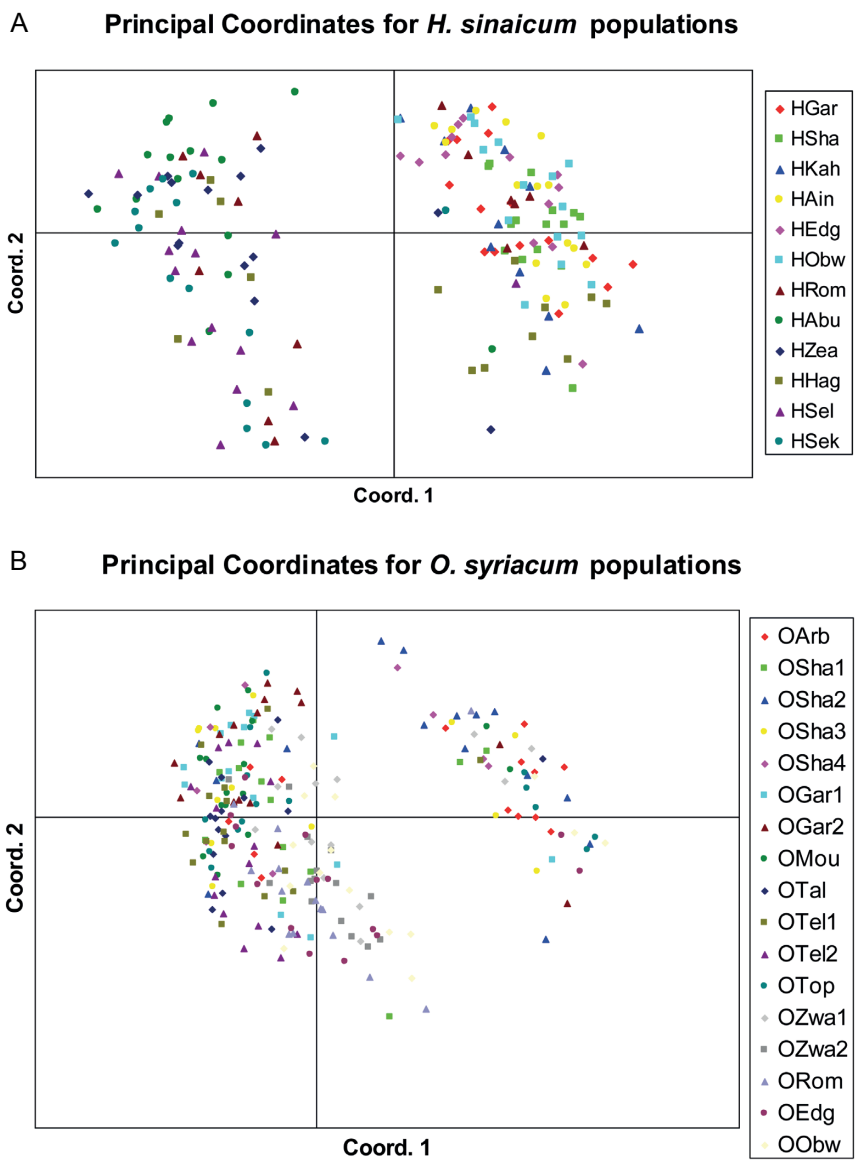

Figure 2 – Principal Coordinate Analysis of studied populations. "H" denotes *H. sinaicum* and "O" denotes *O. syriacum* subsp. *sinaicum* populations.

