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Abstract Genetic variations in *Anastatica hierochuntica* populations in Libya, Egypt and Saudi Arabia were investigated among and within multigenerational population cohort's levels. Considering population demography within-population cohorts, the greatest number of individuals was recruited in the mid-season cohort that received intermediate amount of rainfall compared to the early-season and the late-season population cohorts. Individuals of *A. hierochuntica* belonging to the same population cohort showed that spatial pattern varied between clumped and random distribution, with minimum separation distance not exceeding 10 cm. The spatial pattern within-population cohorts showed decreased overdispersion from the early-season toward the late-season individuals. Considering the spatial relationships between the within-population cohorts, the spatial relationships between early-season and mid-season, and mid-season and late-season cohorts varied between segregation and random distributions. The gene diversity and the number of recruited individuals were found to be not correlated with the amount of rainfall in the study regions. Nei's genetic identity and distance varied among sites and population cohort groups. The overall genetic diversity was lower in the mid-season cohort group than in the early- and late-season cohorts. The variations of genetic characters and spatial patterns among and within-population cohorts of *A. hierochuntica* are regulated by recruitment of individuals from persistent seed output produced from overlapping generations.

Keywords Multigenerational populations · Nei's diversity · Genetic identity · Genetic distance · RAPD-PCR · Seedling recruitment · Canopy seed bank · Seed dispersal

1 Introduction

Anastatica hierochuntica L. (Brassicaceae) is a monocarpic desert plant. The species possesses hygrochastic ombrohydrochoric mechanisms restricting seed dispersal to rain events regulating the timing of germination and deposition of seeds in space and time as well as the yearly seed output (Gutterman 1993; Van Oudtshoorn and Van Rooyen 1999; Hegazy et al. 2006; Hegazy and Kabiel 2007, 2010). As reported by Hegazy et al. (2013), the seed output from the dead plant skeletons (canopy seed bank, hereafter CSB) may extend to three decades according to the plant size and amount of CSB. Seeds are released by "ombrohydrochory", i. e., seed dispersal by rain, where the amount of seeds dispersed is directly proportional to the rain force (Friedman et al. 1978). The dispersed seeds generally germinate in the vicinity of mother or source

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Table 1 Study sites locations and climatological parameters of the study populations of *Anastatica hierochuntica*

Population	GPS location	Elevation (m a s l)	Mean annual rainfall (mm)	Mean maximum annual temperature (°C)	Mean minimum annual temperature (°C)
EW	28° 21′ 49.8″ N 29° 11′ 10.3″ E	212	52.2	30.6	15.3
EH	29° 55′ 08.0″ N 32° 11′ 55.9″ E	239	66.4	28.5	14.2
L	32° 07′ 27.3″ N 11° 46′ 37.0″ E	145	93.2	34.3	14.8
S	25° 14′ 56.9″ N 46° 37′ 42.2″ E	591	101.7	34.2	18.9

L Al-Watya site in Libya, *EW* Bahareya Oasis site in Egypt, *EH* Wadi Hagoul site in Egypt, and *S* Thumamah National Park site in Saudi Arabia

plants (topochory mode of seed dispersal), or carried by water sheets to depressions (hydrochory). The overall spatial pattern of *A. hierochuntica* skeleton resulted from the interaction between seed dispersal, amount of rainfall and soil topography (Hegazy and Kabiel 2007).

The dead skeletons enclosing seeds may remain for several years or even decades as pure patches (Friedman and Stein 1980; Hegazy and Kabiel 2007). It seems that water-soluble inhibitors leached from dead skeletons by rain and dew interception are responsible for this particular pattern (Hegazy et al. 1990, 2005; Hegazy 1999). In addition, due to allelopathic effect, *A. hierochuntica* may prevent or decrease the opportunity of other species to germinate or grow within its patches (Hegazy et al. 1990).

Persistence and evolution of a species depend on the maintenance of genetic variation within and among populations to adapt to new selection pressures as those exerted by environmental changes (Barrett and Kohn 1991; Brinegar 2009; Cohen 2013). Genetic variations (diversity, divergence or structure) within and among populations depends mainly on the population history, life form, seed dispersal mechanism and habitat type of a plant species (Hamrick and Godt 1996; Shikano et al. 2010; Ali et al. 2012).

Previous study by Hegazy and Kabiel (2007) covered the spatial pattern variation among population size classes without considering the successive population cohorts. The possible genetic and spatial pattern variations among or within-population generations or population cohorts of *A. hierochuntica* individuals have yet not been studied. The aim of the present study is to investigate the genetic variations and spatial pattern distribution within and among population cohorts of *A. hierochuntica.*

2 Materials and methods

2.1 Field data

Populations of *A. hierochuntica* were investigated in the runnel microhabitat in Libya (Al-Watya, hereafter L), Egypt (Wadi Hagoul, Eastern Desert, hereafter EH and Bahareya Oasis, Western Desert, hereafter EW) and Saudi Arabia (Thumama National Park, Riyadh, hereafter S). The study site locations are shown in Suppl. Fig. 1 and site characteristics in Table 1.

The study populations were monitored for new cohorts (generations) during the years 2009–2010 in Libya and Saudi Arabia and during the years 2008–2010 in Egypt. Five permanent plots were established in the study sites (2 m length × 1.5 m width) except for the gravel desert (EH) site, where the dimensions of plots were 2 m length × 1 m width as the width of the runnel is generally within the range of 1 m. The plots were established in sites representing the dominant habitat types of *A. hierochuntica* in every study area. For monitoring the seedling emergence and plant establishment, the position of established individuals was marked and mapped. The newly established individuals in the successive generations were recorded and their numbers added to the total number of the population. For the 2010 growing season, in the EH study site, the emerged seedlings were followed each week throughout the season from February to May. The Cartesian coordinates were recorded for the mapped individuals to be used in the pattern analysis (*cf.* Hegazy and Kabiel 2007). At the end of the field experiment, individual plants belonging to each of the multigenerational population cohorts and within-cohort groups were uprooted and seeds extracted from the dry skeletons and used for raising new plants in the greenhouse to obtain material (green leaves) for further DNA analysis.