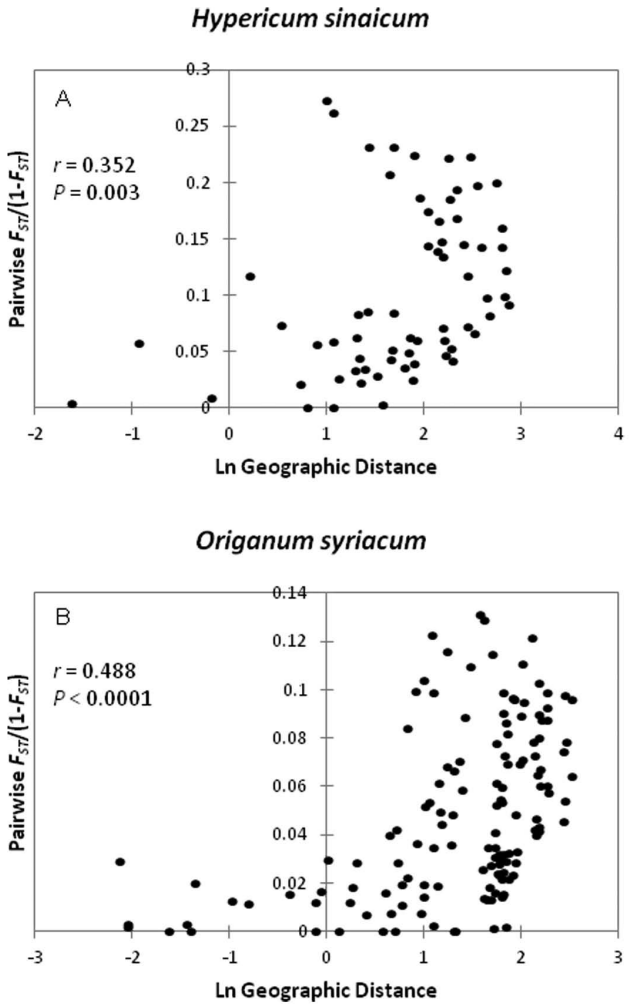

Figure 3 – Mantel test for *Fst*/(1-*Fst*) and logarithm of geographic distance between populations of *H. sinaicum* (A) and *O. syriacum* subsp. *sinaicum* (B).

geographic distribution. Although HAin, HObw, and HEdg are geographically closer to this group, they were grouped in the other cluster together with HGar, HSha, and HKah which are located in the east of the study area (fig. 4A). In *O. syriacum* subsp. *sinaicum*, the UPGMA dendrogram could be considered as being close to a random tree where bootstrap support is above 50% for only 33.3% of the nodes. Nevertheless, OZwa1, OZwa2, OObw, ORom, and OEdg populations were grouped together distinctively from the other populations (fig. 4B). This clustering also matches the geographic distribution.

Bayesian clustering of individuals

Bayesian clustering of *H. sinaicum* and *O. syriacum* subsp. *sinaicum* individuals was successful in identifying several populations with distinct gene pools. Plotting averaged estimated admixture coefficients over the 10% runs with the lowest values of the DIC values for K_{max} ranging from two to twelve in *H. sinaicum* (fig. 5A) and to seventeen in *O. syriacum* subsp. *sinaicum* (fig. 5B) indicated that the K_{opt} is six for *H. sinaicum* and four for *O. syriacum* subsp. *sinaicum*.

In *H. sinaicum*, the regional pattern of genetic structure matched the UPGMA tree based on Nei's genetic distances, where the model clustered HSek, HSel, HZea, and HAbu together in one group and HKah, HAin, HEdg, HObw, HGar, and HSha in another. Although HHag population is different from the two groups, it was grouped with the latter cluster. Also, HRom population seems to be the connection (or divergence) point between the two detected genetic clusters (fig. 1). In *O. syriacum* subsp. *sinaicum*, the model agreed with the UPGMA tree in separating OZwa1, OZwa2, OObw, OEdg, and ORom populations in distinctive gene pools. These populations are geographically separated from other populations (fig. 1).

Table 4 – Pairwise population matrix of Nei unbiased genetic identity (lower diagonal) and geographic distances (km, higher diagonal) for *H. sinaicum*.

Pop.	HGar	HSha	HKah	HAin	HEdg	HObw	HRom	HAbu	HZea	HHag	HSel	HSek
HGar		2.25	3.91	6.16	9.86	9.98	12.56	16.60	17.44	17.84	12.89	10.39
HSha	0.983		3.13	5.37	9.07	9.19	11.77	15.81	16.65	17.05	12.10	9.60
HKah	0.966	0.964		2.96	6.66	6.78	9.36	13.40	14.24	14.64	9.69	7.19
HAin	0.962	0.954	0.981		3.70	3.82	6.40	10.44	11.28	11.68	6.73	4.23
HEdg	0.956	0.948	0.968	0.964		0.20	3.72	7.76	8.60	9.00	5.24	2.74
HObw	0.956	0.950	0.956	0.956	0.974		3.78	7.82	8.66	9.06	5.44	2.94
HRom	0.945	0.940	0.950	0.955	0.946	0.933		4.04	4.88	5.28	6.99	6.46
HAbu	0.903	0.885	0.903	0.897	0.907	0.891	0.962		0.84	1.24	2.95	5.45
HZea	0.921	0.912	0.925	0.908	0.911	0.895	0.977	0.976		0.40	2.11	4.61
HHag	0.931	0.926	0.930	0.919	0.903	0.905	0.947	0.919	0.948		1.71	4.21
HSel	0.890	0.880	0.888	0.879	0.887	0.872	0.948	0.957	0.971	0.944		2.50
HSek	0.900	0.887	0.893	0.881	0.865	0.867	0.954	0.947	0.972	0.941	0.959	
Mean Identity	0.938	0.930	0.939	0.932	0.930	0.923	0.951	0.922	0.938	0.929	0.916	0.915
Mean Geo-distance	10.90	10.18	8.36	6.62	6.05	6.15	6.84	7.85	8.16	8.37	6.21	5.48

Table 5 – Pairwise population matrix of Nei Unbiased genetic identity (lower diagonal) and geographic distances (km, higher diagonal) for *O. syriacum* subsp. *sinaicum*.

Pop.	OArb	OSha1	OSha2	OSha3	OSha4	OGar1	OGar2	OMou	OTal	OTel1	OTel2	OTop	OZwa1	OZwa2	ORom	OEdg	OObw
OArb		2.92	3.04	3.17	3.30	4.09	3.19	3.98	2.76	2.52	2.97	2.09	3.04	4.41	8.54	5.77	5.72
OSha1	0.947		0.12	0.25	0.38	1.80	0.90	1.95	5.68	5.44	5.89	5.01	5.96	7.33	11.46	8.69	8.64
OSha2	0.974	0.964		0.13	0.26	1.92	1.02	2.07	5.80	3.04	3.49	5.13	6.08	7.45	11.58	8.81	8.76
OSha3	0.963	0.977	0.973		0.13	2.05	1.15	2.20	5.93	5.69	6.14	5.26	6.21	7.58	11.71	8.94	8.89
OSha4	0.946	0.965	0.958	0.966		2.18	1.28	2.33	6.06	5.82	6.27	5.39	6.34	7.71	11.84	9.07	9.02
OGar1	0.946	0.983	0.956	0.978	0.961		0.90	2.76	6.85	6.61	7.06	6.18	7.13	8.50	12.63	9.86	9.81
OGar2	0.945	0.971	0.962	0.975	0.963	0.981		1.86	5.95	5.71	6.16	5.28	6.23	7.60	11.73	8.96	8.91
OMou	0.938	0.974	0.955	0.968	0.959	0.968	0.967		6.35	6.11	6.56	6.07	7.02	8.39	12.52	9.75	9.70
OTal	0.932	0.974	0.945	0.964	0.952	0.970	0.968	0.979		0.24	0.69	2.54	3.49	4.86	8.99	6.22	6.17
OTel1	0.932	0.968	0.932	0.961	0.952	0.964	0.960	0.968	0.981		0.45	2.78	3.73	5.10	9.23	6.46	6.41
OTel2	0.921	0.971	0.926	0.953	0.943	0.970	0.952	0.970	0.974	0.977		3.23	4.18	5.55	9.68	6.91	6.86
OTop	0.964	0.966	0.972	0.970	0.965	0.962	0.961	0.963	0.966	0.957	0.957		0.95	2.32	6.45	3.68	3.63
OZwa1	0.957	0.966	0.969	0.965	0.956	0.959	0.968	0.951	0.949	0.949	0.937	0.968		1.37	5.50	2.73	2.68
OZwa2	0.932	0.954	0.943	0.950	0.936	0.949	0.934	0.928	0.931	0.932	0.938	0.945	0.969		5.66	1.51	1.31
ORom	0.948	0.959	0.946	0.953	0.938	0.951	0.936	0.935	0.939	0.945	0.945	0.948	0.974	0.981		3.72	3.78
OEdg	0.948	0.956	0.948	0.956	0.941	0.952	0.936	0.930	0.938	0.944	0.937	0.956	0.970	0.979	0.985		0.20
OObw	0.961	0.957	0.955	0.954	0.942	0.949	0.940	0.931	0.943	0.943	0.936	0.959	0.974	0.973	0.982	0.987	
Mean Identity	0.947	0.966	0.955	0.964	0.953	0.963	0.957	0.955	0.957	0.954	0.951	0.961	0.961	0.948	0.954	0.954	0.955
Mean Geo-distance	3.84	4.53	4.29	4.71	4.84	5.65	4.80	5.60	4.91	4.71	5.13	4.12	4.54	5.42	9.06	6.33	6.28