2.2 Pattern analysis

The spatial distribution of *A. hierochuntica* individuals in Egypt (EH and EW) was performed on two levels: (1) univariate analysis, i.e., spatial pattern of plant individuals belonging to each of the study multigenerational population cohorts or within-cohort groups ignoring the presence of others; (2) bivariate analysis, i.e., the spatial

interrelationship between each pair of multigenerational population cohorts or cohort groups. The *L*-function was used to study the spatial pattern of individuals (Ripley 1976; Bessag 1977; Upton and Fingleton 1985; Haase 2001; Hegazy and Kabiel 2007). In the case of univariate analysis, the *L*-function was calculated by the equation $L(d) = \sqrt{k(d)/\pi} - d$, where $K(d)$ is the Ripley's *K*-function: $k(d) = n^{-2}A \sum_{i=1}^{n} \sum_{j=1}^{n} wij \cdot I_d(i,j)$, where n is the number of skeletons in the area A and wij is a weighting factor used to reduce the problem of the edge effect (Haase 1995). The term I_d takes the value 1 if the distance between two individual points, i and j, is less than d, and the value 0 otherwise. The value of $L(d)$ indicates the degree of clumping or overdispersion. Values of $L(d) > 0$ indicates a clumped pattern, i.e., greater number of individuals than would be expected if the individuals were randomly distributed. $L(d) < 0$ indicates an overdispersed pattern, i.e., fewer number of individuals within a scale d than would be expected if the individuals were randomly distributed. In the case of bivariate analysis, the *L*-function was calculated by: $L_{12}(d) = \sqrt{k_{12}(d)/\pi} - d$. The value of $L(d) > 0$ indicates aggregation between individuals belonging to different cohorts or cohort groups and $L(d) < 0$ indicates segregation within the scale d. Monte Carlo simulations were performed to estimate the confidence intervals of the *L*-function at a 0.05 significance level. The spatial pattern analysis was carried out using the computer program (SPPA) following Haase (2002).

2.3 Genetic diversity

2.3.1 DNA analysis

The genetic material (seeds) of each multigenerational population cohorts or within-cohort groups was sampled at the end of the experiment from the corresponding individuals. Seeds from three individuals per each of the five study plots in every site were collected randomly, i.e., from a total of 15 individuals in each study site. Seeds from each multigenerational cohort or cohort group were allowed to germinate in pots containing sandy soil, obtained from the plant's natural habitat, until juvenile leaves appeared. Total genomic DNA was extracted using DNeasy plant mini kit (Qiagen). Ten primers were screened for their amplification (Suppl. Table 1). PCR amplification was performed in a total volume of 25 µl containing 10 × reaction buffer, 2.5 µl dNTPs, 2 µl MgCl2, 3 µl/reaction primer, 10 ng of genomic DNA and 5 U/µl of Taq polymerase (promega, Germany). The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England). After a denaturation step for 5 min at 94 °C, the amplification reactions were carried out for 40 cycles. Each cycle comprised 40 s at 94 °C; 1 min of annealing temperature ranged at 36 °C in the primers used and 1 min at 72 °C. The final elongation step was extended to 7 min. Amplification products were resolved by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5 µg/ml) in 1 × TBE buffer at 95 V. PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000). Amplification products were compared with molecular weight marker *I* × (100–1,000 bp).

2.3.2 Data analysis

To assemble the matrix of the RAPD phenotypes, bands were scored as binary presence (1) or absence (0) characters. The study parameters of genetic diversity and differentiation were percentage polymorphic loci (PPL), observed number of alleles (Na), number of effective alleles (Ne), Nei's gene diversity (*h*), Shannon's diversity index (*I*), genetic identity and genetic distance. These parameters were calculated and analyzed on the basis of gene frequencies using POPGENE 3.2 software (Excoffier et al. 1992; Yeh et al. 1999).

3 Results

3.1 Among multigenerational cohort variations

3.1.1 Genetic diversity

In the study sites of Egypt (EW and EH), the highest values for the Nei's gene diversity and Shannon's diversity index were recorded for 2008 generation in the EW site (0.295 and 0.426, respectively) and for 2010 generation in the EH site (0.303 and 0.442, respectively) (Table 2). The Nei's gene diversity and Shannon's diversity index were greater in the EW site than in the EH site in the 2008 generation. The gene diversity in the L site and S site populations attained higher value in 2010 generation (0.264 and 0.301, respectively) as compared to 2009 generation (0.246 and 0.283, respectively). Weak linear relationship is obtained when the total annual rainfall is plotted against the corresponding Nei's gene diversity in all populations in the different study sites (Suppl. Fig. 2).

When comparing the genetic relationship between 2009 and 2010 population cohorts, the EW and EH populations showed greater values as compared to the L site and S site populations (Table 3). The EH population reached the highest value of Nei's genetic identity (0.985) and the lowest value for Nei's genetic distance (0.015). The genetic relationship between the 2008 and 2009 population cohorts showed greater value for Nei's genetic identity (0.895) and

Table 2 Genetic diversity parameters for *Anastatica hierochuntica* individuals from different population cohorts

Population	Cohort	Na	Ne	*h*	*I*	PL	PPL
EW	2008	1.7	1.533	0.295	0.426	14	70
	2009	1.7	1.420	0.249	0.374	14	70
	2010	1.65	1.454	0.255	0.374	13	65
EH	2008	1.6	1.433	0.244	0.355	12	60
	2009	1.7	1.504	0.284	0.414	14	70
	2010	1.75	1.533	0.303	0.442	15	75
L	2009	1.65	1.419	0.246	0.365	13	75
	2010	1.75	1.452	0.264	0.396	15	80
S	2009	1.75	1.487	0.283	0.419	15	65
	2010	1.8	1.520	0.301	0.446	16	75

Na Observed number of alleles, *Ne* effective number of alleles, *h* Nei's gene diversity, *I* Shannon's diversity index, *PL* number of polymorphic loci, *PPL* percentage of polymorphic loci

Table 3 Nei's genetic identity (GI) and genetic distance (GD) between population cohort pairs of *Anastatica hierochuntica*

Population	Generation (year × year)	GI	GD
EW	2008 × 2009	0.895	0.111
	2009 × 2010	0.974	0.027
	2008 × 2010	0.886	0.121
EH	2008 × 2009	0.888	0.119
	2009 × 2010	0.985	0.015
	2008 × 2010	0.892	0.114
L	2009 × 2010	0.874	0.135
S	2009 × 2010	0.868	0.141

lower value for Nei's genetic distance (0.111) in the EW site as compared to the EH site population.

3.1.2 Demography and spatial pattern

The maximum percentage of recruited individuals was observed in 2008 population cohorts and ranged from 24 to 26 % of the total number of individuals in the EW and EH sites, respectively (Fig. 1). The percentage of recruited individuals decreased in successive years as the CSB increased by the cumulative addition of new individuals to the site. The number of recruited individuals ranged from four to six individuals per square meter in the EW site to six to eight individuals per square meter in the EH site. The linear relationship between the numbers of recruited individuals in each population cohort to the corresponding amount of rainfall showed a weak relationship (Suppl. Fig. 3).

In the EW site, the individuals belonging to the 2008 population cohort demonstrated a random distribution pattern at all scales and a clumped distribution at 15 cm with a neighborhood of 5 cm (minimum separation distance) where no individuals from the same cohort were recorded (Fig. 2a). Random distribution at all scales was observed for individuals belonging to 2009 and 2010 population cohorts with a minimum separation distance of 10 and 5 cm, respectively (Fig. 2c, e). The clumped pattern was more pronounced in the EH site for individuals belonging to the 2008 and 2009 cohorts where clumped distribution was detected at 10 cm for both population cohorts and at the 40–45 cm scale for the 2008 population cohort (Fig. 2b, d). Random distribution at all scales was observed for individuals belonging to the 2010 population cohort with a minimum separation distance of 5 cm (Fig. 2f).

The spatial relationship between individuals belonging to the 2008 and 2009 population cohorts was random at all scales with a marginal aggregation at the 50–60 cm scale (Fig. 3a). Similarly, a random distribution at all scales with an aggregation up to 15 cm scale was demonstrated between individuals belonging to the 2008 and 2010 population cohorts (Fig. 3e). Random distributions at all scales with 5 cm minimum separation distance described the spatial relationship between individuals belonging to the 2009 and 2010 population cohorts in the EW site (Fig. 3c) and for the three study cohort interactions for the EH site (Fig. 3b, d, f).

3.2 Within-cohort variations

3.2.1 Genetic diversity

The genetic diversity parameters showed similar values particularly for early-season (ES) and late-season (LS) cohort groups reaching Shannon's diversity index value of 0.428 and 0.435, respectively (Table 4). Lower values for the genetic diversity parameters were recorded in the mid-season (MS) cohort group with Shannon's diversity index 0.370 and Nei's gene diversity 0.237 as compared to values greater than 0.28 in the ES and LS cohort groups (Table 4). The greatest value for Nei's genetic identity was obtained when the ES and LS cohort groups were compared, which reached 0.727 followed by value 0.707 between the LS and MS cohort groups (Table 5). The lowest value for Nei's genetic identity (0.622) was observed when the ES and MS cohort groups were compared. Alternatively, the Nei's genetic distance attained the lowest value 0.319 in ES and LS cohort groups, but reached the highest value of 0.474 between the ES and MS cohort groups. The ES and MS cohort groups attained a value of 0.347 for Nei's genetic distance.

3.2.2 Demography and spatial pattern

The within-season variations in climatic conditions in the EH site during the growing season 2010 is characterized by a decrease in the total amount of rainfall and the relative humidity per month that was coupled with a slight increase

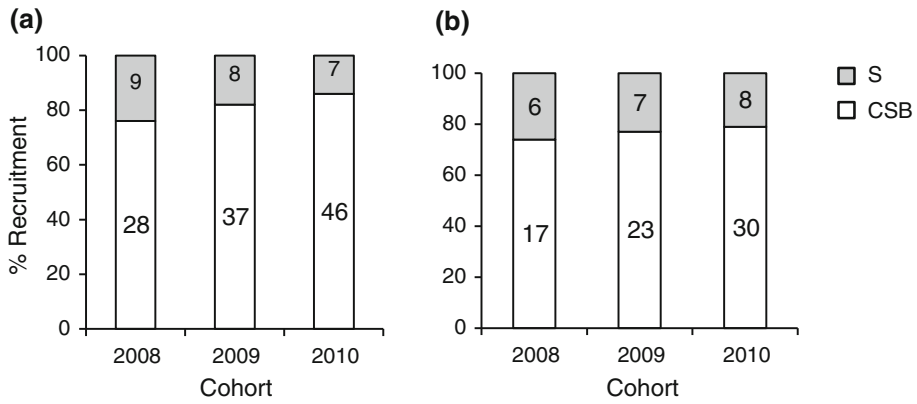

Fig. 1 Percentage of established cohorts (S) in the years 2008–2010 as compared to the canopy seed bank (CSB) in populations of EW site (**a**) and EH site (**b**). The actual numbers of seedlings are indicated in the corresponding *bar*

in temperature as the season proceeded (Fig. 4a). Obviously, the plants in the ES within-cohort group experienced relatively the highest amount of rainfall, which amounted to 2.3 mm/month during a life span that extended for 4 months (Fig. 4a, b). Alternatively, LS individuals received the lowest amount of rainfall (1.1 mm/month) during 2 months (Fig. 4a, b). Five individuals were recorded in the MS within-cohort group (2 mm rainfall/month for 3 months) as compared to only two and one individual in the ES and LS within-cohort groups, respectively (Fig. 4b).

The spatial distribution of *A. hierochuntica* CSB skeletons (old cohorts before the experiment time) showed a clumped distribution up to the 10 cm scale (Fig. 5a). The whole population pattern was also clumped up to the 15 cm scale with the maximum value of $L(d)$ reaching 13.3 at the 5 cm scale (Fig. 5b). The study individuals were randomly distributed at larger scale and showed clumped pattern at the 50 cm scale. The ES and MS within-cohort groups showed a random distribution with a minimum separation distance of 20 and 5 cm, respectively (Fig. 6a, b). Alternatively, the LS within-cohort group showed marginally clumped pattern up to 5 cm scale and random distribution at larger scale (Fig. 6c).

The spatial distribution between the ES and MS and between MS and LS within-cohort groups demonstrated random distributions with minimum separation distances of 25 and 20 cm scales, respectively (Fig. 7a, c). The ES within-cohort group showed an aggregated distribution with the late-season individuals up to the 10 cm scale and a random distribution at larger scale (Fig. 7b).

Comparing the spatial patterns and relationships within and among the within-cohort groups, a maximum association was observed between the ES and LS within-cohort groups where they were closer together than to their own cohort individuals. The separation distance between MS and ES and between MS and LS within-cohort groups was greater than that between their own cohort individuals.

4 Discussion

4.1 Genetic variation

Genetic variations were detected among and within generations in *A. hierochuntica* populations. In spite of the relatively high values of gene diversity in the four study populations (ranging from 0.2440 to 0.3026), no marked differences were observed between the successive cohorts in the same population. Considering the two study populations in Egypt, the genetic distance ranged from 1.5 to 2.7 % between the 2009 and 2010 population cohorts in the EH and EW sites, respectively, to more than 11 % between 2008 and 2009 and between 2008 and 2010 population cohorts. In Libya and Saudi Arabia populations, the genetic distance between 2009 and 2010 cohorts reached 13.5 and 14.1 %, respectively. The Nei's gene diversity of the study population cohorts appears to be not affected by the total annual rainfall in all the study sites. The random and few fluctuations in gene diversity and genetic differentiation among population cohorts irrespective of the amount of rainfall indicate the possible presence of a mechanism homogenizing and preserving the level of gene diversity among generations through years. This mechanism is not dependent on the population size such as the number of established plants in a season depending on the received amount of rainfall (Hegazy and Kabiel 2010). One of the proposed factors that may weaken the genetic differentiation or genetic structure within the same population is the overlapping of seed shadows (Hardesty et al. 2005). Similarly, in a study on *Camellia japonica* L. populations characterized by overlapping generations, Chung et al. (2003) found weak spatial genetic structure within and among age classes and this was rendered to the overlapping seed shadows which overcome limited seed dispersal in the species. In populations where germination was spatially heterogeneous as a result of patchy fire, Ayre et al. (2009) found a lack of intergenerational genetic variation in the