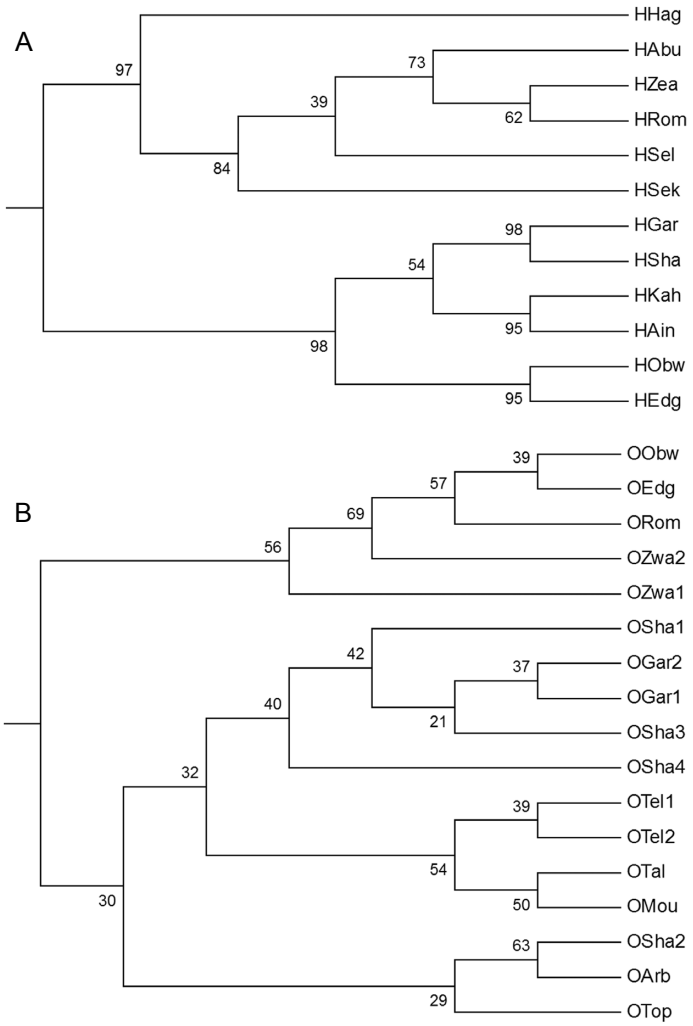

Figure 4 – UPGMA tree, showing bootstrap support, based on Nei's genetic distances for *H. sinaicum* (A) and *O. syriacum* subsp. *sinaicum* (B).