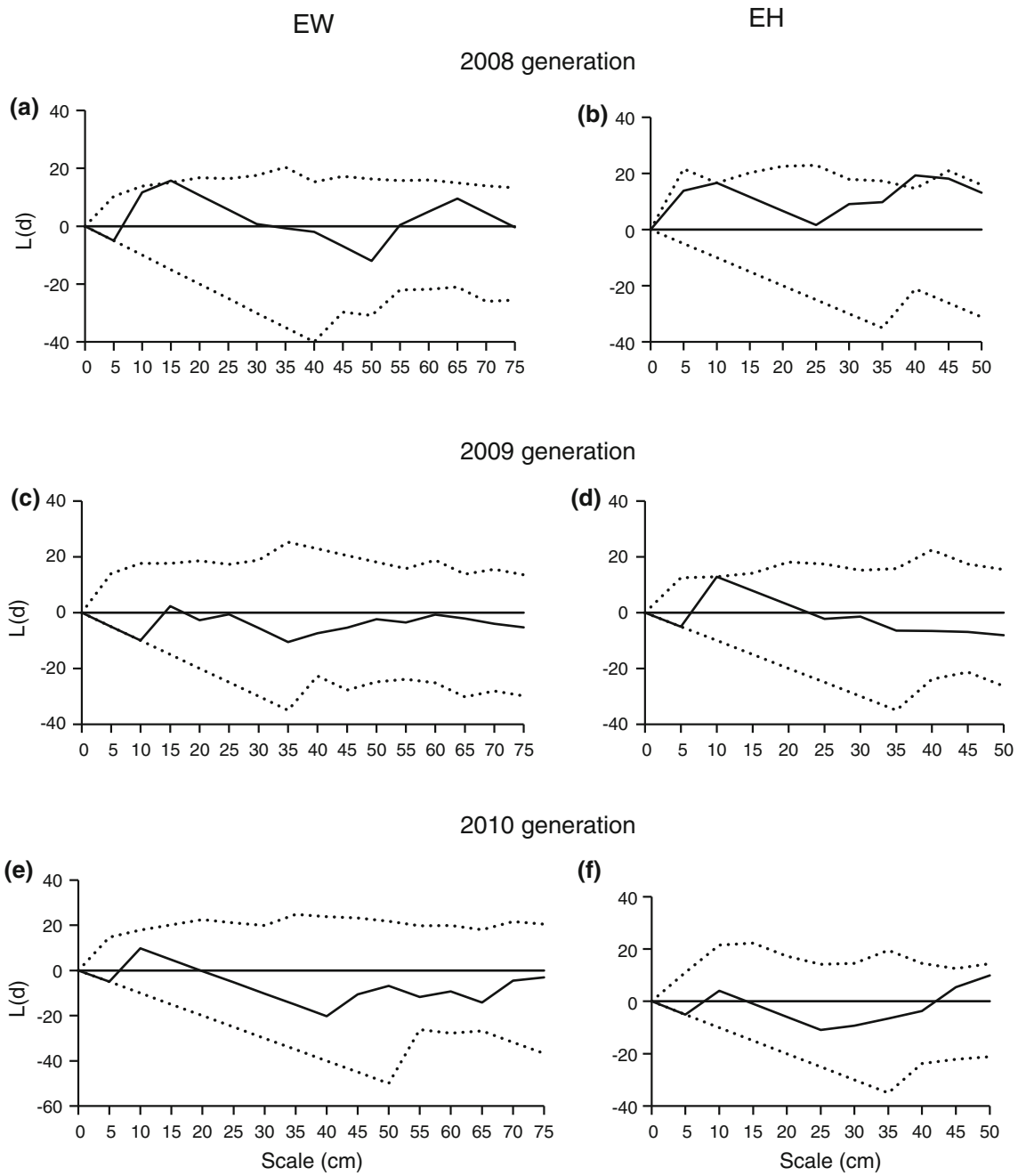

Fig. 2 The statistics $L(d)$ testing the spatial pattern of *Anastatica hierochuntica* at the within-population cohort level in the EW (**a**, **c**, **e**) and EH (**b**, **d**, **f**) sites. *Solid lines* are the values of the statistics calculated from the data. *Dotted lines* delimit the 95 % confidence regions for the random model

successive population cohorts. As suggested by Jacquemyn et al. (2009), the overlapping seed shadows and mixing of genotypes are the major factors explaining the weak spatial genetic structure within populations.

Considering the overall evolutionary situation, the variations of the functional molecular diversity in plants have congruence with the environmental conditions on local, regional and global levels (Schmitt et al. 1999; Ackerly et al. 2000; Matesanz and Valladares 2013). This explains the development of similar molecular adaptive mechanisms in a particular species within its distribution range, an important asset for development of evolutionary traits in the plant lineages. For example, the repeated overlapping generations in *A. hierochuntica* may influence plant fitness to undergo genetic or molecular evolution, leading to local adaptation in the population at microhabitat level in response to

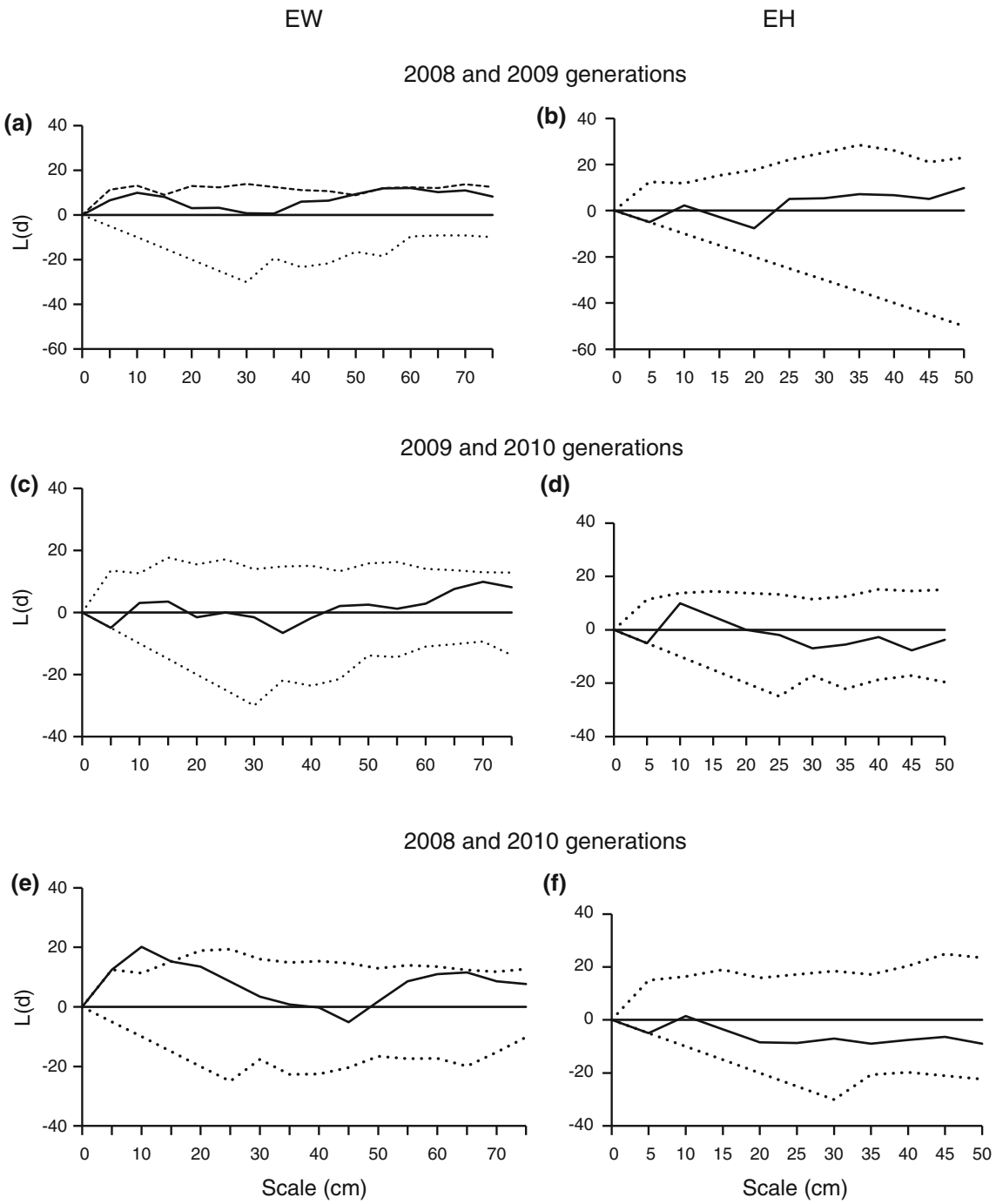

Fig. 3 The statistics $L(d)$ testing the spatial pattern of *Anastatica hierochuntica* at the between-population cohort level in the EW (**a**, **c**, **e**) and EH (**b**, **d**, **f**) sites. *Solid lines* are the values of the statistics calculated from the data. *Dotted lines* delimit the 95 % confidence regions for the random model

environmental variations, at a level just few meters apart (Hegazy and Kabiel 2007; Neel 2008). The heritable differences among populations in different microenvironments in the same growth season within the range of species distribution produce more fit individuals in their respective environments (Schmitt et al. 1999; Brinegar 2009; Ali et al. 2012). Such heritable variations provide the raw material for adaptive evolution of the species.

Fluctuations of higher and lower values of genetic parameters were observed when the within-cohort groups were considered. The genetic diversity parameters were greater in early-season and late-season within-cohort

Table 4 Genetic diversity parameters of *Anastatica hierochuntica* groups within the 2010 cohort in the EH site

Cohort group	Na	Ne	*h*	*I*	PL	PPL (%)
ES	1.800	1.469	0.284	0.428	12	60
MS	1.790	1.374	0.237	0.370	12	60
LS	1.867	1.471	0.281	0.435	13	65

ES Early season, *MS* mid season, *LS* late season, *Na* observed number of alleles, *Ne* effective number of alleles, *h* Nei's gene diversity, *I* Shannon's diversity index, *PL* number of polymorphic loci, *PPL* percentage of polymorphic loci

Table 5 Genetic differentiation parameters of *Anastatica hierochuntica* individual groups within the 2010 cohort in the EH site

Relationship	Nei's genetic identity	Nei's genetic distance
ES × LS	0.727	0.319
ES × MS	0.622	0.474
MS × LS	0.707	0.347

ES early season, *MS* mid season, *LS* late season

groups which are more similar genetically (GI = 0.73) as compared to their relationships with mid-season within-cohort group. This ensures the maintenance of relatively high genetic diversity where the presence of canopy seed bank together with secondary dispersal mechanism in *A. hierochuntica* may result in genetic mixing providing a powerful buffer against demographic and genetic change even in small, isolated and disturbed populations and thus weaken the genetic structure and increase species resilience (Shimono et al. 2006; Ayre et al. 2009). As reported by Parker et al. (2001), among-cohort genetic diversity seems to account for the weak genetic structure in a population.

4.2 Spatial variation

The number of recruited *A. hierochuntica* individuals ranged from four to eight individuals per square meter per population cohort. Considering population demography on the within-cohort level, the greatest number of individuals was obtained in the mid-season cohort group which

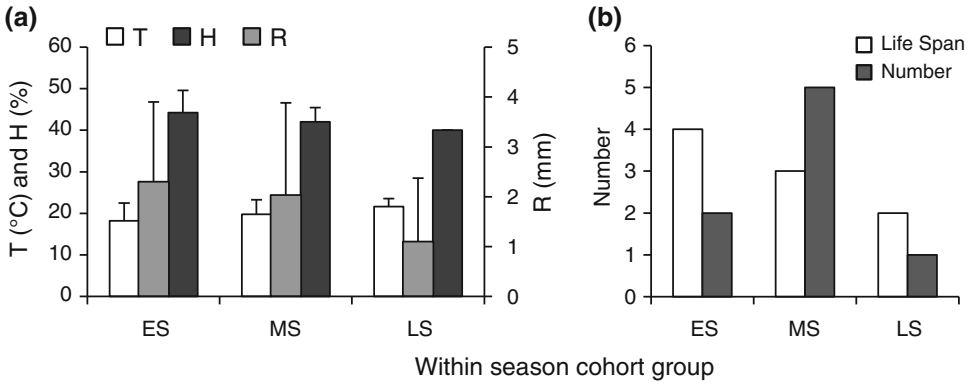

Fig. 4 **a** Within-season variations of climatic conditions during the growing season in the year 2010 for *Anastatica hierochuntica* cohort groups in the EH site. **b** The number of established individuals and the corresponding life span for each cohort group; the early season (ES—February to May), mid season (MS—March to may) and late season (LS—April to May). T = average daily temperature per month (°C) and H = mean relative humidity percent per month on the first axis and R = average total rainfall per month (mm) on the second axis