Table 6 – Comparison of genetic diversity (P_S, *Hes*) and genetic differentiation (G_{ST}) parameters between *H. sinaicum* and *O. syriacum* subsp. *sinaicum* and all plant species, plant species with similar life forms and mating systems and four species which were analysed previously from the Sinai Peninsula.
P_S = proportion of polymorphic loci at species level, *Hes* = unbiased heterozygosity expected under Hardy–Weinberg assumptions at the species level, G_{ST} = the proportion of total genetic diversity found among populations.

Species (genetic marker)	P_S (%)	*Hes*	G_{ST}	Source
All plant species (allozymes)	50.5	0.149	0.224	Hamrick & Godt 1989
All plant species (AFLP)		0.23	0.21	Nybom 2004
Short-lived, outcrossing perennial species (allozymes)	43.7	0.123	0.218	Hamrick & Godt 1996
Short-lived, endemic perennial species (allozymes)	32.1	0.083	0.325	Hamrick & Godt 1996
Acacia tortilis subsp. *raddiana* (allozymes)	68.8	0.213	0.044	Zaghloul et al. 2007
Ballota kaiseri (allozymes)	95.2	0.297	0.099	Zaghloul et al. 2006
B. saxatilis (allozymes)	90.5	0.317	0.069	Zaghloul et al. 2006
B. undulate (allozymes)	95.2	0.195	0.045	Zaghloul et al. 2006
Hypericum sinaicum (AFLP)	59.28	0.223	0.17	This paper
Moringa peregrina (allozymes)	40.9	0.143	0.410	Zaghloul et al. 2012
Origanum syriacum (AFLP)	57.10	0.220	0.10	This paper

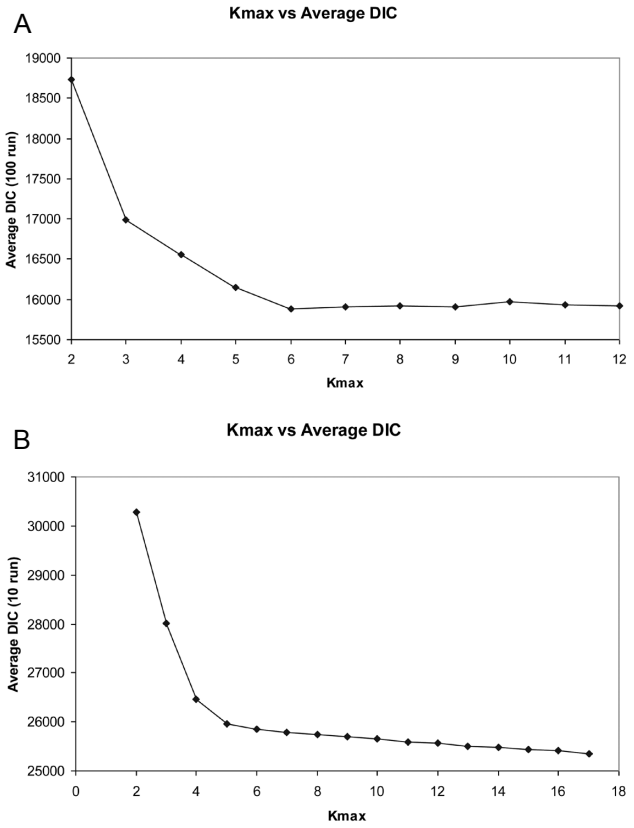

Figure 5 – Deviance information criterion (DIC) as a function of K_{max} in *H. sinaicum* (A) and *O. syriacum* subsp. *sinaicum* (B). K_{max} = the highest number of potentially unobserved parental populations.