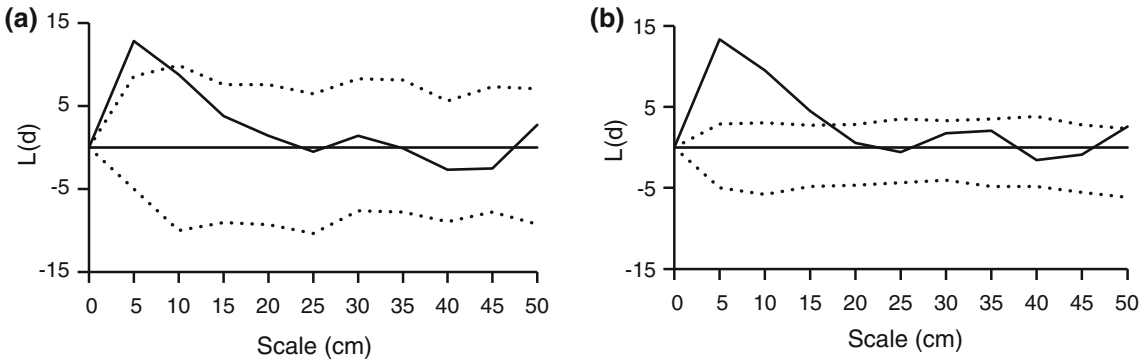

Fig. 5 The statistics $L(d)$ testing the spatial pattern of *Anastatica hierochuntica* at the individual level for CSB skeletons (**a**) and after establishment of the 2010 population cohort (**b**). *Solid lines* are the values of the statistics calculated from the data. *Dotted lines* delimit the 95 % confidence regions for the random model

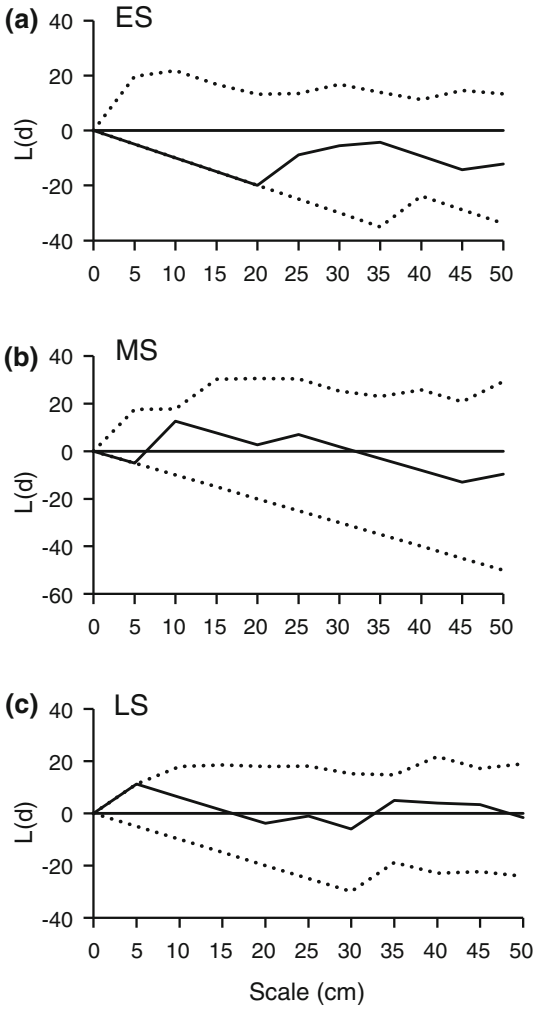

Fig. 6 The statistics $L(d)$ testing the spatial pattern of *Anastatica hierochuntica* at the within-cohort individual level. *ES* early-season, *MS* mid-season and *LS* late-season individuals. *Solid lines* are the values of the statistics calculated from the data. *Dotted lines* delimit the 95 % confidence regions for the random model

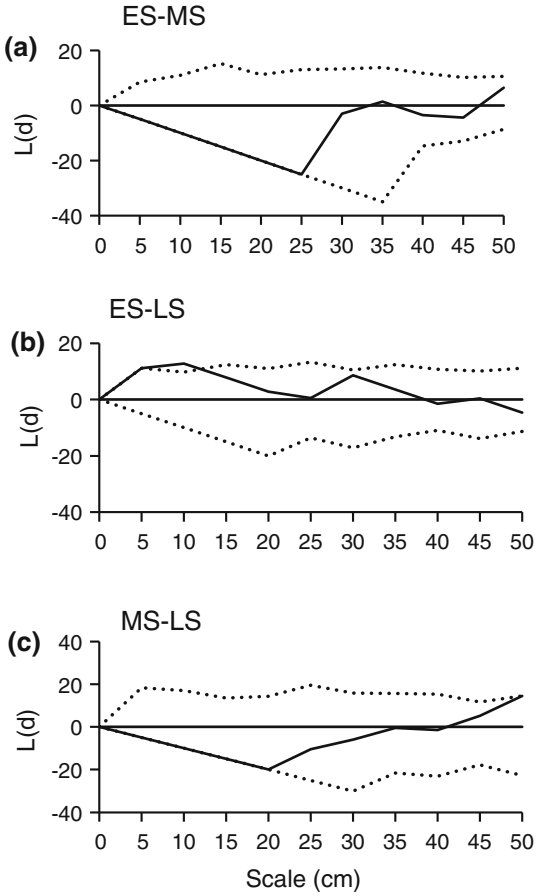

Fig. 7 The statistics $L(d)$ testing the spatial pattern of *Anastatica hierochuntica* at the between and within-cohort groups level. *ES* early-season, *MS* mid-season and *LS* late-season individuals. *Solid lines* are the values of the statistics calculated from the data. *Dotted lines* delimit the 95 % confidence regions for the random model

received comparatively intermediate amount of rainfall between the early-season and late-season cohort groups. The mid-season cohort individuals seem to be dispersed and established as triggered by a rain flash where the established plants benefit from prevailing wet conditions and increased temperature toward the peak growing season (Hegazy 1990).

A clumped pattern of *A. hierochuntica* individuals within the same cohort or aggregation between individuals belonging to two successive cohorts is not well observed. Variations between clumped and random distribution patterns—with minimum separation distance in some cases that did not exceed 10 cm—are shown and random distribution pattern was mostly pronounced. Irrespective of the cohort structure, the spatial pattern of *A. hierochuntica* was described at the population level by Hegazy and Kabiel (2007) to be clumped in the runnel microhabitat types. The present study proves that clumps are multigenerational in origin. The resulting clumped pattern does not mean an aggregation of individuals of one population cohort, where seed dispersal is responsible for such clumped pattern or multigenerational population structure. The topochory mode of seed dispersal is not the sole operating mechanism. Secondary seed dispersal, influenced by environmental variables, was suggested to affect critically the subsequent population and community patterns, particularly in desert ecosystems (Cabin et al. 2000).