DISCUSSION

In this study, AFLP markers have been applied for the first time to assess the genetic variation of species from the Egyptian flora. Our results showed that *H. sinaicum* and *O. syriacum* subsp. *sinaicum* maintain relatively high levels of genetic diversity (table 2). The high genetic diversity observed suggests that these populations should have a high potential to adapt to prospected environmental changes. While allozyme data showed that species with restricted distributions maintain lower genetic diversity than more widespread species (Hamrick & Godt 1989), RAPD-based data showed no association at all, whereas STMS produced a significant result, with lowest values noted for endemic species, followed by narrow-range, widespread and regionally distributed species (Nybom 2004). Our results on *H. sinaicum* and *O. syriacum* subsp. *sinaicum* showed that the molecular variation within populations was not significantly different between both species (T-test: P_{SI} = 0.30, P_{He} = 0.36, P_{UHe} = 0.36, P_{PB} = 0.12). Nevertheless, *H. sinaicum* which is the more locally restricted and endangered species had a higher proportion of polymorphic loci (table 2). This result emphasizes the importance of empirical data for understanding the genetic diversity and structure of specific species. Comparatively recent bottlenecks and the maintenance of genetic diversity within refuge populations have been suggested to be causes of relatively high genetic diversity in rare plant species (Lewis & Crawford 1995). When compared with wide-ranging taxa, rare species are heavily impacted by genetic drift, the founder effect, and directional selection with high levels of inbreeding decreasing genetic diversity by eliminating polymorphic loci and reducing the number of alleles per polymorphic locus (e.g. Dodd & Helenurm 2002, Gitzendanner & Soltis 2000, Sherman-Broyles et al. 1992). However, recent comparative studies of genetic variation between rare and widespread species have demonstrated that several rare species were as polymorphic as their widespread congeners (Dodd & Helenurm 2002, Gitzendanner & Soltis 2000). Thus, it is difficult to state that species with small populations and limited geographic range always have low genetic diversity. While *H. sinaicum* populations had more structured genetic diversity than *O. syriacum* subsp. *sinaicum*, most of the total genetic diversity was found within populations for both species.

Comparing with other short-lived, outcrossing and endemic perennial species (P_S = 43.7% and 32.1, H_{es} = 0.123 and 0.083, and G_{ST} = 0.218 and 0.325, respectively, table 6), *H. sinaicum* and *O. syriacum* subsp. *sinaicum* exhibited much higher genetic diversity and lower genetic differentiation in our study. On the local-scale, three *Ballota* species (Lamiaceae); *B. undulata* (Sieber ex Fresen.) Benth., *B. saxatilis* Sieber ex C.Presl, and *B. kaiseri* Täckh. native to southern Sinai have been analysed for allozyme diversity (Zaghloul et al. 2006). Comparing with these species, which are perennial outcrossing herbs and have approximately the same geographic distribution in southern Sinai, *H. sinaicum* and *O. syriacum* subsp. *sinaicum* had a very similar level of genetic diversity but higher genetic differentiation (table 6). These results indicate that *H. sinaicum* and *O. syriacum* subsp. *sinaicum* display a significant local-scale structure and consistently support the hypothesis that the St. Katherine area has been a centre of diversity and endemism (Ayyad et al. 2000). For comparisons across studies using different genetic markers, estimates of heterozygosity appear to be the most suitable and commonly reported parameters, although these are influenced by choice of bands and loci (Nybom 2004). On the other hand, Percifield et al. (2007) found that only 12% of the variation can be attributed to among-populations differences between *H. perforatum* accessions. In *H. nummularium*, the AMOVA showed that 76% of the total genetic variance was found within populations (Gaudeul 2006).

Variation in genetic diversity found within species representing different phylogenetic clades occurs rather commonly and can result from numerous evolutionary factors (e.g. lineage age, historical hybridization, geographic distribution, and contemporary effective population sizes) (Hamrick & Godt 1996). Meanwhile, habitat fragmentation is viewed as a major force affecting genetic structure of wild populations (Schweiger et al. 2004). Our results show that genetic connectivity among populations of the studied taxa decreases with increasing spatial distance, which could be a result of natural fragmentation. The St. Katherine Protectorate area has a diversity of landforms: slopes, terraces, gorges, plains, ridges, and wadis (Moustafa & Klopatek 1995). Slopes and gorges harbour *H. sinaicum* and *O. syriacum* subsp. *sinaicum* where habitat of smooth faced granite outcrops and terraces with rocky surfaces support sparse species-poor veg-

etation (Ayyad et al. 2000, Zaghloul 1997). As explained by Danin (1983), hard smooth-faced rocks absorb less than two percent of their weight in water and lack small depressions that can store water. Thus, even weak showers result in runoff and may cause flooding of the runnels, gorges and wadis and move seeds for long distances along the route of moving water enabling the studied species to disperse into their habitat within each drainage system. On the other hand, dispersal may be very restricted in non-rainy years. Also, the precipitation in the study area (as a part of a desert) occurs as rainstorms or convective rains which are very local in extent and irregular in occurrence (Danin 1983) and it is normal in the study area to have phases of drought (7–10 years) that alternate with one or more rainy years.

The UPGMA phenograms suggest direct relationships between the studied populations representing each taxon and their geographical origin, similarly as in *Ballota* species from southern Sinai (Zaghloul et al. 2006). Mantel tests confirmed these relationships. Bayesian clustering and plotting averaged estimated admixture coefficients over the 10% runs with the lowest values of the DIC values for K_{max} of *H. sinaicum* and *O. syriacum* subsp. *sinaicum* individuals indicated that the K_{opt} is 6 for *H. sinaicum* and four for *O. syriacum* subsp. *sinaicum*.

Conservation implications

The high allelic diversity and expected heterozygosity within *H. sinaicum* and *O. syriacum* subsp. *sinaicum* populations and the low G_{ST} estimates suggest that historical populations of these species were formerly nearly continuous and that gene exchange among these populations was relatively common. To our knowledge, no information is available on the contemporary gene exchange by insect-mediated pollen flow. Apparently, the loss of genetic variation due to genetic drift has not yet had a major influence on populations of *H. sinaicum* and *O. syriacum* subsp. *sinaicum*. Because both taxa are perennials, recent genetic isolation and reduction of population sizes due to increased human activities (e.g. cutting for medicinal use and habitat destruction) and additional fragmentation due to climate change (Zaghloul et al. 2013) may not have significantly affected genetic diversity yet. Hence, the conservation of genetic diversity naturally occurring in these species should still be possible by a combination of *in situ* and *ex situ* conservation efforts.

Bearing in mind that total genetic variation and structure of the studied species across their entire distribution is not known and since the distribution ranges of the species differ, the sampling in this study may be not representative for both species. Nevertheless, systematic rather than opportunistic selection of populations and areas in St. Katherine Protectorate for *in situ* protection should be based on an understanding of how genetic diversity is distributed within and among wadis (Pressey et al. 1993). The priority of *in situ* conservation for *H. sinaicum* and *O. syriacum* subsp. *sinaicum* should be to conserve a few large well-distributed populations representing different wadis. Special conservation efforts should target HHag and OSha2 and OSha3 populations having the highest diversity in *H. sinaicum* and *O. syriacum* subsp. *sinaicum*, respectively (table 2). Seed collection for *ex situ* conservation should be done across the regional range of the species to ensure a representative sampling of genetic variation.

ACKNOWLEDGMENTS

The authors wish to thank Alexander von Humboldt Foundation for providing funding of this project to the first author under the Georg Forster Fellowship for Experienced Researchers.

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Manuscript received 17 Jan. 2013; accepted in revised version 10 Feb. 2014.

Communicating Editor: Myriam Heuertz.