In the runnel microhabitat, secondary dispersal of *A. hierochuntica* seeds plays a major role in the spatial distribution of individuals which depends on the amount of rainfall determining the strength of water sheets carrying seeds, the retention of seeds in the canopy seed bank (dry skeletons) and the soil microrelief on which seeds may be anchored (Friedman and Stein 1980; Hegazy and Kabiel 2007). The microsite characteristics including microtopography and plant structures have major role in directing

and trapping secondary dispersed seeds and therefore in seedling recruitment (Schupp 1988; Eriksson and Ehrlén 1992; Bullock and Moy 2004; Hampe 2004; Caballero et al. 2005). In addition, the development of mucilage seed coat around *A. hierochuntica* seeds upon wetting plays an important ecological role by adhering seed to the soil microrelief (Gutterman and Shem-Tov 1997).

As new individuals recruited to the population, the intensity of the clumped pattern increased indicating the recruitment of new individuals in the vicinity of the old plant skeletons. As reported by Shimono et al. (2006), new individuals tend to establish successfully near adults resulting in a clumped pattern. Spatial pattern analyses conducted by Jacquemyn et al. (2009) revealed that adults and recruits showed tight spatial aggregation. Alternatively, Ayre et al. (2009), using parentage analysis, found that seedling cohorts clustered under dead adults (from fire disturbance) displayed low spatial genetic variations. Even for plants having persistent soil seed bank, the aboveground population may be limited to sites favorable for soil seed persistence, which showed positive correlation with soil seed germination (Cabin and Marshall 2000). The increase in the intensity of the clumped pattern after the establishment of new individuals does not mean that seeds germinated in the vicinity of mother plant, as our study reveals that clumps are multigenerational. Instead, a secondary dispersal process in which canopy seed bank skeletons and soil microrelief catch and retain seeds are involved.

The spatial pattern of the within-cohort groups showed a decrease in the degree of overdispersion from the early-season to the mid-season then the late-season individuals. The late-season individuals showed marginally clumped pattern which may be due to more pronounced primary seed dispersal mode as compared to secondary dispersal resulting from lower amount of rainfall late in the season. When the spatial relationships between the within-cohort groups was considered, the spatial relationships between early-season and mid-season and mid-season and late-season cohort groups ranged between segregation and random distributions. Maximum association was observed between early-season and late-season individuals as compared to other spatial relationships. This may be explained by the probable presence of an autopathic effect (Hegazy et al. 2005) on juvenile plants before reaching the flowering stage. Consequently, early-season and late-season cohort groups were observed to be more clumped as compared to other spatial relationships between the within-cohort groups.

5 Conclusions

The persistent canopy seed bank in *A. hierochuntica* populations acts to maintain an overall high genetic diversity and to slow genetic differentiation. Establishment of new individuals from a seed pool produced from overlapping generations ensures genetic homogeneity and slow changes in the population resulting from chronically small population size or bottleneck events (McCue and Holtsford 1998). Even in years with very low amount of rainfall where secondary seed dispersal is not effective, the presence of a persistent canopy seed bank reduces the opportunity for accumulation of within-population genetic structure. Limited seed dispersal was found to have no significant influence on the patterns of spatial genetic structure (Vekemans and Hardy 2004).

The number of recruited individuals each year is observed to be few as compared to the relatively high genetic diversity observed in the populations. Dick (2008) suggests that enhanced gene flow may compensate for low population densities in fragmented landscapes. The persistent canopy seed bank in *A. hierochuntica* appears to represent a significant genetic reservoir that may help preserve genetic diversity by acting as a buffer against the genetic consequences of small population size even with limited seed dispersal (McCue and Holtsford 1998; Morris et al. 2002). The role of the random dispersal process where secondary dispersal is involved cannot be ignored. A persistent canopy seed bank can disperse genes through time and is more likely than a transient one to have opportunities for secondary dispersal, such as by water flow or soil disturbance (Shimono et al. 2006).

Fluctuations of higher and lower values of genetic parameters, within or among successive or overlapping population cohorts, without a well-defined trend coupled with a mostly random spatial distribution of individuals seem to be important for the genetic diversity of the species. The pattern of genetic and spatial variations among and within-population cohorts of *A. hierochuntica* may be rendered to a stochastic (random) process constituting the resultant of many interfering (interdependent) factors as secondary seed dispersal, soil microrelief and possible autopathic effect. This complex process seems to weaken the genetic structure within and among population cohorts and creates clumps of multigenerational origin which could be mistakenly considered as clumps of cohorts recruited in the vicinity of the mother plant.

Populations of *A. hierochuntica* are endowed with a mechanism preserving the genetic diversity due to the multigenerational cohorts' pattern. The temporal genetic variations and spatial patterns of populations in different microhabitats are important for understanding its adaptive evolution and divergence of conspecific populations. Further comparative studies on populations within the geographical range of species distribution in North Africa and West Asia are recommended to understand the species pattern of adaptive evolution.

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References

Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lechowicz MJ (2000) The evolution of plant ecophysiological traits: recent advances and future directions. Bioscience 50:979–995

Ali IB, Guetat A, Boussaid M (2012) Genetic diversity, population structure and relationship of Tunisian *Thymus algeriensis* Boiss. et Reut. and *Thymus capitatus* Hoff. et Link. assessed by isozymes. Ind Crop Prod 36:149–163

Ayre DJ, Ottewell KM, Krauss SL, Whelan RJ (2009) Genetic structure of seedling cohorts following repeated wildfires in the fire-sensitive shrub *Persoonia mollis* ssp. *nectens*. J Ecol 97:752–760

Barrett SCH, Kohn JR (1991) Genetics and evolutionary consequences of small population size in plants: implications for conservation. In: Falk DA, Holsinger KE (eds) Genetics and conservation of rare plants. Oxford University Press, New York, pp 3–30

Bessag JE (1977) Comments on ripley's paper. J Royal Stat Soc B 39:193–195

Brinegar C (2009) Assessing evolution and biodiversity in plants at the molecular level. KUSET 5:149–159

Bullock JA, Moy IL (2004) Plants as seed traps: interspecific interference with dispersal. Acta Oecol 25:35–41

Caballero I, Olano JM, Luzuriaga AL, Escudero A (2005) Spatial coherence between seasonal seed banks in a semi-arid gypsum community: density changes but structure does not. Seed Sci Res 15:153–160

Cabin RJ, Marshall DL (2000) The demographic role of soil seed banks. I. Spatial and temporal comparisons of below- and above-ground populations of the desert mustard *Lesquerella fendleri*. J Ecol 88:283–292

Cabin RJ, Marshall DL, Randall DL, Mitchell J (2000) The demographic role of soil seed banks. II. Investigations of the fate of experimental seeds of the desert mustard *Lesquerella fendleri*. J Ecol 88:293–302

Chung MY, Epperson BK, Gi Chung M (2003) Genetic structure of age classes in *Camellia japonica* (Theaceae). Evolution 57:62–73

Cohen JI (2013) A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution. Cladistics (in press, published online)

Dick CW (2008) New interpretations of fine-scale spatial genetic structure. Mol Ecol 17:1873–1876

Eriksson O, Ehrlén J (1992) Seed and microsite limitation of recruitment in plant populations. Oecologia 91:360–364

Excoffier L, Laval G, Schneider S (1992) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Friedman J, Stein Z (1980) The influence of seed dispersal mechanism on the dispersion of *Anastatica hierochuntica* (cruciferae) in the Negev desert. Isr J Ecol 68:43–50

Friedman J, Gunderman N, Ellis M (1978) Water response of the hygrochastic skeletons of the true rose of Jericho (*Anastatica hierochuntica* L.). Oecologia 32:289–301

Gutterman Y (1993) Seed germination in desert plants (adaptation of desert organisms). Springer, Germany, p 253

Gutterman Y, Shem-Tov S (1997) The efficiency of the strategy of mucilaginous seeds of some common annuals of the Negev adhering to the soil crust to delay collection by ants. Isr J Plant Sci 45:317–327

Haase P (1995) Spatial pattern analysis in ecology based on Ripley's K-function: introduction and methods of edge correction. J Veg Sci 6:575–582 (note corrections to eq. 4 and 5 given in J Veg Sci 7: 304)

Haase P (2001) Can isotropy vs. anisotropy in the spatial association of plant species resolve physical vs. biotic facilitation? J Veg Sci 12:127–134

Haase P (2002) Spatial point pattern analysis (SPPA), computer program version 2.0

Hampe A (2004) Extensive hydrochory uncouples spatiotemporal patterns of seedfall and seedling recruitment in a 'bird-dispersed' riparian tree. J Ecol 92:797–807

Hamrick JL, Godt MJW (1996) Conservation genetics of endangered plant species. In: Avise JC, Hamrick JL (eds) Conservation genetics: case histories from nature. Chapman & Hall, London, pp 281–304

Hardesty BD, Dick CW, Kremer A, Hubbell S, Bermingham E (2005) Spatial genetic structure of *Simarouba amara* Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. Heredity 95:290–297

Hegazy AK (1990) Growth, phenology, competition and conservation of two desert hygrochastic annuals raised under different watering regimes. J Arid Environ 19:85–94

Hegazy AK (1999) Allelopathy in desert ecosystems: an overview, problems and prospects. In: Narwal SS (ed) Basic and applied aspects, vol 2., Allelopathy updateOxford and IBH Publishing Co. Pvt. Ltd, India

Hegazy AK, Kabiel HF (2007) Significance of microhabitat heterogeneity in the spatial pattern and size-class structure of *Anastatica hierochuntica* L. Acta Oecol 31:332–342

Hegazy AK, Kabiel HF (2010) Size-class structure and growth traits of *Anastatica hierochuntica* L. populations as rainfall indicators in arid lands. J Adv Res 1:331–340

Hegazy AK, Elfiky A, Kabiel HF (2005) Spatial pattern and mulching effect of *Anastatica hierochuntica* L. on structure and function of some desert plants. Proceeding of the 4th World Congress on Allelopathy, Charles Sturt University, Wagga Wagga, NSW, Australia, 21–26 August 2005, pp 57–63

Hegazy AK, Mansour KS, Abdel-Hady NF (1990) Allelopathic and autotoxic effects of *Anastatica hierochuntica* L. J Chem Ecol 16:2183–2193

Hegazy AK, Barakat HN, Kabiel HF (2006) Anatomical significance of the hygrochastic movement in *Anastatica hierochuntica*. Ann Bot 97:47–55

Hegazy AK, Kabiel HF, Alatar AA, Lovett-Doust J (2013) Dynamics and persistence of A*nastatica hierochuntica* populations under simulated rainfall treatments. Pol J Ecol 61:493–504

Jacquemyn H, Wiegand T, Vandepitte K, Brys R, Roldán-Ruiz I, Honnay O (2009) Multigenerational analysis of spatial structure in the terrestrial, food-deceptive orchid *Orchis mascula*. J Ecol 97:206–216

Matesanz S, Valladares F (2013) Ecological and evolutionary responses of Mediterranean plants to global change. Environ Exp Bot (in press, published online)

McCue KA, Holtsford TP (1998) Seed bank influences on genetic diversity in the rare annual *clarkia springvillensis* (Onagraceae). Am J Bot 85:30–36

Morris AB, Baucom RS, Cruzan MB (2002) Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*:

evidence for historical changes in genetic structure. Am J Bot 89:29–36

Neel M (2008) Patch connectivity and genetic diversity conservation in the federally endangered and narrowly endemic plant species *Astragalus albens* (Fabaceae). Biol Conserv 141:938–955

Parker KC, Hamrick JL, Parker AJ, Nason JD (2001) Fine-scale genetic structure in *Pinus clausa* (Pinaceae) populations: effects of disturbance History. Heredity 87:99–113

Ripley BD (1976) The second-order analysis of stationary processes. J Appl Prob 13:255–266

Schmitt J, Dudley SA, Pigliucci M (1999) Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade avoidance responses in plants. Am Nat 154:S43–S54

Schupp EW (1988) Factors affecting post-dispersal seed survival in a tropical forest. Oecologia 76:525–530

Shikano T, Shimada Y, Herczeg G, Merilä J (2010) History *vs.* habitat type: explaining the genetic structure of European nine-spined stickleback (*Pungitius pungitius*) populations. Mol Ecol 19:1147–1161

Shimono A, Ueno S, Tsumura Y, Washitani I (2006) Spatial genetic structure links between soil seed banks and above-ground populations of *Primula modesta* in subalpine grassland. J Ecol 94:77–86

Upton G, Fingleton B (1985) Spatial data analysis by example. In: Point pattern and quantitative data, vol 1. John Wiley, New York

Van Oudtshoorn RK, Van Rooyen MW (1999) Dispersal biology of desert plants. Springer, Germany 241 pp

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol 13:921–935

Yeh FC, Yang R-C, Boyle T, Ye Z-H, Mao JX (1999) POPGENE: the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada